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Nutrition and oral health: Public health relevance

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Nutrition and oral health: Public health relevance

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Editorial: Nutrition and oral health: Public health relevance

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oral - general health, nutrition, public health, oral microbiota, tea

Editorial on the Research Topic
Nutrition and oral health: Public health relevance

The roles and regulation of various nutrients in systemic health are well known, although the bifacial association among oral health, diet, and nutrition are not studied in similar depth and detail. Nutritional imbalances, including vitamins C and D, magnesium, zinc, calcium, and phosphate, are shown to be associated with the initiation and propagation of various chronic oral diseases (1–5), including gingivitis and dental decay (6, 7). Sugar-sweetened beverages are also linked to a higher risk of developing non-communicable diseases and oral diseases in low-, middle- and high-income countries (8, 9). Oral manifestations are one of the earliest clues of the evolvement of some prevalent systemic diseases, including metabolic disorders (1, 10). This Research Topic is intended to bring together different dental, medical, and nutritional science specialties to share their experiences in reducing oral health burden and improving systemic diseases and beyond; 17 articles were published on this Research Topic to attain the intended goals.

The oral microbiota is vital to the human microbiome (11). Native microbiota of the oral cavity could prevent adhesion and invasion of pathogens on the oral mucosa to facilitate colonization resistance. The balance between bacterial symbiosis, microbial virulence, and host resistance ensures the integrity of the oral cavity. Akimbekov et al. explained how nutritional factors impact the integrity of the oral indigenous microbiota and its contribution to colonization resistance.

Orthodontic patients are at significant risk for developing oral lesions by facilitating increased plaque formation and bacterial dysbiosis in the oral cavity. Oral probiotics with efficacy against caries offer an attractive option to reduce caries risk in these patients. In a randomized controlled trial, Ebrahim et al. determined the effectiveness of a commercially obtainable Lorodent Probiotic Complex at reducing plaque buildup and Streptococcus mutans bacterial accumulations in adolescent orthodontic patients. Although no significant changes in the oral outcome measures were found in the study, the results offer a baseline for subsequent testing of other potential probiotics in adolescents. Koukou et al. reviewed the possible association between fixed orthodontic treatment and the onset of eating disorders. While limited numbers of case reports suggest that patients develop eating disorders after the initiation of fixed orthodontic treatment, well-designed clinical studies are needed to determine whether fixed orthodontic treatment is the cause of eating disorders.

Several articles on this Research Topic focused on the roles and regulations of vitamins, minerals, and sugar-sweetened beverages in the initiation and progression of various oral diseases. In a population-based study in Brazil, Nascimento et al. found a protective effect of

calcium, but not vitamin D, on periodontitis, mainly among women. In another Brazilian study, Feldens et al. provided recommendations to improve the oral health of the populations. The Brazilian Academy of Dentistry recommended no sugar-containing food to less than 2 years of old children and limiting total sugar consumption to <25 gm/day after 2 years of age. In a systematic review, Zupo et al. identified that alcohol, sugary drinks, and coffee consumption were associated with poor oral health outcomes in the elderly population, including periodontal disease, oral dysbiosis, and tooth loss.

Santosh et al. highlighted the global health burden, economic impact, and oral health inequalities, focusing on the Caribbean region. Mahriani et al. reported the relationship between oral hygiene behavior and adolescent girls' oral health status. The authors found that the higher the oral hygiene behavior score, the higher the oral health condition score, which could be seen in association with the family's socioeconomic status. Al Anouti et al. summarized the accessible data on oral health among children and adolescents in the United Arab Emirates (UAE) over the past decade for developing future strategies to effectively implement preventive and interventional programs.

Dai et al. studied the relationship between serum 25-hydroxyvitamin D concentration and all-cause and cause-specific mortality among adult patients with existing cardiovascular disease. In a large prospective cohort study with 37,080 cardiovascular disease patients, the increase in serum 25(OH)D levels were found to be associated with a reduced risk of all-cause and cause-specific mortality, and the decreasing trend of mortality risk reached a plateau at around 50 nmol/L concentration of 25-hydroxyvitamin D. Huang et al. using the National Health and Nutrition Examination Survey (NHANES) database, reported that blood lead and cadmium levels were positively associated with mean clinical attachment loss with periodontitis, while blood selenium was negatively associated with mean clinical attachment loss. Further research is warranted to determine the underlying mechanism of trace minerals dysregulation. Using the China Health and Nutrition Survey (2000–2011), Qi et al. reported that systolic-diastolic hypertension increased with higher carbohydrate energy intake, which was not observed in isolated systolic hypertension, nor in isolated diastolic hypertension in men and women. A cross-sectional study was performed by Majdi et al. to determine the association between habitual and meal-specific carbohydrate quality index and metabolic syndrome among Iranian adults, but no such association was documented. However, the quantity and quality of the food, eating time, and frequency of eating should be considered for developing healthy eating habits. Murererehe et al. elaborated on the protective roles of vitamin C in reducing oral disease burdens, ranging from cariogenesis to carcinogenesis.

Fluorosis is caused by excessive fluoride intake through drinking water, using fluoride supplementation, or using fluoridated toothpaste. In addition, excessive consumption of brick tea in Tibetan areas can also induce fluorosis. The article by Wen et al. reported the prevalence of dental fluorosis and its relationship with brick-tea consumption among the Tibetan residential area population. Of clinical importance, maternal consumption of fluorinated brick tea may be associated with dental fluorosis in children. A higher probability of brick-tea fluorosis is associated with a higher altitude, and based on the results, Wen et al. recommended the restriction of excessive consumption of high-fluoride brick-tea to avoid dental fluorosis. Deng et al. assessed the potential effect of oolong tea consumption on the risk of oral squamous cell cancer, using 744 newly diagnosed oral squamous cell cancer patients and 1,029 healthy controls. Compared to their non-drink counterparts, patients who drank oolong tea demonstrated a reduced risk of oral squamous cell cancer. Moreover, the reduced risk was associated with tea-drinking habits (for detail, please see the article). Furthermore, subgroup analysis revealed that poor oral hygiene was a confounding factor in the negative association of oolong tea drinking with oral squamous cell cancer risk. Aslam et al. used a novel mathematical model of trimmed regression to determine the relationship between dietary fat consumption and prostate cancer. The authors claimed that the proposed model effectively forecasts prostate cancer patients under an indeterminacy setting.

The Global Burden of Disease Study 2017 has estimated nearly 3.5 billion people around the globe are affected by oral infections and other diseases (12). In low- and middle-income countries with rapid urbanization, lifestyle alterations and dietary behaviors are partly contributed to a higher prevalence of oral diseases. Such a higher rate of oral diseases is predominantly due to nutritional inadequacy and lack of access to primary oral health care.

In summary, the aforementioned articles published in this Research Topic highlight the dietary aspects of closely connected oral lesions, such as the harmful effects of brick-tea in dental fluorosis and the potentially beneficial effects of oolong tea in oral tumorigenesis. These articles also help increase the healthcare providers' awareness of the importance of maintaining nutritional balance to prevent or delay the emergence of oral diseases. Together, this Research Topic highlighted the need for discussion within the healthcare providing community to develop effective nutritional strategies, intended to promote healthier food consumption habits by reducing disease burden and improving oral health for all age range.

Author contributions

MR wrote the first draft. MH, AM and MR revised and approved the final submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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Is There an Association Between Fixed Orthodontic Treatment and Initiation of Eating Disorders? A Review of Currently Available Evidence

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Objectives: The aim was to review the available literature regarding the potential association between fixed orthodontic treatment (OT) and the onset of eating disorders (EDs).

Method and Materials: Six indexed databases were searched until November 2020. The inclusion criteria were as follows: (a) patients undergoing fixed OT and (b) EDs in relation to fixed OT. Commentaries, letters to the Editor, reviews, and studies in patients with EDs not undergoing fixed OT were excluded. The pattern of the present review was customized to summarize the pertinent information.

Results: Four out of 10,076 initially-identified studies were included, and all of them were case reports. All patients were females, and the EDs reported were either anorexia nervosa (AN) or bulimia nervosa (BN). In three case reports, patients developed EDs after the initiation of OT. Fixed OT was performed in all the studies, and a variety of oral complications such as sore mouth, gingivitis, tooth surface demineralization, and others were reported.

Conclusion: Based upon the limited available evidence, the association between OT and the onset of EDs remains unclear. Further well-designed observational clinical studies are needed in this regard.

Keywords: orthodontic therapy, eating disorders, anorexia nervosa, bulimia, review

INTRODUCTION

Eating disorders (EDs) are psychiatric disorders characterized by abnormal routine eating-related behaviors [1–3], and patients often correlate them with psychological concerns related to their weight and body image [1, 2]. A mortality rate of up to 25% has been reported for patients with EDs [4–7]; and the prevalence of EDs is higher in females compared with males [4, 5, 8, 9]. The most common forms of EDs are anorexia nervosa (AN) and bulimia nervosa (BN) [7, 10, 11]. The primary characteristic of AN includes restriction of food intake due to a persistent fear of becoming overweight, even though the affected individual is underweight [1, 5, 10, 11]; whereas, BN is characterized by binge eating which, is often followed by self-induced vomiting in order to maintain

a low body weight [1, 5, 6, 10, 12]. It is well-established that AN and BN are serious conditions that jeopardize the general and psychological health status of patients [4, 5]. They make their appearance more usually in adolescence, which is also the most common period during which orthodontic treatment (OT) is initiated [4, 5, 8, 9, 13]. There are a variety of oral symptoms in patients with EDs including enamel erosion, dental caries, dentinal hypersensitivity, enamel demineralization, malocclusion, and xerostomia; with tooth erosion being the most prevalent [6, 10–12, 14–16].

The OT is commonly performed for the correction of craniofacial disharmonies and dental malocclusion, and its success depends on patient compliance and regular follow-up visits [17, 18]. Moreover, oral hygiene maintenance during the course of OT is a critical factor that influences the success of planned OT as these patients are susceptible to gingival inflammation and enamel demineralization [19, 20]. It has also been suggested that psychological stress may negatively influence the outcome of planned OT in susceptible individuals [21]. Furthermore, alternations in dietary intake and weight status have been reported in patients undergoing OT [22]. Mental and physical development occurs at a rapid rate during adolescence. In addition, adolescence is a time period during which individuals are managing issues such as self-esteem and acceptance by peers [7]. It has also been reported that psychiatric disorders such as EDs may manifest during the adolescent years [7]. Due to the nature of OT, which requires regular follow-up appointments, it is postulated that the orthodontist could be the first health care provider to observe such disorders [7, 10, 11, 23]. For instance, in the study by Jaffa [24], it was reported that AN was manifested in an adolescent female after the initiation of OT. Similar results have been reported by Lee et al. [13] In this regard, the authors of the present study hypothesize that there is a potential association between OT and the onset of EDs. To the authors' knowledge, there are no studies in indexed literature that have reviewed the relationship between OT and EDs.

The aim of this study was to review the available literature regarding the potential association between fixed OT and the onset of EDs.

METHOD AND MATERIALS

Focused Question

In the present review, the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines [25] were followed to assess the following focused question: "Can fixed OT trigger the onset of EDs?"

Eligibility criteria

The inclusion criteria were as follows: (a) patients undergoing fixed OT and (b) EDs in relation to fixed OT. Commentaries, letters to the Editor, reviews, and studies on patients with EDs not undergoing fixed OT were excluded. The pattern of the present review was customized to summarize the pertinent information.

TABLE 1 | Keywords and database search strategy.

Keywords	Search strategy
Orthodontic; orthodontics; orthodontic therapy; orthodontic treatment; eating disorders; anorexia; anorexia nervosa; bulimia; bulimia nervosa	([orthodontic[All Fields] AND ("therapy"[Subheading] OR "therapy"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields])] OR [orthodontic[All Fields] AND ("therapy"[Subheading] OR "therapy"[All Fields] OR "treatment"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields])]) AND [("feeding and eating disorders"[MeSH Terms] OR ("feeding"[All Fields] AND "eating"[All Fields] AND "disorders"[All Fields]) OR "feeding and eating disorders"[All Fields] OR ("eating"[All Fields] AND "disorders"[All Fields]) OR "eating disorders"[All Fields]) OR ("anorexia nervosa"[MeSH Terms] OR ("anorexia"[All Fields] AND "nervosa"[All Fields]) OR "anorexia nervosa"[All Fields]) OR ("bulimia"[MeSH Terms] OR "bulimia"[All Fields])]

Information Sources, Search Strategy, and Study Selection

Six indexed databases [EMBASE, PubMed (National Library of Medicine), Google Scholar, ISI Web of Knowledge, OVID, and Scopus] were searched without time and language restrictions up to and including November 2020. A customized search strategy was implemented by one author (MK) (**Table 1**). The titles and abstracts of relevant articles were screened by two authors (MK and DM), and the full-texts of relevant articles were independently read. A hand-search of the reference lists of relevant articles was also performed to collect possible articles that may have been missed in the previous steps. Disagreements were resolved *via* discussion between three authors (MK, DM, and FJ).

Data Collection and Data Items

Data extraction from the included studies was performed independently by two authors (MK and FJ); and pertinent information was collected as follows: (1) reference, (2) study design, (3) number of patients, (4) demographic information, (5) information regarding EDs and its treatment, (6) chronological association between EDs and OT, (7) type of malocclusion, (8) information regarding OT, (9) orofacial complications, and (10) instructions of the orthodontist. Disagreements related to data extraction were resolved through consensus discussion.

RESULTS

Study Selection

Initially, 10,076 studies were identified, and 10,048 studies were excluded due to irrelevant titles and abstracts or duplicate removal. Twenty-four studies were further excluded after a full-text review based on the previously-mentioned inclusion criteria. A total of four studies [6, 13, 15, 24] were included

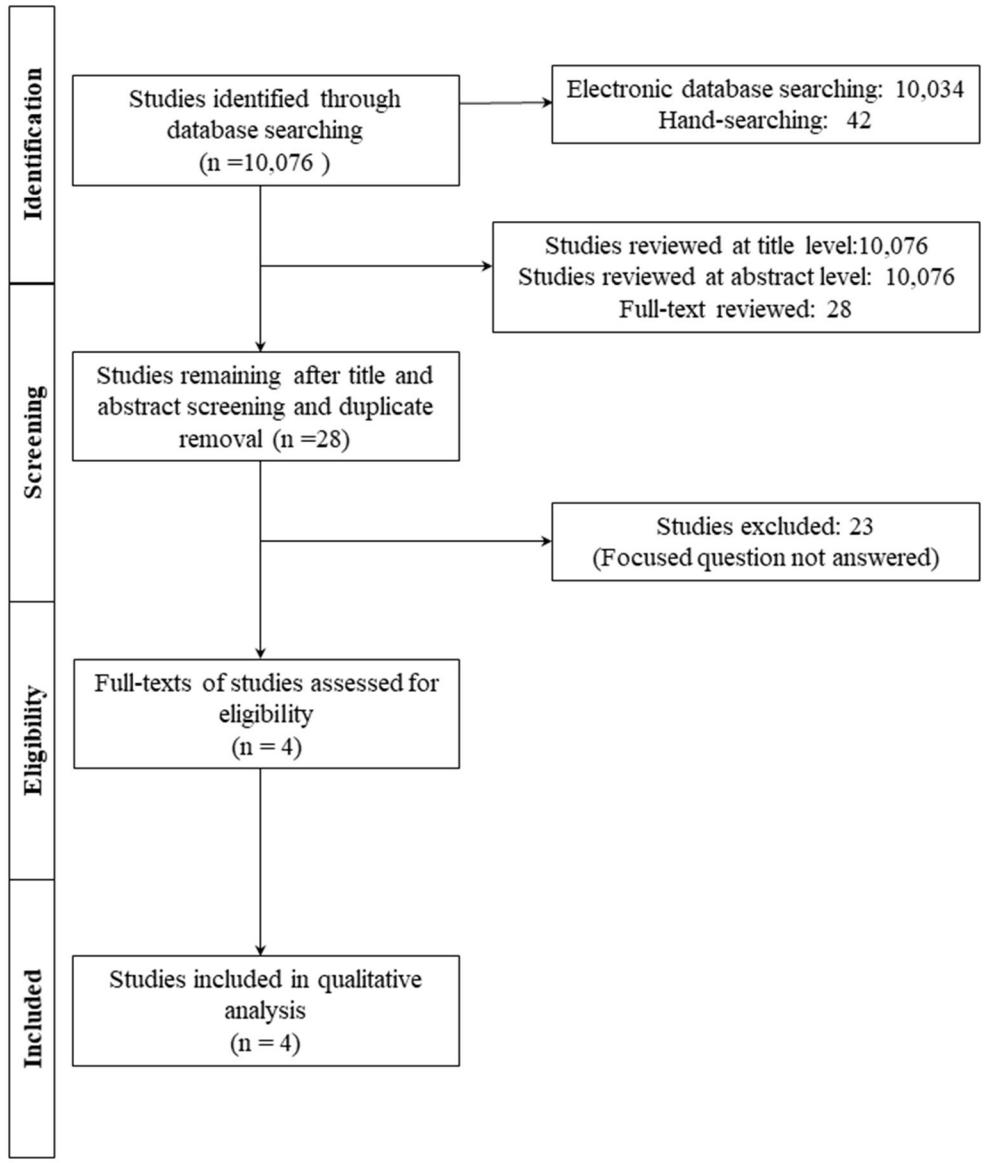

FIGURE 1 | Flowchart of the study.

in this review and analyzed for data extraction (**Figure 1**, **Supplementary Table A**).

General Characteristics of the Included Studies

All studies [6, 13, 15, 24] included in the present review were case reports, and the number of patients ranged between 1 and 3. In the study by Jaffa [24], a total of three patients were included; however, one patient was excluded from the present review since the patient did not undergo OT. In the three case reports [6, 13, 24], the patients were adolescents with their age ranging between 13 and 17 years. In the study by Shaw BM [15], the patient was a 30-year-old adult. All patients described were females [6, 13, 15, 24] (**Table 2**). These case reports were performed in the following countries: United Kingdom [24], Korea [13], France [6], and the United States [15].

Study Characteristics Related to EDs

In two studies [13, 24], patients had AN, and in two studies [6, 15], patients had BN. The onset of EDs in two studies [13, 24] was reported to be at the beginning of OT. In the study by Corega et al. [6], it was reported that the onset of EDs occurred between 4 and 7 months after the initiation of OT. In the study by Shaw BM [15], the patient had undergone OT from 9 to 11 years old, was diagnosed with BN in her teen years, and sought OT again at the age of 30 years after overcoming the ED. Sore mouth after the fitting of the braces, instructions from the orthodontist to avoid sweet foods, and comments/compliments

from peers about the slim body of the patient were reported as potential triggers for the EDs in two studies [13, 24], and two studies [6, 15] did not report such potential triggers. One of the patients [24] seemed to have concerns about her body image before the ED appeared; and three patients [13, 24] did not have any such concerns. In two studies [6, 15], concerns about body image prior to ED were not reported. The diagnosis of EDs was made by a health care professional in two studies [13, 24]. The EDs resulted in the hospitalization of the patients in two studies [13, 24]. The patient in the study by Shaw BM [15] and one of the patients in Corega's report [6] were not hospitalized; while, for the other patient in the Corega's study, information about hospitalization was not reported. Treatment strategies adopted for the management of EDs were hospitalization ranging from 20 days to 4 months [13, 24], medications [13, 24], psychoeducation, supportive treatment, psychiatric treatment or psychotherapy [6, 13, 15, 24], and nutritional rehabilitation [13]. Corega et al. [6] reported two cases in which EDs were manifested in patients undergoing OT; however, in one of the patients, the treatment protocol for the ED was not reported (**Table 3**).

TABLE 2 | General characteristics of the included case reports.

Authors (year)	Number of patients	Age	Gender
Jaffa (2007) [24]	3*	16 years	Female
		14 years	Female
Lee et al. (2015) [13]	2	14 years	Female
		13 years	Female
Corega et al. (2014) [6]	2	13 years	Female
		17 years	Female
Shaw (1994) [15]	1	30 years	Female

**One of the three patients did not undergo orthodontic treatment; and thus, was excluded from the present study based on the inclusion criteria.*

Study Characteristics Related to OT

The type of malocclusion was reported in two studies [6, 15]. Fixed appliances were used in all studies [6, 13, 15, 24]; one patient [6] was scheduled also for orthognathic surgery but OT had to be discontinued at the presurgical phase. In the report by Shaw BM [15], the interproximal surfaces of the mandibular incisors were stripped and 2 months before the fitting of the braces in the lower arch, a removable maxillary bite plane with expansion screws was used. The total duration of OT ranged between 7 and 10 months in the two studies [6, 15] that it was reported. In two studies [13, 24], it was stated that the patients were hospitalized after a period of OT ranging from 7 months to 2 years. Oral complications which were encountered in relation to EDs were sore mouth [13, 24], recurrent oral ulcers [13], generalized gingivitis [6], demineralization [6], white spot lesions [6], diffuse erythema [6], incisal-lingual enamel erosions of the upper incisors, canines and premolars, and lower incisor over-eruption [15]. The OT had to be discontinued in both the patients of the Corega's report [6], it was successfully completed in the report by Shaw BM [15], and in two studies [13, 24], the outcome of OT was not reported. Specific guidelines for the management

TABLE 3 | Study characteristics related to the eating disorder.

Authors (year)	Type of eating disorder	Onset of eating disorder	Potential trigger of abnormal eating behavior	Concerns with body image prior to eating disorder	Diagnosis of eating disorder	Hospitalization due to eating disorder	Treatment of eating disorder
Jaffa (2007) [24]	Anorexia nervosa	At the beginning of OT	Sore mouth after fitting of braces, advice to avoid sweet foods	Yes	By healthcare provider	Yes	Inpatient psychiatric treatment and pharmacological treatment
	Anorexia nervosa	At the beginning of OT	Sore mouth after fitting of braces, comment of friend that braces result in weight lose	No	By healthcare provider	Yes	Inpatient psychiatric treatment
Lee et al. (2015) [13]	Anorexia nervosa	At the beginning of OT	Sore mouth after fitting of braces	No	By healthcare provider	Yes	Psychoeducation, supportive treatment, nutritional rehabilitation
	Anorexia nervosa	At the beginning of OT	Sore mouth after fitting of braces, comments of friends that her slim body looked better	No	By healthcare provider	Yes	Psychoeducation, psychological support, pharmacological treatment
Corega et al. (2014) [6]	Bulimia nervosa	4 months after the initiation of OT	NR	NR	NR	No	Psychiatric treatment
	Bulimia nervosa	7 months after the initiation of OT	NR	NR	NR	NR	NR
Shaw (1994) [15]	Bulimia nervosa	Years before the initiation of OT*	NR	NR	NR	No	Psychotherapy

OT, orthodontic treatment; NR, not reported.

**Patient had a prior history of OT (9–11 years old); and bulimia nervosa was manifested during her teen years. In the present case report, the patient underwent OT again at the age of 30 years when she had overcome the eating disorder.*

TABLE 4 | Study characteristics related to orthodontic therapy.

Authors (year)	Type of malocclusion	Type of OT	Total duration of OT	Oral complications	Treatment of oral complications	Outcome of OT
Jaffa (2007) [24]	NR	Fixed appliances	NR***	Sore mouth	NR	NR
	NR	Fixed appliances	NR***	Sore mouth	NR	NR
Lee et al. (2015) [13]	NR	Fixed appliances	NR****	Sore mouth	NR	NR
	NR	Fixed appliances	NR****	Recurrent oral ulcers, sore mouth	NR	NR
Corega et al. (2014) [6]	Crowding	Fixed appliances	10 months	Generalized gingivitis and denineralizations, WSLs, diffuse erythema	Referral to dentist for restorative treatment	OT was discontinued
	Class II Division 2	Fixed appliances combined with planned orthognathic surgery*	7 months	Generalized gingivitis and denineralizations, WSLs	Referral to dentist for restorative treatment	OT was discontinued
Shaw (1994) [15]**	Class I, anterior closed bite, mandibular anterior crowding, narrow arches	Removable maxillary bite plane with expansion screws, stripping and fixed appliances	∼8 months**	Incisal-lingual enamel erosions of upper incisors, canines and premolars, and lower incisor over-eruption	OT to increase interincisal space followed by placement of porcelain veneer crowns on upper incisors	Successful

OT, orthodontic treatment; NR, not reported; WSLs, white spot lesions.
**The OT was discontinued at the final presurgical phase.*
***The patient also had a prior history of OT when she was 9–11 years old.*
****The author reported time of hospitalization after 2 years of OT.*
*****The authors reported time of hospitalization after 23 months of OT for the first case and after 7 months for the second.*

of patients with ED by the orthodontist were not reported in any of the included studies [6, 13, 15, 24] (**Table 4**).

DISCUSSION

An exhaustive search of indexed literature was conducted to identify studies that addressed the focused question; however, to date, there are no cohort (prospective or retrospective) clinical studies that have assessed the association between OT and the onset of EDs. The only available evidence is in the form of case reports [6, 13, 15, 24]. In this regard, it was difficult to adopt the traditional protocols followed in systematic reviews and meta-analyses. Therefore, the pattern of the present review was customized to primarily summarize the available information.

In summary, results from 75% of the case reports [6, 13, 24] showed that OT triggered the onset of EDs in adolescent female patients. In these studies [6, 13, 24], the patients developed sore mouth after the initiation of fixed OT, and this could have influenced the routine dietary patterns of the patients. It is important to interpret these results with caution as, by no means, do the authors intend to suggest that OT is a risk factor for the onset of EDs. However, these results do suggest that OT may instigate abnormal eating behaviors in susceptible patient populations. In an age and gender-matched controlled cohort study, Shirazi et al. [22] assessed the nutritional intake of adolescents undergoing fixed OT. The results showed that adolescent patients undergoing fixed OT consumed significantly lower amounts of chromium, fiber, and beta-carotene compared with controls [22]. Similarly, Carter et al. [26] investigated the influence of fixed OT on the routine eating habits in teenagers. According to the findings of this study [26] the participants restricted food intake due to factors such as fear of breakage of orthodontic appliances, dietary advice given by their orthodontist, fear of social embarrassment, and alterations in taste perception. Moreover, in this study [26], some participants also reported that fixed OT had a significant impact on their routine dietary habits. Based upon the results reported above [22, 26], it is evident that fixed OT influences the daily eating habits of the patients; however, should these dietary alterations lead to the onset of EDs remains questionable. There is a likelihood that patients undergoing fixed OT would resume their normal eating habits following the completion of OT. However, to date, there are no cohort studies that have assessed the preoperative and postoperative eating habits of patients undergoing OT. Further, studies are needed in this regard.

There is sufficient evidence in indexed literature to confirm that oral and craniofacial health is at risk in patients diagnosed with EDs. Studies have shown that enamel erosion, parotid swellings, alterations in salivary constituents, dry lips, burning tongue, and temporomandibular disorders are more prevalent in patients with than without EDs [27, 28]. Nevertheless, the association between EDs and periodontal diseases remains debatable [28]. Moreover, according to Robinson et al. [29], patients with EDs particularly adolescent females are at increased risk of demonstrating low bone mineral density. In the case reports included [6, 13, 15, 24], oral health-related complications such as sore mouth, recurrent oral ulcers, generalized gingivitis,

demineralizations, white spot lesions, diffuse erythema, and enamel erosions were manifested in all patients undergoing fixed OT. It is noteworthy, that the planned OT was successfully completed in only one case report [15]. However, based on the oral and general health-related complications, OT was discontinued in the study by Corega et al. [6]. Since EDs are a complex issue, a multidisciplinary therapeutic approach is required for the treatment of malocclusion and dentoskeletal deformities in susceptible patient groups. Such an approach may potentially include consultations with Nutritionists, Psychiatrists, Psychologists, Restorative Dentists, Dental Hygienists, and Orthodontists [30].

The risk of bias evaluation is an important aspect of a critical scientific review. Since indexed evidence available to date that addressed our focused question is solely based on case reports, the authors perceive a high risk of bias in these studies [6, 13, 15, 24]. Nevertheless, irrespective of such scientific limitations, the possibility of an existing link between OT and the onset of EDs cannot be overlooked. From an ethical aspect, patients should be informed about possible dietary and oral complications that may be encountered during the course of fixed OT. Likewise, consultations with nutritionists and psychologists for patients planned and/or scheduled to undergo fixed OT might help minimize the risk of the onset of EDs. Routine dental follow-ups in patients undergoing fixed OT may play a role in the early detection of oral complications such as tooth erosion and enamel demineralization that may be potentially induced by latent EDs. Furthermore, psychological stress is a risk factor for the onset of EDs [31] and has also been shown to influence orthodontic tooth movement [21]. The authors of the present study suggest that prescreening of potential candidates for future OT could be done using questionnaires focusing on a history of stress/anxiety disorders as well as EDs. It is, therefore, essential to educate the patients as well as health care providers about the potential bidirectional interaction between EDs and outcomes of OTs and vice versa.

Conclusion

Based on the currently available case reports, the association between fixed OT and the onset of EDs remains unclear. Further well-designed observational clinical studies are needed in this regard.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

FJ was responsible for conceptualization and editing. MK was responsible for writing, methodology, and data extraction. DM was responsible for writing, editing, methodology, and supervision. All authors have read and approved the final draft, contributed equally to the manuscript preparation, and made substantial contributions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/froh.2021.707040/full#supplementary-material

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Association of Serum 25-Hydroxyvitamin D Concentrations With All-Cause and Cause-Specific Mortality Among Adult Patients With Existing Cardiovascular Disease

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Background: Vitamin D insufficiency and deficiency are common in patients with cardiovascular disease (CVD). We aimed to prospectively examine the associations of serum 25-hydroxyvitamin D [25(OH)D] concentrations with all-cause and cause-specific mortality among adult patients with existing CVD.

Methods: We included 37,079 patients with CVD from the UK Biobank study, a prospective cohort of half a million participants aged 40–69 years. We defined patients with CVD as those who suffered coronary heart disease, atrial fibrillation, heart failure, or stroke. The associations of serum 25(OH)D concentration with all-cause and cause-specific mortality were examined by using multivariable Cox regression models and competing risk analyses.

Results: Among 37,079 patients with CVD at baseline, 57.5% were subjected to vitamin D deficiency (i.e., 25[OH]D <50 nmol/L). During a median follow-up of 11.7 years, 6,319 total deaths occurred, including 2,161 deaths from CVD, 2,230 deaths from cancer, 623 deaths from respiratory disease, and 1,305 other-cause deaths. We observed non-linear inverse associations for all-cause, cancer, respiratory disease, and other-cause mortality (*P*-non-linearity <0.01) and approximately linear inverse associations for CVD mortality (*P*-non-linearity = 0.074). Among CVD patients with vitamin D deficiency, per 10 nmol/L increment in serum 25(OH)D concentrations was associated with an 12% reduced risk for all-cause mortality and 9% reduced risk for CVD mortality.

Conclusion: Among patients with existing CVD, increasing levels in serum 25(OH)D were independently associated with a decreased risk of all-cause and cause-specific mortality. These findings suggest that elevated serum 25(OH)D concentration benefits CVD patients with vitamin D deficiency.

Keywords: vitamin D, cardiovascular disease, mortality, cohort study, UK Biobank

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality and poses significant health burdens globally (1). The past few decades have witnessed an escalating research interest in the potential role of vitamin D in CVD prevention (2, 3). Multiple cardiovascular protective mechanisms of vitamin D have been proposed, including suppression of the renin-angiotensin-aldosterone system, increased insulin sensitization, anti-inflammatory actions, inhibition of foam cell formation, and parathyroid hormone (PTH) synthesis (2, 3).

Observational studies have linked low 25-hydroxyvitamin D (25[OH]D) serum levels with increased risk of subsequent CVD (3–8). Despite the accumulating mechanism- and population-based evidence of vitamin D on preventing CVD, recent randomized controlled trials (RCTs) failed to establish the cardiovascular benefits of vitamin D supplementation in the general population or those without a previous history of CVD (9–11). The null findings may be explained by the short-term duration of follow-up, lower event rate than expected, and optimal baseline 25(OH)D levels of population. Although results from these trials are not encouraging, vitamin D status remains an important worldwide public-health concern (12). Vitamin D insufficiency and deficiency are common in patients with CVD (13, 14). Existing evidence from RCTs indicates that vitamin D supplementation exerts beneficial effects on coronary artery disease (CAD) (15), left ventricular structure and function in patients with chronic heart failure (CHF) (16), and inflammatory milieu in patients with CHF (17). However, it does not reduce mortality in patients with advanced heart failure (HF) (18) and does not improve functional capacity or quality of life in older HF patients with vitamin D insufficiency (19).

Evidence from observational studies is limited and inconsistent in this regard (20–27). Prior cohort studies among patients with CVD (20), HF (21–23), suspected coronary artery disease (24), or suspected stable angina pectoris (25) found that plasma 25(OH)D concentrations are inversely associated with all-cause and cardiovascular mortality. However, two cohort studies did not find a significant association between vitamin D levels and all-cause mortality or secondary cardiovascular event incidence (26, 27). Meanwhile, previous studies are subjected to several limitations, including a relatively small sample size, ignoring dietary factors, physical activity, season of vitamin D assessment, and CVD duration and other confounding factors, which might limit the interpretation of the results. Improved understanding of the association between vitamin D status and mortality may shed new light on the potential of vitamin supplementation to provide clinical benefits for patients with CVD in further RCTs. To fill the knowledge gap, we aimed to prospectively examine the associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality among adult patients with existing CVD in a large cohort.

METHODS

Study Population

The UK Biobank is a prospective cohort of half a million participants, aged 40–69 years, recruited between 2006 and 2010 throughout the UK (28). These participants completed extensive touch screen questionnaires, physical and functional measures, and collection of biological samples. Follow-up was conducted through linkages to routinely available national datasets. Ethical approval was obtained by the National Health Service National Research Ethics Service (11/NW/0382) and renewed by the North West–Haydock Research Ethics Committee (16/NW/0274). All participants provided informed written consent. The background information about UK Biobank and details is available on the website http://www.ukbiobank.ac.uk.

Definitions of baseline CVD were constructed using the 9th revision of the International Statistical Classification of Diseases (ICD-9), ICD-10, and Office of Population, Censuses, and Surveys-4 codes as well as self-reported data fields with choice-, disease- or procedure-specific codes. We defined patients with CVD as those who suffered coronary heart disease (CHD), atrial fibrillation (AF), HF, or stroke. Detailed definition is described in **Supplementary Table 1**. In the present study, we excluded participants without CVD at baseline (n = 461,625), leaving 40,849 participants with pre-existing CVD. Participants with missing data on serum 25(OH)D (n = 3,760) and pregnancy (n = 10) were further excluded from analyses. Finally, 37,079 patients with CVD were included (**Supplementary Figure 1**).

Assessment of Vitamin D Status

Vitamin D status was classified according to the serum levels of 25(OH)D. The Endocrine Society Clinical Practice Guidelines define vitamin D severe deficiency as serum 25(OH)D levels <25.0 nmol/L, moderate deficiency as 25.0–49.9 nmol/L, insufficient as 50.0–74.9 nmol/L, and sufficient as ≥75.0 nmol/L (29). In the UK biobank, analysis of serum 25(OH)D concentrations utilized immunoassay analyzers (DiaSorin Liaison XL Analyzer, made in Diasorin S.p.A) by a direct competitive chemiluminescent immunoassay method, with an analytical range of 10–375 nmol/L. A rigorous protocol was adopted to verify the assay and analyzer performance by the following parameters: precision, accuracy (or recovery) and bias, linearity, and reportable range, including the limit of quantification, carryover, and multi-instrument comparison. Additional details of UK Biobank Biomarker Project have been described in the UK Biobank Showcase (http://biobank.ndph.ox.ac.uk/showcase/showcase/docs/serum_biochemistry.pdf).

Assessment of Outcomes

In the UK Biobank study, mortality data of each participant were obtained by linkages to National Health Service (NHS) datasets, including the NHS Digital (for England and Wales) and the NHS Central Register (for Scotland). The date of death and the causes of death were provided and coded using the ICD-10 system. In addition to the all-cause mortality, primary cause of death was extracted from the UK Biobank Cause of Death Registry, including cardiovascular mortality (ICD-10: I00-I99), cancer mortality (ICD-10: C00-C97), respiratory disease mortality (ICD-10: J00-J99), and other-cause mortality (mortality excluding cardiovascular, cancer, and respiratory disease).

Covariates

Information on age, sex, ethnicity, education, household income, and lifestyle behaviors was acquired using touch screen questionnaires. Participants were labeled as never, former, and current smokers according to the summarized smoking status (Field ID: 20116). Daily average alcohol consumption was described depending on the drinking frequency and the number of drink-equivalents/day. Physical activity was classified in accordance with 2018 Physical Activity Guidelines for Americans as inactive (those with no documented leisure time physical activity), insufficient (<150 min/week of moderate activity and <75 min/week of vigorous activity), and active (≥150 min/week of moderate activity and/or ≥75 min/week of vigorous activity) (30). We constructed a healthy diet score with reference to the dietary priorities for cardiometabolic health recommended by American Heart Association (31). Definitions of each component of a healthy diet score are shown in **Supplementary Table 2**. The scale of the healthy diet score ranged from 0 to 10, and a higher score equates to a much healthier dietary pattern. Adherence to a healthy diet was defined as participants who had at least 5 scores of healthy diet components. Socioeconomic status was indicated by Townsend deprivation index scores, and higher Townsend scores equate to higher levels of socioeconomic deprivation (32). Details regarding health conditions and drug use were ascertained by touch screen questionnaires, face-to-face interviews, and linkage to electronic health records. A trained nurse measured the height, weight, and blood pressure during the initial assessment. Serum was collected through venipuncture, and biomarkers including glycated hemoglobinA1c (HbA1c), lipids, and C-reactive protein (CRP) were measured. The estimated glomerular filtration rate (creatinine-cystatin C equation, eGFRcr-cys) was calculated from serum creatinine and cystatin C (33). Further details of these measurements are available on the UK Biobank website (http://www.ukbiobank.ac.uk).

Statistical Analysis

Multivariable Cox regression models to the estimate hazard ratios (HRs) and 95% confidence intervals (CIs) were used for the associations of the serum 25(OH)D concentration with all-cause mortality. Competing risk analyses were conducted using the cause-specific hazard function model to estimate hazards for CVD, cancer, respiratory disease, and other-cause mortality (34, 35). The time to events was calculated from the date of the blood sample collection to the death or the censoring date (31, December, 2020), whichever came first. Participants with severe deficiency of vitamin D (serum 25[OH]D <25.0 nmol/L) were selected as the reference group. Models were successively adjusted for age (continuous), sex (male, female), ethnicity (White, mixed, Asian, Black, Chinese, others), education (college or university, vocational qualification, upper secondary, lower secondary, others), Townsend deprivation index (in quintiles), household income (<18,000; 18,000–30,999; 31,000–51,999; 52,000–100,000; >1,00,000 £), smoking status (never smoker, former smoker, current smoker), alcohol consumption (0, 0.1–4.9, 5.0–14.9, 15.0–19.9, 20.0–29.9, ≥30.0 g/day), physical activity (inactive, insufficient, active), healthy diet score (in quintiles), BMI (<18.5, 18.5–22.9, 23.0–24.9, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m^2), $eGFR_{cr-cys}$ (<30.0, 30.0–60.0, 60.0–90.0, ≥90.0 mL min^{-1} per 1.73 m^2), CRP (in quintiles), anti-hypertensive medication use, cholesterol lowering medication use, diabetes medication use (none, only oral medication, only insulin, or insulin and oral medication), history of cancer, diabetes, hypertension, and CVD duration (<1.0, 1.0–4.9, 5.0–9.9, ≥10.0 years). Missing values of covariates were treated as dummy variables. The dose-response curves presenting the hazard of serum 25(OH)D were fitted by using the restricted cubic spline model with four knots (rms, hmisc, lattice, and survival packages in R software).

Several sensitivity analyses were performed to test the robustness of our results. We performed subgroup analyses across age, sex, BMI, smoking status, physical activity, dietary supplement use, antihypertensive treatment, cholesterol lowering medication, and CVD duration. The joint test was used to obtain a *P*-value for interaction for examining the statistical significance of the difference between subgroups. Considering the influence of seasonal fluctuations and time spent outdoors on circulating 25(OH)D concentrations, we further adjusted for the month of blood collection (January through December, categorical), time spent outdoors in summer (continuous), and time spent outdoors in winter (continuous) in Cox regression models. We further adjusted for blood pressure, HbA1c, lipids, and dietary supplement use, including vitamin D supplements, multivitamin supplements, mineral supplements, fish oil, and glucosamine. We also excluded patients with diagnosed thyroid and parathyroid diseases. In view of the unavailable data on blood parathyroid hormone levels in UK Biobank, we adjusted for the serum levels of calcium and phosphate (36). We also considered energy intake and dietary vitamin D intake of 12,505 patients who completed 24h dietary recalls between April 2009 and June 2012. To minimize the potential reverse causation bias, we excluded patients who died within 4 years and re-examined the association between serum 25(OH)D and mortality.

Statistical analyses were performed between 1 November, 2020 and 13 July, 2021. SAS version 9.4 (SAS Institute, USA) and R software (The R Foundation, http://www.r-project.org, version 4.0.2) were utilized for analyses and plotting with a two-sided significance threshold of $P < 0.05$.

RESULTS

Baseline Characteristics

A total of 37,079 patients with CVD (mean age, 61.4 years [SD, 6.4 years]; 12,668 [34.2%] females) were included in the present analysis. **Table 1** shows the baseline characteristics according to serum 25(OH)D levels. Among these patients, 5,773 (15.6%); 15,557 (42.0%); 11,451 (30.9%); and 4,298 (11.6%) were in the vitamin D status of severe deficiency, moderate deficiency, insufficient, and sufficient, respectively. Compared with patients with vitamin D deficiency, those with higher serum 25(OH)D concentrations were more likely to be older, male, non-smoker, and to have lower levels of socioeconomic deprivation, drink more alcohol, adhere to a healthy diet pattern, exercise more, use more

TABLE 1 | Baseline characteristics of 37,079 patients with cardiovascular disease in UK Biobank.

Characteristics	Serum 25(OH)D concentrations, nmol/L			
	<25 nmol/L (*n* = 5,773)	**25–49.9 nmol/L (*n* = 15,557)**	**50–74.9 nmol/L (*n* = 11,451)**	**≥75 nmol/L (*n* = 4,298)**
Age, mean (SD), years	59.8 (7.0)	61.2 (6.4)	62.1 (6.0)	62.2 (6.0)
Male, *n* (%)	3,604 (62.4%)	10,101 (64.9%)	7,754 (67.7%)	2,952 (68.7%)
Socioeconomic status, median (IQR)	0.2 (−2.6 to 3.3)	−1.4 (−3.3 to 1.8)	−2.2 (−3.7 to 0.4)	−2.4 (−3.7 to −0.1)
Education, *n* (%)				
College or university	1,244 (21.5%)	3,569 (22.9%)	2,513 (21.9%)	934 (21.7%)
Vocational qualification	750 (13.0%)	2,233 (14.4%)	1,717 (15.0%)	628 (14.6%)
Upper secondary	527 (9.1%)	1,316 (8.5%)	982 (8.6%)	350 (8.1%)
Lower secondary	1,239 (21.5%)	3,444 (22.1%)	2,585 (22.6%)	1,039 (24.2%)
Others	1,880 (32.6%)	4,717 (30.3%)	3,463 (30.2%)	1,272 (29.6%)
Unknown	133 (2.3%)	278 (1.8%)	191 (1.7%)	75 (1.7%)
Ethnicity, *n* (%)				
White	4,946 (85.7%)	14,675 (94.3%)	11,179 (97.6%)	4,247 (98.8%)
Mixed	47 (0.8%)	78 (0.5%)	24 (0.2%)	7 (0.2%)
Asian	464 (8.0%)	323 (2.1%)	79 (0.7%)	16 (0.4%)
Black	154 (2.7%)	240 (1.5%)	66 (0.6%)	12 (0.3%)
Chinese	19 (0.3%)	26 (0.2%)	10 (0.1%)	0 (0.0%)
Others	97 (1.7%)	131 (0.8%)	45 (0.4%)	5 (0.1%)
Unknown	46 (0.8%)	84 (0.5%)	48 (0.4%)	11 (0.3%)
Household income, £				
<18,000	2,295 (39.8%)	5,051 (32.5%)	3,446 (30.1%)	1,232 (28.7%)
18,000–30,999	1,090 (18.9%)	3,533 (22.7%)	2,767 (24.2%)	1,061 (24.7%)
31,000–51,999	725 (12.6%)	2,366 (15.2%)	1,960 (17.1%)	744 (17.3%)
52,000–100,000	434 (7.5%)	1,417 (9.1%)	1,054 (9.2%)	419 (9.7%)
>100,000	89 (1.5%)	357 (2.3%)	273 (2.4%)	131 (3.0%)
Unknown	1,140 (19.7%)	2,833 (18.2%)	1,951 (17.0%)	711 (16.5%)
Smoking status, *n* (%)				
Never smoker	2,119 (36.7%)	6,342 (40.8%)	4,843 (42.3%)	1,796 (41.8%)
Former smoker	2,356 (40.8%)	7,168 (46.1%)	5,564 (48.6%)	2,154 (50.1%)
Current smoker	1,238 (21.4%)	1,947 (12.5%)	935 (8.2%)	312 (7.3%)
Unknown	60 (1.0%)	100 (0.6%)	109 (1.0%)	36 (0.8%)
Alcohol consumption, median (IQR), g/day	8.0 (0–23.1)	10.2 (2.2–22.4)	11.9 (3.6–23.0)	13.0 (5.1–25.5)
Adherence to a healthy diet*, *n* (%)	1,130 (19.6%)	3,118 (20.0%)	2,501 (21.8%)	981 (22.8%)
Physical activity, *n* (%)				
Inactive group	1,619 (28.0%)	3,360 (21.6%)	1,850 (16.2%)	640 (14.9%)
Insufficient group	1,617 (28.0%)	4,136 (26.6%)	2,876 (25.1%)	960 (22.3%)
Active group	1,903 (33.0%)	6,738 (43.3%)	5,935 (51.8%)	2,457 (57.2%)
Unknown	634 (11.0%)	1,323 (8.5%)	790 (6.9%)	241 (5.6%)
Dietary supplement use, *n* (%)				
Vitamin D supplements	104 (1.8%)	434 (2.8%)	517 (4.5%)	261 (6.1%)
Multivitamin supplements	489 (8.5%)	2,262 (14.5%)	2,508 (21.9%)	1,049 (24.4%)
Mineral supplements	472 (8.2%)	1,406 (9.0%)	1,477 (12.9%)	711 (16.5%)
Fish oil	926 (16.0%)	4,302 (27.7%)	4,701 (41.1%)	1,935 (45.0%)
Glucosamine	470 (8.1%)	2,065 (13.3%)	2,250 (19.6%)	889 (20.7%)
BMI, mean (SD), kg/m^2	30.4 (6.2)	29.6 (5.2)	28.5 (4.5)	27.4 (4.0)
Antihypertensive medication, *n* (%)	3,638 (63.0%)	9,522 (61.2%)	6,767 (59.1%)	2,517 (58.6%)
Cholesterol lowering medication, *n* (%)	4,173 (72.3%)	11,084 (71.2%)	8,162 (71.3%)	3,217 (74.8%)
Diabetes medication use, *n* (%)				
None	4,747 (82.2%)	13,590 (87.4%)	10,480 (91.5%)	4,001 (93.1%)

(Continued)

TABLE 1 | Continued

Characteristics	Serum 25(OH)D concentrations, nmol/L			
	<25 nmol/L (*n* = 5,773)	**25–49.9 nmol/L (*n* = 15,557)**	**50–74.9 nmol/L (*n* = 11,451)**	**≥75 nmol/L (*n* = 4,298)**
Only oral medication	668 (11.6%)	1,298 (8.3%)	687 (6.0%)	191 (4.4%)
Only insulin	154 (2.7%)	285 (1.8%)	124 (1.1%)	59 (1.4%)
Insulin and oral medication	204 (3.5%)	384 (2.5%)	160 (1.4%)	47 (1.1%)
Hypertension, *n* (%)	4,823 (83.5%)	12,736 (81.9%)	9,093 (79.4%)	3,397 (79.0%)
Diabetes, *n* (%)	1,507 (26.1%)	2,981 (19.2%)	1,561 (13.6%)	471 (11.0%)
Cancer, *n* (%)	733 (12.7%)	2,024 (13.0%)	1,579 (13.8%)	633 (14.7%)
Duration of CVD, years, *n* (%)	613 (10.6%)	1,879 (12.1%)	1,459 (12.7%)	528 (12.3%)
<1				
1–4.9	1,699 (29.4%)	4,735 (30.4%)	3,593 (31.4%)	1,377 (32.0%)
5–9.9	1,583 (27.4%)	4,245 (27.3%)	3,075 (26.9%)	1,156 (26.9%)
≥10	1,878 (32.5%)	4,698 (30.2%)	3,324 (29.0%)	1,237 (28.8%)
eGFRcr-cys, mean (SD), ml min^{-1} per 1.73 m^2	81.5 (17.9)	81.8 (16.4)	82.1 (15.1)	82.2 (15.2)
C-reactive protein, median (IQR), mg/L	2.1 (1.0–4.5)	1.7 (0.8–3.5)	1.5 (0.7–3.0)	1.3 (0.6–2.6)

Data were expressed as the mean (SD), median (IQR), or n (%).

[*] Adherence to a healthy diet was defined as participants who had at least 5 score of healthy diet components.

BMI, body mass index; eGFRcr-cys, estimated glomerular filtration rate (creatinine–cystatin C equation); CVD, cardiovascular disease; IQR, interquartile range; SD, standard deviation.

TABLE 2 | Adjusted means of cardiometabolic markers according to serum 25(OH)D concentrations among 37,079 CVD patients.

Characteristics	Serum 25(OH)D concentrations, nmol/L				P_{trend}
	<25.0 nmol/L (*n* = 5,773)	**25.0–49.9 nmol/L (*n* = 15,557)**	**50.0–74.9 nmol/L (*n* = 11,451)**	**≥75.0 nmol/L (*n* = 4,298)**	
Triglycerides, mg/dL	185.6 (183.2, 187.9)	171.4 (170.0, 172.8)	159.2 (157.6, 160.9)	138.7 (136.0, 141.4)	<0.001
Total cholesterol, mg/dL	181.0 (180.2, 181.9)	176.4 (175.9, 176.9)	173.5 (172.9, 174.1)	166.5 (165.5, 167.5)	<0.001
High-density lipoprotein cholesterol, mg/dL	47.2 (46.9, 47.4)	46.5 (46.4, 46.7)	46.8 (46.6, 47.0)	46.9 (46.5, 47.2)	0.721
Low-density lipoprotein cholesterol, mg/dL	109.9 (109.2, 110.5)	107.3 (106.9, 107.7)	105.3 (104.9, 105.8)	100.2 (99.5, 101.0)	<0.001
Lipoprotein(a), nmol/L	47.9 (46.3, 49.5)	48.9 (47.9, 49.8)	47.3 (46.2, 48.3)	46.4 (44.6, 48.2)	0.048
Apolipoprotein-A, g/L	1.45 (1.44, 1.46)	1.44 (1.43, 1.44)	1.44 (1.44, 1.44)	1.43 (1.42, 1.43)	<0.001
Apolipoprotein-B, g/L	0.92 (0.91, 0.92)	0.90 (0.89, 0.90)	0.88 (0.88, 0.89)	0.84 (0.84, 0.85)	<0.001
Systolic blood pressure, mmHg	139.6 (139.1, 140.0)	138.9 (138.7, 139.2)	138.6 (138.3, 138.9)	137.7 (137.2, 138.2)	<0.001
Diastolic blood pressure, mmHg	80.9 (80.7, 81.2)	80.3 (80.1, 80.5)	80.3 (80.1, 80.4)	79.7 (79.4, 80.0)	<0.001
HbA1c, mmol/mol	40.0 (39.8, 40.2)	39.5 (39.4, 39.6)	39.2 (39.1, 39.4)	39.0 (38.8, 39.2)	<0.001
C-reactive protein, mg/L	3.5 (3.4, 3.7)	3.2 (3.1, 3.2)	3.1 (3.0, 3.2)	3.2 (3.0, 3.3)	<0.001
eGFRcr-cys, ml min^{-1} per 1.73 m^2	81.9 (81.6, 82.3)	82.0 (81.8, 82.2)	82.0 (81.7, 82.2)	81.2 (80.8, 81.6)	0.03

Data were expressed as the adjusted mean (95% CI) and adjusted for age (continuous), sex (male, female), ethnicity (White, mixed, Asian, Black, Chinese, others, or unknown), education (college or university, vocational qualification, upper secondary, lower secondary, others, or unknown), Townsend deprivation index (in quintiles), household income (<18,000, 18,000–30,999, 31,000–51,999, 52,000–1,00,000, >1,00,000 £, or unknown), smoking status (never smoker, former smoker, current smoker, or unknown), alcohol consumption (0, 0.1–4.9, 5.0–14.9, 15.0–19.9, 20.0–29.9, ≥30.0 g/day, or unknown), physical activity (inactive group, insufficient group, active group, or unknown), healthy diet score (in quintiles), BMI (<18.5, 18.5–22.9, 23.0–24.9, 25.0–29.9, 30.0–34.9, or ≥35.0 kg/m^2), antihypertensive medication use (yes, no), cholesterol lowering medication use (yes, no), diabetes medication use (none, only oral medication, only insulin, or insulin and oral medication), history of cancer, diabetes, hypertension, and duration of CVD (<1.0, 1.0–4.9, 5.0–9.9, or ≥10.0 years). CVD, cardiovascular disease; eGFRcr-cys, estimated glomerular filtration rate (creatinine–cystatin C equation); HbA1C, glycated hemoglobinA1c.

dietary supplements, have a lower BMI, and have lower prevalence of hypertension and diabetes but a higher prevalence of cancer.

The adjusted means (95% CIs) of cardiometabolic markers were presented according to serum 25(OH)D concentrations (**Table 2**). Higher levels of serum 25(OH)D were significantly associated with lower levels of triglycerides, total cholesterol, low-density lipoprotein direct, lipoprotein(a), apolipoprotein-A, apolipoprotein-B, systolic blood pressure, diastolic blood pressure, HbA1c, CRP, and eGFRcr-cys (all $P_{trend} < 0.05$). However, levels of high-density lipoprotein cholesterol did not change significantly across serum 25(OH)D categories ($P_{trend} = 0.721$).

TABLE 3 | Associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality among 37,079 CVD patients.

	Serum 25(OH)D concentrations, nmol/L				Per 10 nmol/L increment in 25(OH)D	
	<25.0 nmol/L (*n* = 5,773)	25.0–49.9 nmol/L (*n* = 15,557)	50.0–74.9 nmol/L (*n* = 11,451)	≥75.0 nmol/L (*n* = 4,298)	Patients with 25(OH)D <50.0 nmol/L (*n* = 21,331)	Patients with 25(OH)D ≥50.0 nmol/L (*n* = 15,749)
All-cause mortality						
Incident rate per 1,000 person-year (cases)	21.99 (1,368)	15.55 (2,687)	13.04 (1,679)	12.11 (585)	17.26 (4,055)	12.79 (2,264)
Model 1	1 (ref.)	0.61 (0.58, 0.66)	0.47 (0.44, 0.51)	0.43 (0.39, 0.48)	0.78 (0.76, 0.80)	0.97 (0.94, 0.996)
Model 2	1 (ref.)	0.75 (0.70, 0.80)	0.65 (0.60, 0.70)	0.61 (0.56, 0.68)	0.86 (0.83, 0.89)	0.98 (0.95, 1.01)
Model 3	1 (ref.)	0.78 (0.73, 0.84)	0.70 (0.65, 0.76)	0.66 (0.59, 0.73)	0.88 (0.85, 0.90)	0.98 (0.95, 1.002)
Cardiovascular disease mortality						
Incident rate per 1,000 person-year (cases)	7.76 (483)	5.41 (935)	4.44 (571)	3.56 (172)	6.03 (1,418)	4.20 (743)
Model 1	1 (ref.)	0.61 (0.55, 0.68)	0.46 (0.41, 0.52)	0.37 (0.31, 0.44)	0.80 (0.76, 0.84)	0.92 (0.88, 0.97)
Model 2	1 (ref.)	0.75 (0.67, 0.84)	0.64 (0.56, 0.72)	0.53 (0.44, 0.64)	0.88 (0.84, 0.93)	0.94 (0.89, 0.99)
Model 3	1 (ref.)	0.79 (0.71, 0.89)	0.71 (0.63, 0.81)	0.59 (0.49, 0.70)	0.91 (0.86, 0.96)	0.93 (0.89, 0.98)
Cancer mortality						
Incident rate per 1,000 person-year (cases)	6.65 (414)	5.57 (962)	4.84 (623)	4.78 (231)	5.86 (1,376)	4.82 (854)
Model 1	1 (ref.)	0.71 (0.64, 0.80)	0.57 (0.50, 0.65)	0.55 (0.47, 0.65)	0.83 (0.78, 0.87)	0.99 (0.95, 1.04)
Model 2	1 (ref.)	0.86 (0.77, 0.97)	0.75 (0.66, 0.86)	0.74 (0.63, 0.88)	0.90 (0.85, 0.95)	1.00 (0.96, 1.04)
Model 3	1 (ref.)	0.88 (0.79, 0.995)	0.78 (0.69, 0.89)	0.77 (0.65, 0.91)	0.90 (0.86, 0.95)	1.00 (0.95, 1.04)
Respiratory disease mortality						
Incident rate per 1,000 person-year (cases)	2.60 (162)	1.51 (261)	1.10 (141)	1.22 (59)	1.80 (423)	1.13 (200)
Model 1	1 (ref.)	0.50 (0.41, 0.61)	0.33 (0.27, 0.42)	0.37 (0.27, 0.50)	0.68 (0.62, 0.75)	1.06 (0.98, 1.15)
Model 2	1 (ref.)	0.69 (0.57, 0.85)	0.53 (0.42, 0.67)	0.59 (0.43, 0.81)	0.80 (0.72, 0.88)	1.07 (0.98, 1.16)
Model 3	1 (ref.)	0.72 (0.59, 0.89)	0.58 (0.46, 0.74)	0.64 (0.47, 0.87)	0.81 (0.74, 0.90)	1.06 (0.97, 1.15)
Other mortality						
Incident rate per 1,000 person-year (cases)	4.97 (309)	3.06 (529)	2.67 (344)	2.55 (123)	3.57 (838)	2.64 (467)
Model 1	1 (ref.)	0.54 (0.47, 0.63)	0.44 (0.38, 0.51)	0.42 (0.34, 0.51)	0.73 (0.68, 0.78)	0.96 (0.90, 1.02)
Model 2	1 (ref.)	0.65 (0.56, 0.75)	0.58 (0.50, 0.69)	0.57 (0.46, 0.71)	0.80 (0.74, 0.85)	0.96 (0.90, 1.02)
Model 3	1 (ref.)	0.67 (0.58, 0.77)	0.64 (0.54, 0.75)	0.62 (0.50, 0.77)	0.81 (0.76, 0.87)	0.95 (0.90, 1.02)

Model 1: adjusted for age (continuous), sex (male, female), and ethnicity (White, mixed, Asian, Black, Chinese, others, or unknown).

Model 2: adjusted for model 1 plus education (college or university, vocational qualification, upper secondary, lower secondary, others, or unknown), Townsend deprivation index (in quintiles), household income (<18,000, 18,000–30,999, 31,000–51,999, 52,000–1,00,000, >1,00,000 £, or unknown), smoking status (never smoker, former smoker, current smoker, or unknown), alcohol consumption (0, 0.1–4.9, 5.0–14.9, 15.0–19.9, 20.0–29.9, ≥30.0 g/day, or unknown), physical activity (inactive group, insufficient group, active group, or unknown), healthy diet score (in quintiles), and BMI (<18.5, 18.5–22.9, 23.0–24.9, 25.0–29.9, 30.0–34.9, or ≥35.0 kg/m^2).

Model 3: adjusted for model 2 plus eGFRcr-cys (<30.0, 30.0–60.0, 60.0–90.0, ≥90.0 ml min^{-1} per 1.73 m^2), C-reactive protein (in quintiles), antihypertensive medication use (yes, no), cholesterol lowering medication use (yes, no), diabetes medication use (none, only oral medication, only insulin, or insulin and oral medication), history of cancer, diabetes, hypertension, and duration of CVD (<1.0, 1.0–4.9, 5.0–9.9, or ≥10.0 years). BMI, body mass index; CVD, cardiovascular disease; eGFRcr-cys, estimated glomerular filtration rate (creatinine–cystatin C equation).

Associations of Serum 25(OH)D Concentrations With All-Cause and Cause-Specific Mortality

During 412,046 person-years of follow-up (median 11.7 years), 6,319 total deaths occurred, including 2,161 deaths from CVD, 2,230 deaths from cancer, 623 deaths from respiratory disease, and 1,305 other-cause deaths. In Cox regression analyses, compared with patients in severe deficiency of vitamin D (serum 25[OH]D <25 nmol/L), those with moderate deficiency, insufficient, or sufficient vitamin D status showed a decreased hazard for all-cause and cause-specific mortality (**Table 3**). These associations remained robust after stepwise adjustment for confounders. In the fully adjusted model, the HRs and 95% CIs from the lowest to the highest serum 25(OH)D categories (<25.0, 25.0–49.9, 50.0–74.9, and ≥75.0 nmol/L) were 1.00 (reference), 0.78 (0.73, 0.84), 0.70 (0.65, 0.76), and 0.66 (0.59, 0.73), respectively, for all-cause mortality; 1.00 (reference), 0.79 (0.71, 0.89), 0.71 (0.63, 0.81), and 0.59 (0.49, 0.70), respectively, for CVD mortality; 1.00 (reference), 0.88 (0.79, 0.995), 0.78 (0.69, 0.89), and 0.77 (0.65, 0.91), respectively, for

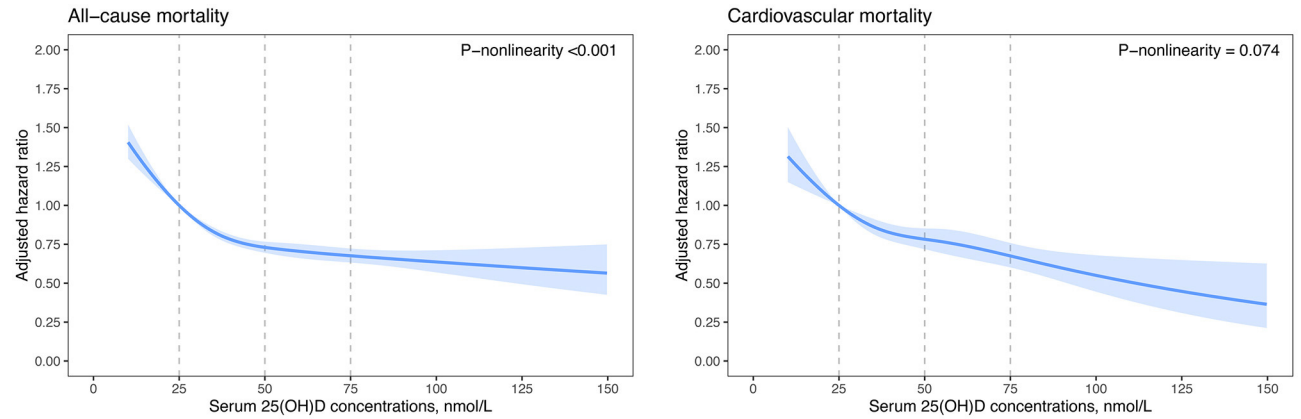

FIGURE 1 | Dose-response curves for serum 25(OH)D concentrations and all-cause and cardiovascular mortality. Hazard ratios (blue lines) and 95% confidence intervals (light blue shade) were adjusted for age (continuous), sex (male, female), and ethnicity (White, mixed, Asian, Black, Chinese, others, or unknown), education (college or university, vocational qualification, upper secondary, lower secondary, others, or unknown), Townsend deprivation index (in quintiles), household income (<18,000, 18,000–30,999, 31,000–51,999, 52,000–1,00,000, >1,00,000 £, or unknown), smoking status (never smoker, former smoker, current smoker, or unknown), alcohol consumption (0, 0.1–4.9, 5.0–14.9, 15.0–19.9, 20.0–29.9, ≥30.0 g/day, or unknown), physical activity (inactive group, insufficient group, active group, or unknown), healthy diet score (in quintiles), and BMI (<18.5, 18.5–22.9, 23.0–24.9, 25.0–29.9, 30.0–34.9, or ≥35.0 kg/m^2), eGFRcr-cys (<30.0, 30.0–60.0, 60.0–90.0, ≥90.0 ml min^{-1} per 1.73 m^2), C-reactive protein (in quintiles), antihypertensive medication use (yes, no), cholesterol lowering medication use (yes, no), diabetes medication use (none, only oral medication, only insulin, or insulin and oral medication), history of cancer, diabetes, hypertension, and duration of CVD (<1.0, 1.0–4.9, 5.0–9.9, or ≥10.0 years). BMI, body mass index; CVD, cardiovascular disease; eGFRcr-cys, estimated glomerular filtration rate (creatinine–cystatin C equation).

cancer mortality; 1.00 (reference), 0.72 (0.59, 0.89), 0.58 (0.46, 0.74), and 0.64 (0.47, 0.87), respectively, for respiratory disease mortality; and 1.00 (reference), 0.67 (0.58, 0.77), 0.64 (0.54, 0.75), and 0.62 (0.50, 0.77), respectively, for other-cause mortality (Model 3 in **Table 3**).

We further fitted smoothing splines to present the dose-response relationship between serum 25(OH)D concentrations and risk of mortality. All-cause and CVD mortality decreased with increasing serum 25(OH)D concentrations and then reached a plateau at around 50 nmol/L 25(OH)D (**Figure 1**). The test of non-linearity was statistically significant (*P*-non-linearity <0.001) for all-cause mortality but not significant for CVD mortality (*P*-non-linearity = 0.074). Similar non-linear trends were observed for cancer, respiratory disease, and other-cause mortality (**Supplementary Figure 2**). Among patients with vitamin D deficiency (serum 25(OH)D <50.0 nmol/L), per 10 nmol/L increment in serum 25(OH)D concentrations was associated with a 12% (HR, 0.88 [95%CI: 0.85, 0.90]) reduced risk for all-cause mortality, 9% (HR, 0.91 [95%CI: 0.86, 0.95]) reduced risk for CVD mortality, 10% (HR, 0.90 [95%CI: 0.86, 0.95]) reduced risk for cancer mortality, 19% (HR, 0.81[95%CI: 0.74, 0.90]) reduced risk for respiratory disease mortality, and a 19% (HR, 0.81 [95%CI: 0.76, 0.87]) reduced risk for other-cause mortality (**Table 3**). However, among patients with 25(OH)D concentrations ≥50.0 nmol/L, increment in 25(OH)D concentrations was not significantly associated with a decreased risk for all-cause and cause-specific mortality, excluding CVD mortality.

We also investigated the associations of vitamin D status with all-cause, CVD, cancer, respiratory disease, and other-cause mortality of various CVD types, including CHD, AF, HF, and stroke (**Table 4**). Among patients with stroke, the HRs and 95% CIs from the lowest to the highest serum 25(OH)D categories were 1.00 (reference), 0.80 (0.70, 0.92), 0.69 (0.59, 0.80), and 0.59 (0.48, 0.73), respectively, for all-cause mortality and 1.00 (reference), 0.87 (0.69, 1.10), 0.73 (0.55, 0.96), and 0.45 (0.29, 0.70), respectively, for CVD mortality.

Sensitivity Analyses

In stratified analyses, the associations of serum 25(OH)D concentration with all-cause and CVD mortality were robust across the strata of age, sex, BMI, smoking status, physical activity, dietary supplement use, antihypertensive treatment, cholesterol lowering medication, and CVD duration (**Supplementary Table 3**). We further plotted smoothing splines to present the influence of season variations and outdoor exposure time on serum 25(OH)D. Serum 25(OH)D concentrations increased with prolonged time spent outdoors, and the seasonal changes in 25(OH)D concentration showed a peak during the summer months and a trough during the winter months (**Supplementary Figure 3**). Additional adjustment of the confounders of the month of blood collection and time spend outdoors did not change the associations between serum 25(OH)D and mortality risk (**Supplementary Table 4**, Model 1). Further sensitivity analyses with additional adjustment of dietary supplement use, mean arterial pressure, HbA1c, triglycerides, and high-density lipoprotein cholesterol produced similar results (**Supplementary Table 4**, Models 2 and 3). These associations did not change appreciably with further adjustment of serum calcium and phosphate after excluding participants with a history of thyroid or parathyroid diseases (**Supplementary Table 5**). In addition, we excluded participants who died within 2 years

TABLE 4 | Hazard ratios for all-cause and cause-specific mortality by serum 25(OH)D among patients with CVD subtypes.

		Serum 25(OH)D concentrations, nmol/L			
	Incident rate per 1,000 person-year	**<25.0 nmol/L**	**25.0–49.9 nmol/L**	**50.0–74.9 nmol/L**	**≥75.0 nmol/L**
Coronary heart disease (*n* = 26,359)					
All-cause mortality	15.86	1 (ref.)	0.79 (0.73, 0.85)	0.70 (0.64, 0.77)	0.67 (0.60, 0.76)
CVD mortality	5.69	1 (ref.)	0.78 (0.69, 0.89)	0.70 (0.6, 0.81)	0.59 (0.48, 0.72)
Cancer mortality	5.39	1 (ref.)	0.93 (0.81, 1.07)	0.79 (0.68, 0.93)	0.82 (0.67, 1.002)
Respiratory disease mortality	1.58	1 (ref.)	0.70 (0.56, 0.89)	0.59 (0.44, 0.77)	0.60 (0.41, 0.87)
Other-cause mortality	3.19	1 (ref.)	0.66 (0.55, 0.78)	0.65 (0.53, 0.79)	0.66 (0.51, 0.85)
Atrial fibrillation (*n* = 6,914)					
All-cause mortality	18.06	1 (ref.)	0.80 (0.69, 0.94)	0.76 (0.64, 0.90)	0.73 (0.58, 0.91)
CVD mortality	7.19	1 (ref.)	0.80 (0.63, 1.03)	0.78 (0.60, 1.03)	0.71 (0.50, 1.01)
Cancer mortality	5.37	1 (ref.)	0.80 (0.59, 1.08)	0.76 (0.55, 1.04)	0.75 (0.50, 1.11)
Respiratory disease mortality	1.65	1 (ref.)	0.96 (0.58, 1.60)	0.75 (0.41, 1.34)	0.99 (0.49, 2.02)
Other-cause mortality	3.84	1 (ref.)	0.76 (0.54, 1.05)	0.76 (0.53, 1.10)	0.64 (0.39, 1.04)
Heart failure (*n* = 2,133)					
All-cause mortality	37.05	1 (ref.)	0.78 (0.65, 0.94)	0.72 (0.58, 0.90)	0.61 (0.45, 0.82)
CVD mortality	19.12	1 (ref.)	0.78 (0.60, 1.01)	0.80 (0.59, 1.09)	0.65 (0.43, 0.996)
Cancer mortality	7.27	1 (ref.)	0.81 (0.49, 1.33)	0.57 (0.31, 1.05)	0.56 (0.27, 1.17)
Respiratory disease mortality	4.20	1 (ref.)	0.56 (0.33, 0.97)	0.61 (0.32, 1.18)	0.69 (0.30, 1.56)
Other-cause mortality	6.47	1 (ref.)	1.14 (0.72, 1.81)	0.85 (0.48, 1.50)	0.79 (0.38, 1.62)
Stroke (*n* = 8,059)					
All-cause mortality	17.07	1 (ref.)	0.80 (0.70, 0.92)	0.69 (0.59, 0.80)	0.59 (0.48, 0.73)
CVD mortality	5.43	1 (ref.)	0.87 (0.69, 1.10)	0.73 (0.55, 0.96)	0.45 (0.29, 0.70)
Cancer mortality	6.23	1 (ref.)	0.90 (0.72, 1.13)	0.80 (0.62, 1.03)	0.68 (0.47, 0.97)
Respiratory disease mortality	1.65	1 (ref.)	0.62 (0.40, 0.94)	0.64 (0.40, 1.04)	0.70 (0.39, 1.28)
Other-cause mortality	3.75	1 (ref.)	0.65 (0.49, 0.86)	0.52 (0.38, 0.73)	0.58 (0.38, 0.90)

Data are presented as hazard ratio (95% confidence interval) and adjusted for age (continuous), sex (male, female), ethnicity (White, mixed, Asian, Black, Chinese, others, or unknown), education (college or university, vocational qualification, upper secondary, lower secondary, others, or unknown), Townsend deprivation index (in quintiles), household income (<18,000, 18,000–30,999, 31,000–51,999, 52,000–1,00,000, >1,00,000 £, or unknown), smoking status (never smoker, former smoker, current smoker, or unknown), alcohol consumption (0, 0.1–4.9, 5.0–14.9, 15.0–19.9, 20.0–29.9, ≥30.0 g/day, or unknown), physical activity (inactive group, insufficient group, active group, or unknown), healthy diet score (in quintiles), BMI (<18.5, 18.5–22.9, 23.0–24.9, 25.0–29.9, 30.0–34.9, or ≥35.0 kg/m^2), eGFRcr-cys (<30.0, 30.0–60.0, 60.0–90.0, ≥90.0 ml min^{-1} per 1.73 m^2), C-reactive protein (in quintiles), antihypertensive medication use (yes, no), cholesterol lowering medication use (yes, no), diabetes medication use (none, only oral medication, only insulin, or insulin and oral medication), history of cancer, diabetes, hypertension, and duration of CVD (<1.0, 1.0–4.9, 5.0–9.9, or ≥10.0 years). BMI, body mass index; CVD, cardiovascular disease; eGFRcr-cys, estimated glomerular filtration rate (creatinine–cystatin C equation).

of follow-up and re-examined the associations. Results did not alter the significance of the associations between 25(OH)D concentration and all-cause and cause-specific mortality (**Supplementary Table 6**). Similar results were also obtained with further adjustment of energy intake and dietary vitamin D intake (**Supplementary Table 7**).

DISCUSSION

In this large prospective cohort study, near 60% of patients with CVD were subjected to vitamin D deficiency (serum 25[OH]D <50 nmol/L). Among patients with existing CVD, increasing levels in serum 25(OH)D were independently associated with a decreased risk of all-cause and cause-specific mortality. Such associations presented non-linear trends for all-cause, cancer, respiratory disease, and other-cause mortality, and a linear trend for cardiovascular mortality. Our findings underscore the importance of optimizing vitamin D status for patients with existing CVD, especially for those subjected to vitamin D deficiency.

Although numerous observational studies have examined the association of vitamin D with all-cause and cause-specific mortality, most studies focused on the general population and deliberately excluded patients with known CVD (37). Existing evidence for the long-term association between vitamin D status and adverse outcomes in CVD patients is inconsistent and insufficient (20–27). One cohort study including 1,125 German patients with stable CHD found no significant association of serum 25 (OH)D levels with secondary cardiovascular event incidence and all-cause mortality (27). In another longitudinal study with 946 stable CHD patients in the San Francisco Bay Area, Welles et al. found that 25(OH)D levels under 50 nmol/L remain independently associated with cardiovascular events, but the independent association is no longer present (adjusted HR, 1.11 [95% CI: 0.85, 1.44]) after further adjustment for potential biological mediators [i.e., blood pressure, lipids, HbA1c,

CRP, and PTH; (26)]. Three small-sample studies found that vitamin D deficiency is associated with an increased risk of ischemic heart disease events among 244 patients with prior CVD (including ischemic heart disease, peripheral vascular disease, and stroke) (20). In addition, a low 25(OH)D concentration is associated with a poor prognosis in patients with HF ($n = 148$ and $n = 548$) (22, 23). A clinic-based study in Israel found that vitamin D deficiency is more prevalent in HF patients ($n = 3{,}009$) than in the control group ($n = 46{,}825$) (21). HF patients with severe deficiency of vitamin D (25[OH]D <25.0 nmol/L) have higher risk of mortality than those with serum 25(OH)D ≥75.0 nmol/L (adjusted HR, 1.61 [95% CI: 1.08, 2.41]). The authors observed that vitamin D supplementation is associated with reduced mortality in HF patients with an (adjusted HR, 0.68 [95% CI: 0.54–0.85]). However, the clinic-based design and relatively short follow-up duration (median 518 days) might limit the generalizability of these findings to the broader population of CVD patients and long-term outcomes. Similar limitations also remained in the hospital-based single-center study comprising 3,316 patients of White ethnicity referred for coronary angiography (24). They also had no clear diagnoses of CVD and subtypes. In another study among 4,114 white patients suspected of having stable angina pectoris, Degerud et al. found that plasma 25(OH)D concentrations are non-linearly (U-shaped) associated with all-cause mortality; near 70 nmol/L 25(OH)D corresponds to the lowest mortality risk, and their analysis suggested increased all-cause mortality at concentrations >100 nmol/L (25). However, our findings were not consistent with this trend. All-cause mortality decreased with increasing serum 25(OH)D concentration and then reached a plateau at around 50 nmol/L 25(OH)D. A small amount could potentially decrease the risk of cardiovascular mortality with higher 25(OH)D concentrations (>100 nmol/L) in our analyses. This continuing downward trend of all-cause and cardiovascular mortality was more pronounced among patients with stroke. Further clinical trials are warranted to determine the benefits of vitamin D supplementation among patients with existing CVD and subtypes, and the threshold range of 25(OH)D is applicable to vitamin D supplementation.

To the best of our knowledge, this dataset is the largest to examine the associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality among adult patients with existing CVD. Compared with previous studies, the present study considered much more comprehensive confounders, conducted numerous sensitivity analyses, and included various CVD subtypes. Higher serum 25(OH)D levels were significantly associated with lower all-cause and cause-specific mortality among patients with CVD. Such associations presented non-linear trends for all-cause, cancer, respiratory disease, and other-cause mortality and an approximately linear trend for cardiovascular mortality. These results suggest that CVD patients with vitamin D deficiency (25[OH]D <50 nmol/L) are more likely to benefit from optimizing vitamin D status than those without vitamin D deficiency. An interesting finding was that the analysis of patients with stroke showed impressive reductions in CVD mortality for the group with sufficient vitamin D (25[OH]D ≥75.0 nmol/L), with an adjusted HR of 0.45 (95% CI: 0.29, 0.70). This finding implied that stroke patients might derive additional cardiovascular benefits from maintaining more adequate vitamin D status. Although insufficient evidence has been proposed in this regard, our findings provided a novel perspective of vitamin D status and cardiovascular morbidity and mortality in patients with stroke. Nowadays, several RCTs are underway to assess the effect of vitamin D supplementation on patients with heart failure (i.e., NCT03416361, NCT03289637), idiopathic cardiomyopathies (i.e., NCT02517814), and coronary artery disease (i.e., NCT01570309, NCT02996721). In the future, these clinical trials may further demonstrate our findings.

Several possible mechanisms could be involved in explaining the effects of vitamin D in CVD. Vitamin D receptors (VDRs) are widely expressed in the cardiovascular system (cardiomyocytes, vascular endothelial cells, vascular smooth muscle cells, fibroblasts, pericytes, platelets, macrophages, and other immune cells), and activated vitamin D binding to VDRs regulates multiple cardiovascular mechanisms (2). In the blood vessels, vitamin D may modulate vascular tone via regulation of calcium influx and stimulation of nitric oxide production in smooth muscle cells (38, 39). In addition, it also exhibits anti-inflammatory actions, inhibition of foam cell formation, and reduction of thrombogenicity and vascular calcifications (39, 40). Vitamin D deficiency accelerates CAD progression by increasing karyopherin α4 and nuclear factor kappa beta levels (41). In the heart, vitamin D treatment may inhibit cardiac hypertrophy, modulate cardiac contractility, regulate extracellular matrix turnover, and attenuate left ventricular abnormalities (42, 43). The cardiovascular system appears to be highly sensitive to vitamin D deficiency (38). In humans, vitamin D deficiency could be linked to vascular dysfunction, arterial stiffness, reduced coronary flow, subclinical atherosclerosis, and left ventricular hypertrophy (44, 45). All the mentioned studies demonstrate that vitamin D treatment could have salutary effects in the development of CVD. Given that the mechanism of whether and how vitamin D deficiency exacerbates the progression of CVD is limited, additional studies are warranted to clarify the potential mechanisms.

The strengths of our study include the large number of participants and the wide range of serum 25(OH)D levels. The high-quality data from UK Biobank favor the definition and classification of various CVD subtypes and allow us to examine the long-term association of vitamin D status with all-cause and cause-specific mortality. Associations were robust after adjustment for a wide range of potential confounding factors, including socioeconomic status, dietary and lifestyle factors, comorbidities, and CVD duration. In addition, the robustness of these findings was confirmed by comprehensive sensitivity analyses. Several potential limitations need to be considered. First, while our research cannot make a causal inference because of the observational nature, it provides evidence to support the importance of conducting future studies using a more rigorous study design, including RCTs. Second, although we considered a wide range of covariates and performed adequate sensitivity analyses, residual confounders and potential bias may still be present. Third, since the UK Biobank cohort included the largely white population, the results from this study may not be directly generalizable to other populations. Fourth, we were unable to access the relationship between dynamic

changes in 25(OH)D concentrations and mortality because the 25(OH)D measurements were not repeated. Additionally, a single measurement of serum 25(OH)D might not represent long-term vitamin D status, but 25(OH)D values do appear to remain relatively stable over time (46). Fifth, although the competing risk of cancer death existed in CVD patients, it appeared to be a minor effect, and the cause-specific hazard function model was used in our cox regression analyses.

CONCLUSION

In the UK Biobank, nearly 60% of CVD patients were subjected to vitamin D deficiency. Among patients with existing CVD, increasing levels in serum 25(OH)D were independently associated with a decreased risk of all-cause and cause-specific mortality in a non-linear and dose-response manner. Compared with CVD patients with serum 25(OH)D $\geq$50 nmol/L, those subjected to vitamin D deficiency benefited more from an increment in serum 25(OH)D. Our findings provided novel clues awaiting further validation in clinical trials.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethical approval was obtained by the National Health Service National Research Ethics Service (11/NW/0382) and renewed by the North West–Haydock Research Ethics Committee (16/NW/0274). All participants provided informed written consent. The background information about UK Biobank and details is available on the website http://www.ukbiobank.ac.uk. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LC conceived and designed the study, revised it critically for important intellectual content, and attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. LD and ML analyzed and interpreted data. LD drafted the manuscript. All authors provided final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.740855/full#supplementary-material

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SYSTEMATIC REVIEW
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Oral Health of Children and Adolescents in the United Arab Emirates: A Systematic Review of the Past Decade

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Oral diseases are a universal public health problem with serious health and economic burdens. These diseases are a major concern in the pediatric population specifically. In the United Arab Emirates (UAE), among all the diseases that affect children, oral diseases, particularly early childhood caries, are the most common despite improvement in the provision of oral health services. Enhancing oral health status is one of the key public health goals in the country. This current systematic review aims to summarize the available data on oral health among children and adolescents in the UAE over the past decade (2011–2021). The review was conducted following a predefined protocol and in concordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement. PubMed, the Cumulative Index to Nursing and Allied Health Literature (CINAHL) *via* EBSCO, EMBASE *via* Ovid, the Cochrane Library, and the Index Medicus for the Eastern Mediterranean Region (IMEMR) databases, and the gray literature were searched for original studies reporting on oral health in the pediatric population in the UAE, without applying any language restriction. Twenty-nine studies were included reporting on a total of 43,916 participants; they were mostly cross-sectional, and emirate-based; they were mostly limited by their setting and convenient sampling. Among the general pediatric population, results showed a high prevalence of dental caries across different emirates. Nevertheless, it was difficult to provide a predictable profile of caries, as risk factors were not well-explored and inconsistent across studies. Suboptimal oral hygiene practices were also prevalent, in addition to a low utilization of dental services. Furthermore, included studies showed a high level of oral problems in children with different disease (down syndrome, cerebral palsy, thalassemia, autism…) and special conditions (children in prison nurseries); yet, in general, treatment indices were lower than their healthy counterparts. This review suggests that dental caries is a major pediatric health problem in the UAE. Risk factors included higher consumption of snacks, being in public schools, lower maternal education level, and socioeconomic status. Nevertheless, risk factors were not well-explored and inconsistent across studies. Suboptimal oral hygiene practices and a low utilization of dental services were also

identified, in addition to a high level of oral problems in children with different disease coupled with lower treatment indices in comparison with their healthy counterparts. This systematic review provides crucial information for planning and evaluating effective oral health programs, identifies gaps in the recent research in this field, and paves the way for preventive and interventional studies targeting oral health in pediatrics in the UAE. Immediate oral health promotion strategies are needed to address this public health problem early in its course by creating conditions that promote oral health, and increasing uptake of dental services. Intensifying research to draw temporal trends, understand the profile of childhood caries in the UAE, and explore cost-effective national community prevention programs are also needed.

Keywords: oral health, child, adolescent, United Arab Emirates, systematic review

INTRODUCTION

Oral diseases encompass a wide range of conditions including dental caries, periodontal disease, tooth loss, and birth defects with dental disease associations such as cleft lip and palate [1]. These diseases affecting 3.5 billion people [2], and posing serious health and economic burdens [3] are undoubtedly a universal public health problem [3]. Fortunately, oral diseases are largely preventable [3], since their modifiable risk factors include mainly unhealthy diets high in simple sugars [1] within an environment of enamel adherent, acid-producing bacteria [4]. Furthermore, the evidence pinpoints an underlying influence of psychosocial, economic, environmental, as well as political factors in oral health, highlighting the importance of addressing underlying social determinants to promote sustainable oral health and reduce inequalities [5].

Oral diseases in the pediatric population are a major concern. Specifically, dental caries is the most common chronic disease in childhood [6], with global rates ranging from 12 to 98% among 4-year-old children [4]. Although the prevalence and severity of dental caries among 5–12-year-olds have declined over the last four decades, the decay-component remains very high [7]. While considered as none life-threatening, oral diseases are however associated with a wide range of clinical consequences amongst children, ranging from pain, discomfort, and lack of sleep [8], to negative impact on self-esteem, eating ability, compromised nutrition, and health [9], to increased absences from school and decreased academic performance [10], as well as decreased quality of life for both the children and their caregivers [3]. Furthermore, childhood caries experience is associated with increased risk of adulthood caries [11]. Oral health is thus a cornerstone for well-being, health, and quality-of-life in the pediatric population.

In high-income countries, the current approach to tackle oral diseases is trapped in a treatment-dominated, high-technology interventionist cycle that does not address the determinants of the disease [12]. Even with a decreased overall disease prevalence in the pediatric population, the progressive and cumulative nature of oral diseases into adult life remains a main issue [12].

In the United Arab Emirates (UAE), among all the diseases that affect children, oral disease, specifically early childhood caries, is the most common [13]. Available evidence indicates that, despite improvement in the provision of oral health services, dental caries remains a pediatric national health problem [14, 15]. Other oral diseases were seldom investigated; nevertheless, available evidence suggests a high prevalence of these diseases. For example, a national survey published in 2009 showed that only 37% of 15-year-olds schoolchildren had healthy periodontal tissues [15]. Enhancing oral health status is one of the key public health goals in the country [16, 17]. So far, numerous public health initiatives, such as the Community Dental Services (CDS)'s national oral diseases preventive program "Dubai Smiles Healthy" have been launched in schools and health centers with the aim of improving pediatric oral health in the UAE [18]. Additionally, the "Abu Dhabi Smiles" [16] is a school-based program launched in 2012 to provide awareness and advice on good oral hygiene practices for both parents and children whose ages range between 5 and 11 years [16]. The latest available literature review on this topic was published in 2014. It included studies published up to the year 2011, was limited to few databases, and focused only on dental caries in children younger than 13 years of age [14].

The current systematic review aims to summarize the available data on oral health among children and adolescents in the UAE over the last decade and hence could be valuable for policy and research considerations. Providing such information is crucial for planning and evaluating effective oral health programs. The review also aims to identify gaps in the recent research related to the pediatric oral health status in the UAE.

MATERIALS AND METHODS

Protocol Registration

A predefined protocol for this systematic review was registered at the OSF registries (DOI 10.17605/OSF.IO/Y7PTZ).

Criteria for Study Inclusion

In terms of Population, Exposure, Comparator, and Outcomes (PECO), our research question was defined as follows: P: pediatric population in the UAE, as well as their caregivers; E: including but not limited to sociodemographic and dietary factors, oral hygiene practices…; C: unspecified, such as poor

dietary practices, suboptimal oral hygiene practices…; and O: including but not limited to prevalence and patterns of oral diseases, determinants and implications of poor oral health, oral hygiene knowledge and practices, utilization of dental services, as well as the effectiveness of programs and interventions aiming to improve oral health.

Hence, studies reporting on the national or Emirate-specific prevalence and patterns of oral diseases in children and adolescents, including dental caries, periodontal disease, tooth loss, birth defects with dental disease associations [1] were included. In addition, studies addressing the determinants of poor oral health, pertaining clinical, quality-of-life, and economic implications, oral hygiene knowledge and practices of children and their caregivers, utilization of dental services among healthy children and adolescents, and those with disease, and the effectiveness of programs and interventions aiming to improve oral health in the target population were also included.

Included studies had to be original articles (non-original studies such as editorials, case reports, case series, and reviews were excluded), address oral health in the pediatric population (i.e., in people aged <19 years), report data specific for UAE's citizens irrespective of their nationality (nationals and/or expatriates), sex (female or male), or health status and irrespective of the design (observational or interventional). Studies published after 2010 were included, without any language restriction.

Search Strategy

The searches were run on PubMed, the Cumulative Index to Nursing and Allied Health Literature (CINAHL) *via* EBSCO, EMBASE *via* Ovid, the Cochrane Library, and the Index Medicus for the Eastern Mediterranean Region (IMEMR) databases. The bibliographies of included articles and previous relevant reviews were also hand-searched for eligible studies.

The search strategy considered three key terms: [1] United Arab Emirates, [2] children and adolescents, and [3] oral health. For each term, controlled vocabulary terms and text words were mapped. The electronic search strategy (**Supplementary Material 1**) was validated by a medical information specialist, and the search was run of May 4th 2021.

Study Selection

One pair of authors (SH and RR) screened the titles and/or abstracts from electronic scientific databases using EndNote, version X6, and identified studies that potentially meet the inclusion criteria outlined above. Two pairs of authors (FA and RR; SH and NM) then screened the full texts of potentially eligible studies in addition to the records identified through the gray literature search. Calibration exercises were conducted before each step of this process; furthermore, study selection was conducted independently and in duplicate. Discrepancies were solved through discussions or with the help of a third reviewer.

Data Extraction

Following a calibration exercise, two pairs of authors (FA and RR; SH and NM) extracted data from eligible studies using a data extraction form. This was done independently by each pair of authors and in duplicate, whereby discrepancies were solved through discussions or with the help of a third reviewer.

Data Synthesis

A narrative synthesis of the findings from the studies was provided including the author-recorded characteristics of the study, details of the population included, as well as the study's methodology and main findings.

Risk of Bias Assessment

Two pairs of authors (FA and RR; SH and NM) independently and in duplicate performed risk of bias assessment of the studies using the Newcastle—Ottawa Quality Assessment Scale adapted for cross sectional studies [19]. This scale assesses the appropriateness of representativeness of the sample, sample size, response rate, comparability in different outcome groups, and exposure and outcome assessment. Discrepancies between pairs of authors were solved through discussions or with the help of a third reviewer.

Quality of Reporting

The authors followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses literature search extension (PRISMA-S) checklist for the literature searching component of the systematic review [20], and the PRISMA statement for the reporting of the systematic review [21].

RESULTS

Search Results

The details of the search process are detailed in **Figure 1**. A total of 29 studies were included in the systematic review [13, 22–49], reporting on a total of 43,916 participants.

Characteristics of the Studies

Included studies were mostly cross-sectional [13, 22, 23, 25, 30, 31, 34–36, 38–47, 49], most of them ($n = 10$) took place in Dubai [22, 24, 25, 29, 31–33, 37, 42, 48], and only two were national [27, 49]. The sample size ranged between 54 [30] and 24,220 [49] participants. The populations studied included healthy preschool [13, 31, 35, 36, 38], school-aged [22–25, 34, 39–42, 45, 49], and secondary school children [44]. Also, included studies assessed preschool children of incarcerated mothers and their caregivers [27], preterm children [33], children diagnosed with leukemia [26], children with cerebral palsy [29], special health care needs children [30], beta thalassemia major children [32], children with down syndrome [37], children with autism spectrum disorder [43, 48], and mothers of preschool children [47].

The details of included studies (design, sample, methodology, results, and conclusion) are available in **Supplementary Material 2**.

Outcomes of the Studies

Information related to caries' prevalence, indices, and risk factors from included studies are available in **Table 1**.

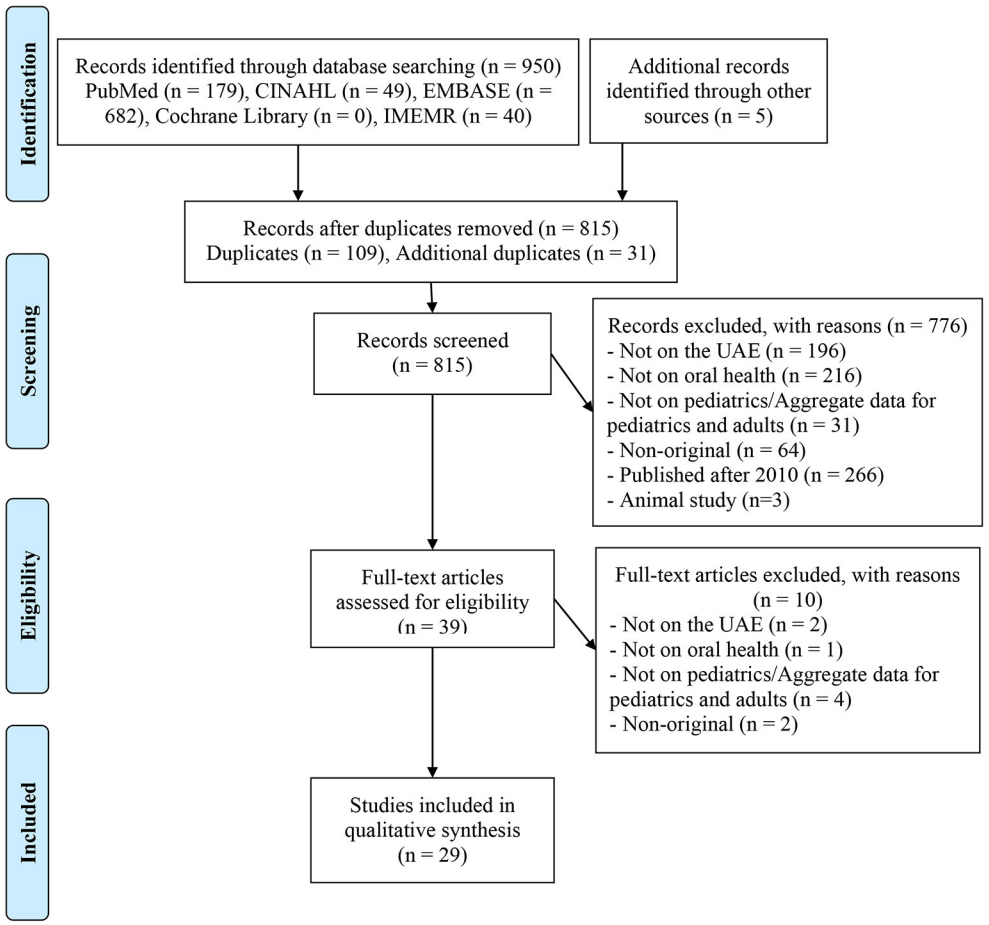

FIGURE 1 | PRISMA diagram of study selection.

Dental Caries

The majority of studies [13, 25, 31, 35, 36, 38–40, 44, 45] used the World Health Organization criteria for assessing caries. The prevalence of caries ranged from 22% in public day cares in Sharjah [35] to 99.4% in children attending a pediatric dentistry department in Al Ain, Abu Dhabi [46]. As for severity of caries, reported mean decayed, missing, and filled primary teeth (dmft) values ranged between 0.75 [36] and 10.9 [46]. Specifically, among Emiratis, mean dmft varied from 3.07 in children in Ras Al Khaima nurseries [13] to 10.9 in children attending a pediatric dentistry department in Al Ain, Abu Dhabi [46]. Only the study by Elamin et al. [36] conducted among children attending nurseries in Abu Dhabi presented data per nationality and highlighted significantly higher mean dmft indices in Emirati children than non-Emirati (2.60 vs. 0.75). Furthermore, this study showed significantly higher dmft values in Emirati children residing in rural areas than in urban or suburban areas [36]. Decayed, missing, and filled permanent teeth (DMFT) values ranged from 1.48 in healthy children attending a pediatric dentistry clinic in Sharjah [39] to 3.19 healthy secondary school children in Sharjah [44], with the decay component accounting for the majority of the DMFT values. No study presented data per nationality.

Risk Factors for Dental Caries

Sociodemographic variables: Majority of the studies reported a lack of significant association between dental caries and age [35, 36, 38, 40]. Regarding the association between the family's socioeconomic status and dental caries, Hashim et al. [40, 41] reported contradictory results; whereby Hashim et al. [40] found an inverse association between severe early childhood caries families' monthly income, whereas Hashim et al. [41] did not find a significant association between monthly income and DMFT. Moreover, Ahmad et al. [22] reported significantly higher DMFT values in schools with low socioeconomic classes. As for parental educational level, it was demonstrated that the mother's educational level was not associated with child's caries in the studies by Hashim et al. [40, 41], whereas father's educational level and DMFT values were negatively associated in one study by Khadri et al. [44].

Dietary factors: Soft drink consumption [44] and high extent of snacking [40, 41] were main dietary factors affecting

dental caries. Interestingly, Kowash et al. [13] did not find a significant association between frequency of sweet consumption and dmft values.

Oral hygiene practices: Higher frequency of tooth brushing was a protective factor against caries in the study by Kowash et al. [13], and Hashim et al. [41]. A contradictory finding was reported by Hashim et al. [40]; also a non-significant effect of time of brushing was identified by Kowash et al. [13].

Utilization of dental services: Both Kowash et al. [13] and Hashim et al. [40] found that a higher frequency of dental visits was protective against caries.

Other Oral Diseases

Apart from caries, other assessed oral disease included dental erosion [38], molar-incisor hypomineralisation (MIH) [22, 42], fluorosis [22], tooth wear [23], and gingivitis [39, 44], the results of which along with the risk factors are presented in **Table 2**.

Among 5 year-olds in Sharjah, Gopinath et al. [38] reported a high level of dental erosion (58.8%) and dissolution of enamel (55%). Predictors of dental erosion included Arab non-Emirati nationalities, caries experience, and consumption of sugary or carbonated beverages.

In school-aged children in Dubai, Ahmad et al. [22] and Hussain et al. [42] assessed MIH. The former reported a low prevalence of MIH (7.59%), which was mostly found in children attending low socioeconomic class schools, while the latter reported a higher prevalence reaching 27.2%, which was found especially in females (32.6%) compared with males (18.1%). When MIH was present, it was mostly mild (53%).

Al Halabi et al. [23] demonstrated a high prevalence of tooth wear in primary teeth (97.6%) among school children in Abu Dhabi. The authors also found the highest prevalence and severity of tooth wear to be at the level of the upper incisor and upper canine segments. Risk factors related to tooth wear included older age, mother's employment, mouth breathing, and anterior deep bite.

Gopinath et al. [39] reported high gingivitis and plaque scores among 3–12 year-olds attending a pediatric dentistry teaching clinic in Sharjah. Increase in dmft/DMFT values corresponded to increase in plaque and gingival scores. Also, the authors found that female gender and children more than 6 years were more likely to have gingivitis. No significant associations were identified between tooth brushing frequency and the physical structure of the diet, and plaque and gingival scores.

Hashim et al. [41] identified risk factors for plaque in 5–6 year-olds in Ajman, whereby high frequency of eating per day and high snack consumption level were both significantly associated with higher plaque scores. In contrast, children who brushed their teeth twice or more per day were found to have lower mean plaque score than those who brushed their teeth less than daily. The authors did not find any association between age, sex, mother's education, monthly income, snacks between meals per day and plaque score. The authors also suggested that children with high plaque scores were more likely to experience caries.

Finally, in secondary-aged children, Khadri et al. [44] identified a substantially elevated visibility of plaque/gingivitis in at least on region of the oral cavity, reaching 95% of the sample, as well as a high visibility of plaque in all regions (69.5%). The authors also reported a high prevalence of unhealthy gums/gingivitis in all regions (68.9%).

Knowledge and Practices

Children

Among preschoolers, Kowash et al. [46] reported a high level of poor oral hygiene reaching 63%, with 58% of the sample never or rarely brushing their teeth: 58%. In the same study, only 1 child out of the assessed 176 children was given fluoride.

In the recent national study conducted among a representative sample of school-aged children, Pengpid et al. [49] reported high levels of inadequate oral hygiene. In details, the prevalence of tooth brushing less than once daily mounted among males to 46.1% in 2016 without a change in comparison with values reported in 2010 (46.8%), nor in 2005 (48.6%). In females, although inadequate oral hygiene was relatively still high in 2016 reaching 28.3%; it reflected a significant reduction over time (37.9% in 2005, and 31.4% in 2010).

Conversely, Khadri et al. [45] reported high levels of daily tooth brushing reaching 93% among school-aged children in Sharjah. Hygiene practices were also found to be better among females, in terms of daily brushing (98 vs. 89%), brushing three times daily (19.6 vs. 13.8%), as well as tooth brushing at morning and evening (76.8 vs. 60.1%).

In his single-school study in Ajman, Dakhili et al. [34] reported good knowledge and practice regarding frequency of brushing, frequency of changing the brush, interdental cleaning, and cleaning the tongue. Nevertheless, the authors reported poor knowledge and practices regarding duration of brushing, method of brushing, use of mouthwash, frequency of tongue cleaning, and materials to clean the tongue were observed. Interestingly, a significant association was noted between correct knowledge and practices of dental hygiene, interdental cleaning and use of mouth wash, and tongue hygiene, as well as a significant positive correlation between knowledge and practice on oral hygiene.

Finally, only Khadri et al. [44] assessed oral hygiene practices among secondary-aged healthy children. The authors reported regularity in good hygiene in the sample, especially among females (97.5 vs. 89.4%). The percentage of children who brushed three times daily was low with highest value being reported for UAE nationals (22.8 vs. other Arabs: 14%; Indian subcontinent: 7.4%; Others: 16.9%). Also, more UAE nationals consumed fluoride supplements compared with participants from other nationalities (30.8 vs. other Arabs: 16.9%; Indian subcontinent: 8.1%; Others: 10.8%). Lastly, visiting the dentist in the past 12 months was low, and was least among Indian participants (25.2 vs. UAE nationals: 49.5%, other Arabs: 50.7%; Others: 53.8%).

Mothers

Knowledge, attitude, and practices of mothers were seldom assessed in the included studies. The study by Mahmoud et al. [47] among mothers of preschool children in Sharjah showed higher than average knowledge and excellent attitude toward their children's oral health, but mostly improper practices, and low levels of utilization of dental services, except during problems. Mothers' knowledge and practices were significantly

TABLE 1 | Summary of caries-related data reported in included studies.

References	Emirate, setting	Design	Population	Criteria	Prevalence	Indices	Risk factors
Preschoolers							
AlKhayat [31]	Dubai, private preschools and kindergarten	Cross-sectional	Sample size: 2,957 Age group: 3–5 years %Males: 51.6% %Emirati: NR	WHO (1987)	38.5%	Mean(SD) dmft: 1.55(2.75) SIC: 4.55(3.12)	Caries prevalence and SIC index increased as the age increased NS gender-based differences
El Batawi and Fakhruddin [35]	Sharjah, day cares	Cross-sectional	Sample size: 435 Mean(SD) age: 1.2(3.6) years %Males: 55% %Emirati: 51.4%	WHO (2013)	22% in public day cares 77% in private day cares	Not assessed	NS difference between mean dmft score of the boys and of girls enrolled either in private or public daycare centers Sig. higher mean dmft score in residents enrolled in private daycares than nationals in public daycares (8.1 vs. 0.8) Sig. association between dmft scores and mothers' employment status in private centers (lowest scores among children of working mothers) Low dmft scores associated with high level of knowledge of caregivers in public centers
Elamin et al. [36]	Abu Dhabi, nurseries	Cross-sectional	Sample size: 186 Age group: 18 months−4 years; Mean age: 2.46 years %Males: 59.1% %Emirati: 46.2%	WHO (2013)	41%	Total sample: Mean(SD): dmft: 1.68(2.8); dt: 1.70(2.8); ft: 0.02(0.1); mt: 0 Emirati: dmft: 2.60(3.2); dt: 2.57(3.2); ft: 0.03(0.2); mt: 0 Non-Emirati: dmft: 0.75(1.8); dt: 0.75(1.8); ft: 0(0); mt: 0 SIC: Emirati: 50.8%; Non-Emirati: 15.9% Children residing in rural areas: 59.5%; Suburban: 36.5%; Urban: 12%	Sig. higher dmft and dt in Emirati children than non-Emirati Sig. higher dmft in Emirati children residing in rural areas than in urban or suburban areas NS difference in mean dmft between boys and girls SIC: Sig. higher among Emirati children than non-Emirati Sig. higher in children residing in rural areas NS difference between genders, or age groups Low maternal education, rural nursery location, infrequent tooth-brushing, frequent consumption of high-sugar food items and Emirati nationality were significantly associated with dental caries
Gopinath [38]	Sharjah, kindergarten	Cross-sectional	Sample size: 403 Age group: 5 years %Males: 48.1% %Emirati: 31.2%	WHO (1997)		Males: Mean(SD) dmft: 6.41(5.04); dt: 6.05(4.9); mt: 0.05(0.54); ft: 0.28(0.94) Females: Mean(SD) dmft: 6.01(4.95); dt: 5.61(4.88); mt: 0.19(1.20); ft: 0.24(0.84)	NS gender-based differences
Kowash et al. [13]	Ras Al Khaima, nurseries	Cross-sectional	Sample size: 540 Age group: 4–6 years; Mean(SD) age: 5.1(0.71) years %Males: 54.1% %Emirati: 100%	WHO (1997)	74.1%	Mean(SD) dmft: 3.07(0.13) (95% CI: 2.81–3.34) SIC: 13.3 (very high) Care index: 3.8% (very low)	NS association between time of tooth brushing, brushing time, frequency of sweet consumption, mother's dental knowledge and presence of caries

(Continued)

TABLE 1 | Continued

References	Emirate, setting	Design	Population	Criteria	Prevalence	Indices	Risk factors
							Sig. association between frequency of visits to dentist, frequency of tooth brushing and presence of caries
Kowash [46]	Al Ain, Abu Dhabi, pediatric dentistry department	Cross-sectional	Sample size: 176 Age group: 1.5–5 years; Mean age: 3.7 years %Males: 57.3% %Emirati: 100%	BASCD criteria	99.4%	Mean: dmft: 10.9 (very high); dt: 10.2; mt: 0; ft: 0.7; dmfs: 32.1; ds: 30.3; ms: 0; fs: 1.8 Care Index: 6.4 % (very low) Restorative Index: 6.4% (very low)	Not assessed
School-aged children							
Ahmad et al. [22]	Dubai, governmental schools	Cross-sectional	Sample size: 779 Age group: 6–10 years; Mean(SD) age: 8.1(0.8) years %Males: 33.8% %Emirati: NR	EAPD criteria		Mean(SD) DMFT: Total sample: 2.41(1.70) (high) Low socioeconomic classes: 2.66(1.78) Middle class: 2.26(1.77) High class: 2.10(1.6)	Sig. higher in schools with low socioeconomic classes as compared to middle and high classes
Al Mashhadani et al. [25]	Dubai, public and private schools	Cross-sectional	Sample size: G1: 1,317 G2: 2,237 Age group: G1: 5–7 years G2: 12–14 years %Males: G1: 43.3% G2: 48.5% %Emirati: NR	WHO (2013)	G1: 60.3% diagnosed with decay 7.5% with missing teeth 21.6% with fillings ≈60% had untreated caries G2: 41.6% diagnosed with decay 7% with missing teeth 29.4% with fillings ≈41% had untreated caries	G1: dmft>0: 65.1% Mean dmft/DMFT: 3.87 G2: dmft>0: 59.2% Mean dmft/DMFT: 1.83	Not assessed
Gopinath et al. [39]	Sharjah, pediatric dentistry teaching clinic	Cross-sectional	Sample size: 405 Age group: 3–12 years; Mean(SD) age: 8.47(1.65) years %Males: 57% %Emirati: NR	WHO (1997)	Not assessed	≤6 years: mean(SD): dmft: Males: 7.86(3.35); Females: 6.97(3.38) DMFT: Males: 0.04(0.28); Females: 0.03(0.17) >6 years: mean(SD): dmft: Males: 6.00(3.55); Females: 5.79(3.84) DMFT: Males: 1.48(2.14); Females: 1.79(2.21)	Not assessed

(Continued)

TABLE 1 | Continued

References	Emirate, setting	Design	Population	Criteria	Prevalence	Indices	Risk factors
Hashim et al. [40]	Ajman, public and private schools	Cross-sectional	Sample size: 1,036 Age group: 5–6 years %Males: 49.7% %Emirati: NR	WHO (1997)	s-ECC: 31.3% (95% CI: 23.6, 38.9)	Not assessed	Sig. associations between s-ECC and monthly income (OR: 1.43; 95% CI: 1.11, 1.85 for children from low-income families compared with children from high income families); high level of snack consumption (OR: 1.80 (1.26, 2.58) compared with using children with low snacking level); and dental visiting (OR for those visited a dentist because of a problem: 1.92 (1.49, 2.49) compared with those who had not visited the dentist in the previous year) NS association between s-ECC and age, gender, mother's education level, frequency of snacks between meals per day, and frequency of brushing
Hashim et al. [41]	Ajman, public and private schools	Cross-sectional	Sample size: 1,036 Age group: 5–6 years %Males: 50% %Emirati: 68.6%		Not assessed	Not assessed	Sig. associations between DMFT and frequency of snacking (aRR: 1.25; 95% CI: 1.00–1.57 for children who snacked 3 or more times daily compared with those who had snacked once daily), and level of snack consumption (aRR: 1.46; 95% CI: 1.26–1.70 for children with high snack consumption level compared with low consumption of snack), frequency of tooth brushing (aRR: 0.8; 95% CI: 0.64–0.93 for children who brushed their teeth ≥ twice daily compared with those who brushed their teeth less than daily) NS association between child and family characteristics and DMFT
Secondary-aged children							
Khadri et al. [44]	Sharjah, public/private schools	Cross-sectional	Sample size: 803 Age group: 11–17 years; Mean(SD) age: 12.8(1.4) years %Males: 50.4% %Emirati: 40.5%	WHO (1997)	Caries: 75.5% Decayed: 71.6% Missing: 4% Filled: 17.9%	Mean(SD) DMFT: 3.19(2.9)	Sig. association between DMFT and father's education level (−0.35; 95% CI: −0.53; −0.17), adolescent's age (0.42; 95% CI: 0.27–0.567), gender (0.41; 95% CI: 0.01–0.81), Arab ethnicity (0.74; 0.32–1.17), and soft drink consumption (0.31; 95% CI: 0.14–0.47)

(Continued)

TABLE 1 | Continued

References	Emirate, setting	Design	Population	Criteria	Prevalence	Indices	Risk factors
Al Mashhadani et al. [25]	Dubai, public and private schools	Cross-sectional	Sample size: 2,063 Age group: 15–17 years %Males: 33.4% %Emirati: NR	WHO (2013)	42.4% diagnosed with decay 15.6% with missing teeth 39.3% with fillings 42.4% had untreated caries	dmft>0: 65.9% Mean dmft/DMFT: 2.70	Not assessed

aRR, Adjusted Relative Ratio; BASCD, British Association for the Study of Community Dentistry; CI, Confidence Interval; dmfs, Decayed, Missing, and Filled Surfaces; DMFT, Decayed, Missing, and Filled Permanent Teeth; dmft, Decayed, Missing, and Filled Primary Teeth; DS, Down Syndrome; EAPD, European Academy of Pediatric Dentistry; G, Group; NR, Not Reported; NS, Not Significant; OR, Odds Ratio; SD, Standard Deviation; s-ECC, Severe Early Childhood Caries; SIC, Significant Caries Index; Sig, Significant; WHO, World Health Organization.

associated with mothers' occupation and education. There was significant association between knowledge of mothers with their educational level; mothers with primary level of education had the highest scores, followed by those having a secondary education, then a university qualification, and finally by illiterate mothers. Also, practices of mothers were associated with their educational level; they were best among mothers with secondary level of education, and poorest among illiterate mothers. Interestingly, knowledge and attitude of mothers were associated with their occupation, with highest scores reported among employed mothers. Source of knowledge of mother's dental information included mostly relatives (27%), friends (23%), TV/Radio media (20%), reading (17%), and finally educational programs (13%).

Kowash et al. [13] explored the dental knowledge of mothers of Emirati preschool children in Ras Al Khaimah. The authors showed remarkable levels of poor knowledge among mothers of children with caries compared with mothers of caries-free children. For example, in that study, 13.7% of mothers thought that fluoride did not help in the prevention of tooth decay. Interestingly, 79% of those mothers had children with caries. Moreover, 3.5% of mothers did not consider that a balanced diet was important for the child's dental health and prevention of tooth decay; with 78% of those mothers had children with caries.

Caregivers

Only El Batawi and Fakhruddin [35] assessed caregivers in day care centers in Al Sharjah, and reported a significantly higher knowledge regarding oral health in those operating in public centers compared with private ones.

Utilization of Dental Services

Only the studies by Kowash et al. [13, 46] assessed the utilization of dental services among healthy Emirati preschoolers. The first study conducted in Abu Dhabi among children presented to a pediatric dentistry department [46] reported that two-thirds of children never visited a dentist, with very low care index and restorative index of 6.4%. Similarly, the second study [13], reported a very low care index of 3.8% among preschoolers in Ras Al Khaimah, despite the high mean dmft score of 3.07 reported by the authors.

Implications of Poor Oral Health/ or Dental Interventions

Khadri et al. [45] showed that school-aged healthy children with higher self-esteem scores brushed their teeth more often. The authors did not report any association between the presence of caries and self-esteem.

On the other hand, Alantali et al. [28] in the study conducted among preschool children showed that restorative dental general anesthesia resulted in significant improvement in child and family physical, psychological, and social aspects of quality-of-life, with a large change noted specifically in both the child and family impact sections.

Children With Disease/Special Conditions

Alnuaimi et al. [26] investigated the oral health of children with leukemia. The authors reported a 60% prevalence of oral problems, mostly consisting of oral mucositis and ulceration followed by dental caries and oral candidiasis. The prevalence of these problems was not associated with age, gender, nationality, nor family history; yet it was higher among patients who received treatment and follow-up locally within the country (75 vs. 2.8 in those treated abroad, and 22.2% treated in both locations). The peak occurrence of most oral problems was during phase IV (maintenance).

Al Hashmi et al. [29] explored oral health status among children with cerebral palsy in Dubai. More than half of the sample were diagnosed with caries (53%), with a mean(SD) DMFT index of 2.83(2.86), an oral health index of score of 1.68(1.34), and a 58.8% prevalence of gingivitis. These findings were similar to the age- and gender-matched control group. Patients with cerebral palsy presented a significantly higher calculus index [0.56(0.78) vs. 0.07(0.27)], as well as a higher rate of occlusal and oral soft tissues' anomalies, in addition to more a greater proportion and severity of erosion compared with healthy controls. Nevertheless, provision of services was low for cerebral palsy children, as exhibited by the restorative index (1.9), and was lower than the healthy counterparts (4.7), despite a higher

TABLE 2 | Summary of oral-disease-related data reported in included studies.

References	Emirate, Setting	Design	Population	Result	Risk factors
Preschoolers					
Gopinath [38]	Sharjah, kindergarten	Cross-sectional	Sample size: 403 Age group: 5 years %Males: 48.1% %Emirati: 31.2%	Dental erosion: 58.8% Dissolution of enamel: 55.0% Exposed dentin: 3.7%	Predictors of dental erosion: Arab non-Emirati nationalities (OR: 0.27; 95% CI: 0.18–0.42); Caries experience (OR: 0.28; 95% CI: 0.16–0.51); Drinking sugary or carbonated beverages compared with water (OR: 0.30; 95% CI: 0.19–0.41)
School-aged children					
Ahmad et al. [22]	Dubai, governmental schools	Cross-sectional	Sample size: 779 Age group: 6–10 years; Mean(SD): 8.1(0.8) years %Males: 33.8% %Emirati: NR	MIH: 7.59% (low) Fluorosis: 10.9%, mostly very mild or mild	NS difference between genders (Male: 7.58%; Females: 7.57%) Prevalence of MIH: twice higher in low socioeconomic class schools (11.31%) as compared to high class (4.58%) Sig. higher mean DMFT in children with MIH than those without MIH [3.5(1.7) vs. 2.3(3.1); 49.5% of MIH cases had DMFT from 3 to 5 and 16.8% had DMFT ≥6] NS difference between genders, MIH status, and socioeconomic levels of school
Al Halabi et al. [23]	Abu Dhabi, schools	Cross-sectional	Sample size: 506 children; 9,213 teeth Age group: 2.6- 6.8 years; Mean(SD): 4.92(0.841) years %Males: 51.8% %Emirati: NR	Tooth wear: 97.6% Females: 49.39%; Males: 53.03% Severity in examined teeth: No tooth wear: 41.6%; Mild: 42.1%; Moderate: 16.2%; Severe: 0.15% Highest prevalence and severity of tooth wear in upper incisor and upper canine segments	Sig. associations between attrition and older age (95% CI: 1.07–2.06), mouth breathing (95% CI: 1.05–1.70), harder type of tooth brush (95% CI 1.03–1.69), mother's employment (95% CI: 1.14–1.88), and anterior deep bite (95% CI: 1.03–1.69)
Gopinath et al. [39]	Sharjah, pediatric dentistry teaching clinic	Cross-sectional	Sample size: 405 Age group: 3–12 years; Mean(SD): 8.47(1.65) years %Males: 57% %Emirati: NR	Plaque index: ≤6 years: Males: 1.63(0.75); Females: 1.33(0.74) >6 years: Males: 1.54(0.76); Females: 1.76(0.67) Gingival index: ≤6 years: Males: 1.06(0.81); Females: 1.06(0.79) >6 years: Males: 1.18(0.70); Females: 1.31(0.61)	Increase in dmft/DMFT values corresponds to increase in plaque index and gingival index scores Males were less likely to have gingivitis compared with females (OR: 0.47; 95% CI: 0.24–0.93), and children aged ≤6 years were less likely to have gingivitis than those aged >6 years (OR: 0.33, 95% CI: 0.17–0.62) NS association between tooth brushing frequency and plaque and gingival score values NS association between the physical structure of the diet with dmft/DMFT and plaque/gingival scores
Hashim et al. [41]	Ajman, public and private schools	Cross–sectional	Sample size: 1,036 Age group: 5–6 years %Males: 50% %Emirati: 68.6%	Mean(SD) plaque score: 0.67(0.32)	Children who had a high snack consumption level had higher mean plaque score than those with low snack consumption (adjusted difference: 0.13; 95% CI: 0.02–0.24) Children who brushed their teeth twice or more per day had lower mean plaque score than those who brushed their teeth less than daily (adjusted difference: −0.09; 95% CI: −0.17; −0.00) NS association between age, sex, mother's education, monthly income, snacks between meals per day, and plaque score Sig. association between plaque and DMFT (aRR for highest plaque category: 4.77; 95% CI: 3.67–6.19, compared with those in the lowest category)

(Continued)

TABLE 2 | Continued

References	Emirate, Setting	Design	Population	Result	Risk factors
					Children who snacked 3 or more times per day had higher mean DMFT and plaque than those who had snacked once per day (aRR: 1.19; 95% CI: 1.00–1.42) Children who had a high snack consumption level had higher mean DMFT and plaque than children with low consumption of snack (aRR: 1.21; 95% CI: 1.06–1.38) Children who brushed their teeth twice or more per day had lower mean DMFT and plaque than those who brushed their teeth less than daily (aRR: 0.85; 95% CI: 0.71–1.00) NS association between child and family characteristics and DMFT with plaque
Hussain et al. [42]	Dubai, public schools	Cross-sectional	Sample size: 342 Age group: 8–12 years; Mean(SD): 9.4(1.2) years %Males: 37.1% %Emirati: NR	MIH: 27.2% Females: 32.6%; Males: 18.1% Severity: mild: 53%; moderate: 17%; severe: 30% Incisor most frequently affected: Maxillary left central incisor (11.1%); and least frequently affected: mandibular left lateral incisor (0.6%)	High prevalence of MIH in school children, mainly with a mild severity Prevalence of MIH and MH was sig. higher in females, and related to location of tooth in the oral cavity
Secondary-aged children					
Khadri et al. [44]	Sharjah, public/private schools	Cross-sectional	Sample size: 803 Age group: 11–17 years; Mean(SD) age: 12.8(1.4) years %Males: 50.4% %Emirati: 40.5%	Plaque/gingivitis visible in at least 1 region of the oral cavity: 95% Plaque visible in all regions: 69.5% Unhealthy gums/gingivitis in all regions: 68.9%	Not assessed

aRR, Adjusted Relative Ratio; CI, Confidence Interval; DMFT, Decayed, Missing, and Filled Permanent Teeth; dmft, Decayed, Missing, and Filled Primary Teeth; MH, Molar Hypomineralization; MIH, Molar-Incisor Hypomineralisation; NR, Not Reported; NS, Not Significant; OR, Odds Ratio; SD, Standard Deviation; Sig, Significant.

treatment need, as exhibited by the met need index in the study group (0.32 vs. 0.24).

The oral health and problems of Emirati and non-Emirati children with β-thalassemia major in Dubai were explored by Al-Raeesi et al. [32], in comparison with healthy Emirati children. The overall prevalence of caries in children with thalassemia amounted to 68.4%, and the mean(SD) DMFT reached 1.49(2.67). Both findings were higher than values reported for the control group [48.7%; 0.21(0.56)]. Oral hygiene status was similar across study groups. More children with thalassemia had calculus than controls (28.9 vs. 7.9%), but less had gingivitis than controls (44.7 vs. 69.7%). More children with thalassemia than controls had gingival pigmentation (23.7 vs. 0%); no other differences were noted regarding soft-tissue anomalies and orofacial manifestations. Finally, the restorative care and treatment were lower in children with thalassemia.

Ghaith et al. [37] conducted a study on children with Down Syndrome. More than half of these children had dental decay (57.6%), and this rate was not different than that reported for healthy controls (57.6%). Yet, children with down syndrome had a higher mean(SD) DMFT [2.73(0.22) vs. 1.65(2.46)]. The oral health index score was similar between groups. This was also noted regarding the prevalence of gingivitis (65.4 vs. 70.4% in controls). However, the calculus index was higher among children with Down Syndrome [0.25(0.52)] compared with controls [0.07(0.27)]. Also, the prevalence of erosion (34 vs. 15.3%); severity of erosion (1.9 vs. 0%); prevalence of erosion into enamel (19.8 vs. 11.3%); and prevalence of erosion into enamel and dentine were higher among children with Down Syndrome (12.3 vs. 4%). Children with Down Syndrome had higher malocclusion problems, higher proportion of open bite, crossbite, scissor bite, anterior spacing, and posterior spacing, and more Class III molar relationship, in addition to a higher proportion of all oral soft tissues problems compared with healthy children. Nevertheless, children with Down Syndrome received more restorations and dental treatment and had better access to dental care (RI: 26.81 vs. 11.76; MNI: 35.6 vs. 23.6).

Both Jaber et al. [43] and Mansoor et al. [48] investigated children with Autism Spectrum Disorder. The first study [43] explored oral health status, and reported a prevalence of dental caries of 77% (males: 73.3%; females: 87.5). Mean(SD) dmft of the primary and early, mixed dentition years was: 2.2(1.77), and those of the late, mixed dentition and permanent dentition were 1.8(1.67) and 4.0(1.44), respectively. All children had gingivitis

(generalized: 70.4%, or localized: 29.6%). The oral hygiene was mostly poor (59%), and finally, both the met need index and restorative index of the studied autistic children were low. Information regarding challenges to oral health among children with Autism Spectrum Disorder were provided by Mansoor et al. [48]. Regarding oral care at home, 83.3% reported that their children needed assistance in brushing their teeth, and 24.5% reported that their children always resisted tooth brushing. Around half of the parents reported that their children disliked the feeling of the toothpaste and toothbrush in his/her mouth (45.8 and 53%, respectively). These findings were higher than what was reported among healthy school-aged children. Around two-thirds of children with Autism Spectrum Disorder (65%) have visited a dentist, and this was not different than their healthy counterparts. Most common reason for not visiting a dentist was child being uncooperative, followed by child being afraid, having no complaint, and finally not finding a dentist who can handle the child. More than one-third (37%) of parents rated their child's experience as negative in the last dental visit, and 32.7% reported feeling more afraid or extremely afraid if their child had to go to the dentist tomorrow. Dislikes at the dentist included dentist drill, leaning back in the dental chair, loud sounds, bright light, and smell. Dislikes also included feeling of the toothbrush and toothpaste within mouth.

Finally, Alkhabuli et al. [30] assessed a group of Arab children with special healthcare needs in Ras Al Khaimah, and reported a high prevalence of dental caries (85.2%), especially among children with Down Syndrome and mental disability (62%), and a high mean(SD) dmft/DMFT [5.67(4.69)]. Around two-thirds of the sample had good oral hygiene (64.8%), without age- or gender-differences, but with differences across disabilities, where good oral hygiene was highest among children with autism (100%), and lowest in children with multiple disabilities (25%). Need for oral treatment was high, especially for restorations (89%), oral prophylaxis (41%), and orthodontic treatment (20%).

Alshehhi et al. [33] investigated oral health among preterm children when reaching an average age of 8, in comparison with a sample of full-term children. The authors reported a significantly higher mean(SD) DMFT [1.00(1.55) vs. 0.38(0.99)], and a 4 times higher prevalence of enamel defects (58.15 vs. 24.2%; OR = 4.33, 95% CI: 2.01–9.36) in preterm children, with the highest proportion found among those with abnormal birth weights. In addition to low birth weight, cesarean delivery and intubation were significantly related to the occurrence of enamel defects. In contrast, no relationship was reported between the prevalence of enamel defects and diseases during pregnancy, hospitalization in early life, systemic disease and antibiotic exposure in the first 3 years of life, and history of previous dental trauma. Finally, the most common type of enamel defects in the study group included white or creamy demarcated opacities, and post-eruptive breakdown.

In his case-controlled study, Al Salami et al. [27] reported a prevalence of caries of 89.8%, and a mean(SD) dmft score of 4.97(3.61) among children of incarcerated mothers. Both findings were similar to their counterparts. Nevertheless, the authors reported a worse oral hygiene (6.2%; 18.2%), a higher score of debris/plaque (93.8 vs. 81.8%), and a higher prevalence of calculus in the study group (3.1%; 0.4%). Utilization of dental services were lower in the study group, as exhibited by the lower restorative and care indices (4.2%; 4.43%, and 0.3%; 0.34%), despite a higher treatment need index in the study group (14.74%; 1.91%). The caregivers of prison nurseries showed unsatisfactory oral-health knowledge and attitude, especially knowledge on the effect of fluoride and its dental benefits, in addition to dietary habits. Knowledge and attitude of caregivers were in association, whereby 80% of caregivers with a satisfactory level of knowledge presented a positive attitude toward oral health while the level of the positive attitude of caregivers with poor knowledge reduced to 48%.

Oral Health Interventions

Al Mashhadani [24] investigated the effects of a 3-month school-based intervention consisting of daily tooth brushing with fluorinated toothpaste after mealtime among 1,500 students aged 4–6 years in 7 randomly chosen private and governmental schools. The intervention was multilevel. School nurses (or oral health coordinators) were trained to ensure proper tooth brushing technique, hygiene standards, and give oral health awareness tips to students, and equipped with special charts to follow up on students, and materials such as brushes and toothpaste. Also, parents were involved in the intervention, as they were introduced on the tooth brushing scheme, provided with information on healthy diet and good oral hygiene habits and tooth brushing, and charts to help follow up on the brushing at home. The evaluation based on a pre and post visible plaque index examination by dentists and dental hygienists, an interview with the involved school nurse, and a feedback questionnaire for the parents.

In post-intervention, the presence of visible plaque index decreased from 76.8 to 36.7%. Children accepted the activity, enjoyed participating daily, and had higher awareness of the importance of daily brushing and consequences of poor oral hygiene. The success of the activity increased when the school administration and nurse embraced and accepted having the students brushing daily in schools. Nevertheless, main barriers included: storage of the toothbrushes and maintenance of infection control standards; allowing students to leave the class for the activity; long-term cooperation of class teachers and administration. Parental feedback showed positive behavioral change toward tooth brushing (86%) with positive influence on siblings, established good oral hygiene routine (83%), increased interest to have more oral health sessions (72%), desire to have children continue to brush at school (79%), but a concern with infection control regarding storage of toothbrushes in schools (21%). This experience suggests that tooth brushing in schools might encourage and enforce good oral hygiene habits and improve children's oral hygiene status and the attitudes of their parents. This program showed promising results and could be implemented with the possibility of setting up policies and guidelines to govern its application in all schools.

Risk of Bias Assessment

The detailed results of risk of bias assessment are available in **Supplementary Material 2**. The assessment of the outcome

was optimal in the majority of the studies. Nevertheless, major shortcomings pertained to the representativeness of the sample and its limited size, as well as the absence of information related to the response rate.

DISCUSSION

The aim of this systematic review was to summarize the available data on oral health among children and adolescents in the UAE over the past decade in order to provide important information that could be utilized by stake holders for future planning of effective preventive and interventional program. The main findings shed light on the high prevalence of dental caries among pediatric population including those with special conditions and diseases across all emirates of the UAE. Moreover, oral hygiene practices and utilization of dental services were suboptimal and low.

Maintaining oral health and hygiene starting from an early age is crucial for maintaining well-being throughout life span. According to WHO and the American Academy of Pediatric Dentistry, the best time to for a child's visit to a dental care provider is at the age of 1-year-old past the emergence of the first tooth [50, 51]. The global prevalence of childhood dental caries- one of the most common oral diseases, has ranged between 12 and 98%, with about 600 million children being affected worldwide [4]. When children develop oral diseases like dental caries or gingivitis, this might be accompanied by pain, discomfort, lack of sleep, an inability to ingest food, and more visits to the dentists [8]. Consequently, this will interfere with their daily life activities, nutrient and food intake, energy levels and might even deter them from learning [8]. Moreover, Rebelo et al. [10] showed that untreated dental caries has an inverse relationship with school performance and attendance among children. According to Al-Bluwi [52], about 50 million hours of school is lost every year due to oral health issues. The burden of untreated oral diseases does not only impact the academic and social life of children, but also surpasses to financial tolls. For instance, in 2010, 298 billion US dollars were spent globally in treating dental caries [53].

Among all the diseases that affect children in the UAE, early childhood dental caries is considered the most common [13]. Recent updates about oral health status for children and adolescents in the UAE are lacking. A previous informative review on oral health among children in the UAE highlighted studies published up to the year 2011. However, the review was limited to few databases and focused only on dental caries in children younger than 13 years of age [14]. The current systematic review aims to first summarize the available data on oral health among children and adolescents in the UAE over the past decade and second to highlight gaps pertaining to this area and provide valuable implications for future policy and research considerations. Despite the significant advancement in dental services in the UAE, dental caries remains highly prevalent among children in the UAE. Although El Nadeef et al. [15] and Al-Bluwi [14] recommended to conduct routine epidemiological studies to assess oral health and understand its determinants, to the best of our knowledge, there are no new nation-wide studies to underscore the prevalence of caries and DMFT among children and adolescents. Being a country with multicultural diversity poses a major challenge to research aiming to explore risk factors and intervention-based treatments [14].

The high prevalence of dental caries documented in our study is similar to the results reported by researchers from the Eastern Mediterranean Region, reaching among 12-year-olds a prevalence of 70% in Bahrain, 62% in Iraq, 86.9% in Lebanon, and 90.2% in Yemen [54].

The only comprehensive national survey of oral health of children in the UAE conducted by El Nadeef et al. [15] determined the prevalence of dental caries among 12 and 15-year-old children ($N = 2{,}651$) indicated 54 and 65% prevalence, respectively [15]. The results were comparable to those reported by researchers from the neighboring country Saudi Arabia, indicating a prevalence of approximately 80% for dental caries among Saudi children for primary teeth and 70% for permanent teeth [55].

The study by Elamin et al. [36] investigated the influence of socioeconomic factors, oral hygiene practices and eating habits on the prevalence of dental caries in preschool children living in Abu Dhabi. Lower maternal educational levels along with lack of regular tooth brushing and elevated intake of sugar rich foods were the most prominent risk factors. Moreover, Emiratis had a more significant prevalence of dental caries as compared with non-Emiratis [36]. These results highlighted the need for effective interventions to improve dental habits in children and regular dental screenings for this age group. The high intake of sugary foods and drinks being readily available and affordable to purchase in the UAE for both Emirati and non-Emirati children warrants more investigation before conclusive results are inferred about the socioeconomic status. The contradictory results reported by Hashim et al. [40, 41] and Ahmad et al. [22] regarding the relation between the family's socioeconomic status and dental caries could be due to the distinctive socioeconomic factors of the country since income alone does not determine the socioeconomic level in the UAE.

Our results demonstrate a high prevalence for dental caries among both children and adolescents in the UAE. The study by Elamin et al. [36] highlighted significantly higher prevalence of dental caries among Emirati children as compared with non-Emirati (dmft 2.60 vs. 0.75). This was the only study that discerned differences in pediatric oral health status based on nationality, hence solid conclusions cannot be inferred. Nevertheless, the findings might be attributed to the more frequent checkups and availability of subsidized dental health services to UAE nationals. Ironically, Emirati children especially in rural areas have a tendency to consume traditional foods whereas expatriate non-Emirati children tend to consume more fast food and sweetened snacks. Also, the difference in access of dental care and possibly insurance coverage between the two groups should be noted. In general, specific risk factors implicated with higher dental caries scores were higher consumption of snacks, being in public schools, lower maternal education level, and socioeconomic status. Specifically, parents of higher socioeconomic status have a higher level of oral

health awareness and tend to be aware about the importance of early dental checkups [56]. Contradicting results however were revealed by other researchers for children with higher income families who were shown to have higher scores for dental caries. In addition, a robust correlation existed between knowledge and practice in different areas such as brushing techniques and flossing [34]. Mahmoud et al. [47], later demonstrated that interestingly the levels of knowledge and attitude of the interviewed mothers were sufficient, but their practices concerning their children's oral health were inappropriate. The results reported by Mahmoud et al. [47] regarding the high knowledge of mothers with primary level of education is worth further investigation. This association should be explored in the context of other factors including the mother's occupation, age, employment, source of knowledge about dental information and socioeconomic status. In addition, the presence of an educated care giver in the house as is common with many Emirati families might play a major role in this sense by offering information and resources about dental hygiene and oral health for the children.

Abu Gharbieh et al. [56] reported significant differences in the oral health knowledge score in terms of age, gender, and nationality. Emirati parents showed a higher level of knowledge about their children's oral health as compared to non-Emirati parents. The researchers suggested that the significant prevalence of diabetes mellitus among Emirati parents might have played a role in increasing the level of awareness about oral health and hygiene in general since these individuals receive more frequent educational information during medical visits to health professional to manage their diabetic condition. Moreover, the researchers concluded that females were more knowledgeable and practicing better oral health behavior than males.

On a relevant context, a more recent study by Pengbid et al. [49] demonstrated the presence of inadequate oral hygiene knowledge and practices among adolescents in a cross-sectional national survey in 2005, 2010 and finally 2016 as part of the UAE Global School-Based Student Health Survey (GSHS). This highlights the importance of school-based interventions in improving knowledge and practice and the need for public health professionals to focus on specific factors when designing oral health educational programs to increase levels of knowledge and help individuals develop healthy oral habits. In addition, it is imperative to carefully design oral health education programs to include not only parents but also school teachers and care givers especially that home caregivers could be domestic helpers recruited from abroad and hence might have cultural gaps.

One particular category pertains to children with special conditions and diseases. There is a high level of oral problems in children with different disease (down syndrome, cerebral palsy, thalassemia, autism) and special conditions (children in prison nurseries); yet, in general, treatment indices are lower [26, 29, 37, 43, 48]. Despite the availability of services, utilization level by children with different diseases remains low. This might be attributed to the fact that such services might require more specific tailoring to match the needs of children with special conditions.

The implications of our study could guide future intervention studies which are very limited and lacking. The role of public health professionals in conducting educational programs to not only raise awareness but to ensure that knowledge is indeed translated into practice is of utmost importance.

The main focus of this review was on dental caries as a major and highly prevalent oral health disease, however the high prevalence of other oral health diseases is documented by few researchers. Although such studies are very heterogeneous and limited in number, the contributions of other diseases like dental erosion [38], fluorosis [22], tooth wear [23], and gingivitis [39, 44] to the scope of oral health among children and adolescents in the UAE should not be ignored. While the prevalence of dental erosion was associated with the consumption of sugary carbonated beverages, the prevalence of tooth wear was correlated with mother's employment and mouth breathing. Moreover, frequent snacking was linked to plaque development among children and adolescents as this prolonged exposure to food remains within the mouth.

It is worth mentioning that most included studies emanated mainly from the largest emirates within the country: Abu Dhabi, Dubai, and Sharjah. Additional research from other emirates is urgently needed. Moreover, a solid predictive model with significant risk factors for caries might be very challenging because of the vast differences within the UAE as a country with a multicultural background [14]. Finally, the results of some studies cannot be extrapolated to the target population because of using specific conditions such as limited setting and convenient sampling.

Strengths and Limitations

To our knowledge, this is the first review to systematically explore the epidemiology of oral health among healthy children and adolescents and those with special conditions in the UAE over the past decade. This review was conducted according to a pre-defined protocol and following standard methods for reporting systematic reviews [20, 21]. We searched numerous databases, as well as the gray literature to increase the exhaustiveness of the search. Yet, as for all systematic reviews, this quality of evidence of this work is inherently limited by the design and quality of included individual studies, whereby the majority of the studies were conducted in only one Emirate, and or limited by their convenient sampling technique, bivariate statistical analysis methods, and generalizability of their results. Within this scope, as the included studies were of significant heterogeneity in terms of exposure and outcome assessment methods, and also study population, we were not able to meta-analyze available data especially those related to the dental caries indices or factors contributing to the development of dental caries and other oral diseases. Nevertheless, our work holds important implications for dental health policy in the UAE. By focusing on modifiable risk factors in the pediatric population and utilization of dental health services in this population, this review can guide informed dental public health policies, and subsequently targeted prevention and intervention programs, thereby decreasing inequities in dental health. Finally, by mapping existing epidemiological and interventional studies, this review highlights the gaps in pertaining research in the country. Research should be intensified to draw temporal trends and understand the profile of childhood

caries in the UAE, as well as to explore cost-effective national community prevention and intervention programs.

CONCLUSION

Since the oral cavity is the first station of the digestion process, where different types of food and drinks are ingested, it is important to maintain its hygiene and health starting from an early age using the correct methods. The multifactorial nature of the oral health diseases among children makes the theoretical pathways that link the relationships among predictor variables and risk factors with oral health outcomes children very complex. Specifically, biological pathways play crucial roles in susceptibility and resistance to this complex disease. These factors include most importantly the dietary features, i.e., cariogenic potential of foods (e.g., sucrose), the frequency of eating, and the physical state of the diet, which can affect individually or jointly the carious process. Other physiological factors, include salivary flow and composition especially in calcium and phosphates, tooth morphology, whereby malposition of the teeth or deep anatomy grooves can hinder tooth brushing, and fluoride penetration, as well as enamel and dentin formation processes, which are genetically-regulated through interactions with oral bacteria to affect caries susceptibility and/or enhancement of the enamel thickness/fluoride concentration, hence protecting teeth against caries [57].

Within the limitations of this review, it can be suggested that dental caries is still a major pediatric health problem in the UAE. Suboptimal oral hygiene practices, coupled with a low utilization of dental services, were reported. Moreover, a high level of oral problems in children with different disease was noted; yet, in general, treatment indices in these patient populations were lower than those of their healthy counterparts. Accordingly, immediate oral health promotion strategies are needed to address this public health problem early in its course by creating conditions that promote oral health, and increasing uptake of dental services. Our findings echo the call of Al Mashhadani [24] for a mandatory law requiring a dental health clearance (status and certification) for student registration, a referral system for students requiring immediate dental care, and a policy to enforce supervised tooth brushing in schools [24]. Ongoing culturally-sensitive and practical awareness and education programs are also needed to change the behavior of parents or caregivers, toward enhanced oral health practices in children. Finally, more studies reporting on national dental caries and exploring determinants of this disease are needed to obtain an accurate picture of pediatric dental caries in the country.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

FA and RR were involved in the concept and design. SH performed the searches. SH and RR conducted the title and abstract screening. FA, RR, SH, and NM conducted the full text screening, performed the data extraction, and risk of bias assessment. All authors contributed to writing the draft manuscript and have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/froh.2021.744328/full#supplementary-material

Supplementary Material 1 | Search Strategy.

Supplementary Material 2 | Details of included studies.

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Beverages Consumption and Oral Health in the Aging Population: A Systematic Review

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Little study has yet been made of the effect of different beverages on oral health outcomes in the aging population. The purpose of this systematic review is to evaluate the association between different beverages, including alcohol intake, coffee, milk, tea, and sugary drinks, and a cluster of oral health outcomes, including periodontal disease, oral dysbiosis, and tooth loss in older adults. The literature was screened from the inception up to May 2021 using six different electronic databases. Two independent researchers assessed the eligibility of 1308 retrieved articles regarding inclusion criteria; only 12 fitted the eligibility requirements, representing 16 beverage entries. A minimum age of 60 was the inclusion criterion. No exclusion criteria were applied to outcomes assessment tools, recruiting facilities (hospital or community), general health status, country, and study type (longitudinal or cross-sectional). The consumption of alcoholic beverages was expressed as alcohol intake in all eligible studies, thereby replacing alcoholic beverages in the analysis. The quality of evidence was judged as moderate for alcohol and low or very low for beverages. In regard to oral health in the elderly, the review identified information on alcohol (56.25%), followed by coffee (18.75%), milk (12.50%), tea (6.25%), and sugary drinks (6.25%). Alcohol, sugary drinks, and coffee were found to be related to tooth loss. Periodontal disease was inversely related to coffee and milk, but fostered by alcohol consumption. In one article, tea but not coffee seemed to improve oral microbiota. In summary, alcohol seems to be a driver for tooth loss and periodontal disease in the aging population. However, more research is needed to gain a more solid knowledge in this research area.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, PROSPERO, Identifier: CRD42021256386.

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INTRODUCTION

Healthy aging is critical to a good quality of life in older adults, and to reducing the healthcare system burden. In this context, the deterioration of oral function, most often combined with an altered perception of taste and thirst, is a well-known but often overlooked adverse feature of the aging process (1). Tracing back along the etiopathogenic trajectories of age-related oral

deterioration, the evidence suggests that a pattern consisting of poor dentition, tooth decay, altered microbiota, and periodontal disease delineates a causal path linked to immune and cellular senescence and subsequently to physical and cognitive deterioration (2, 3). Recently, concerns about poor oral health are in the spotlight when considering the aging population, especially those frail. Oral health represents an essential aspect of health, life satisfaction, quality of life, and self-perception, and this feature in turn may indirectly affect aging trajectories through biological interaction paths with several functional domains (4).

There is further evidence for lifestyle components associated with an acceleration of poor oral health, where diet is a key factor (5). Diet could be seen to contribute to the etiology of poor oral health, also as a consequence of a dysfunctional oral health pattern, particularly in older adults. This can lead to feeding difficulties, resulting in changes in food choices likely to predispose to an increased risk of malnutrition and sarcopenia, resulting in loss of muscle mass, muscle strength, and physical performance (6–9).

Dietary intake of all types of fluids, including water and various beverages such as tea, coffee, milk, sugary drinks, fruit juices, and alcoholic drinks, serves to prevent dehydration, another possible problem of aging due to an altered thirst perception (1, 10). This generally positive role of the intake of fluids could be counterbalanced by adverse effects caused by the mixed composition of popular beverages. These affect oral health, inducing problems such as dental caries and erosion. In this context, sugary beverages have been identified as drivers of the risk, showing a clear dose-response relationship (11). Alcohol has also been extensively explored, and a recent meta-analysis of observational studies has suggested excessive consumption to be associated with a higher incidence of periodontitis, thus advocating the inclusion of this dietary item as a noteworthy yardstick in periodontal risk management (12). Against this preventive backdrop, the aging population comes to the forefront again if considering that heavy alcohol consumption is more prevalent among middle-aged and older people compared to the youngest (13).

The level of exposure to beverage consumption, known as a potentially modifiable lifestyle factor, and the potential interaction with oral health outcomes so far refereed by scientific evidence leaves an exploratory window for conceptual synthesis, useful in terms of risk management. The potential interaction between nutrition and oral health prompted us to systematically evaluate the literature on the association between exposure to different beverages and poor oral health outcomes in the aging population.

METHODS

Search Strategy and Data Extraction

The present systematic review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines, adhering to the PRISMA 27-item checklist (14). An *a priori* protocol for the search strategy and inclusion criteria was established and registered, without particular amendments to the information provided at registration, on PROSPERO, a prospective international register of systematic reviews (CRD42021256386). We performed separate searches in the US National Library of Medicine (PubMed), Medical Literature Analysis and Retrieval System Online (MEDLINE), EMBASE, Scopus, Ovid, and Google Scholar databases to find original articles inquiring into any association between the exposure to different beverages and oral health outcome(s). Thus, the main goal was to evaluate the association between exposure to different beverages and poor oral health outcomes in the aging population. We also considered the gray literature using the largest archive of preprints https://arxiv.org/ in the study selection phase, and http://www.opengrey.eu/ database to access conference remarkable conference abstract and other not peer reviewed material. Since we chose only observational studies to be included, the search strategy followed PECO (Populations, Exposure, Comparator, and Outcomes) concepts (15), including populations (60+ years of age), exposure (alcohol and beverages such as milk, tea, coffee, and sugar-sweetened drinks), comparators (exposure levels), and outcomes (oral health outcomes). Exposure factors were selected to include major groups of beverages, i.e., alcoholic beverages (as alcohol intake), coffee, milk, tea, and sugary drinks, regardless of the assessment tool(s) employed. Outcomes included all types of deteriorating oral health conditions, i.e., periodontal disease, oral dysbiosis (gingivitis), and tooth loss. It should be borne in mind that in articles regarding oral health, the consumption of alcoholic beverages is usually calculated as alcohol intake [except for a single report that also looks at alcoholic beverages (16)], so this was the exposure taken into account in this study.

The search strategy used in PubMed and MEDLINE and adapted to the other four electronic sources is shown in detail in **Supplementary Table 1**. In the literature search, no time limit was set and articles were retrieved until June 1st, 2021. No language limitation was introduced. Two researchers (RZ, VD) searched the papers, reviewed titles and abstracts of articles retrieved separately and in duplicate, checked full texts, and selected the articles for inclusion in the study. Technical reports, letters to the editor, and systematic and narrative review articles were excluded. Inter-rater reliability (IRR) was used to estimate inter-coder agreement, and then the κ statistic as a measure of accuracy and precision. A coefficient k of at least 0.9 was obtained in all data extraction steps, both according to PRISMA concepts and along with the quality assessment steps (17).

Inclusion Criteria, Data Extraction, and Registration

Exposure and outcome had to be referred to a population aged 60 years or older. No criterion was applied to the recruitment settings (hospital or community) or health status of the study population (general population or groups with specific characteristics). Potentially eligible articles were identified by reading the abstract and, if eligible, reading the full-text version of the articles. For each article selected, the best statistical approach in respect to confounding as applied in evaluating the magnitude of the effect size for associations was considered.

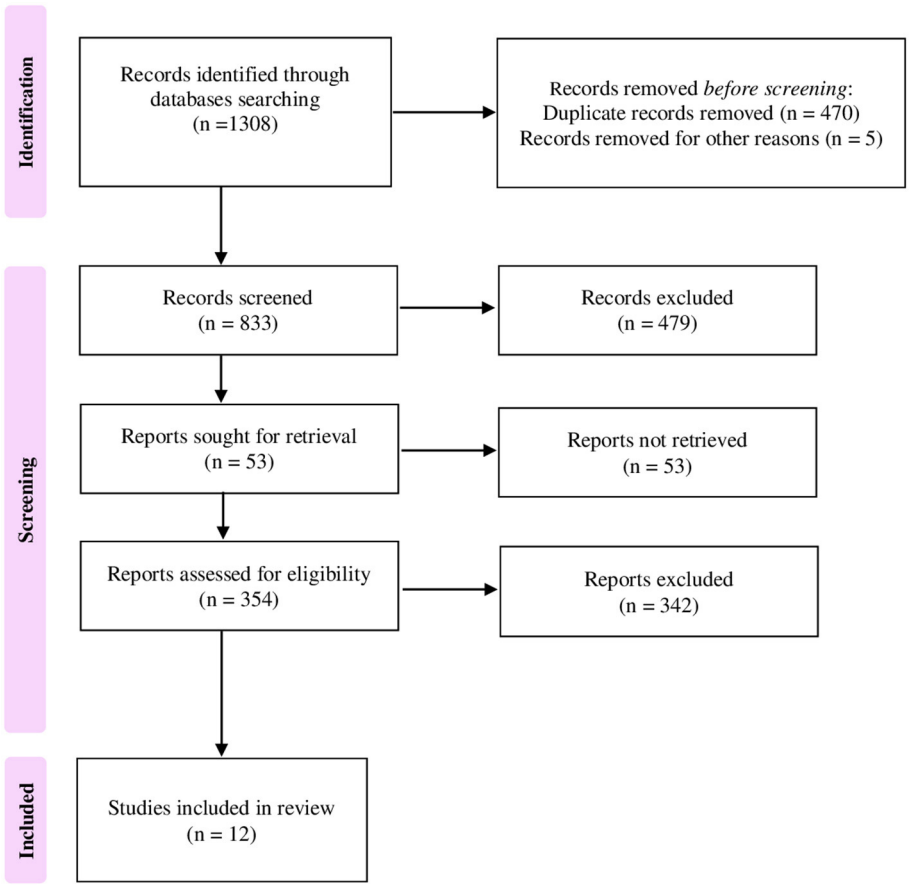

FIGURE 1 | Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow chart illustrating the number of studies at each stage of the review.

Data were cross-checked, any discrepancies were discussed, and disagreements were resolved by a third investigator (RS).

The following information was extracted by two investigators (RZ, VD) separately and in duplicate in a piloted form: (1) general information about single studies (author, year of publication, country, settings, design, sample size, age); (2) type of beverage (including alcohol) exposure; (3) outcome(s) regarding oral health conditions, including periodontal disease, oral dysbiosis, and tooth loss; (4) type of oral health outcome assessment tool(s); (5) main finding(s); (6) effect size of the association between exposure and outcome.

All references selected for retrieval from the databases were managed with the MS Excel software platform for data collection. Lastly, data extracted from selected studies and stored in the database were structured as tables of evidence.

Quality Assessment Within and Across Studies and Overall Quality Assessment

The methodological quality of the included studies was independently appraised by paired investigators (VD, FC), using the National Institutes of Health Quality Assessment Toolkits for Observational Cohort and Cross-Sectional Studies (18, 19). The ratings: high (good), moderate (fair), or poor were assigned to studies according to the criteria stated in the toolkit. This tool contains 14 questions that assess several aspects associated with the risk of bias, type I and type II errors, transparency, and confounding factors, i.e., study question, population, participation rate, inclusion criteria, sample size justification, time of measurement of exposure/outcomes, time frame, levels of the exposure, defined exposure, blinded assessors, repeated exposure, defined outcomes, loss to follow-up, and confounding factors. Items 6, 7, and 13 do not refer to cross-sectional studies, and the maximum possible scores for cross-sectional and prospective studies were 8 and 14, respectively. Disagreements regarding the methodological quality of the included studies between the two investigators were resolved through discussion until a consensus was reached together with a third investigator (RS). A modified version of the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) rating system was used to assess the overall quality of evidence of the studies included in the present systematic review (16, 20). The following factors were considered: the strength of association for beverage consumption and related oral health outcomes, methodological quality/design of the studies, consistency, directedness, precision, size, and (where possible) dose-response gradient of the estimates of effects across the

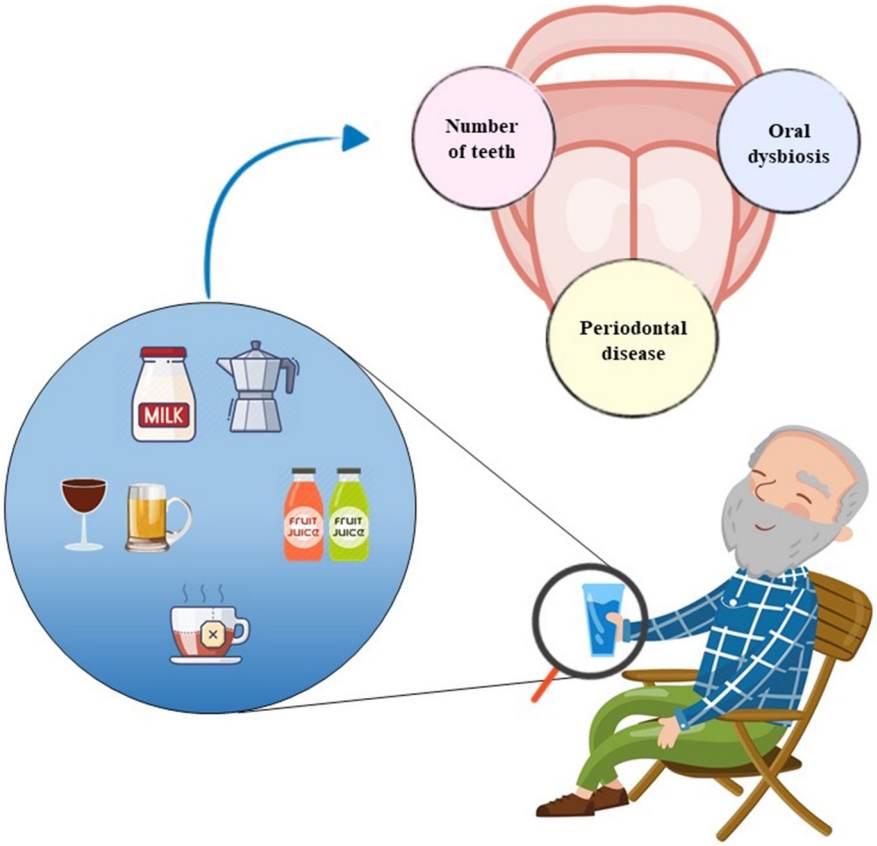

FIGURE 2 | Graphic overview of the findings.

evidence base. Evidence was graded as very low, low, moderate, and high, as in the GRADE rating system.

RESULTS

The preliminary systematic search of the literature yielded 1,308 records. After excluding duplicates, 833 were considered potentially relevant and retained for the analysis of titles and abstracts. Then, 479 were excluded for not meeting the characteristics of the approach or the review goal. After reviewing the full text of the remaining 354 records, only 12 met the inclusion criterion of age and were included in the final qualitative analysis (21–32). The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow chart illustrating the number of studies at each stage of the review is shown in **Figure 1**. The final study base included 12 articles reporting on four different beverages, i.e., coffee, milk, tea, and sugary drinks, as well as alcohol intake. **Figure 2** shows a graphic overview of the findings.

Details of the design (cohort or cross-sectional), sample size (N) and sex ratio (%), minimum or and mean (SD) age or age range, setting (community or hospital), and country of individual studies are shown in **Table 1**. The cross-sectional design (75%, $N = 9$) predominated over the longitudinal design (25%, $N = 3$). Recruitment settings were mostly community-based (91.7%; 11 out of 12), but also included one hospital-based study. The geographic distribution of the studies extended over Asia, Europe, and America ($N = 4$ of 12 each, so 33.3% per continent). In accordance with the inclusion criterion, subjects were aged over 60 and were predominantly 65+ years. Among all 12,223 subjects of the studies, gender was balanced (approximately 50% each). Most studies focusing on alcohol intake found a positive association with tooth loss (3 out of 5 selected studies) (16–19, 25) and periodontal disease (3 out of 4 selected studies) (20, 22, 23, 26). Overall, alcohol intake was the most represented item of the findings (56.25%, $N = 9$ of 16), followed by coffee (18.75%, $N = 3$ of 16), milk (12.50%, $N = 2$ of 16), tea (6.25%, $N = 1$ of 16), and sugary drinks (6.25%, $N = 1$ of 16).

Regarding the measurements, alcohol intake was reported as grams per day or week or per body weight of the enrolled subjects. Tea, coffee, milk, and sugary beverages were quantified as daily cups. The three outcomes recorded in the studies referred to tooth loss, periodontal disease, and oral dysbiosis. The assessment method for tooth loss was consistent across the studies, detected either by questionnaire or clinical assessment, whereas oral dysbiosis was estimated by 16S rRNA gene sequencing. Conversely, assessment measures for periodontal disease included pocket depth on probing (PPD),

TABLE 1 | Selected studies investigating beverage consumption and oral health status in older age (*N* = 12).

Author, Year (Ref.)	Beverage	Oral Health Item	Oral Health Assessment Tool	Design (follow-up)	Setting	*N*	Age	Sex	Country	Findings
Drake et al. (1995) (21)	Alcohol	Number of teeth	Interview (questionnaire)	Longitudinal, 3-year	Community	810	65+	ND	America (USA)	Increased alcohol consumption was associated with tooth loss in Whites
Norlén et al. (1996) (22)	Coffee Alcohol	Number of teeth	Interview (questionnaire)	Cross-sectional	Community	483	68	100% M	Europe (Sweden)	High consumption of coffee or alcohol were associated with fewer remaining teeth
Hanioka et al. (2007) (23)	Alcohol	Number of teeth	Interview (questionnaire)	Cross-sectional	Community	6,805	70+	39% M, 61%F	Asia (Japan)	The prevalence of tooth loss was lower in current drinkers and the relationship approached significant levels in females
Yoshihara et al. (2009) (24)	Milk alcohol	Periodontal disease	Root caries and periodontal disease. Evaluation of the clinical attachment level (CAL)	Longitudinal, 6-year	Community	600	70	51% M, 49% F	Asia (Japan)	There was a significant negative relationship between the amount of daily milk intake and the number of root caries. The number of root caries events during the 6 years was significantly lower among subjects with a greater intake of milk products. Alcohol consumption was positively associated with the number of periodontal disease events during the same time period
Heegaard et al. (2011) (25)	Alcohol	Number of teeth	Number of remaining teeth, including third molars, dichotomized as ≥20 vs. <20 remaining teeth	Cross-sectional	Community	783	65+ (65–95)	ND	Europe (Denmark)	Alcohol consumption, wine drinking, and wine and spirits preference among women were associated with a higher number of teeth compared with abstainers
Adegboye et al. (2012) (26)	Milk	Periodontal disease	Number of teeth with attachment loss ≥3 mm	Cross-sectional	Community	135	65+	47% M, 53% F	Europe (Denmark)	Dairy calcium, particularly from milk and fermented products, may protect against periodontitis
Machida et al. (2014) (27)	Coffee Alcohol	Periodontal disease	Probing pocket depth (PPD) and clinical attachment level (CAL), bleeding on probing (BOP), plaque levels evaluation	Cross-sectional	Hospital	414	66.4 ± 9.9	20.8% M, 79.2% F	Asia (Japan)	There appears to be an inverse association between coffee consumption (≥1 cup/day) and the prevalence of severe periodontitis
Hach et al. (2015) (28)	Alcohol	Periodontal disease	Pocket depths, clinical attachment loss, distance from the enamel–cementum junction to the bottom of the periodontal pocket evaluation	Longitudinal, 20-year	Community	168	65+	45.8% M, 54.2% F	Europe (Denmark)	Early consumption of alcohol may increase the odds of having periodontitis 20 years later. The results of long-term alcohol consumption, from 1981 to 2003, and periodontitis showed that heavy drinkers tended to have a higher odds ratio for periodontitis compared to light drinkers
Tiwari et al. (2016) (29)	Sweet beverages	Number of teeth	Interview (questionnaire)	Cross-sectional	Community	308	65+	47.3 M, 52.7% F	America (USA)	Tooth loss was significantly associated with the consumption of one or more than one sweet beverage per day

(Continued)

TABLE 1 | Continued

Author, Year (Ref.)	Beverage	Oral Health Item	Oral Health Assessment Tool	Design (follow-up)	Setting	*N*	Age	Sex	Country	Findings
Laguzzi et al. (2015) (30)	Alcohol	Number of teeth	Interview (questionnaire)	Cross-sectional	Community	341	65+	63% M, 37% F	America (Uruguay)	Lack of functional dentition, severe tooth loss, and edentulism were found to be associated with frequent consumption of alcohol
Suwama et al. (2018) (31)	Alcohol	Periodontal disease	Probing pocket depth (PPD) and clinical attachment level (CAL)	Cross-sectional	Community	438	73	53.8% M, 46.2% F	Asia (Japan)	An increased mean CAL was significantly associated with heavy alcohol drinking in community-dwelling elderly Japanese
Peters et al. (2018) (32)	Coffee Tea	Oral microbiota	16S rRNA gene sequencing	Cross-sectional	Community	938	65+	ND	America (USA)	Higher tea intake was associated with greater oral microbiota richness and diversity, and shifts in overall community composition. Coffee was not associated with these microbiome parameters

ND, not defined; N, number of study participants; M, males; F, females; CAL, clinical attachment level; PPD, probing pocket depth; BOP, bleeding on probing.

clinical attachment level (CAL), bleeding on probing (BOP), or assessment of the distance from the enamel-cement junction to the bottom of the periodontal pocket.

Of the studies investigating alcohol, 5 of 9 investigated tooth loss as the oral outcome (21–23, 25, 30), while the remaining four investigated periodontal disease (24, 27, 28, 31). Three of five studies of alcohol and tooth loss found a negative relation, while the other two studies found the opposite, so a beneficial relation (23, 25). A single study has extended the analysis also to specific alcoholic beverage consumption, concluding that more than six glasses of wine (in females) and beer (in males) were associated with lower odds of having fewer teeth (16), confirming their results on alcohol. The studies about alcohol and periodontal disease showed an inverse relation (24, 27, 28, 31), except for a single study (22).

Regarding beverages, the conclusion was hampered by the small number of studies. Coffee and milk were found to be inversely related to periodontal disease (24, 26, 27), while sugary beverages and coffee were associated with tooth loss (22, 29). Tea but not coffee appeared to improve oral microbiota, although only one study has been recorded to date (32).

We found a moderate ($N = 9$) to low or ($N = 3$) very low ($N = 4$) methodological quality of the studies (**Table 2**). An overview of quality ratings within (panel A, **Supplementary Material**) and across studies (panel B, **Supplementary Material**) is provided in **Table 2**, highlighting areas with higher or lower ratings. Bias was seen primarily in the domains of sample size justification (selection bias) and blinded assessors (detection bias) (100%, 12/12 studies for both domains), and to a lesser extent in the domains of the participation rate (3/12 studies, 25% of studies with a higher risk of bias), different levels of exposure (2/12 studies, 17% of studies with a higher risk of bias), outcome measurement (3/12 studies, 25% of studies with a higher risk of bias), and statistical correction for confounding factors (3/12 studies, 25% of studies with a higher risk of bias) (**Table 2**, panel B, **Supplementary Material**).

DISCUSSION

The present systematic review aimed to address the conceptual hypothesis of the existence of a link between beverage consumption and oral health in the aging population. To this end, the body of evidence on alcohol intake and beverage consumption as milk, tea, coffee, and sugar-sweetened drinks was examined in relation to poor oral health outcomes, including periodontal disease, oral dysbiosis, and tooth loss. A major result of this systematic review was the lack of good studies filling the gap of knowledge in regard to the research question. Nevertheless, some interesting findings were revealed, such as a potential role of high alcohol intake in the development of periodontal disease and probably tooth loss. Tooth loss could also be associated with the consumption of sugary beverages and coffee, although only one eligible study of each had been retrieved (18, 24). Periodontal disease, on the other hand, seems to be potentially inversely affected by the high consumption of beverages such as milk or coffee. Only one study investigated the

TABLE 2 | Summary of findings about different beverages associated to oral health items in older age.

Exposure	Evidence base	Strength of association	Strength of evidence (GRADE)
Alcohol	Nine studies	Number of alcoholic drinks/week and loss of at least one tooth in 3 years: logistic regression estimate of 0.483 (OR 1.62), significant	⊕⊕⊕ Moderate
	$n = 10,842$	(21)	
		Number of teeth within three categories of increasing alcohol consumption. Less teeth were observed with increasing alcohol intake. ANOVA of lowest (0–110 g alcohol/week) and highest (>250 g/week) category was significant $p < 0.05$	
		(22)	
		Males: Tooth loss with current alcohol consumption (>20 g of alcohol/day for 3 or more days/week) vs. never: OR 0.71, 95% CI 0.47–1.09. Females: Tooth loss with current alcohol consumption (>20 g of alcohol/day for 3 or more days/week vs. never): OR 0.25, 95% CI 0.07–0.84	
		(23)	
		Regression analysis between alcohol consumption (g/kg) and periodontal disease events over 6 years: Positive regression coefficient of 1.87, 95% CI 0.08–3.66, $p = 0.041$	
		(24)	
		OR for having a low number of teeth (>20) with moderate or heavy alcohol drinking vs. abstainers: females OR 0.40, 95% CI 0.22–0.76 for moderate drinking and OR 0.34, 95% CI 0.16–0.74 for heavy drinking. Similar estimates for males	
		(25)	
		Relation between alcohol consumption (never/former vs. current) and severe periodontitis (presence/absence): OR: 1.45, 95% CI 0.82–2.57 (non-significant)	
		(27)	
		Various follow-up periods and drinking information were analyzed. In the most reasonable analysis (5 years' follow-up time with high respondence to follow-up), heavy alcohol consumption (>7 units per week for women and >14 units per week for men) vs. light consumption (0–3 units per week for women and 0–7 units per week for men) showed an OR of 4.64, 95% CI 1.1; 19.42 for periodontitis	
		(28)	
		Frequent and infrequent alcohol consumption (daily/weekly intake vs. no/annual/monthly intake) and every tooth loss: Prevalence OR 1.54, 95% CI 1.20–1.56	
		(30)	
		Heavy alcohol consumption (≥40 g for men, ≥20 g for women vs. non-drinking) and risk of periodontal disease (assessed by clinical attachment level, CAL): OR: 2.44, 95% CI 1.03–5.78	
		(31)	
Coffee	Three studies	Number of teeth within four categories of increasing coffee consumption (0 cups/day, 1–2 cups/day, 3–6 cups/day, ≥7 cups/day). Less teeth were observed with increasing coffee intake. ANOVA of lowest (0 cups/day) and highest (≥7 cups/day) category was significant $p < 0.001$	⊕ Low
	$n = 1835$	(22)	
		Logistic regression analysis between coffee consumption (≥1 cup/day vs. <1 cup/day) and risk of severe periodontitis: OR: 0.55, 95% CI 0.32–0.92	
		(27)	
		Regression analysis between coffee consumption (cups/day) and oral microbiota richness: negative regression coefficient of −0.216, 95% CI −1.038 to 0.606, $p = 0.606$	
		Regression analysis between coffee consumption (cups/day) and microbiota diversity: positive regression coefficient of 0.002, 95% CI −0.013 to 0.018, $p = 0.77$	
		Regression analysis between coffee (cups/day) and oral microbiota evenness: positive regression coefficient of 0.001, 95% CI −0.001 to 0.002, $p = 0.52$	
		(32)	
Milk	Two studies	Regression analysis between Milk and Milk Products (MMP) (g/Kg) and periodontal disease events over 6 years: negative regression coefficient of −0.10, 95% CI 0.20–0.07, $p = 0.035$	⊕ Very low
	$n = 735$	(24)	
		Logistic regression analysis between total dairy calcium (> recommended mg/day vs. < recommended mg/day) and risk of periodontitis: OR: 0.76, 95% CI 0.58–0.99, $p = 0.04$	
		Logistic regression analysis between total dairy whey (≥ 9.6 g/day vs. <9.6 g/day) and risk of periodontitis: OR: 0.75, 95% CI 0.58–0.97, $p = 0{\cdot}03$	
		(26)	

(Continued)

TABLE 2 | Continued

Exposure	Evidence base	Strength of association	Strength of evidence (GRADE)
Tea	One study	Regression analysis between tea (cups/day) and oral microbiota richness: positive regression coefficient of 1.473, 95% CI 0.015–2.931, $p = 0.05$	⊕ Very low
	$n = 938$	Regression analysis between tea (cups/day) and microbiota diversity: positive regression coefficient of 0.039, 95% CI 0.011–0.067, $p = 0.006$	
		Regression analysis between tea (cups/day) and oral microbiota evenness: positive regression coefficient of 0.004, 95% CI 0.001–0.007, $p = 0.002$	
		(32)	
Sugary beverages	One study	Logistic regression analysis between sugary beverages consumption (>1drink/day vs. ≤1drink/day) and risk of tooth loss: OR: 4.52; $p = <0.01$	⊕ Very low
	$n = 308$	(28)	

OR, odds ratio; CI, confidence interval; p, p-value; OR, odds ratio; CI, confidence interval.

role of beverages (tea, coffee) in the microbiota composition of the oral cavity in the elderly. The overall quality of the evidence was rated as moderate for alcohol intake but low to very low for beverages.

Several biological pathways have been proposed to explain the association between alcohol and oral health. These pathways were based on intrinsic features of alcoholic beverages. Alcoholic (carbonated) drinks may lead to a rise in salivary acid levels of the mouth, resulting in a drop in salivary pH and the consequent risk of tooth erosion (33, 34). Among alcoholic beverages, wine is particularly low in pH, and this makes erosions quite common among drinkers (35), whereas beer is carbonated. Furthermore, alcohol can damage the soft tissues of the mouth, leading to periodontal disease. Moreover, alcohol slows down the salivary flow, which might explain why alcohol drinkers experience an increase in dental plaque, and thus a higher risk of both tooth decay and discoloration, and of receding gums (33). The saliva flow helps to neutralize acids released by plaque, which further fights tooth decay. A lack of saliva allows acids to accumulate and cause gum disease and tooth decay, along with periodontal disease (36).

The current review identified sugary drinks and coffee as fostering tooth loss. A recent meta-analysis reporting on the association between sugary beverages and dental caries or erosion found a positive dose-response gradient for caries (11). In general, sugar is a substrate supporting bacterial flora that breaks down enamel and dentin and promotes the development of caries, and this may support biological plausibility of our findings. Due to the cross-sectional design of most of the studies included in this review, no claims about temporality can be made, but the positive dose-response relationship of the aforementioned meta-analysis supports a potential causative link. Moreover, coffee is often sweetened with sugar or syrup, leading to bacterial fermentation and disruption of the enamel surface.

Other effects of beverages on oral health could be related to bone metabolism in general. Caffeine consumption has been reported to be involved in altering calcium metabolism and reducing bone mineral density, possibly due to its ability to inhibit osteoblast development and thus the expression of vitamin D receptors on osteoblast surfaces (37). As such, heavy coffee consumption has been reported to be associated with a higher risk of osteoporosis and osteoporotic fractures (38, 39). The observed small beneficial association of milk regarding periodontal disease in adults is in line with literature considering milk and dairy products, containing micro and macronutrients such as calcium and casein, to be helpful in preserving tooth mineralization and protecting against the early onset of cariogenic bacteria (40). In this regard, a large Danish cross-sectional study supported the hypothesis that higher intakes of calcium, casein, and whey may contribute to a lower risk of periodontitis over a wide age range, although causality cannot be inferred (26). Aligned with these latter points, an earlier observation conducted by Barrett-Connor and colleagues indicated that lifelong caffeinated coffee intake only preserved bone mineral density in regular milk drinkers. As such, it seems that caffeine- or coffee-induced calcium and bone loss may be compensated by adequate calcium intake from milk (41). By contrast, the weak positive role of coffee on periodontal disease is supported by a recent report showing that coffee consumption may be protective against periodontal bone loss in adult males (42). These authors found a small protective association of coffee consumption (≥1 cup/day) with periodontal health, particularly in reducing the number of teeth suffering from alveolar bone loss; they attributed this finding to antioxidant nitrogen compounds, vitamins, minerals, and phenols to be found in coffee.

Data on tea were confined to the report by Peters et al. (32), observing an overall improved richness and composition of the oral microbiota in tea drinkers. This observation is in line with other findings suggesting a beneficial role of green tea on oral bacterial networks estimated in the stool of healthy subjects (43). Also, considering existing scientific assumptions about caries and periodontal disease conditions as being associated with reduced oral microbiome diversity (44), this would suggest a potential pathway to good oral health driven by tea consumption.

In the present study, the limited data and the heterogeneity of exposure variables in relation to oral health outcomes lower the reliability in quantitative terms of this narrative

and qualitative meta-analysis. Some other limitations should also be taken into account. Firstly, the designs were different across the selected studies, the cross-sectional design being more common, thus leaving little space for discussion of a causal inference. The statistical method of elucidating effects (association) of alcohol and different beverages consumption on oral health, even if the definition was the same, was different across the studies in terms of both the assessment tools used and of the specific description of the beverage type. Especially concerning alcohol, the exposure of interest was the amount as such, rather than the type of alcohol beverage consumed. Also, selected studies differed in sample size and number of beverages considered, alcohol intake being the major focus.

The present systematic review highlighted the importance of considering beverages consumption, in particular alcohol, in protocols to preserve oral health and prevent oral frailty during aging. More research into other beverages and oral cavity outcomes is needed, not covered here due to lack of studies in the elderly, as well as more and better studies on the relations addressed here. We see this review as a first step toward a more thorough acquisition of evidence about this research question (20), which is a prerequisite of an informed decision-making process in public health nutrition. Nowadays, the preventive management of geriatric syndromes is becoming increasingly imperative in a multidisciplinary setting.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RZ and FC: conceptualization, research, resource provision, data collection, writing original version, and visualization. HB: review and correction. RZ, VD, and ML: research and data collection. FP, GG, and GD: conceptualization, validation, review, and correction. RS and HB: conceptualization, validation, review and correction, and visualization. All authors contributed to the article and approved the submitted version.

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Tropical Oral Disease: Analysing Barriers, Burden, Nutrition, Economic Impact, and Inequalities

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Traditionally, a healthy mouth is a good indicator of good general health. Poor oral hygiene reflects the health of the oral cavity and is a risk factor for overall health. Although oral diseases like dental decay and periodontitis are prevalent, awareness of oral diseases is still limited. Oral disorders include a wide range of diseases that may not be confined to the oral anatomical structures but may be manifestations of systemic diseases. Identification of the risk factors of dental and oral diseases, including socio-economic determinants, plays a major role in the type of oral health care, and in the promotion of dental health awareness. This article reviews oral diseases in the Caribbean and aims to raise awareness of this subject while suggesting a research agenda for the region.

Keywords: oral disease, dental caries, periodontitis, oral cancer, health care, nutrition, tropical, Caribbean

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INTRODUCTION

Oral diseases can be broadly categorized into developmental, microbial, inflammatory, cystic, neoplastic, and oral manifestations of generalized or systemic diseases. The developmental pathologies may affect the soft and hard tissues of the oral cavity. Developmental malformations are the abnormalities that result from disturbances of growth and development (1, 2). The jaw bone, palate, dentition, and salivary glands may be involved in a number of disturbances that affect the shape and form of oral structures. Developmental alterations of the teeth and other structures lead to functional disturbances (3). However, greater levels of functional and aesthetic disturbances are associated with cleft lip and cleft palate. Some of the developmental disturbances are hereditary or familial due to mutations or genetic abnormalities, whereas other developmental disturbances are caused by local abnormalities or environmental influences (4). Studies on the frequency of developmental disorders of teeth show a wide range between 1.73 and 34.28% (5, 6). WHO report on the global burden of cleft-lip and/or palate data shows that an incidence of 3.74 per 1,000 live birth in Americans, 1:700 in Europeans,.82 and 4.04 per 1,000 live birth in Asians,.6 and 2.69 per 1,000 live birth in Caucasians, and 0.18 and 1.67 per 1,000 live births in Africans (7, 8). The highest incidence is found in Americans and the lowest in Africans, with an intermediate incidence in Caucasians.

The most frequent dental problem is dental caries which is microbial in origin. Dental caries result in the demineralization of inorganic constituents, whereas organic substances experience destructive damages. Infections in enamel and dentin tissue may further progress into the pulp and periapical tissues and cause serious dental problems such as pulpal and periapical inflammation (9). Periodontal diseases are infectious and inflammatory conditions primarily involve the periodontal tissues (10). These infections may spread into the jaw bone and soft tissues in favorable circumstances (11), for example, low tissue resistance, poor immune mechanism,

or malnutrition. The presence of epithelial tissue within the bone marrow of the maxillae and mandible is one of the numerous dissimilarities between the jaws and other bones of the skeleton. The source of this epithelium is both odontogenic and non-odontogenic, which predisposes the jaws to the development of cystic pathology (12).

Neoplastic diseases of the oral cavity are a broader category. Neoplastic diseases are classified as odontogenic, non-odontogenic, and of salivary gland tissue origin (13). Oral tissues can develop benign or malignant neoplasms like any other body tissue, and oral cancer is of most concern to dental surgeons. The most frequent neoplasm in the mouth is oral squamous cell carcinoma (OSCC). OSCC is the fifth most frequent cancer globally with an estimated incidence of 400,000 new cases annually (14). Benign conditions such as ameloblastoma are also a concern, as they may also cause severe destruction of the jaw bone.

Oral manifestations of systemic disease may be caused by vitamin deficiencies, blood dyscrasias, metabolic disturbances, endocrine disturbances, granulomatous diseases, dermatological and mucous membrane diseases, bone diseases, and poisoning due to metals (15). Oral health providers must maintain alertness to these manifestations as the systemic disease may first present in this form. Close consideration should therefore be given to the exact pathology of oral disorders. This includes a broad spectrum of diseases ranging from modest to severe tissue damage, which will pose management challenges. Raising the awareness of oral diseases will increase the focus on the dental needs of society. To summarize, the major risk factors for oral diseases can be broadly put into three categories: dental caries, periodontal diseases, and neoplasms of the oral cavity.

RISK FACTORS

Dental Caries

Initially, the factors such as diet, microbial flora, and susceptible teeth were linked to the initiation and progression of dental caries (16). However, recent concepts state that caries is a resultant of factors such as a susceptible host, cariogenic micro-organisms, suitable substrate (sucrose sugar/carbohydrate), and duration of exposure (17). The four factors in the etiology of dental caries are influenced by local and general factors. Tooth alignment, salivary rate, and oral hygiene are local risk factors. Whereas, parameters such as gender, age, ethnicity, geographic variations, and socio-cultural practices are general risk factors for caries development. Diet is the most dominant variable and risk factor in establishing the prevalence and incidence of dental caries. The evidence suggests that microbial flora have a higher risk influence in the initiation and progression of dental caries. Studies on the localization of microbial flora related to dental caries suggested *Streptococcus mutans* as a pioneer bacteria in dental caries pathology (17). Based on the production of bacteriocins and mutacins, *Streptococcus mutans* are classified into four types, namely, I, II, III, and IV. The antigenicity and virulence of *Streptococcus mutans* are evaluated by the serotypic classification (18). Based on the chemical constituents of the cell surface in *Streptococcus mutans*, strains are classified as c, e, and f serotypes (19). *Streptococcus mutans* adhere to host tissue, i.e., tooth surface through the enzymatic action of gluocsyltransferase (GTF) enzymes. Thus, the pathogenic nature is influenced by the enzymatic factor and thus linked with the serotype of the organism (20, 21). A study that investigated the diversity, commonality, and stability of *Streptococcus mutans* genotypes associated with dental caries among children, identified the high caries risk *S. mutans* genotypes (22). Based on the studies with *Streptococcus mutans* genotypes, the suggestion is that epidemiological correlation with high caries risk in the community should be researched to identify the high-risk genotypes of *Streptococcus mutans* in the community.

Periodontal Diseases

Periodontal disease is considered a chronic disease and frequently affects all age groups, i.e., children, adolescents, adults, and the elderly (23). The periodontal structures which support teeth are important as they hold them in their anatomical position. It is not just infection that causes periodontal morbidity but other factors have a significant role in periodontal disease initiation and progression. Risk factors have a substantial impact on the response of an individual to periodontal infection. These risk factors include age, tobacco use, smoking, alcohol consumption, brushing habits, lifestyle, genetic influences, diabetes mellitus, obesity, metabolic syndrome, osteoporosis, and vitamin deficiencies (24–26). Modification of these risk factors allows us to control periodontal diseases. Understanding risk factors and the early identification of vulnerable individuals will assist a dental surgeon in planning prevention and treatment strategies for periodontal diseases (25). Based on our understanding, some of these risk factors are independent and modifiable, such as smoking and alcohol consumption. Generating awareness about modifiable risk factors and educating the public about the other risk factors in the community may raise the importance of oral health, and the rate of tooth loss may be reduced (27). Smoking is the most well-established modifiable risk factor for periodontitis. However, evidence to support a relationship between periodontal disease and nutrition, alcohol consumption, socioeconomic status, and stress levels have not been clearly established (28).

Periodontitis has been reported to affect 11% of the global population and is listed as the sixth most frequent condition in the world (29). Globally, symptoms of periodontal disease were frequently observed in adults (30, 31). A severe form of periodontal disease, aggressive periodontitis, is noted to affect 2% of teenagers worldwide. Severe periodontitis was ranked 77th among the detailed causes of Disability Adjusted Life Years (DALYs). Severe periodontitis is mentioned as a leading cause of DALYs in 9 regions of the world, namely, Australasia, Sub-Saharan Africa East, Central, East, and Southeast Asia, and Southern, Central, Tropical, and Latin America (31). It is noteworthy to mention that data on the frequency, type, and associated risk factors of periodontal disease in the Caribbean region needs to be researched.

Oral Cancer

Tumors of the head and neck comprise an important group of neoplastic conditions of the body. The incidence of head and neck cancers is increasing in many parts of the world (32). This increase remains high despite all the advances in modern medicine. These malignancies are more prevalent in the developing world and, unfortunately, have not received satisfactory attention as have the more prevalent cancers of the developed world, like lung, breast, and colon cancer (7).

According to the World Health Organization (WHO), the most commonly diagnosed cancers in males worldwide were those of the lung, prostrate, colorectal, stomach, bladder, and oral cavity, whereas in females, it is breast, colorectal, lung, stomach, uterus, cervix, ovary, bladder, liver, and oral cavity. The data on cancer statistics suggests that oral cancer is the sixth most common cancer among men and the tenth most common cancer in women. Oral cancers are reported to be more prevalent in developing countries of the world. However, oral cancers have not received satisfactory attention as compared with other common cancers (7, 31, 33–35).

More than 95% of the carcinomas of the oral cavity are of the squamous cell type in nature. They constitute a major health problem in developing countries, representing a leading cause of death. The survival index continues to be small (50%) as compared with the progress in diagnosis and treatment of other malignant tumors (7). The risk factors include tobacco chewing, smoking, alcohol consumption, immunosuppressed condition, and diets with low levels of vitamins A and C. Inadequate consumption of vegetables and fruits may contribute to the risk of oral cancer (23, 36, 37). In the Western world, the use of tobacco and alcohol is considered to be the greatest risk factor (38). These risk factors are independent and inter-dependent. Ogden (2000) suggested that tobacco smoking is associated with 75% of overall oral cancer cases. Further, it was mentioned that tobacco smoking individuals have a 6-fold risk of developing oral cancer when compared to non-smoking individuals. The ratio was similar with persons who drink alcohol and non-drinkers. The combination of tobacco and alcohol use poses a 15-fold risk of oral cancer development in comparison with non-users (39). While tobacco and alcohol use are traditionally the greatest risk factors, other known risk factors are betel quid chewing, areca nut, narcotics, epigenetic factors, and viral infections such as Human papilloma virus (HPV), Epstein Barr virus (EBV), and Hepatitis C virus. However, certain lesions are considered to be precursor lesions to oral cancer, and these include leukoplakia, erythroplakia, actinic cheilitis, lichen planus, sideropenic dysphagia (Plummer-Vinson syndrome), submucous fibrosis, dyskeratosis congenita, and discoid lupus erythematosus (40). Recently, these lesions are termed potentially malignant disorders (41). Cancer awareness programs should be targeted at different levels of the population. One suggestion would be to target schools, individuals with occupational risks, and persons with precursor lesions to prevent the further development of precursor lesion to cancer and to prevent the occurrence of both precancer and cancer among school children individuals who have smoking or other deleterious habits.

TROPICAL ENVIRONMENT AND ORAL DISEASE

The term tropical denotes a climatic feature with which other aspects such as soil and vegetation are correlated. The tropical geographical location is that portion of the globe where the sun passes directly overhead. The tilt in the earth's axis extends between 23°-30° latitude north and south of the equator, and it covers 38% of the total land surface (42). The influencing factors in tropical countries are major climatic subdivisions, socio-economic status, environment, food, water, and nutrition. Oral diseases that can result from exposure to sunlight are pigmentation, actinic chelitis, squamous cell carcinoma, keratocanthoma, basal cell carcinoma, and malignant melanoma (42). Traditionally, cultural and religious rituals involving the teeth and oro-facial soft tissues also have an impact on the oral and para-oral structures such as mutilations of teeth and oral soft tissues, tooth crown, and soft tissues (43). The significance of oral diseases in tropical environments should be focused primarily on nature and society (cultural and religious rituals). To summarize, the tropical phenomenon does not restrict the scope of these inquiries solely to this zone. It can also be said that physical processes, human characteristics, national borders, and the distribution of oral diseases in tropical countries do not coincide with lines of latitude.

Inter-relation of Oral Disease With Systemic Health

Infectious oral diseases predispose to systemic involvement and potential complications due to the hematogenous spread which can result from focal infections (44). Common inflammatory conditions of the oral tissues such as dental caries, gingivitis, and periodontitis are usually precipitated by the formation of dental plaque (45). The dilation of pulpal and periodontal vasculature due to the sequelae of inflammation provides a greater surface area that facilitates the entry of microorganisms into the bloodstream. Often, the bacteremia is transient with the highest intensity limited to the first 30 min after a triggering episode (46). On occasions, this may lead to the seeding of the microorganisms in different target organs and result in systemic infections (46). It is a well-recognized concept that oral infections, especially periodontitis may affect systemic health and contribute to systemic disease development and progression. This includes cardiovascular disease, cavernous venous thrombosis, bacterial pneumonia, diabetes mellitus, and low birth weight (47). The focus on periodontal disease is due to the fact that the periodontium can serve as a reservoir for mediators such as cytokines and interleukins which can enter the systemic circulation and induce the disease process (48–50). Based on our understanding, a large body of literature has suggested that oral infections may contribute to poor systemic health and disease development. Among the systemic developments, endocarditis has been extensively studied. A future goal in this area is to identify the epidemiological data in cases of oral infections that resulted in systemic complications.

Prevention of Oral Diseases

Preventive oral diseases programs should be aimed at the three conventional methods, namely, primary, secondary, and tertiary (51). The preventive programs should focus on the pre-pathogenic period prior to the onset of smoking habits among early adolescents with tobacco related health hazards and social problems. Prevention strategies should also focus on the early pathogenic period with prompt referral counseling centers, whereas tertiary prevention strategies are to focus on the prevention of complicating sequelae in the disease process (51, 52). Preventive programs should be targeted at various levels to improve oral cancer awareness. School children should be educated about oral health behavior, potential damages of oral tissues, and general health due to smoking and drinking. A plan may be proposed to the school education system about oral health awareness and the impact of compromised dental and oral health may probably motivate student learning and result in positive oral behavior (53). Films about neglected oral health and its impact on general health can be shown in an educational institution to promote oral health (54). Social stigmas related to oral conditions such as cleft lip and cleft palate should be identified and special care should be provided for these students.

Global Burden of Oral Diseases

The burden of oral and dental disease is high, especially in the lower socioeconomic groups and in challenged individuals in both developing and developed nations across the globe. Pathological conditions in the oral cavity such as dental decay, periodontitis, tooth loss, trauma to tooth and jaw, oropharyngeal cancers, oral mucosal lesions due to systemic manifestations, HIV related oral manifestations, and periodontal tissue damage due to diabetes are the major oral health problems worldwide (55). Poor oral hygiene and poor health have the greatest influence on the quality of life of a person (56). The varied nature of oral disease patterns across nations needs to be identified and should be used in the planning of preventive oral health care programs. Identifying the risk factors locally will help in implanting proper preventive measures for oral health.

Dental caries is still a major health problem in most industrialized countries and it affects 60–90% of school aged children (57). Worldwide, the prevalence of dental caries among adults is high as the disease affects nearly 100% of the population in the majority of countries. The data published by WHO show high Decayed, Missing, and Filled—Tooth (DMFT) values in Latin America. In several industrialized countries, older people have often had their teeth extracted due to the disease process. The proportion of edentulous adults aged 65 years is high in Albania (69%) and in the USA (26%) (7, 57). Establishing oral health awareness and the importance of teeth may increase the demand for dental treatment.

The locations characterized by high incidence rates for oral cancer (excluding the lip) are found in South and Southeast Asia (e.g., Sri Lanka, India, Pakistan, and Taiwan), parts of Western (France) and Eastern Europe (Hungary, Slovakia, and Slovenia), parts of South America (Brazil, Uruguay), the Caribbean (Puerto Rico), and in the Pacific (Papua New Guinea and Melanesia). In the Caribbean, Puerto Rico has the highest reported incidence of oral cancer (>15 per 100,000). In terms of worldwide levels, Cuba has an intermediate incidence range of cancers of the oral cavity. A Cuban study that investigated the impact of heavy cigar smoking on the population reported a smoking incidence of 7.2 per 100,000 population. The data presented was stable for over the past decade (58).

The Economic Impact of Oral Disease

Conventionally, dental treatment is sought by persons in higher socio-economic levels, as the costs associated with treating dental and oral diseases are high. Dental Caries is recognized to be the fourth most expensive disease to treat in industrialized countries. Dental practitioners provide their treatment with or without third party payment schemes and, in most developing countries, investment in oral health care is low (59). This makes the development of preventive oral awareness and preventive care programs mandatory if we are to reduce the prevalence of oral disease. Roby et al. (60) mentioned that industrialized countries like Israel spend 12.5%, Germany 8.6%, Sweden 8%, the USA 4.2%, and the UK and Sri Lanka 3.5% of their health funds for dental care (60). Identifying the "*partnership networking*" for oral health care is suggested as a key to reducing the economic barrier. *Partnership Networking* is aimed at bringing high-level healthcare professionals through a combination of regional and international experts to collaborate with local Ministries of Health and dentists to address health care gaps and elevate preventive oral health awareness, through campaigns, and outreach health services. Finding a partnership for oral health care in terms of prevention and oral health education should be a discussion point in dental society meetings, continuing dental education programs, and conferences.

Global Oral Health Inequalities

A major global problem for oral disease care is the failure to implement preventive programs and a failure to understand the social determinants of oral disease. Gaps in knowledge, the separation of oral health from general health, and inadequate evidence-based data are known to be barriers that have led to global oral health inequalities. The International Association of Dental Research (IADR) addressed these three barriers and suggested that the critical gaps in knowledge be identified as this perhaps may bring oral health concepts into the public domain (61). Developing and implementing the partnership with cognate organizations, a knowledge base that uses a standard set of reporting criteria and includes a registry of implementation trials should assist in reducing the inequalities. Emphasis should be placed on identifying the significance of social determinants of oral health. Emphasis should also be placed on the importance of integrating research on oral health inequalities with the wider goal of reducing health inequality as a whole. Emphasis should also be placed on the importance of multi, inter, and trans-disciplinary research and translational research using inter- and multi-sectorial approaches. Disease prevention strategies should be developed based on upstream prevention. Strategies should be developed that is capable of local interpretation in a way that respects cultural sensitivities and socio-economic constraints. Local, regional, and country level systems should

be developed for oral health promotion and healthcare that are appropriate and recognize resource implications. The issue of oral health inequalities should be raised in wider public debates with specific emphasis on underprivileged communities (60). Reducing the barriers and proposing research driven programs. Capacity building research strategies and standardized systems for measuring oral health should raise the level of oral health awareness in society.

Oral Disease Scenario in the Caribbean

Bönecker et al. (61) revealed that evidence of a decrease in dental caries in Latin American and Caribbean children had been noted among 5–6 and 11- to 13-year-old children. Further, they mentioned that the decrease in dental caries was less prominent in the past few years (62). A national survey in St. Vincent and the Grenadines reported a high prevalence of calculus and bleeding, especially among older children. The proportion of children with healthy periodontium ranged from 51% among 7-year old and 12% in 15 to 19 years old (62, 63). As mentioned, Puerto Rico has the highest reported incidence of oral cancer in the Caribbean and Cuba has an intermediate incidence of oral cancers (59). A database search of Caribbean studies revealed that research was focused on the Epidemiology of cariology, periodontal disease, and hygiene or home care practices. In addition, other research areas found in the database were implantology, patient education, preventive dentistry, and dental education. The research findings from Caribbean studies are summarized in **Table 1**.

Nutrition and Oral Disease

Nutrition significantly influences the development and progression of dental and oral tissues (101). The nutritional impact on dental and oral diseases can result from either high sugar content or malnutrition. Dental caries is the most common condition that arises due to the nutritional status of a person. However, other factors also play a role in the initiation and development of carious teeth (2, 93). Nutrition-related pathologies that affect oral tissues are dental caries, periodontal diseases, erosions, fluorosis, acute necrotizing ulcerative gingivitis (102) or periodontitis or oral manifestations of avitaminoses, and micro- or macro-mineral deficiencies. Malnutrition also influences the development and growth of the dentition (103). Dental caries results from acids synthesized by cariogenic bacteria and carbohydrate sources. Oral manifestations in Vitamin B deficiency show glossitis due to loss of papilla over the dorsum of the tongue. Atrophy of fungiform and filliform papillae is observed in folic acid deficiency (104). Vitamin C deficiency presents with bleeding and spongy appearance of the gingiva. Vitamin A and D deficiencies may present as enamel hypoplasia. Vitamin A influences the turnover rate of keratinized cells. Thus, vitamin A deficiency may affect the exfoliation of oral epithelial cells and ulcerations. Vitamin K deficiency presents with wider pre-dentin thickness over the tooth (105). Minerals such as zinc, calcium, manganese, copper, magnesium, and selenium also show oral manifestations. Burning sensations of the tongue or oral cavity are associated with zinc deficiencies (106). Calcium deficiency during the growth or eruption of teeth may result in enamel hypoplasia (107). Other micro mineral deficiencies may show oral ulcerations and impaired wound healing. Acute necrotizing ulcerative gingivitis or periodontitis are usually observed in individuals with malnutrition (102).

Future Directions

Data about the global burden of oral diseases is well-documented but finding data on the oral disease status in Caribbean populations is difficult to identify among those published documents. Epidemiological research (cross sectional and longitudinal studies) on oral diseases needs to be documented on Caribbean populations. Conducting a survey with dental surgeons about their practice and experience of dental disease in the country may generate immediate documentation about the oral disease prevalence. A special focus should be made on dental caries, periodontal diseases, fluorosis, edentulouness, and oral cancer. A survey of dental surgeons that focuses on oral cancer patients in their care, similarly, ENT practitioners and medical hospitals may also assist in generating needed data on the oral cancer burden in the Caribbean population. The survey questionnaire should also include questions on habits such as smoking, alcohol, marijuana (ganja) usage, and other relevant data. The data on habits may be useful for sub-analysis of the survey questions with the disease-like risk factors. Data from cancer registries will be helpful in analyzing the risk factors for oral cancer. The need for epidemiological and surveillance studies to determine the scope of oral health problems and their impact on future dental services needs to be stressed to oral health care workers in meetings. The National Institute of Cancer in the United States of America conducts a Surveillance, Epidemiology, and End result or "SEER" program. A proposal for SEER like programs needs to be planned in the Caribbean and by developing such programs, oral cancer data will be generated in a continuous mode. Interdisciplinary studies such as oral health in HIV/AIDS, oral health in psychiatric patients, oral health in physically compromised individuals, oral mucosal lesions in patients with dermatological diseases, and periodontal health status in Type II Diabetic patients should be carried out. Studies need to be proposed for hospital-based patients such as "oral hygiene evaluation in physically and mentally challenged individuals." Documentation of oral findings in systemic disease may strengthen the trans or multi-disciplinary approach to oral health care. Such trans or multi-disciplinary studies should be promoted in dental clinics with the medical hospital or educational institutional setups. Creating knowledge about "the importance of the primary dentition" in schoolage children between the ages of 5–13 may reverse the trend in dental diseases in the future. In school based oral health programs, information about diet and its role in dental and oral health have to be included, and the same information should be made available to parents. Conducting surveys relating to the "knowledge about oral cancer and its awareness" in individuals in the age group of 16–25 years across these educational institutions can be planned to determine the level of "awareness." Based on the results of these survey reports, oral health care providers may understand the level of awareness, and this will be a helpful tool in revising

TABLE 1 | Published research findings from the Caribbean region.

Publication year	Country	Research hypothesis	Research conclusion	Area of research	Type of publication	References
(2014)	Puerto Rico	Epidemiology of hypodontia in 10–14 years	The prevalence of hypodontia in Puerto Rico was 6.02%.	Epidemiology – Oral Disease	Original research	(64)
(2015)	Puerto Rico	Efficacy of CPC and essential oils mouthwashes compared to a negative control mouthwash in controlling dental plaque and gingivitis	CPC mouthwash showed a reduction of gingival index scores and gingival interproximal index scores. However, these reductions were not considered clinically significant.	Preventive dentistry - Periodontology	Original research	(65)
(2018)	Puerto Rico	To investigate frequency, severity, and risk factors associated with gingival inflammation in adult populations from Kingston (Jamaica), Santo Domingo (Dominican Republic), and San Juan (Puerto Rico).	Gingival inflammation was highly prevalent, but most study subjects presented a moderate level of gingival inflammation.	Periodontology	Original research	(27)
(2013)	Puerto Rico	Estimation of Dento-Gingival Complex dimension variation based on gingival biotype.	Dentogingival complex dimensions are different for thin, mixed, and thick gingival biotypes.	Periodontology	Original research	(66)
(2015)	Puerto Rico	Evaluate the clinical efficacy of two commercially available, fluoride-free, alcohol-free mouthwashes containing either 0.075% or 0.07% cetylpyridinium chloride (CPC) in controlling established dental plaque and gingivitis compared to a non-antibacterial control mouthwash.	Participants rinsing mouth rinse containing Cetylpyridiium chloride exhibited statistically significant reductions in all the gingivitis and plaque parameters. Whereas, in those using the non-antibacterial mouthwash, significant reductions were only observed in whole mouth and interproximal plaque scores.	Preventive dentistry - Periodontology	Original research	(67)
(2013)	Puerto Rico	Evaluate the efficacy of 0.8% arginine, potassium nitrate, and sodium fluoride mouthwashes on dentine hypersensitivity reduction.	Mouthwash containing arginine provides a significant and superior reduction in dentine hypersensitivity compared to potassium nitrate	Preventive dentistry - Restorative dentistry	Original research	(68)
(2013)	Puerto Rico	Evaluate the efficacy of three regimens integrating toothpaste, toothbrush, and mouthwash in reducing dentine hypersensitivity.	Arginine regimen provided the greatest reduction in Tactile and Air-Blast dentine hypersensitivity compared to potassium. It also provides faster dentine hypersensitivity relief than the potassium regimen.	Preventive dentistry - Restorative dentistry	Original research	(69)
(2016)	Puerto Rico	To identify the types, food sources, and patterns of carbohydrates that significantly contribute to dental caries in Puerto Rican children.	Total carbohydrates, total sugars, $\geq$10% kilocaloric energy from total sugars, and sucrose, fructose, and inositol intake significantly increased caries risk	Preventive dentistry - Cariology	Original research	(70)
(2010)	Puerto Rico	Comparison of the efficacy of two commercially available dentifrices for the control of supragingival plaque and gingivitis.	Dentifrice containing 0.3% triclosan, 2.0% PVM/MA copolymer, and 0.243% sodium fluoride provides a significant reduction in established supragingival plaque and gingivitis	Preventive dentistry- Periodontology	Original research	(71)
(2009)	Puerto Rico	Clinical investigation of the efficacy of a commercial mouth rinse containing 0.05% cetylpyridinium chloride in reducing dental plaque.	Mouthrinse containing 0.05% CPC provides significantly greater efficacy for reducing dental plaque 12 h after use than does a control mouth rinse without 0.05% CPC.	Preventive dentistry- Periodontology	Original Research	(72)
(2016)	Puerto Rico	To estimate caries levels of 12-year-old school Puerto Ricans in 2011, and to compare results to data obtained in 1997 to explore any possible change in caries outcomes after a government health insurance (GHI) reform was implemented.	Dental caries prevalence was high and the health disparity persists between children enrolled in public and private schools after more than a decade of the GHI implementation.	Preventive dentistry- Cariology	Original research	(73)

(Continued)

TABLE 1 | Continued

Publication year	Country	Research hypothesis	Research conclusion	Area of research	Type of publication	References
(2008)	Puerto Rico	To assess the efficacy of a dentifrice containing 0.3% triclosan/2.0% polyvinylmethyl ether/maleic acid (PVM/MA) copolymer/0.243% sodium fluoride in a 17% dual silica base for controlling established supragingival plaque and gingivitis	The dentifrice containing 0.3% triclosan/2.0% PVM/MA copolymer/0.243% sodium fluoride in a 17% dual silica base is efficacious for the control of established supragingival plaque and gingivitis.	Preventive dentistry-Periodontology	Original Research	(74)
(2002)	Puerto Rico	To compare the long-term caries increment associated with the use of two dentifrices: (1) a test dentifrice containing 0.836% sodium monofluorophosphate (1,100 ppm F) in a dicalcium phosphate dihydrate base plus 10% xylitol; and (2) a positive control dentifrice containing 0.836% sodium monofluorophosphate (1,100 ppm F) in a dicalcium phosphate dihydrate base.	For both DFS and DFT, the increments associated with the test dentifrice containing 10% xylitol were statistically significantly lower than those associated with the positive dentifrice without xylitol ($P < 0.05$), with the observed reductions in caries increment exceeding 10% in for both parameters.	Preventive dentistry-Cariology	Original Research	(75)
(2016)	Puerto Rico	To evaluate the association between vitamin D levels and periodontal disease in Puerto Rican adults.	Lower serum vitamin D levels are significantly associated with periodontitis in Puerto Rican adults.	Periodontal medicine	Original Research	(76)
(2006)	Puerto Rico	To estimate the prevalence of pit and fissure sealants on first permanent molars in 12 years old living in Puerto Rico and to further evaluate dental sealant prevalence by (1) urban/rural and public/private school status as well as (2) gender;	The prevalence of dental sealants in the first permanent molars of 12-year olds living in Puerto Rico during 1997 (4.3%) is lower than that reported in the United States (18.5%). Sealant prevalence was higher in males and students attending (urban) private schools.	Preventive dentistry - Cariology	Original Research	(77)
(2003)	Puerto Rico	To assess the prevalence of dental caries amongst 12-year-old Puerto Ricans.	The mean DMFS for 12 years old is higher than the mean DMFS of 4.2–4.7, reported for 12–17 years olds in the USA. Dental caries is a highly prevalent disease amongst 12-year-old in Puerto Rico.	Epdiemiology - Cariology	Original Research	(78)
(2018)	Puerto Rico	To estimate the prevalence of gingivitis and calculus among 12-year-old Puerto Ricans	Gingivitis prevalence is higher among 12-year-old Puerto Ricans compared to data reported for U.S. adolescents.	Epidemiology - Periodontology	Original Research	(79)
(2017)	Puerto Rico	To estimate the prevalence of gingivitis in 35- to 70-year-olds residing in San Juan, Puerto Rico, and assess the differences in gingivitis distribution between age and gender groups.	Gingivitis was observed in all participants. Men had significantly higher GI, compared to women. The prevalence of gingivitis was higher in Puerto Rico than in the US.	Epidemiology - Periodontology	Original Research	(80)
(2014)	Puerto Rico	Details the strategies for engaging Caribbean dental researchers		Public health	Review	(81)
(2015)	Trinidad and Tobago	to compare the effect of Motivational Interviewing, in contrast to traditional dental health education (DHE), on oral health knowledge, attitudes, beliefs, and behaviors among parents and caregivers of preschool children in Trinidad.	Using a Motivational Interviewing approach when delivering oral health information had a positive effect on parent/ caregiver oral health knowledge, attitudes, and behaviors compared to traditional Dental Health Education.	Preventive dentistry-Patient education	Original Research	(82)
(2013)	Trinidad and Tobago	To describe the prevalence and severity of early childhood caries in preschool children in a region of central Trinidad and to explore its	The prevalence and severity of ECC in central Trinidad were related to oral health behaviors and access to dental care.	Epidemiology - Cariology	Original Research	(83)

(Continued)

TABLE 1 | Continued

Publication year	Country	Research hypothesis	Research conclusion	Area of research	Type of publication	References
		relationship with social and behavioral factors				
(2015)	Trinidad and Tobago	To describe the prevalence of missing teeth, use of bridges and dentures, and unmet dental needs among those aged 60 years and above.	The prevalence of missing teeth, use of bridges and dentures, and unmet dental needs were high in the SABE cities in 1999–2000.	Epidemiology – Geriatrics	Original Research	(84)
(2012)	Trinidad and Tobago	To explore and understand parents and caregivers' experience of oral healthcare for their preschool aged children and how, within their own social context, this may have shaped their oral health attitudes and behaviors	Parents and caregivers in this qualitative study showed generally positive attitudes toward oral health but appear to have encountered several barriers and challenges to achieving ideal preventive care for their child, with respect to a healthy diet, good oral hygiene, and dental attendance.	Preventive dentistry-Patient education	Original Research	(85)
(2016)	Trinidad and Tobago	The purpose of this study was to describe the prevalence of developmental defects of enamel (DDE) and their relationship with early childhood caries (ECC) among preschool children in Trinidad.	Developmental Defects of Enamel are prevalent among this group of preschool children in Trinidad and are risk factors for Early childhood caries, which emphasizes the importance of preventive oral health care in early childhood for these high-risk children.	Epidemiology – Oral disease	Original Research	(86)
(2016)	Trinidad and Tobago	To describe the relationship between oral health-related quality of life (OHRQoL) and Early Childhood Caries (ECC) among preschool children in a Caribbean population.	The study sample of preschool children OHRQoL was associated with ECC	Preventive dentistry	Original Research	(87)
(2002)	Trinidad and Tobago	To investigate sources of stress and psychological disturbance in dental students across the 5 years of undergraduate study at a dental school in Trinidad.	A psychological disturbance was significantly associated with stress levels for male students but not generally for female students.	Dental Education	Original Research	(88)
(2004)	Trinidad and Tobago	To describe levels of self-rated competency of dental graduates from the University of the West Indies (UWI) and to investigate relationships with gender and the effect of curriculum change	Female graduates rated four competencies significantly higher than males. Graduates exposed to the new curriculum perceived greater overall preparedness for a general dental practice, suggesting the change to a competency-based curriculum was effective.	Dental Education	Original Research	(89)
(2003)	Trinidad and Tobago	To determine the views of dental students concerning the acceptability of the use of sedation in the management of dentally anxious children.	Dental students' perceptions of the acceptability of interventions for use with dentally anxious patients are related to the effectiveness of the intervention. Sedation, regardless of the outcome, is seen as less acceptable than the use of rewards and relaxation.	Dental Education	Original Research	(90)
(2008)	Trinidad and Tobago	To describe parents' views on the dental health of pre-school children in Trinidad.	The generally inaccurate factual knowledge and low awareness of preventive care among parents suggest the need for accurate information about factors influencing the dental health of pre-school children.	Preventive Dentistry	Original Research	(91)

(Continued)

TABLE 1 | Continued

Publication year	Country	Research hypothesis	Research conclusion	Area of research	Type of publication	References
(2013)	Dominican Republic	To evaluate the clinical efficacy of a single professional application of a Pro-Relief desensitizing fluoride-free paste containing 8% arginine and calcium as compared to a fluoride-free prophylaxis paste on dentin hypersensitivity	Single professional application of Pro-Relief desensitizing fluoride-free paste containing 8% arginine and calcium carbonate provided a greater level of instant relief of dentin hypersensitvity that differs significantly from that of a fluoride free prophy paste.	Preventive dentistry - Restorative dentistry	Original research	(92)
(2020)	Dominican Republic	To achieve consensus on the learning domains of cariology education among undergraduate dental schools in the Caribbean countries.	The consensus was obtained from 15 participating dental schools in the Caribbean region.	Restorative dentistry and dental education	Original research	(93)
(2014)	Dominican Republic	To evaluate the healing of extraction sockets after implantation of biphasic calcium sulfate (CS) alone or in combination with a gamma-radiated human mineralized allograft.	Biphasic CS used alone or in combination with an allograft resulted in the same amount of NB formation in alveolar ridge preservation procedures.	Implantology	Original research	(94)
(2013)	Dominican Republic	To evaluate the histometric characteristics of the peri-implant mucosa of human subjects that received textured implant abutments with conventional (implant and abutment with the same diameter) or platform-switched (implant diameter wider than that of the abutment) configurations	The different configurations between the groups tested, the apical extension of the junctional epithelium, an apical extension of the inflammatory cell infiltrate, and maximum occupied by inflammatory cells did not differ between groups.	Implantology	Original research	(95)
(2005)	Dominican Republic	To estimate the prevalence of periodontal attachment loss among Dominican adolescents.	Clinical attachment loss is common in adolescents in Santo Domingo, Dominican Republic, suggesting the necessity for improved standards of prevention, diagnosis, and treatment of these lesions.	Epidemiology Periodontology	Original research	(96)
(2019)	Dominican Republic	To investigate the oral health related quality of life associated with gingival parameters on the Caribbean adult population.	The study identified modifiable risk factors associated with poor oral health related quality of life among participants from the Caribbean region.	Periodontology	Original research	(56)
(2018)	Dominican Republic	To investigate the efficacy of commercial Chlorhexidine mouth rinses available from the Dominican Republic. The efficacy was investigated focusing on antibacterial, anti-inflammatory, and matrix metalloproteinases-8 (MMP-8) action.	Commercial Chlorhexidine digluconate mouth rinses demonstrated inhibition of plaque in concentrations of 0.12 and 0.15%.	Periodontology	Original research	(10)
(2016)	Dominican Republic	To compare the periodonto-pathogen prevalence and tetracycline resistance genes in Dominican patients with different periodontal conditions.	Red complex bacteria and D. pneumosintes were significantly the most prevalent species among periodontitis patients. T. forsythia was the most frequently detected in this population	Periodontology	Original research	(97)
(2015)	Dominican Republic	To evaluate the effect of the platform-switching phenomenon, the use of a smaller diameter abutment on a larger diameter implant platform.	Histological findings for both conventional and platform-switched implant-abutment configurations showed a similar composition of the soft tissue	Implantology	Original research	(98)
(2019)	Jamaica	To investigate the frequency and compare HPV strains in HIV and non-HIV Jamaicans.	HPV prevalence was 8.65%. HPV 84 was the most common type in both HIV and non HIV patients.	Microbiology	Original research	(99)

(Continued)

TABLE 1 | Continued

Publication year	Country	Research hypothesis	Research conclusion	Area of research	Type of publication	References
(2016)	Jamaica	To investigate frequency and gender disparity of torus (palatinus and mandibularis) among UWI medical and dental students.	The frequency of torus was 27.76%. The prevalence rate is comparatively higher than neighboring countries in the Caribbean region.	Oral disease	Original research	(100)
(2020)	Jamaica	To investigate the frequency of smoking practice and gingival inflammation in three nations (Jamaica, Dominican Republic, and Puerto Rico) from the Caribbean region.	Smoking was most prevalent among Jamaicans and least in Dominican republicans. The study findings also showed that smokers have a 4-fold increased risk for developing severe gingival inflammation; and 2-fold increased risk for developing moderate gingival inflammation.	Periodontology	Original research	(26)

existing oral cancer awareness programs. A number of studies have been made on tobacco smoking or chewing and oral cancer.

Jamaica has a longstanding reputation for ganja usage. Ganja is widely used for recreational, medicinal (folk medicine), and religious purposes in Jamaica. A report by the National Commission on Ganja in Jamaica suggested that one-third of ganja users started their habit at the age of 19 or below (41). Studies need to be proposed to know the "effect on the oral mucosa from smoking ganja." The suggestion of oral mucosal evaluation in ganja smokers is being made because it is not just the carcinogens in the tobacco or any agent that is responsible for cancer formation, but the heat generated during smoking may result in a genetic assault resulting in cancer formation. Soyibo et al. mentioned that the use of drugs is relatively common among high school students in Jamaica (108). Awareness measures, such as screening camps and health talks, need to be promoted to school children at their educational institutions. The inclusion of educational courses on general and oral health in the school education system at all levels may reinforce the benefits of good oral health. Statistics on oral and maxillofacial injuries due to road traffic accidents may be helpful in creating trauma care centers.

Research needs to be funded properly in order for proper research to be carried out. Promoting research also needs funding sources, and hence, the identification of associations or institutions that can provide research funding is essential. Whereas, local associations would be ideal, many of these have depleted resources due to the impact of the local economic climate. Thus, the next area of possible funding is from regional or international associations. Finding a regional source in such associations would greatly support and enhance research activities. Globally, major dental research projects are conducted with the grant support of the International Association of Dental Research (IADR). Recently, a proposal for new regional developments in the IADR has been approved for the Caribbean region (109). IADR collaboration in the Caribbean region may assist researchers to collaborate and develop future dental research standards in this region. This is an opportunity that Caribbean researchers must take as it will provide an additional funding branch for their Research program. Thus, a directed approach to research not only increases the statistical data about dental and oral diseases in the Caribbean population but also raises awareness among the Caribbean population.

CONCLUSION

Despite a large number of data available on the global burden of oral diseases, the data on the Caribbean population is less. It is important to document the prevalence of various oral diseases in the Caribbean population for making oral health care policies. The data generated may be helpful to determine the oral health care required and, thus, eventually raise the concept of awareness in oral diseases. Directing the oral health care awareness program in a specific way will reduce the burden of oral diseases in the Caribbean population.

AUTHOR CONTRIBUTIONS

ABRS made substantial contributions to the concepts, design, and intellectual content of the study and manuscript, involved in the preparation, editing, and review of the manuscript. TJ participated in manuscript writing concept, design, intellectual content of the study literature data acquisition, manuscript writing, and manuscript review. All authors contributed to the article and approved the submitted version.

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Associations of Carbohydrate Intake With New-Onset Hypertension Subtypes: Results From the China Health and Nutrition Survey (2000–2011)

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Background: The effects of carbohydrate intake on hypertension (HTN) subtypes are scarce. We examined the association of carbohydrate intake with new-onset HTN subtypes in Chinese adults.

Methods: Chinese Health and Nutrition Survey (CHNS) 2000–2011, 22,418 individuals were recorded using a 24-h recall method over three consecutive days. We excluded those who were pregnant women, lactating mothers, age <18 years, baseline age, blood pressure, and energy intake deficiency, extreme energy intake (male > 6,000 kcal or < 800 kcal; female > 4,000 kcal or < 600 kcal), and pulse pressure difference (Systolic Blood Pressure [SBP] - Diastolic Blood Pressure [DBP]) <10 mm Hg, HTN at baseline and data from only one survey. The total number of subjects who participated in at least two surveys was 7,930. The main outcome was new-onset HTN subtypes over 6.9 person years of follow-up.

Results: 2,521 participants were found to be HTN, which included 1,318 males (52.3%), 1,203 females (47.7%), 721 had systolic-diastolic hypertension (SDH, 28.6%), 655 had isolated systolic hypertension (ISH, 26.0%), and 993 had isolated diastolic hypertension (IDH, 39.4%). Compared with extreme quintiles of carbohydrate, multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) for new-onset HTN, SDH, ISH and IDH associated with carbohydrate intake were 1.12 (0.97–1.30), 1.54 (1.18–2.00), 0.89 (0.67–1.19) and 1.15 (0.91–1.45), respectively. The HR of SDH compared with extreme quintiles of carbohydrates was 1.56 (95% CI, 1.08–2.25; $P_{trend} = 0.04$) in men and 1.52 (95% CI, 1.02–2.26; $P_{trend} = 0.02$) in women.

Conclusion: Carbohydrates were related to a higher risk of SDH, which were not observed with HTN, ISH, and IDH.

Keywords: carbohydrate intake, hypertension subtypes, China Health and Nutrition Survey, ISH, IDH

INTRODUCTION

Hypertension (HTN), as a major modifiable risk factor for cardiovascular disease, accounts for approximately 45% of global cardiovascular disease morbidity and mortality (1). Globally, the overall prevalence in adults ≥25 years of age was 40% in 2008, with the highest being 46% in Africa (2, 3). Previous studies in China have suggested that the prevalence of HTN in adults was from 14.5% in 1991 to 34.0% in 2012 (4–8). According to the China Health and Nutrition Survey (CHNS), the incidence of HTN is 4.4 per 100 person-years in the Chinese adults (9). Reducing the burden of diseases associated with HTN has been identified as a public health priority in the world as well as in China.

The causes of HTN include the intricate interplay between behavioral, environmental (including dietary factors), physiological, genetic, social, and economic factors (10). Evidence suggests that dietary factors are determinants of HTN (11). Some studies have found that a healthy and reasonable diet (such as the Mediterranean diet, Dietary Approaches to Stop Hypertension (DASH diet) can decrease blood pressure (12–14). Compared with Western countries, Chinese who eat carbohydrate-rich, low-fat diets have lower blood pressure levels, but the results are inconsistent. A cross-sectional study found that high-carbohydrate intake was associated with HTN by increasing inflammatory factors in rural areas in China (15). However, a prospective cohort study found that carbohydrate supply was not associated with the risk of developing HTN (16). There was no significant association between the risk of hypertension and intake of total carbohydrates ($P = 0.9$) among 80,426 French adults who participated in the NutriNet-Santé cohort study. In addition, studies have found that partial replacement of carbohydrates with protein or monounsaturated fat can lower blood pressure (17, 18). It was noteworthy that OmniHeart Randomized Trial extended previous knowledge about observations on the effects of protein and unsaturated fat on blood pressure. Compared with the carbohydrate diet, both protein and unsaturated fat diets significantly lowered systolic and diastolic blood pressure in all participants (17).

Few studies are investigating the relationship between carbohydrate intake and the incidence of HTN in China. However, most of them are cross-sectional studies and very few of them are large-scale prospective cohort studies. Since hypertension is associated with wide phenotypic variability, it can be divided into the following subtypes: isolated systolic hypertension (ISH), isolated diastolic hypertension (IDH), and systolic-diastolic hypertension (SDH) (19, 20). These subtypes may provide important information concerning the causation of hemodynamic and/or structural abnormalities that contribute to hypertension. No study has been found to investigate the relationship between HTN subtypes and carbohydrate intake. However, the dietary patterns and disease characteristics of the Chinese population are quite different from those of the Western population. Therefore, in this cohort study, the data of the China Health and Nutrition Survey (CHNS) from 2000 to 2011 was used to explore the relationship between carbohydrate intake and the risk of new incidence of HTN subtypes.

MATERIALS AND METHODS

Study Design and Study Population

The CHNS is a representative sample and is the only large-scale longitudinal, household-based survey in China. This study was based on CHNS data completed in 2000, 2004, 2006, 2009, and 2011. In each survey, only adults aged ≥ 18 years and their data on sex, age, urban-rural status, body mass index (BMI), educational levels, lifestyle factors, and physical examinations (height, weight, as well as waist circumference) were extracted.

To determine incident hypertension and subtypes, we identified a dynamic cohort study covering five time periods, which included the CHNS in 2000 and consequent follow-up surveys. The consequent follow-up surveys were considered as follow-up surveys of the former time period, and the surveys simultaneously added normotensive participants as new baseline surveys of follow-up time periods. To limit the biases caused by pre-existing factors, we excluded participants with ineligible factors in their baseline surveys, which is summarized in **Figure 1**. Pregnant or lactating women were excluded, along with incomplete data, blood pressure difference (Systolic Blood Pressure [SBP]–Diastolic Blood Pressure [DBP]) <10 mm Hg, implausible or extreme BMI (< 15.0 kg/m^2 or > 40.0 kg/m^2) or height (< 120.0 cm) or extreme or implausible energy intake (male > 6,000 kcal or < 800 kcal; female > 4,000 kcal or < 600 kcal). All the participants were free of HTN at baseline.

According to the inclusion exclusion criteria, a total of 7,930 subjects (free of HTN) participated in at least two surveys, including 3,744 males and 4,186 females. The process of this study is summarized in a flow chart in **Figure 1**.

Calculation of Follow-Up Time

We calculated the IRs (per 100 person-years) of hypertension and subtypes according to the number of person-years of follow-up. For participants who did not develop hypertension at a later follow-up survey, the follow-up time was calculated from the date of the baseline survey to the date of later survey of follow-up. For participants who developed hypertension, the date of the onset of hypertension was assumed to be the midpoint between the two surveys because of the insidious onset of hypertension. For participants who did not complete the follow-up survey, the loss-to-follow-up date was also assumed to be the midpoint between the two surveys. Thus, the follow-up time was estimated to be the entire time during which the subjects remained free of hypertension plus half of the follow-up time during which hypertension developed or they were lost in the follow-up survey. This is a common method to deal with missing values (21).

Definition of Hypertension and Subtypes

According to the 1999 regulations by the World Health Organization (WHO) (22) and 2018 Chinese Guidelines for Prevention and Treatment of Hypertension (23), hypertension is defined as: SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg and/or a history of hypertension, or currently using antihypertensive drugs. According to the mechanism of hypertension, hypertension is divided into three subtypes: (1) Isolated Systolic Hypertension (ISH), defined as SBP ≥140 mm

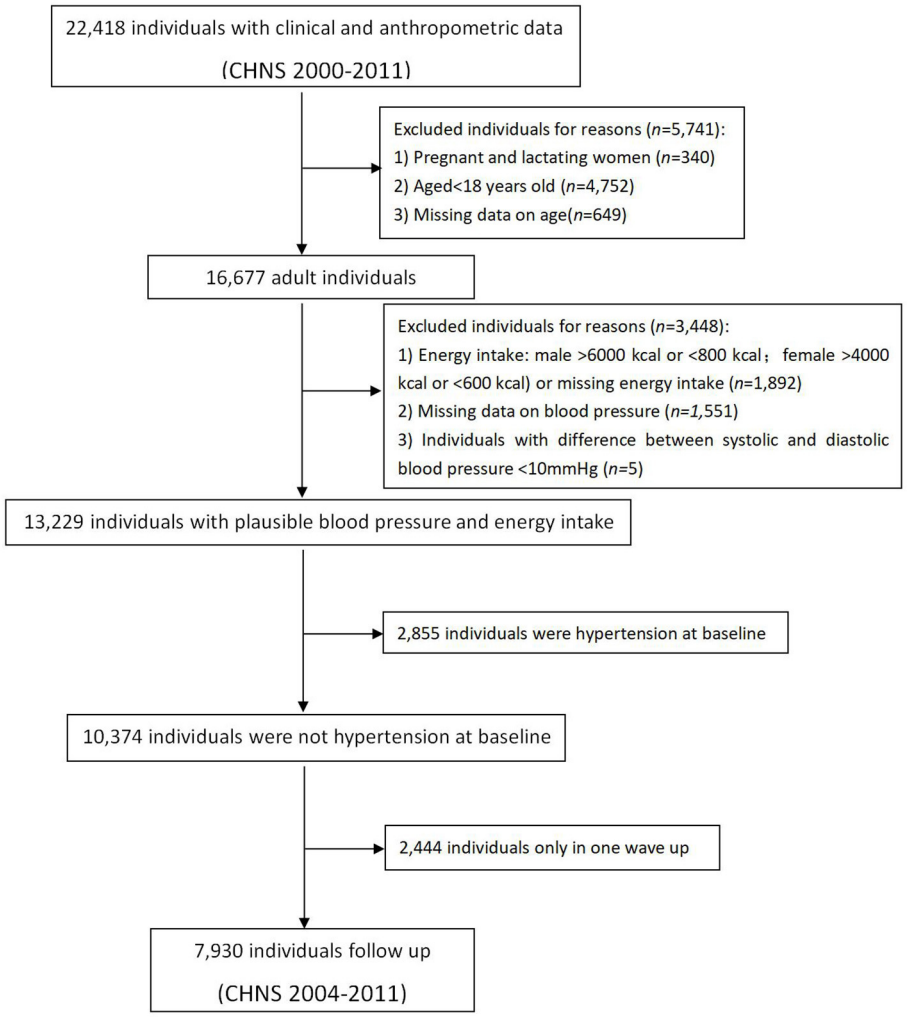

FIGURE 1 | Flow chart illustrating the sample selection procedure.

Hg and DBP <90 mm Hg; (2) simple Isolated Diastolic Hypertension (IDH), defined as SBP <140 mm Hg and DBP ≥90 mm Hg; (3) Systolic-Diastolic Hypertension (SDH), defined as SBP ≥ 140 mm Hg and DBP ≥ 90 mm Hg.

Dietary Assessment

Dietary assessment was based on three consecutive 24-h dietary recalls and a household-level food inventory record during the same time. The dietary data of the CHNS were obtained through a three-day (two weekdays and one weekend day), consecutive 24-h dietary recalls in each wave of the CHNS and were collected by trained investigators who weighed all the available foods in the participants' homes. Household food consumption was assessed by weighing all food consumed by the household (including cooking oil, salt, and other condiments) from the beginning to the end of each day during the survey period. Food stock at the initiation of the survey, food purchases, and/or production and food waste during the survey period were weighed and included in the calculation of household food consumption. In each survey cycle, individual dietary intake was assessed *via* three consecutive 24-hour dietary recalls (24), which is a validated method for assessing dietary intake (25, 26). Days for 24-h dietary recalls were randomly selected from Monday to Sunday and included at least 1 weekend day. All eligible participants were asked to follow their typical daily dietary patterns. All household members reported all detailed food items consumed at home and away from home (i.e., food items consumed at restaurants, canteens, and other locations) to well-trained interviewers during the same survey period. Interviewers recorded the type and amount of all food items consumed, as well as the type of meal and location of food consumption during the 24 h of the previous day. The amount of food in each dish was estimated from the household inventory, and the proportion of each dish consumed was reported by each person. Data for soft drink, sugared-sweetened fruit drink, and alcohol consumption during the past year were obtained by a food frequency questionnaire with five

categories (almost every day, three to four times per week, once or twice per week, once or twice per month, or less than once per month). More details about dietary data have been reported elsewhere (27). Dietary assessment was the same in all surveys.

To reduce recall bias, self-report forms of dietary records were distributed to household units to help the participants record daily food intake and to serve as supplementary materials for formal dietary surveys. The quantity of cooking oil and condiments were simultaneously measured before and after 3 days of household investigation, and the quantities of purchases and waste were noted when entering the household every day. The amount of cooking oil and condiments that was consumed was obtained by weighing food and was divided according to the energy intake of other foods of each family member. The average daily dietary nutrient intake of each family member was calculated by using the three-day consecutive 24-h diet reviewed survey data and the proportionally distributed household cooking oil and condiments consumption data combined with the Chinese Food Composition Table (2002 and 2004 editions). Calculation of the mean average daily nutrients intake per person according to the Chinese Food Composition Table (FCTS) (28): including energy, carbohydrates, protein, fat, dietary fiber, vitamins, etc., and calculate carbohydrate percentage of energy intake (% E) = {([carbohydrate (G) x 4] / total energy intake [kcal]) x 100}.

Covariates

For this cohort study, we also assessed hypertension-related factors: age, urban sites, education level, waist circumference, physical activities, smoke status, alcohol drinking status, personal income, and total energy intake.

Measuring equipment was provided by the project team; blood pressure, height, weight, and waist circumference were measured by trained health workers following the WHO's standardized program. Blood pressure (BP) was based on the mean of 3 consecutive measurements collected after 10 min of seated rest using standard mercury sphygmomanometers (22). The bodyweight of participants dressed in light clothing was measured without shoes to the nearest 0.1 kg with a calibrated beam scale (Seca North America 260). The height of barefoot subjects was measured to the nearest 0.1 cm using a portable stadiometer (Seca North America 880). The body mass index (BMI) was calculated as: BMI = weight (kg) / [height $(m)^2$]. The result of waist circumference was the average of the two measurements. Urbanization was categorized as urban and rural. Education level was categorized as low-level (illiterate and primary school degree), middle-level (lower middle school degree) and high-level (upper–middle school degree, technical/vocational degree, university or college degree and master's degree or higher). Total energy intake was calculated from three-day dietary-recall food composition tables. The metabolic equivalent index (MET) codes and detailed questions of the physical activity survey were also described before (29). Smoking status was categorized as either ever or never smoked. Alcohol consumption status was categorized as either drinker or abstainer. Personal income questions summarized each part of an individual's income listed in the questionnaire. The study was approved by the ethics review committees of the Chinese Center for Disease Control and Prevention and University of North Carolina at Chapel Hill. Written informed consent was obtained from all participants. This study was registered at ClinicalTrials.gov (NCT04104308).

Statistical Analysis

Continuous and categorical variables were described as means (95% confidence intervals [CIs]) and percentages (95% CIs). Overall, participants were categorized into five groups by quintile according to the percentage of energy (E) provided by carbohydrates, which was the total daily energy intake divided by the carbohydrate's energy (E = ([carbohydrate (g) ×4]/total energy intake [kcal]) ×100). The interval of follow-up was defined as the time between baseline and the earliest moment when participants were diagnosed as HTN subtypes, lost to or unavailable for follow-up, or at the end of follow-up.

Hazard ratios (HRs) and 95% CIs of HTN subtypes were calculated in accordance with individual carbohydrate intake (divided into five groups) in a time dependent Cox proportional hazards regression model with follow-up duration as the time scale. To quantify the trend, a median in each quintile was assigned and modeled constantly with statistical significance checked using the Wald test.

Subgroup analyses with multiplicative interaction terms were performed to show whether the associations of carbohydrate intake levels with the risk of HTN subtypes varied by age (<50 or ≥ 50 y), urban sites (yes or no), educational levels (low or high), waist circumference (<80 or ≥ 80 cm in women; <85 or ≥ 85 cm in men), BMI (<24 or ≥24 kg/m^2), energy intake (<2,100 or ≥ 2,100 kcal in women; <2,400 or ≥ 2,400 kcal in men), physical activity (<200 or ≥ 200 METs-hour/week), smoking status (ever or never), and alcohol consumption status (drinker or abstainer). *P* value was determined using the Wald test.

Statistical analyses were done with SPSS, version 20.0. All *P* values were 2-tailed; $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of Participants at Baseline

The mean SBP and DBP were 116.2 (115.9–116.5) mm Hg, 75.8 (74.5–76.0) mm Hg for men, and 112.7 (112.3, 113.1) mm Hg, 73.5 (73.2, 73.7) mm Hg for women at baseline (**Tables 1**, **2**). The average baseline energy intake was 2,455.9 (2,435.0–2,476.9) kcal/d for men and 2,077.3 (2,060.8–2,093.9) kcal/d for women. The average intake of carbohydrates was 357.0 (353.3–360.7) g/d for men and 300.3 (297.4–303.2) g/d for women (**Tables 1**, **2**).

Incidence Rates of Hypertension and Subtypes

The average follow-up time of this study was 7.3 person-years. During the follow-up, 2,521 people were found to have hypertension (HTN), including 1,318 (52.3%) males and 1,203 females (47.7%), 655 had ISH (26.0%), 993 had IDH (39.4%), and 721 had SDH (28.6%) (**Figure 2**). The age-adjusted incidence of HTN was 4.86 (95% CI, 4.68-5.04)/100 person-years, 5.48

TABLE 1 | Characteristics of the study participants at the baseline ($n = 7,930$).

Variable	Quintile of Percentage energy from carbohydrate					Total
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Age (years)[a]	45.1 (44.4–45.8)	43.7 (43.0–44.3)	43.2 (42.6–43.9)	42.8 (42.1–43.4)	41.7 (41.0–42.3)	43.3 (43.0–43.6)
Male (%)[b]	47.5 (45.1–50.0)	44.9 (42.5–47.3)	45.5 (43.0–47.9)	48.9 (46.5–51.4)	49.2 (46.8–51.7)	47.2 (46.1–48.3)
Urban location (%)[b]	56.3 (53.8–58.7)	44.5 (42.0–46.9)	34.2 (31.8–36.5)	21.8 (19.8–23.8)	14.2 (12.5–16.0)	34.2 (33.1–35.2)
SBP (mmHg)[a]	115.2 (114.6–115.7)	114.6 (114.0–115.1)	114.2 (113.6–114.7)	114.2 (113.7–114.8)	113.6 (113.0–114.2)	114.4 (114.1–114.6)
DBP (mmHg)[a]	75.3 (74.9–75.7)	74.7 (74.3–75.1)	74.6 (74.2–74.9)	74.3 (73.9–74.7)	74.0 (73.6–74.4)	74.6 (74.4–74.7)
Waist (cm)[a]	80.2 (79.7–80.7)	79.2 (78.7–79.6)	78.5 (78.0–78.9)	78.4 (77.9–78.8)	78.1 (77.7–78.6)	78.9 (78.7–79.1)
BMI (kg/m^2)[a]	22.9 (22.7–23.0)	22.5 (22.3–22.7)	22.4 (22.2–22.6)	22.3 (22.1–22.5)	22.1 (22.0–22.3)	22.4 (22.4–22.5)
Ever smoking (%)[b]	31.7 (29.4–34.0)	30.0 (27.8–32.3)	29.9 (27.6–32.1)	32.5 (30.2–34.8)	32.1 (29.8–34.4)	31.3 (30.2–32.3)
Alcohol intake (%)[b]	39.3 (36.9–41.7)	33.2 (30.9–35.6)	32.2 (29.9–34.5)	32.7 (30.4–35.0)	32.5 (30.2–34.8)	34.0 (32.9–35.0)
Education levels						
Primary/illiterate (%)[b]	30.7 (28.41–33.0)	32.8 (30.4–35.1)	39.2 (36.8–41.6)	44.7 (42.3–47.2)	51.7 (49.2–54.2)	39.8 (38.7–40.9)
Middle school (%)[b]	31.5 (29.2–33.8)	34.3 (31.9–36.6)	34.5 (32.2–36.8)	34.2 (31.8–36.5)	33.4 (31.0–35.7)	33.6 (32.5–34.6)
High/above (%)[b]	35.6 (33.3–38.0)	31.6 (29.3–33.8)	25.2 (23.0–27.3)	19.0 (17.1–20.9)	12.8 (11.2–14.4)	24.8 (23.9–25.8)
Physical Activity (MET–h/wk)[a]	223.3 (213.7–232.8)	253.8 (243.2–264.4)	294.5 (282.9–306.1)	350.4 (338.2–362.6)	402.8 (391.6–414.1)	304.9 (299.7–310.1)
Income (yuan/year)[a]	4787.6 (4191.8–5383.4)	3591.3 (3184.2–3998.5)	2967.9 (2549.9–3385.9)	1842.9 (1609.6–2076.3)	1288.1 (970.3–1605.9)	2895.3 (2709.0–3081.6)
Energy intake (kcal/d)[a]	2339.7 (2305.9–2373.6)	2270.0 (2240.1–2299.8)	2243.7 (2213.8–2273.6)	2220.1 (2191.0–2249.2)	2207.0 (2175.6–2238.4)	2256.1 (2242.3–2269.9)
Carbohydrate intake (g/d)[a]	246.0 (242.1–249.8)	297.1 (293.1–301.0)	328.4 (324.0–332.7)	358.8 (354.1–363.5)	405.0 (399.1–410.9)	327.1 (324.7–329.4)
Fat intake (g/d)[a]	111.4 (109.5–113.4)	87.1 (85.8–88.3)	72.2 (71.1–73.3)	58.2 (57.3–59.1)	37.9 (37.2–38.6)	73.4 (72.6–74.2)
Protein intake (g/d)a	75.5 (74.1–77.0)	70.2 (69.1–71.3)	67.3 (66.3–68.4)	64.1 (63.2–65.1)	61.1 (60.1–62.1)	67.7 (67.1–68.2)

[a] *Mean with 95% confidence interval (95% CI).*
[b] *Percentage with 95% confidence interval (95% CI).*
MET, metabolic equivalents.

TABLE 2 | Characteristics of the study participants at the baseline among Chinese men and women.

Variable	Quintile of Percentage energy from carbohydrate (Men)					Quintile of Percentage energy from carbohydrate (Women)					Total
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Age (years)[a]	45.2 (44.2–46.2)	43.6 (42.6–44.7)	43.1 (42.1–44.1)	41.9 (40.9–42.9)	41.0 (40.0–41.9)	44.9 (43.9–45.9)	44.0 (43.0–44.9)	43.2 (42.3–44.1)	43.5 (42.6–44.3)	42.5 (41.6–43.4)	43.0 (42.5–43.4)
Urban location (%)[b]	55.7 (52.2–59.3)	42.6 (39.1–46.2)	34.1 (30.7–37.5)	22.3 (19.3–25.3)	12.8 (10.4–15.2)	56.5 (53.2–59.9)	45.7 (42.3–49.1)	35.4 (32.2–38.7)	20.6 (17.8–23.3)	15.7 (13.3–18.2)	33.5 (32.0–35.0)
SBP (mmHg)[a]	117.2 (116.4–118.0)	116.2 (115.4–116.9)	116.1 (115.3–116.8)	115.8 (115.0–116.5)	115.8 (115.0–116.6)	113.3 (112.5–114.1)	113.3 (112.5–114.1)	112.5 (111.8–113.3)	113.0 (112.2–113.8)	111.4 (110.6–112.2)	116.2 (115.9–116.5)
DBP (mmHg)[a]	76.8 (76.3–77.3)	76.3 (75.7–76.8)	75.6 (75.1–76.2)	75.3 (74.8–75.9)	74.8 (74.2–75.4)	73.9 (73.3–74.4)	73.5 (72.9–74.0)	73.6 (73.0–74.1)	73.4 (72.8–74.0)	73.1 (72.6–73.7)	75.8 (74.5–76.0)
Waist (cm)[a]	82.8 (82.1–83.5)	81.2 (80.6–81.9)	80.2 (79.6–80.9)	79.3 (78.7–80.0)	79.3 (78.7–80.0)	77.8 (77.2–78.4)	77.5 (76.9–78.1)	77.2 (76.5–77.8)	77.1 (76.5–77.7)	77.0 (76.4–77.6)	80.6 (80.3–80.9)
BMI (kg/m^2)[a]	23.0 (22.7–23.2)	22.5 (22.2–22.7)	22.3 (22.1–22.6)	22.0 (21.7–22.2)	22.1 (21.8–22.3)	22.8 (22.5–23.0)	22.5 (22.2–22.8)	22.5 (22.3–22.7)	22.6 (22.3–22.8)	22.2 (22.0–22.5)	22.4 (22.4–22.5)
Ever smoking (%)[b]	62.4 (59.0–65.9)	61.1 (57.6–64.6)	62.9 (59.5–66.4)	61.0 (57.5–64.5)	61.5 (58.1–65.0)	3.9 (2.6–5.3)	4.4 (3.0–5.8)	2.4 (1.4–3.4)	4.5 (3.1–6.0)	4.3 (2.9–5.7)	61.8 (60.3–63.4)
Alcohol intake (%)[b]	67.9 (64.6–71.3)	59.2 (55.7–62.7)	61.5 (58.0–64.9)	57.9 (54.4–61.5)	57.9 (54.4–61.5)	13.4 (11.1–15.7)	12.1 (9.9–14.3)	8.5 (6.6–10.4)	7.7 (5.9–9.5)	8.0 (6.2–9.8)	60.9 (59.3–62.5)
Education levels											
Primary/illiterate (%)[b]	26.1 (22.9–29.2)	24.2 (21.1–27.3)	32.7 (29.3–36.0)	34.7 (31.3–38.1)	41.8 (38.3–45.3)	34.9 (31.7–38.1)	39.8 (36.5–43.2)	42.8 (39.5–46.2)	54.8 (51.4–58.2)	62.0 (58.7–65.3)	31.9 (30.4–33.4)
Middle school (%)[b]	33.4 (30.0–36.8)	38.9 (35.4–42.4)	38.3 (34.8–41.8)	40.3 (36.8–43.8)	39.5 (36.0–43.0)	29.9 (26.8–33.0)	30.1 (27.0–33.3)	33.2 (30.0–36.4)	27.9 (24.8–30.9)	26.5 (23.5–29.4)	38.1 (36.5–39.6)
High/above (%)[b]	39.2 (35.7–42.7)	35.4 (32.0–38.9)	28.0 (24.8–31.2)	23.1 (20.1–26.1)	17.2 (14.5–19.9)	32.4 (29.2–35.5)	28.3 (25.3–31.4)	22.8 (20.0–25.6)	15.0 (12.5–17.4)	8.8 (6.9–10.7)	28.6 (27.1–30.0)
Physical Activity (MET–h/wk)[a]	227.5 (213.5–241.4)	257.1 (241.8–272.4)	295.7 (278.7–312.7)	346.4 (329.5–363.2)	391.8 (375.7–407.9)	219.8 (206.7–232.9)	252.9 (238.2–267.6)	287.4 (271.8–303.1)	357.3 (339.8–374.7)	411.7 (395.9–427.5)	303.7 (296.4–311.1)
Income (yuan/year)[a]	6454.8 (5313.0–7596.6)	4753.9 (4028.8–5479.0)	3940.6 (3213.7–4667.5)	2531.9 (2125.0–2938.7)	2035.1 (1387.4–2682.8)	3293.2 (2833.1–3753.3)	2638.5 (2181.2–3095.9)	2173.8 (1766.0–2581.7)	1095.5 (877.5–1313.5)	595.4 (445.6–745.2)	3942.4 (3596.2–4288.5)
Energy intake (kcal/d)[a]	2542.6 (2490.9–2594.3)	2484.4 (2439.4–2529.4)	2458.3 (2414.5–2502.0)	2542.6 (2490.9–2594.3)	2484.4 (2439.4–2529.4)	2155.4 (2114.6–2196.2)	2099.6 (2062.9–2136.3)	2061.3 (2026.0–2096.6)	2052.6 (2018.1–2087.1)	2017.9 (1981.2–2054.6)	2455.9 (2435.0–2476.9)
Carbohydrate intake (g/d)[a]	265.4 (259.5–271.2)	326.4 (320.4–332.4)	362.1 (355.7–368.6)	390.1 (382.7–397.5)	440.7 (431.8–449.7)	228.3 (223.6–233.1)	273.8 (269.0–278.6)	299.9 (294.8–305.0)	330.0 (324.4–335.6)	369.3 (362.4–376.3)	357.0 (353.3–360.7)
Fat intake (g/d)[a]	117.5 (114.4–120.6)	93.3 (91.4–95.2)	77.2 (75.6–78.8)	61.3 (59.9–62.7)	40.5 (39.3–41.6)	105.9 (103.4–108.3)	82.2 (80.6–83.9)	68.1 (66.8–69.4)	55.1 (54.0–56.1)	35.2 (34.3–36.1)	77.9 (76.7–79.1)
Protein intake (g/d)[a]	81.3 (79.0–83.5)	76.0 (74.3–77.8)	73.6 (72.1–75.2)	69.7 (68.2–71.1)	66.4 (64.8–67.9)	70.4 (68.6–72.3)	65.4 (64.0–66.9)	61.7 (60.4–63.0)	59.2 (58.0–60.3)	55.9 (54.7–57.0)	73.4 (72.6–74.2)

[a] *Mean with 95% confidence interval (95% CI).*

[b] *Percentage with 95% confidence interval (95% CI).MET=metabolic equivalents.*

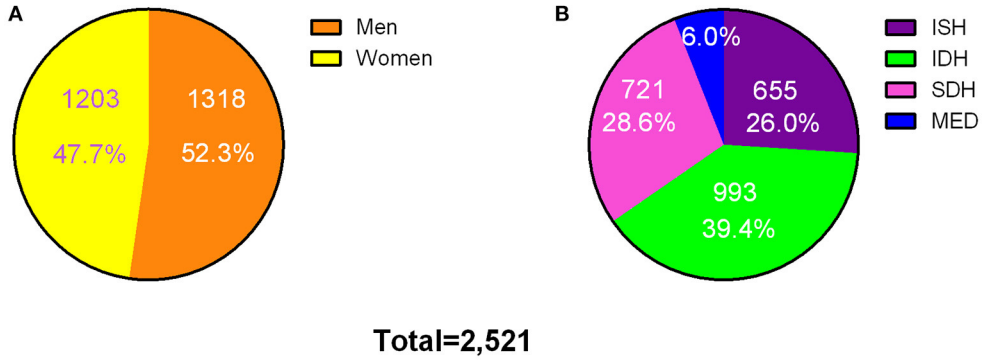

FIGURE 2 | Incident cases of hypertension and subtypes among the participants. Incident cases of hypertension by gender **(A)** and subtypes **(B)**. ISH, isolated systolic hypertension; IDH, isolated diastolic hypertension; SDH, systolic-diastolic hypertension; MED, currently using antihypertensive medication.

(95% CI, 5.20–5.76)/100 person-years for males and 4.31 (95% CI, 4.08–4.55)/100 person-years for females (**Table 3**). The age-adjusted morbidity rates of ISH, IDH, and SDH were 1.48 (95% CI, 1.38–1.59)/100 person-years, 1.69 (95% CI, 1.59–1.80)/100 person-years and 1.38 (95% CI, 1.28–1.47)/100 person-years (**Table 3**). The incidence of SDH increased with the increase of carbohydrate energy intake ($P_{trend} < 0.05$), which was not observed in HTN, ISH, and IDH in men or women (**Table 3**).

Associations Between the Intake of Carbohydrates and Hypertension and Subtypes

Subjects were divided into five groups according to the quintile of baseline carbohydrate energy supply ratio: Quintile 1 (43.7%, 95% CI = 39.3–46.5%), Quintile 2 (52.4%, 95%CI = 50.7–54.0%), Quintile 3(58.5%, 95% CI = 57.1–60.1%), Quintile 4 (64.5%,95%CI = 63.1–66.1%), Quintile 5 (72.7%,95% CI = 70.2–75.9%). Higher carbohydrate intake was related to a higher risk of SDH in the potential confounding variables-adjusted models of men (quartile 5 vs. quartile 1, HR 1.50 (1.03–2.20), $P_{trend} = 0.04$) and women (quartile 5 vs. quartile 1, HR 1.52 (1.02–2.26), $P_{trend} = 0.02$) (**Figures 3**, **4**), The results showed that the risk of SDH increased with the increase of carbohydrate However, it was not associated with HTN, ISH, and IDH (**Figures 3**, **4**).

Subgroup Analyses

To gain a further understanding of the relationship between the risk of hypertension and subgroups and the intake of carbohydrates, a subgroup analysis was carried out. In men, positive associations between carbohydrate intake and SDH were found in the individuals who lived in rural areas, had a higher educational level and lower energy intake (all $P_{trend} < 0.05$, $P_{interaction} > 0.05$) (**Table 4**). In women, positive associations between carbohydrate intake and SDH were found in older individuals (≥ 50 years) who lived in rural areas, had a higher energy intake, and a larger waist circumference (all $P_{trend} < 0.05$, $P_{interaction} > 0.05$) (**Figure 5**).

DISCUSSION

Over the past two decades, with the rapid economic growth, the food consumption patterns and dietary behavior of Chinese residents have undergone great changes (30). Nutrition-related noncommunicable diseases, including obesity, type 2 diabetes, hypertension, cardiovascular disease, and some tumor epidemics, continue to challenge the Asian health sector (31–33). This study used data from CHNS 2000–2011 to explore the relationship between carbohydrate intake and new-onset hypertension and subtypes in Chinese adults. In this study, the age-adjusted incidence rate of HTN was 4.86 (95% CI, 4.68–5.04)/100 person-years, and the age-adjusted incidence rates of ISH, IDH, and SDH were 1.48 (95% CI, 1.38–1.59), 1.69 (95% CI, 1.59–1.80) and 1.38 (95% CI, 1.28-1.47)/100 person-years, respectively. The incidence of SDH increased with increasing carbohydrate intake in both men and women. This study revealed that high carbohydrate intake was related to a positive impact on SDH, and we found no relationship between carbohydrate intake and HTN, ISH, and IDH.

This study additionally found that during the mean follow-up period of 6.9 person-years, the age-adjusted HTN incidence was 4.86 (95% CI 4.68-5.04)/100 person-years, similar to previous CHNS study data (9, 34), higher than Canadian residents (3.21/100 years) (35), and higher than the incidence rate of residents in the Middle East (3.36/100 person-years) (36). Furthermore, we found that the incidence of ISH was also higher than in Taiwan (37) (1.48 vs. 1.18/100 person-years), and the incidence of IDH was more than twice that in Taiwan (1.69 vs. 0.63/100 person-years). Our study found that the age-adjusted incidence rate of SDH was 1.38/100 person-years, higher than a previous study (rough incidence rate of 1.1/100 person-years) (9) and the Middle East (36) (crude incidence rate of 0.63/100 person-years). The difference of the results may be related to the difference of the study population, follow-up time and the different lifestyles, mainly in the dietary habits.

This study shows that age-adjusted incidence of SDH increased with the increase of carbohydrate intake. At present, there is still much controversy about the relationship between

TABLE 3 | Incident rates of hypertension and subtypes in participants by quantiles of percentage energy from carbohydrate.

Variable	Incidence (per 100 person–years; 95% CI)*					Total
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
HTN						
All						
Median (IQR)	43.7 (39.3–46.5)	52.4 (50.7–54.0)	58.5 (57.1–60.1)	64.5 (63.1–66.1)	72.7 (70.2–75.9)	58.5 (50.7–66.1)
Cases/n	475	494	474	538	540	2521
Person–years	9997.7	10330.8	11129.3	11454.1	11549.7	54461.6
IR	4.71 (4.30–5.13)	5.00 (4.58–5.42)	4.66 (4.27–5.05)	4.92 (4.53–5.32)	5.13 (4.73–5.54)	4.86 (4.68–5.04)
Men						
Median (IQR)	43.4 (39.0–46.4)	52.6 (51.0–54.3)	59.0 (57.5–60.4)	64.9 (63.6–66.6)	72.8 (70.5–76.2)	59.0 (50.9–66.6)
Cases/n	257	262	258	262	279	1318
Person–years	4567.2	4813.7	5163.7	5279.0	5291.6	25115.2
IR	5.83 (5.15–6.51)	5.52 (4.87–6.16)	5.43 (4.82–6.05)	5.32 (4.72–5.93)	5.49 (4.87–6.10)	5.48 (5.20–5.76)
Women						
Median (IQR)	44.0 (39.6–46.7)	52.2 (50.6–53.8)	58.2 (56.7–59.8)	64.2 (62.8–65.8)	72.6 (70.0–75.8)	58.2 (50.6–65.8)
Cases/n	217	238	217	269	262	1203
Person–years	5444.3	5520.9	5911.7	6183.5	6253.8	29346.3
IR	4.03 (3.51–4.56)	4.61 (4.06–5.16)	4.12 (3.61–4.62)	4.81 (4.27–5.34)	4.82 (4.29–5.35)	4.31 (4.08–4.55)
ISH						
All						
Cases/n	130	130	137	129	129	655
Person–years	9997.7	10330.8	11129.3	11454.1	11549.7	54461.6
IR	1.39 (1.16–1.62)	1.51 (1.28–1.75)	1.58 (1.35–1.81)	1.43 (1.22–1.65)	1.59 (1.36–1.82)	1.48 (1.38–1.59)
Men						
Cases/n	61	51	64	50	57	283
Person–years	4567.2	4813.7	5163.7	5279.0	5291.6	25115.2
IR	1.57 (1.21–1.93)	1.23 (0.92–1.54)	1.66 (1.31–2.01)	1.27 (0.97–1.58)	1.43 (1.11–1.75)	1.42 (1.28–1.57)
Women						
Cases/n	68	79	73	80	72	372
Person–years	5444.3	5520.9	5911.7	6183.5	6253.8	29346.3
IR	1.36 (1.05–1.67)	1.77 (1.42–2.11)	1.60 (1.28–1.92)	1.61 (1.30–1.93)	1.66 (1.34–1.97)	1.53 (1.39–1.67)
IDH						
All						
Cases/n	181	194	186	207	225	993
Person–years	9997.7	10330.8	11129.3	11454.1	11549.7	54461.6
IR	1.69 (1.44–1.95)	1.81 (1.56–2.07)	1.56 (1.33–1.79)	1.58 (1.35–1.81)	1.82 (1.58–2.07)	1.69 (1.59–1.80)
Men						
Cases/n	111	113	118	118	126	586
Person–years	4567.2	4813.7	5163.7	5279.0	5291.6	25115.2
IR	2.35 (1.91–2.79)	2.23 (1.82–2.65)	2.10 (1.71–2.49)	1.98 (1.60–2.35)	2.23 (1.83–2.62)	2.16 (1.98–2.34)
Women						
Cases/n	70	83	75	83	96	407
Person–years	5444.3	5520.9	5911.7	6183.5	6253.8	29346.3
IR	1.19 (0.91–1.48)	1.46 (1.14–1.78)	1.21 (0.93–1.49)	1.24 (0.97–1.52)	1.44 (1.14–1.73)	1.29 (1.16–1.42)
SDH						
All						
Cases/n	116	143	122	176	164	721
Person–years	9997.7	10330.8	11129.3	11454.1	11549.7	54461.6
IR	1.16 (0.95–1.36)	1.36 (1.14–1.59)	1.21 (1.01–1.42)	1.63 (1.40–1.87)	1.53 (1.31–1.76)#	1.38 (1.28–1.47)
Men						
Cases/n	62	84	67	86	87	386
Person–years	4567.2	4813.7	5163.7	5279.0	5291.6	25115.2
IR	1.36 (1.02–1.69)	1.73 (1.36–2.10)	1.42 (1.09–1.74)	1.88 (1.52–2.25)	1.66 (1.32–2.01)#	1.60 (1.44–1.75)
Women						
Cases/n	54	60	54	85	82	335
Person–years	5444.3	5520.9	5911.7	6183.5	6253.8	29346.3
IR	1.04 (0.77–1.31)	1.04 (0.77–1.31)	1.04 (0.78–1.30)	1.49 (1.18–1.79)	1.54 (1.23–1.84)#	1.18 (1.06–1.30)

**Age adjusted using the direct method to the year 2010 census population.*

IR, incidence rate, per 100 person-years; CI, confidence interval.

#P for trend (P_{trend} < 0.05).

ISH, isolated systolic hypertension; IDH, isolated diastolic hypertension; SDH, systolic-diastolic hypertension; HTN, hypertension.

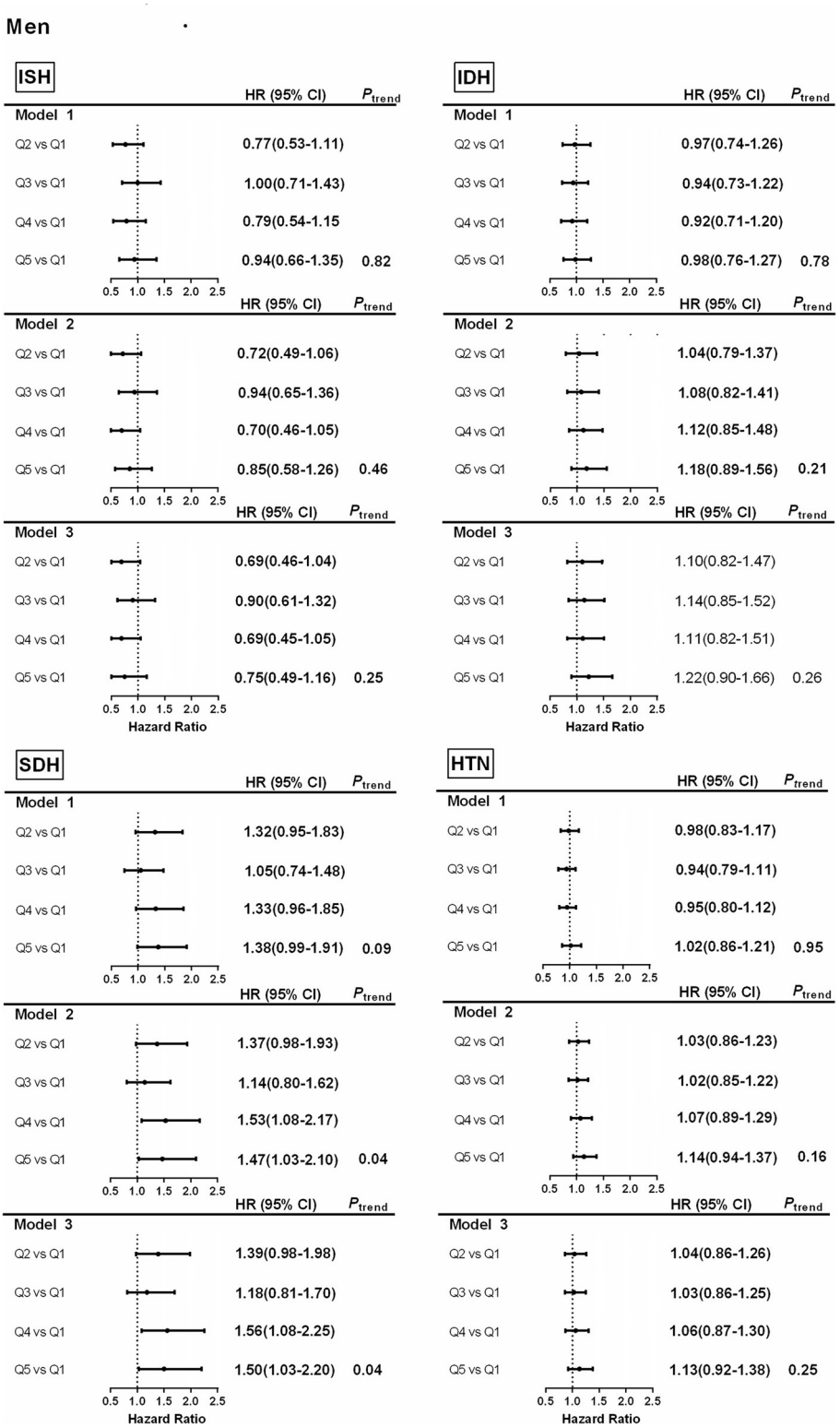

FIGURE 3 | Associations between percentage energy from carbohydrate by quantiles and hypertension and subtypes in men. Model 1. Hazard ratios and 95% CIs are adjusted for age. Model 2. Hazard ratios and 95% CIs are adjusted for age, urban or rural location, education level, waist circumference, ever smoking (never, ever), alcohol drinking (abstainer or drinker), personal income, BMI (underweight, normal weight, overweight, and obesity). Model 3. Hazard ratios and 95% CIs are adjusted for age, urban or rural location, education level, waist circumference, ever smoking (never, ever), alcohol drinking (abstainer or drinker), personal income, BMI (underweight, normal weight, overweight, and obesity), physical activity and energy intake. ISH, isolated systolic hypertension; IDH, isolated diastolic hypertension; SDH, systolic–diastolic hypertension; HTN, hypertension.

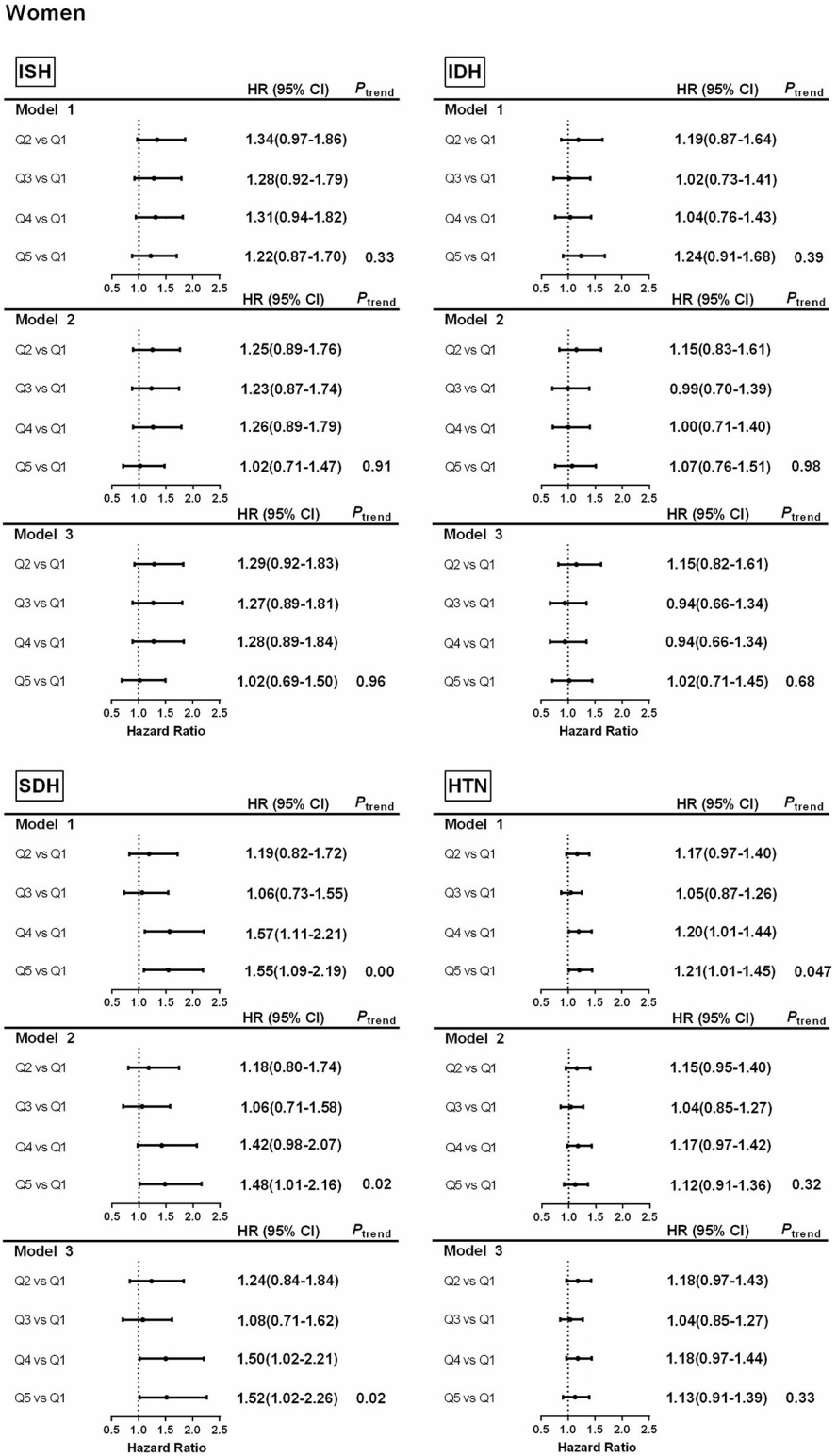

FIGURE 4 | Associations between percentage energy from carbohydrate by quantiles and hypertension and subtypes in women. Model 1. Hazard ratios and 95% CIs are adjusted for age. Model 2. Hazard ratios and 95% CIs are adjusted for age, urban or rural location, education level, waist circumference, ever smoking (never, ever), alcohol drinking (abstainer or drinker), personal income, BMI (underweight, normal weight, overweight, and obesity). Model 3. Hazard ratios and 95% CIs are adjusted for age, urban or rural location, education level, waist circumference, ever smoking (never, ever), alcohol drinking (abstainer or drinker), personal income, BMI (underweight, normal weight, overweight, and obesity), physical activity and energy intake. ISH, isolated systolic hypertension; IDH, isolated diastolic hypertension; SDH, systolic–diastolic hypertension; HTN, hypertension.

TABLE 4 | Hazard ratios (95% CIs) for HTN and SDH risk for quintiles of carbohydrate in subgroups of men.

Subgroups	HTN							SDH						
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}	$P_{interaction}$	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}	$P_{interaction}$
Region Urban	1 (Reference)	1.03 (0.78–1.37)	0.99 (0.73–1.33)	1.09 (0.77–1.53)	1.30 (0.86–1.96)	0.35	0.81	1 (Reference)	1.18 (0.71–1.99)	0.96 (0.54–1.70)	1.38 (0.76–2.51)	1.03 (0.44–2.41)	0.62	0.88
Rural	1 (Reference)	1.03 (0.80–1.33)	1.04 (0.81–1.34)	1.03 (0.80–1.32)	1.07 (0.84–1.38)	0.61		1 (Reference)	1.65 (0.99–2.76)	1.42 (0.84–2.39)	1.76 (1.06–2.93)	1.76 (1.06–2.93)	0.06	
Age < 50 years	1 (Reference)	1.07 (0.83–1.37)	1.00 (0.77–1.29)	1.09 (0.84–1.42)	1.21 (0.93–1.57)	0.17	0.67	1 (Reference)	1.32 (0.82–2.12)	0.97 (0.59–1.62)	1.45 (0.89–2.35)	1.44 (0.87–2.38)	0.17	0.84
≥ 50 years	1 (Reference)	1.03 (0.77–1.36)	1.03 (0.78–1.37)	1.00 (0.74–1.36)	0.90 (0.65–1.25)	0.56		1 (Reference)	1.47 (0.86–2.51)	1.33 (0.78–2.29)	1.53 (0.88–2.69)	1.34 (0.74–2.40)	0.39	
Education levels Low	1 (Reference)	0.98 (0.70–1.37)	1.06 (0.77–1.45)	0.92 (0.66–1.27)	1.11 (0.81–1.52)	0.60	0.71	1 (Reference)	1.90 (1.03–3.47)	1.55 (0.84–2.85)	1.17 (0.62–2.22)	1.76 (0.96–3.23)	0.41	0.06
High	1 (Reference)	1.08 (0.86–1.36)	1.02 (0.80–1.30)	1.16 (0.90–1.49)	1.14 (0.87–1.49)	0.29		1 (Reference)	1.21 (0.78–1.88)	1.00 (0.62–1.62)	1.89 (1.20–2.96)	1.35 (0.80–2.26)	0.06	
Energy intake < 2,400 kcal	1 (Reference)	1.01 (0.77–1.33)	1.09 (0.83–1.43)	1.00 (0.83–1.32)	1.09 (0.82–1.45)	0.64	0.82	1 (Reference)	1.40 (0.82–2.41)	1.50 (0.87–2.56)	1.77 (1.03–3.05)	1.92 (1.11–3.32)	0.02	0.72
≥ 2,400 kcal	1 (Reference)	1.09 (0.84–1.42)	1.12 (0.86–1.47)	1.08 (0.82–1.42)	1.26 (0.96–1.68)	0.16		1 (Reference)	1.53 (0.95–2.46)	0.87 (0.51–1.54)	1.53 (0.93–2.52)	1.14 (0.65–1.99)	0.71	
Waist circumference <85cm	1 (Reference)	1.09 (0.86–1.40)	1.08 (0.84–1.39)	1.10 (0.85–1.43)	1.26 (0.98–1.62)	0.11	0.65	1 (Reference)	1.14 (0.71–1.84)	1.05 (0.64–1.72)	1.53 (0.96–2.46)	1.38 (0.85–2.25)	0.09	0.96
≥ 85cm	1 (Reference)	1.14 (0.84–1.54)	1.21 (0.90–1.63)	1.02 (0.75–1.39)	1.12 (0.81–1.55)	0.79		1 (Reference)	1.21 (0.71–2.08)	1.02 (0.58–1.78)	1.35 (0.79–2.28)	1.33 (0.75–2.36)	0.30	
BMI <24 kg/m^2	1 (Reference)	1.21 (0.94–1.55)	1.12 (0.87–1.44)	1.11 (0.87–1.41)	1.07 (0.83–1.37)	0.15	0.99	1 (Reference)	1.30 (0.80–2.11)	1.17 (0.71–1.91)	1.60 (0.99–2.59)	1.39 (0.84–2.31)	0.14	0.73
≥24 kg/m^2	1 (Reference)	1.00 (0.75–1.34)	0.93 (0.68–1.26)	0.98 (0.71–1.35)	0.99 (0.71–1.40)	0.90		1 (Reference)	1.44 (0.86–2.43)	1.16 (0.66–2.04)	1.38 (0.78–2.44)	1.52 (0.85–2.74)	0.27	
Physical activity < 200 (METs–hour/week)	1 (Reference)	1.20 (0.91–1.57)	1.00 (0.74–1.34)	1.06 (0.76–1.48)	1.12 (0.78–1.62)	0.83	0.42	1 (Reference)	1.67 (0.99–2.81)	1.49 (0.86–2.56)	1.75 (0.98–3.13)	1.49 (0.75–2.94)	0.19	0.59
≥ 200 (METs–hour/week)	1 (Reference)	0.91 (0.70–1.18)	1.06 (0.82–1.36)	1.03 (0.80–1.33)	1.10 (0.86–1.41)	0.23		1 (Reference)	1.14 (0.70–1.84)	0.99 (0.60–1.63)	1.37 (0.85–2.20)	1.40 (0.87–2.24)	0.09	
Smoking status Never	1 (Reference)	1.16 (0.85–1.56)	1.13 (0.82–1.54)	1.05 (0.76–1.46)	1.20 (0.86–1.68)	0.48	0.51	1 (Reference)	1.96 (1.02–3.78)	1.72 (0.85–3.46)	1.72 (0.85–3.49)	2.05 (1.00–4.22)	0.17	0.36
Ever	1 (Reference)	1.00 (0.78–1.27)	1.00 (0.79–1.27)	1.08 (0.84–1.38)	1.10 (0.85–1.42)	0.36		1 (Reference)	1.23 (0.80–1.89)	1.05 (0.68–1.63)	1.49 (0.97–2.30)	1.34 (0.85–2.12)	0.13	
Alcohol drinking status Abstainer	1 (Reference)	1.11 (0.80–1.54)	1.00 (0.71–1.41)	1.14 (0.81–1.59)	1.02 (0.72–1.45)	0.90	0.54	1 (Reference)	1.88 (0.97–3.63)	1.21 (0.58–2.54)	1.91 (0.96–3.81)	1.80 (0.88–3.68)	0.19	0.75
Drinker	1 (Reference)	1.01 (0.80–1.28)	1.07 (0.85–1.35)	1.02 (0.79–1.30)	1.21 (0.95–1.55)	0.19		1 (Reference)	1.22 (0.79–1.86)	1.20 (0.79–1.84)	1.42 (0.92–2.21)	1.42 (0.90–2.23)	0.14	

Hazard ratios and 95% CIs are adjusted for age, urban or rural location, education, waist circumference, ever smoking (never, ever), alcohol drinking (abstainer or drinker), personal income, physical activity, and energy intake.
P_{trend}, p for trend; $P_{interaction}$, p for interaction.

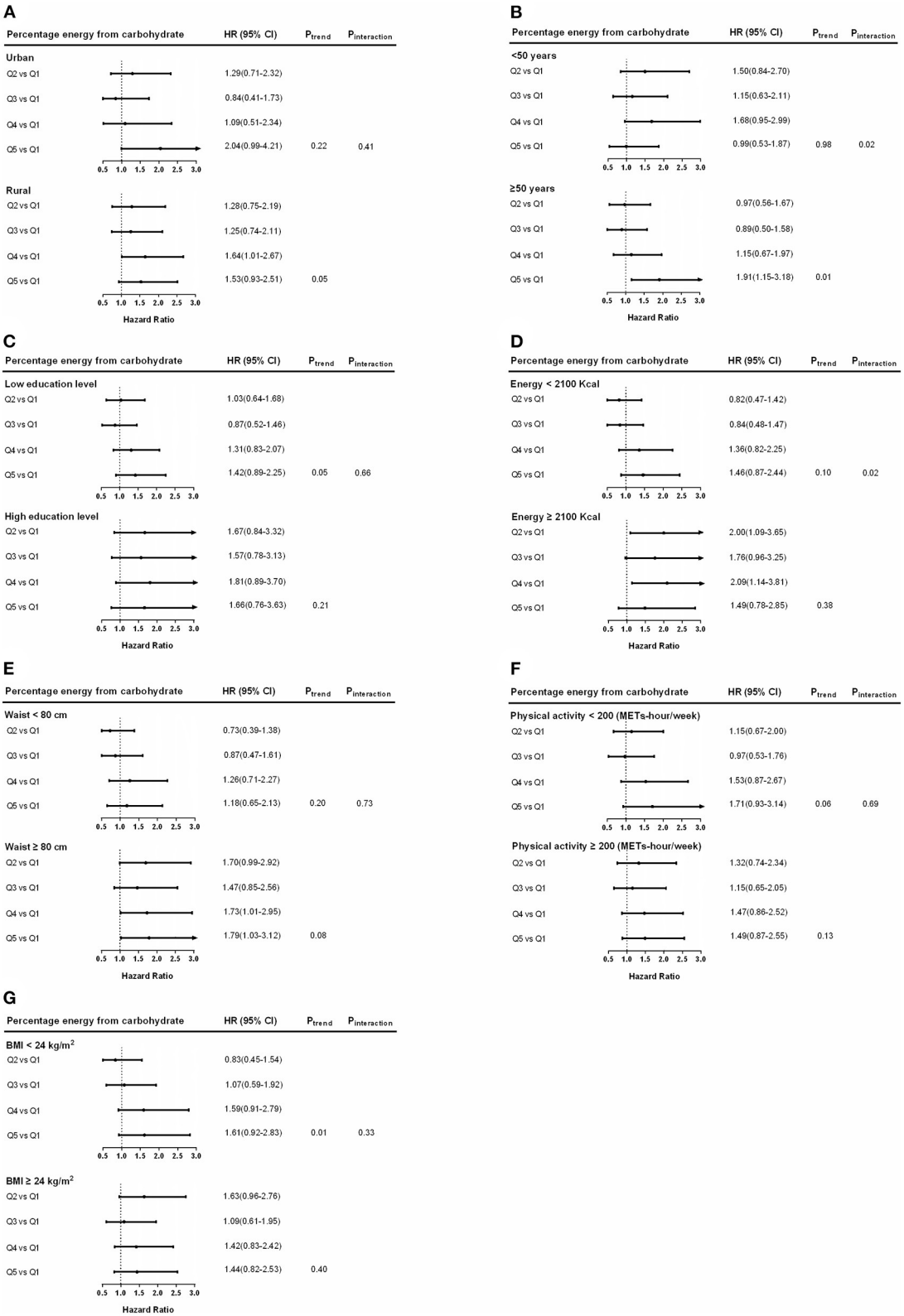

FIGURE 5 | Hazard ratios (HRs) for SDH risk for percentage energy from carbohydrate in subgroups among women. Hazard ratios and 95% CIs are adjusted for age, urban or rural location, education, waist circumference, ever smoking (never, ever), alcohol drinking (abstainer or drinker), personal income, BMI (underweight, normal weight, overweight, and obesity), physical activity, and energy intake. Hazard ratios (95% CIs) of hypertension among women by region **(A)**, age **(B)**, education levels **(C)**, energy intake **(D)**, waist circumference **(E)**, physical activity **(F)**, and BMI **(G)** are shown. Ptrend, P for trend; Pinteraction, P for interaction.

carbohydrate intake and the incidence of hypertension. Carbohydrates are the main source of energy in the diet in China. High intake of carbohydrates and low intake of fat and protein were associated with insulin resistance and hypertension, possibly by increasing inflammatory factors in the rural population of China (15). Several studies have demonstrated positive associations between dietary carbohydrate intake and HTN (15, 38, 39), but these studies were mainly concerned with the relationship between daily intake of carbohydrates and hypertension, blood pressure, and most of them were cross-sectional studies. Appel et al. found that in prehypertension or hypertensive patients, partial replacement of carbohydrates with protein or monounsaturated fat can further lower blood pressure (17, 40), thus reducing carbohydrate intake rather than increasing protein, or monounsaturated fat intake may be a dietary factor that lowers blood pressure. Systematic reviews and meta-analyses have shown that high carbohydrate intake is associated with a significant increase in blood pressure (41, 42). However, there are still some inconsistent conclusions. Lelong et al. found that carbohydrates were not associated with the risk of hypertension, but the mean follow-up time of that study was only 3.4 years (16). Another cohort study of middle-aged men found that total carbohydrate intake was inversely and significantly related to an average annual change in systolic pressure (43). However, the dietary assessment method for that study varied from our study.

The relationship between carbohydrate intake and hypertension was different in men and women subgroups, considering that sex differences in SDH can be explained by the influence of sex hormone types, dietary intake and environment. Recent studies have shown that there were gender differences in the relationship between dietary factors and the risk of chronic diseases such as hypertension (44, 45). Some studies have shown that the link between carbohydrate intake and metabolic diseases is stronger in women (46). Further studies will be required in the future to investigate the relationship between these factors and the incidence of ISH and IDH.

Studying the relationship between carbohydrate intake and the incidence of hypertension is of great significance for the prevention and treatment of hypertension in China. A deeper understanding of these relations may elucidate novel lifestyle approaches to prevent HTN. Understanding the specific effects of carbohydrate nutrition on BP will help people to facilitate a personalized and targeted lifestyle that addresses the support optimal BP control. Since more individuals now meet the criteria for prehypertension and HTN according to the new BP guidelines, such diet management is likely to have a high public health impact. Therefore, given the changes in dietary nutrition and related epidemics, the government needs to take immediate action to implement effective interventions to promote healthy diets and reduce the burden of chronic non-communicable diseases.

The advantages of this study include a prospective design, a large sample size, and control of a high number of potential confounders. Additionally, this is the first study in China on the relationship between carbohydrate intake and the subtypes of hypertension. Our study has potential limitations. First, only baseline levels of carbohydrate intake are considered and dietary changes may occur during follow-up. However, even if significant dietary changes occur after baseline assessment, they may weaken the observed connections. Furthermore, the number of follow-up participants gradually increased in the cohort study, which will have a certain impact on the representation of people of all ages. In addition, levels of dietary intake (e.g. solid or liquid carbohydrates, whole or refined grains, sodium, and crude fiber intake) were estimated based on 24-hour dietary recalls, which might not have representativeness of a subject's typical intake. Therefore, this cohort study did not analyze the association between carbohydrates types and hypertension.

CONCLUSIONS

The incidence of SDH increased with the increase of carbohydrate energy intake, which was not observed in HTN, ISH, and IDH in men and women. High carbohydrate consumption was related to a positive impact on SDH, but there were no similar associations between carbohydrate intake and new-onset HTN, ISH, and IDH in male and female Chinese adults.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Beneficial Effects of Vitamin C in Maintaining Optimal Oral Health

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Vitamin C, also known as ascorbic acid, is typically obtained from the diet. Small amounts of water-soluble vitamin C are needed to maintain normal body functions (1–3). Vitamin C has been broadly recognized as the most important hydrophilic antioxidant and is a specific cofactor for many enzymatic reactions. Most plants and animals can synthesize vitamin C from D-glucose and D-galactose. However, due to the absence of the enzyme L-gulonolactone oxidase (GLO) in humans and some animal species such as monkeys, guinea pigs, bats, and birds, they are unable to generate endogenous forms of vitamin C (2, 4). For that reason, humans must obtain vitamin C from their diet or take supplements because a total deficiency of vitamin C in humans can result in spongy swollen bleeding gums, dry skin, open sores on the skin, fatigue, impaired wound healing, and depression. Scurvy can occur when healthy individuals consume <10 mg of vitamin C per day. Additionally, some cancers, anemias, and infections have been linked to a vitamin C deficiency (3, 5, 6). Ascorbic acid is sensitive to air, light, and heat, thus can be destroyed by overcooking and storing food for prolonged periods. In addition, vitamin C is not stored in the body, which is the reason why it must be regularly consumed.

The highest levels of vitamin C can be found in the brain and neuroendocrine tissues (7). Ascorbic acid is essential for the maintenance of collagen, which represents almost one-third of the body's total proteins. Collagen is a constituent protein of bones, cartilages, ligaments, cornea and eye lenses, skin, intervertebral discs, teeth, tendons, gums, blood vessels, and heart valves. Ascorbic acid is also essential for the synthesis of muscle carnitine (β-hydroxybutyric acid), which is necessary for the transport of fatty acids in mitochondria for energy production. Ascorbic acid is needed to synthesize catecholamines and ensure optimal functions of oxytocin, vasopressin, cholecystokinin, and alpha-melanotropin (8). Small amounts of ascorbic acid can prevent against the development of scurvy, and the accumulation of high levels of ascorbate in the plasma and tissues has been found to protect against oxidative damage and limit inflammation (8, 9). As previously mentioned, vitamin C concentration is higher in the brain compared to other organs; therefore, it is likely to contribute to maintaining cognitive functions (10). In newborns, vitamin C deficiency leads to memory impairments due to the decreased neurons in the hippocampus (11). In the elderly, optimum vitamin C levels can help reduce the intensity of many degenerative diseases such as Parkinson's disease, perhaps by manipulating dopamine regulation (12, 13). Vitamin C deficiency also contributes to a higher risk of stroke and associated complications in the elderly (10, 11). It has also been found that smokers are at a higher risk of developing a vitamin C deficiency and may require an additional 35 mg/day of vitamin C to be able to maintain proper vitamin C functions (13).

Another clinically important finding is that vitamin C is associated with reduced mortality in certain populations. Prospective studies found that plasma concentration of ascorbic acid was inversely related to mortality from all causes, including an array of cardiovascular diseases and ischemic heart disease (14, 15). When vitamin C was combined with vitamin E, selenium, β-carotene, and zinc, it reduced total mortality, and this mortality reduction was especially

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pronounced in men (16). Furthermore, prospective studies of critically ill patients showed that intravenous ascorbic acid at 3 g/day reduced multiple organ failure, ICU stay lengths as well as mortality rate (6, 17). This paper will briefly discuss the recommended amounts of vitamin C, its regulation in humans, and the role of vitamin C in oral health in an attempt to highlight the importance of vitamin C in oral health.

RECOMMENDED AMOUNTS, SOURCES, AND REGULATION OF VITAMIN C

The vitamin C requirement in a healthy adult is the amount that will compensate for metabolic losses. An average fasting vitamin C plasma level should be around 50 μmol/l (18). Daily recommendations for vitamin C are based on the individual and are different for infants, adolescents, women, men, pregnant, and lactating women (18). The Recommended Dietary Allowance (RDA) of vitamin C for adult men is 90 mg/day, and the RDA for adult women is 75 mg/day, with the upper limit being 2,000 mg/day (18).

Vitamin C is widely distributed in fresh vegetables such as broccoli, green and red pepper, tomatoes, green leafy vegetables, cauliflowers, and cabbage (1, 2, 7, 19). Fruits that are rich in vitamin C are oranges, pineapples, papaya, raspberries, lemons, strawberries, cherries, cantaloupes, grapefruits, and watermelon (1, 2, 7, 19). Potatoes have also been found to be a source of vitamin C (1, 2, 7, 19).

Under healthy conditions, the plasma concentration of vitamin C depends on dietary intake of ascorbic acid or its reversible oxidized metabolite [dehydroascorbic acid (DHAA)] (20). DHAA can be an adequate dietary source of vitamin C in humans because cellular mechanisms for transport allow DHAA to be converted into ascorbate. Additionally, DHAA has a similar bioavailability to ascorbic acid. Enterocytes absorb ascorbic acid and DHAA in the lumen of the small intestines (**Figure 1**). Human enterocytes have reductases that have the ability to convert DHAA into ascorbic acid. Sodium-independent carriers take up DHAA by facilitated diffusion, while ascorbic acid uses sodium-ascorbate cotransporters which are different from the ones used for DHAA. There are two isoforms of sodium-ascorbate cotransporters, SVCT1 and SVCT2 (21). SVCT1 has been shown to have a higher capacity for ascorbate transport than the other isoform due to its inherent protein structure and size (20). These transporters show relevance because SVCT1 has reduced expression in the elderly, in turn, making their daily requirements higher than younger individuals. It is also important to note that chronic inflammation of the gastric mucosa can decrease the concentration of ascorbate; therefore, patients with chronic gastritis should be potentially monitored for possible vitamin C deficiencies.

After the ascorbic acid is absorbed from the small intestines, it will enter the bloodstream and be rapidly taken up by GLUT1 transporters on erythrocytes (20–22). Ascorbic acid will then be distributed from the blood to different organs; however, the brain is a special exception in that it has a blood-brain barrier and lacks SVCT2 expression, which prevents direct transfer of ascorbic acid. Thus, ascorbic acid makes its way to the brain through the cerebrospinal fluid by going through SVCT2 receptors in the choroid plexus (21).

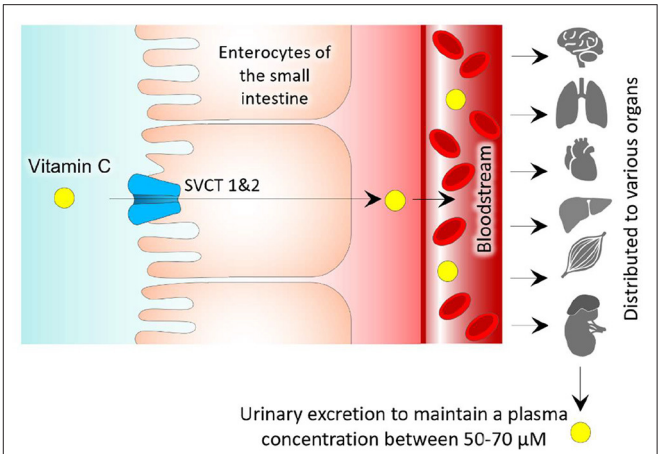

FIGURE 1 | Simplified steps of regulation of vitamin C.

Because of the relatively low hydrophobicity, simple diffusion of vitamin C across the plasma membrane plays a minor role in its regulation. The transport and metabolism of vitamin C are important regulatory functions that can help concentrate intracellular levels of vitamin C and enhance its function as an enzyme cofactor and antioxidant. In humans, the plasma saturation of vitamin C is around 70 μM, and when the dose is above saturation, urinary excretion is increased, and oral bioavailability decreases, in turn permitting a sustained steady-state equilibrium (21, 22).

Adequate amounts of vitamin C in the body allow for the biosynthesis of collagen, catecholamines, and carnitine (18). Vitamin C can also help with the absorption of iron and can provide antioxidant protection. Several proteins have been identified as critical regulators of vitamin C homeostasis. For example, vitamin C transporters regulate its bioavailability in plasma and tissues. Additionally, vitamin C is used in the reduction of active redox metal factors and plays an important role in a host of endogenous stresses such as oxidative stress, infection, and inflammation (20).

VITAMIN C AND ORAL HEALTH

The essential role of vitamin C in health and disease is well-studied; however, its role in oral health is not studied in similar depth and detail (23). A study done by Eydou et al. has shown that vitamin C can play a vital role in preventing the development of dental caries (24). This study revealed that there is a concentration-dependent inhibitory effect between vitamin C and *Streptococcus mutans*. S. *mutans* is a key bacterium that has been linked to the development of dental caries. As mentioned, vitamin C contributes to collagen synthesis, which is an important protein for providing tooth structure, support, and maintenance (25, 26). Vitamin C induces calcium deposition,

mineralization, and reduces the risk of developing secondary caries in children (27). In a meta-analysis conducted to review dietary factors associated with dental erosion, Li et al. revealed that chewing vitamin C tablets was significantly associated with tooth wear development (28) partly due to its low pH. Despite the beneficial effect of vitamin C on dental health, oral health educators should reinforce the important oral health practices such as decreasing the time that soft drinks, fruits, and other vitamin C-containing staff remain in the mouth (28). The literature also highlights that erosive tooth wear is associated with frequent consumption of acidic fruits, juices, and chewable vitamin C with a pH lower than the normal oral pH (2 <5.5) (29).

There is scientific evidence linking periodontal disease and vitamin C deficiency (30, 31). Clinical studies have found that vitamin C depletion can cause gingival bleeding regardless of oral hygiene (32). Individuals with lower blood levels of vitamin C has presented with severe periodontal diseases compared to individuals with higher vitamin C concentration in their plasma (30). Vitamin C released from chewing gum used in healthy individuals can result in lessened supragingival calculus deposition (33). Vitamin C reduces the inflammation reaction in periodontal disease, and the administration of vitamin C supplements has been shown to improve periodontal conditions (30, 34). The periodontal healing activity is attributed to the antioxidant activity of vitamin C, and its role in collagen biosynthesis that facilitates wound healing (34). A vitamin C deficiency can result in scurvy which commonly manifests with bleeding gums and increased tooth mobility due to weakened collagen that constitutes periodontal ligament, and leads to atrophic changes of ameloblasts and odontoblasts. A clinical trial conducted by Shimabukuro and colleagues on patients with gingivitis found that spontaneous bleeding and redness of the gum could be reduced by the use of vitamin C (35). A similar reduction of gingival inflammation and bleeding following the use of vitamin C is also documented in patients with chronic gingivitis, chronic periodontitis, and type 2 diabetes (30, 36).

Vitamin C is an antioxidant that is capable of inhibiting the initiation of carcinogenesis and can help to neutralize the transformation of cells (37). Vitamin C is believed to play a protective role in patients with oral cancers. A study involving patients with oral cancers showed that patients with oral cancers had decreased saliva levels of vitamin C compared to the control group (38). A case-control study found that dietary intake of vitamin C was associated with a reduced risk of oral premalignant lesions (39). In addition, a high intake of vitamin C from natural sources (i.e., fruits, vegetables) was associated with a significantly lower risk of head and neck cancer (40). A study conducted among patients with oral cell carcinoma grade I and II revealed a marked decrease in vitamin C levels among oral cancer patients compared to the control group (37). Hence, a vitamin C deficiency is regarded as a risk factor for oral carcinogenesis. Thus, vitamin C is currently recommended as a therapeutic measure to minimize the initiation and progression of oral cancer (37, 41).

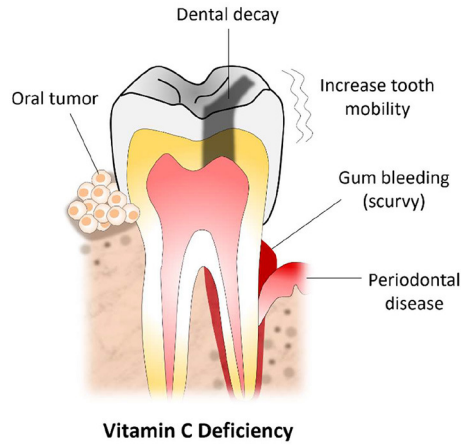

FIGURE 2 | Vitamin C deficiency and commonly encountered oral lesions.

VITAMIN C OVERDOSE

Even though the side effects of normal vitamin C intake are minor or inexistent, at large doses, calcium oxalate stones can be formed because vitamin C converts to oxalate during the elimination process. Patients with renal dysfunctions are more prone to developing calcium oxalate stones; however, it can still happen to healthy individuals at a daily dose of greater than one gram (14). The most common negative effects of high doses of vitamin C are gastrointestinal distress such as gastric pain and flatulence, nausea as well as diarrhea which appeared at the oral ingestion of a single dose of 5–10 g or daily consumption of 2 g. These symptoms usually disappear within 1–2 weeks after reducing the consumption (13, 14). Vitamin C increases iron absorption and transportation across the epithelium of the small intestines (13). This poses an additional risk to patients with sickle cell anemia, hemochromatosis, beta-thalassemia major, or sideroblastic anemia with iron overload. Additionally, in the case of a glucose-6-phosphate dehydrogenase deficiency, there is a higher risk of hemolysis (13, 14). It is advised to split the amount of ingested vitamin C into multiple doses to maintain a sustained release of the formulations in order to maintain a protective level in the plasma and reduce gastric complications. Even though vitamin C tends to be well-tolerated, it should not exceed tolerable upper intake levels, which is two grams per day for adults (13, 42).

SUMMARY

In all, this paper elaborated the recommended amounts of vitamin C, its regulation in humans, and the role of vitamin C in oral health (**Figure 2**). It was determined that the RDA of vitamin C for adult men is 90 mg/day, and the RDA for adult women is 75 mg/day. Vitamin C is abundant in many fresh vegetables such as broccoli, green pepper, tomatoes, and green leafy. It is also found in an array of fruits such as oranges, pineapples, papaya, and lemons.

Additionally, this paper revealed that humans could use both forms of vitamin C, ascorbate, and DHAA; however, their transport mechanisms and regulations differ slightly. It was also found that adequate amounts of vitamin C in the body are vital for the synthesis of collagen, catecholamines, and carnitine. Also, it was determined that there is a concentration-dependent inhibitory effect between vitamin C and *Streptococcus mutans*. A vitamin C deficiency can result in scurvy which can present with bleeding at the gums and increased tooth mobility. Vitamin C also plays a major role in reducing the severity of gingivitis and advanced stages of periodontal diseases. In addition, this paper highlighted that while an overdose on vitamin C is rare, it can still occur in individuals with renal dysfunctions and can potentially cause calcium oxalate stones. However, most individuals that exceed the upper limit of the RDA of vitamin C are likely to experience gastrointestinal disturbances such as gastric pain, flatulence, nausea, and diarrhea.

A future area of study includes expanding the scope of the investigation to evaluate the underlying molecular mechanism of how vitamin C reduces disease burden in chronic disorders ranging from vascular to skeletal, metabolic, neurogenerative and oral diseases.

AUTHOR CONTRIBUTIONS

JM, AU, and PN collected information and drafted the manuscript. JP edited the manuscript and added aditional info. MR conceptualized and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Dietary Fat and Prostate Cancer Relationship Using Trimmed Regression Under Uncertainty

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In this paper, a new trimmed regression model under the neutrosophic environment is introduced. The mathematical model of the new regression model along with its neutrosophic form is given. The methods to find the error sum of square and trended values are also given. The trimmed neutrosophic correlation is also introduced in the paper. The proposed trimmed regression is applied to prostate cancer. From the analysis, it is concluded that the proposed model provides the minimum error sum of square as compared to the existing regression model under neutrosophic statistics. It is found that the proposed model is quite effective to forecast prostate cancer patients under an indeterminacy setting.

Keywords: regression, correlation, classical statistics, neutrosophic statistics, cancer data analysis

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INTRODUCTION

The regression analysis has been applied to study the relationship between two variables that are correlated in a variety of fields such as business, medical science, and weather forecasting. One of the main objectives of the regression model is the forecasting of the response variable using the information of the independent variable. For example, the relationship between prostate cancer and dietary fat can be studied using the regression model. On the other hand, the correlation analysis is done to see the degree of relationship between two correlated variables. In the regression models, a mode is selected which has the minimum error sum of square. Abdul-Wahab et al. (1–4) discussed the applications of the regression models in various fields.

Prostate cancer kills around 34,130 people in the USA every year where more than 248,530 patients are coming every year (https://www.cancer.org/cancer/prostate-cancer/about/key-statistics.html). Smoking is one of the main reasons for this type of cancer and patient has to bear a costly treatment of this disease. Jemal et al. (5) studied the relationship between age and prostate cancer. Rahib et al. (6) expected an increase in deaths due to cancer. Arnold et al. (7) found a strong relationship between smoking and cancer. Torre et al. (8) studied prostate cancer patients in the UK. According to Lin et al. (9), cancer patients remain uncomfortable during the rest of life. Siegel et al. (10) reported the yearly deaths due to cancer in the USA. Prostate cancer is very common in men and can be controlled if it is diagnosed at an early stage. According to Cao et al. (11) "Prostate cancer is the third highest cause of male mortality in the developed world." Lin et al. (9) pointed out the high death rate due to prostate cancer. Scarton et al. (11, 12) studied various factors that cause cancer. Applegate et al. (13) presented a study on the relationship between soy food and cancer. Aslam and Albassam (14) studied the relationship between prostate cancer and dietary fat. More information can be seen in (15).

As mentioned earlier, the regression models are applied for the purpose of the estimation and forecasting of different fields. The presences of the extreme values in the data affect the forecasting and estimation significantly. Several methods are applied to remove these extreme or outliers from the data for a better analysis of the data at hand. The idea of a trimmed average is applied to calculate the average of the observations after removing a specific percentage of the extreme values from the data. The use of the trimmed method is helpful in minimizing the variation in the data. Oten and de Figueiredo (16–22) presented various trimmed methods in various fields.

The regression models and trimmed mean are applied under the assumption that there is no uncertain observation in the data. In practice, as mentioned by (23), the observations can be uncertain, imprecise, and in the interval. For this kind of data, the existing regression model and trimmed mean cannot be applied. To deal with this type of data, statistical methods developed under fuzzy logic are applied. The fuzzy logic-based statistical methods provide the results in an uncertain environment. Saritas et al. (24–28) analyzed prostate cancer data using fuzzy logic. Vela-Rincón et al. (29) presented the idea of a trimmed average under the fuzzy logic.

Smarandache (30) gave the idea of neutrosophic statistics and declared it as the generalization of classical statistics. Neutrosophic statistics has advantages over classical statistics as it gives additional information about the measure of indeterminacy. The neutrosophic statistics is applied when the data is vogue, unclear, imprecise, and indeterminate, Chen et al. (31–35).

As mentioned before that the idea of a trimmed average is applied to remove the extreme values from the data. By exploring the literature and according to the best of our knowledge, there is no work on trimmed regression under neutrosophic statistics. In this article, we will present the trimmed neutrosophic regression originally. We will present the trimmed neutrosophic regression, trended values, and error sum of square in the indeterminate environment. The application of the proposed regression model is given in prostate cancer data. It is expected that the proposed regression will be efficient than the existing regression model. In addition, it is expected that the proposed model will be quite effective to be applied for forecasting and prediction of prostate cancer.

THE PROPOSED REGRESSION MODEL

Suppose that we have $n_N \epsilon [n_L, n_U]$ pairs of observations as $(x_{1N}, y_{1N}), \ldots, (x_{nN}, y_{nN})$, where x_{nN} is an independent variable and y_{nN} be a dependent variable. It is assumed that both neutrosophic variables are correlated. For the implementation of the proposed trimmed regression model, the neutrosophic data is arranged in ascending order in variable x_{nN} or y_{nN}. Let ω be the percentage of the removed values from both variables. For example, if $\omega = 6$ and we arranged the data in ascending according to the variable x_{nN}, it means that three pairs of observations will be removed from the starting and three pairs of observations will be removed from the end of the data. Using this information, the trimmed regression model under neutrosophic statistics, say $Y_{NT} \epsilon [Y_{LT}, Y_{UT}]$ is given as

$$Y_{NT} = a_{NT} + b_{NT} X_{NT}; a_{NT} \epsilon [a_{LT}, a_{UT}], b_{NT} \epsilon \left[b_{LT}, b_{UT}\right] \quad (1)$$

where $a_{NT} \epsilon [a_{LT}, a_{UT}]$ and $b_{NT} \epsilon \left[b_{LT}, b_{UT}\right]$ are slope and rate of change of the proposed trimmed regression model.

The proposed trimmed regression in neutrosophic form can be written as

$$\hat{Y}_{NT} = (a_{LT} + a_{UT} I_{NaT}) + \left(b_{LT} + b_{UT} I_{NbT}\right)(X_{LT} + X_{UT} I_{NXT}); \\ I_{NaT} \epsilon [I_{LaT}, I_{UaT}], I_{NbT} \epsilon [I_{LbT}, I_{UbT}], I_{NXT} \epsilon [I_{LXT}, I_{UT}] \quad (2)$$

where a_{LT}, b_{LT}, X_{LT} be the lower values of indeterminate interval and known as determinate values under classical statistics. On the other hand, $a_{UT} I_{NaT}; I_{NaT} \epsilon [I_{LaT}, I_{UaT}]$, $b_{UT} I_{NbT}; I_{NbT} \epsilon [I_{LbT}, I_{UbT}]$, $X_{UT} I_{NXT}; I_{NXT} \epsilon [I_{LXT}, I_{UT}]$ are indeterminate values of indeterminate interval. The proposed trimmed regression model is a generalization of the regression model proposed by Aslam and Albassam (14). The operations of neutrosophic numbers can be seen in Chen et al. (31, 32). The trimmed neutrosophic correlation, say $r_{NT} \epsilon [r_{LT}, r_{UT}]$ is defined as

$$r_{NT} = \frac{n_{NT} \sum X_{NT} Y_{NT} - \sum X_{NT} \sum Y_{NT}}{\sqrt{\left\{n_{NT} \sum (X_{NT})^2 - \left(\sum X_{NT}\right)^2\right\}\left\{n_{NT} \sum (Y_{NT})^2 - \left(\sum Y_{NT}\right)^2\right\}}} \quad (3)$$

The neutrosophic form of $r_{NT} \epsilon [r_{LT}, r_{UT}]$ is given by

$$r_{NT} = r_{LT} + r_{UT} I_{NrT}; I_{NrT} \epsilon [I_{LrT}, I_{UrT}] \quad (4)$$

where r_{LT} is a determinate part and $r_{UT} I_{NrT}; I_{NrT} \epsilon [I_{LrT}, I_{UrT}]$ is an indeterminate part. The proposed trimmed neutrosophic correlation reduces to (14) if no pair of observations is trimmed from the data. The following steps can be applied to run the proposed regression model on real data.

1. Arrange the data of X_N or Y_N in ascending order.
2. Fix the trimmed value ω. Indicate ω extreme values in X_N. Remove $\frac{\omega}{2}$ pair of (X_N, Y_N) from the start and $\frac{\omega}{2}$ pair from the end.
3. Fit the proposed regression on trimmed data.
4. Determine the neutrosophic trended values and neutrosophic error sum of square.

APPLICATION FOR PROSTATE CANCER

In this section, the application of the proposed regression model is applied to the prostate cancer data of 30 countries. The present case study is based on two variables namely dietary fat and death rate. The decision-makers are interested to see the relationship between these two variables. For this study, dietary fat is considered as the independent variable, and the death rate is labeled as the dependent variable. Let X_N denote the variable dietary fat and Y_N denotes the death rate. The purpose of this study is to determine the effect of dietary effects on the

TABLE 1 | Prostate cancer death rate of 30 countries.

County No.	Diet Fat	D-rate	County No.	Diet Fat	D-rate
1	(38,38)	(0.9,1.1)	16	(97,97)	(10.1,10.3)
2	(29,31)	(1.3,1.3)	17	(73,75)	(11.4,11.4)
3	(42,42)	(1.6,1.6)	18	(112,112)	(11.1,11.1)
4	(57,57)	(4.5,4.5)	19	(100,100)	(13.1,13.3)
5	(96,98)	(4.8,4.10)	20	(134,134)	(12.9,13.1)
6	(47,49)	(5.4,5.6)	21	(142,142)	(13.4,13.4)
7	(67,67)	(5.5,5.5)	22	(119,119)	(13.9,14.2)
8	(72,74)	(5.6,5.6)	23	(137,137)	(14.4,14.4)
9	(93,93)	(6.4,6.6)	24	(152,152)	(14.4,14.6)
10	(58,58)	(7.8,7.8)	25	(129,129)	(15.1,15.3)
11	(95,95)	(8.4,8.6)	26	(156,156)	(15.9,15.9)
12	(67,69)	(8.8,8.8)	27	(147,147)	(16.3,16.4)
13	(62,62)	(9,9)	28	(133,133)	(16.8,16.9)
14	(96,96)	(9.1,9.1)	29	(132,132)	(18.4,18.4)
15	(86,87)	(9.4,9.4)	30	(143,144)	(12.4,12.6)

TABLE 2 | Trimmed Prostate cancer death rate of 30 countries.

County No.	Diet Fat	D-rate	County No.	Diet Fat	D-rate
1	(57,57)	(4.5,4.5)	13	(97,97)	(10.1,10.3)
2	(96,98)	(4.8,4.1)	14	(73,75)	(11.4,11.4)
3	(47,49)	(5.4,5.6)	15	(112,112)	(11.1,11.1)
4	(67,67)	(5.5,5.5)	16	(100,100)	(13.1,13.3)
5	(72,74)	(5.6,5.6)	17	(134,134)	(12.9,13.1)
6	(93,93)	(6.4,6.6)	18	(142,142)	(13.4,13.4)
7	(58,58)	(7.8,7.8)	19	(119,119)	(13.9,14.2)
8	(95,95)	(8.4,8.6)	20	(137,137)	(14.4,14.4)
9	(67,69)	(8.8,8.8)	21	(129,129)	(15.1,15.3)
10	(62,62)	(9,9)	22	(133,133)	(16.8,16.9)
11	(96,96)	(9.1,9.1)	23	(132,132)	(18.4,18.4)
12	(86,87)	(9.4,9.4)	24	(143,144)	(12.4,12.6)

TABLE 3 | The trended values and error sum of square for two regression models.

Existing regression		Proposed regression	
$\hat{Y}_N$	$\sum_{i=1}^{n_N}\left(Y_N-\hat{Y}_N\right)^2$	$\hat{Y}_N$	$\sum_{i=1}^{n_N}\left(Y_N-\hat{Y}_N\right)^2$
(3.15, 3.24)	(5.49, 4.24)		
(2.22, 2.35)	(0.85, 1.11)		
(3.69, 3.61)	(4.40, 4.07)		
(5.39, 5.34)	(0.80, 0.71)	(6.11, 6.04)	(2.59, 2.37)
(9.81, 10.05)	(25.19, 35.44)	(10.13, 10.34)	(28.47, 38.99)
(4.26, 4.42)	(1.28, 1.38)	(5.08, 5.20)	(0.10, 0.15)
(6.53, 6.49)	(1.06, 0.98)	(7.14, 7.09)	(2.70, 2.52)
(7.09, 7.29)	(2.24, 2.87)	(7.65, 7.82)	(4.24, 4.95)
(9.47, 9.47)	(9.48, 8.29)	(9.82, 9.81)	(11.73, 10.36)
(5.51, 5.45)	(5.23, 5.48)	(6.21, 6.14)	(2.51, 2.73)
(9.70, 9.70)	(1.70, 1.23)	(10.03, 10.02)	(2.66, 2.04)
(6.53, 6.72)	(5.14, 4.31)	(7.14, 7.30)	(2.74, 2.24)
(5.96, 5.91)	(9.21, 9.50)	(6.62, 6.56)	(5.62, 5.92)
(9.81, 9.82)	(0.51, 0.52)	(10.13, 10.13)	(1.07, 1.07)
(8.68, 8.78)	(0.51, 0.37)	(9.10, 9.18)	(0.08, 0.04)
(9.93, 9.93)	(0.02, 0.13)	(10.23, 10.23)	(0.01, 0.00)
(7.21, 7.41)	(17.54, 15.91)	(7.76, 7.93)	(13.22, 12.03)
(11.63, 11.66)	(0.28, 0.31)	(11.78, 11.81)	(0.47, 0.51)
(10.27, 10.28)	(7.99, 9.09)	(10.54, 10.55)	(6.50, 7.53)
(14.12, 14.19)	(1.50, 1.18)	(14.05, 14.12)	(1.33, 1.04)
(15.03, 15.10)	(2.66, 2.92)	(14.88, 14.88)	(2.19, 2.44)
(12.42, 12.46)	(2.17, 3.00)	(12.50, 12.54)	(1.93, 2.72)
(14.46, 14.53)	(0.00, 0.01)	(14.36, 14.43)	(0.00, 0.00)
(16.16, 16.25)	(3.12, 2.75)	(13.54, 13.59)	(2.43, 2.89)
(13.56, 13.61)	(2.37, 2.83)	(13.95, 14.01)	(8.10, 8.29)
(16.62, 16.71)	(0.51, 0.66)	(13.85, 13.91)	(20.69, 20.12)
(15.60, 15.68)	(0.48, 0.51)	(14.98, 15.17)	(6.68, 6.62)
(14.01, 14.07)	(7.76, 7.97)		
(13.90, 13.96)	(20.24, 19.71)		
(15.14, 15.33)	(7.54, 7.50)		
Sum	[147.41, 155.11]		[128.18, 137.70]

death rate. The neutrosophic data of variables X_N and Y_N is selected from Aslam and Albassam (14) and shown in **Table 1** for easy reference. From **Table 1**, it can be seen that the given data is given in indeterminate intervals; therefore, the classical regression model under classical statistics cannot be applied to study the relationship between death rate and dietary fat. Aslam and Albassam (14) presented the neutrosophic regression analysis for the same data. We now apply the idea of trimmed regression on the same data. The proposed regression analysis can be applied as follows

5. Arrange the data of X_N and Y_N in ascending order.
6. Fix the trimmed value $\omega = 3\%$. Indicate the six extreme values in X_N. Remove three pairs of (X_N, Y_N) from the start and end three pairs of (X_N, Y_N) from the end.
7. Fit the proposed regression on this trimmed data.
8. Determine the neutrosophic trended values and neutrosophic error sum of square $\sum_{i=1}^{n_N}\left(Y_{NT}-\hat{Y}_{NT}\right)^2$

The trimmed data of X_{NT} and Y_{NT} is shown in **Table 2**. The neutrosophic trimmed regression model using the data given in **Table 2** is given by

$$\hat{Y}_{NT} = [0.2306, 0.0567] + [0.1032, 0.1049]\, X_{NT} \tag{5}$$

The neutrosophic form of $\hat{Y}_{NT}$ for the cancer data is given by

$$\hat{Y}_{NT} = (0.2306 - 0.0567I_{NaT}) + (0.1032 + 0.1049I_{NbT})X_{NT};$$
$$I_{NaT}\epsilon\,[0, 3.06]\,, I_{NbT}\epsilon\,[0, 0.02\,] \tag{6}$$

The proposed regression model can be interpreted as when $X_{NT}\epsilon\,[0, 0]$, the death rate will be from 0.2306 and 0.0567. The rate of change in the death rate due to the dietary fat is from 0.1032 to 0.1049. The neutrosophic correlation between dietary fat and death rate is from 0.7996 and 0.7910. From this study, it can be noted that the proposed regression analysis provides the values of intercept and rate of change values in indeterminate

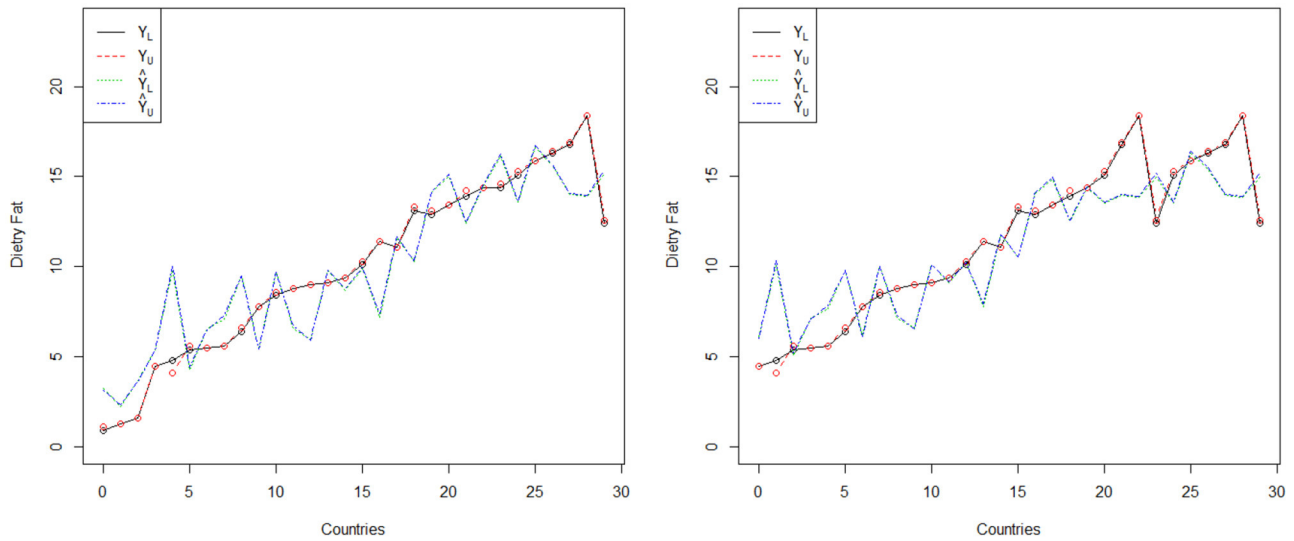

FIGURE 1 | The trended lines of two regression models.

intervals rather than the exact values as in regression under classical statistics. Therefore, the proposed regression analysis is quite effective to be applied to study the relationship between dietary fat and death rate under indeterminacy.

COMPARATIVE STUDIES BASED ON CANCER DATA

Aslam and Albassam (14) applied the neutrosophic regression model on the prostate cancer data. As mentioned earlier, in the regression theory, a regression model having the smaller values of the error sum of square is minimum is called an efficient regression model. We now compare the efficiency of the proposed regression model with Aslam and Albassam (14) regression model in terms of neutrosophic error sum of square $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$, where Y_{NT} and $\hat{Y}_{NT}$ are original values and trend values, respectively. The values of $\hat{Y}_{NT}$ and $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$ for both regression models are shown in **Table 3**. From **Table 3**, it can be seen that the values of $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$ are smaller for the proposed regression model as compared to the existing values of $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$. For example, the values of the error sum of square is $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2 = [147.41, 155.11]$ from the existing regression proposed by (14). The values of the error sum of square are $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2 = [128.18, 137.70]$ from the proposed regression model. By comparing the values of $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$ of both regressions, it is concluded that the proposed model is better than the existing model proposed by Aslam and Albassam (14). Therefore, the proposed model can be used for the forecasting of prostate cancer under the presence of uncertainty.

COMPARISON IN TRENDED VALUES BASED ON CANCER DATA

In this section, the comparison of the proposed regression model is given with the existing model proposed by Aslam and Albassam (14) in terms of trended values. The trended values of both models are presented in **Table 3**. The trended lines of both regression models are shown in **Figure 1**. From **Figure 1**, it can be noted that the trended values are close to actual values of prostate cancer for the proposed regression model. On the other hand, the trended values are away from the actual values of prostate cancer for the existing regression model proposed by Aslam and Albassam (14). From this comparative study, it can be concluded that the proposed model is quite suitable to apply for the forecasting of prostate cancer patients as compared to the existing regression model under the presence of uncertainty.

MEASURES OF INDETERMINACY BASED ON CANCER DATA

In this section, we will present the neutrosophic forms along with the measures of indeterminacy of the values of $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$. The neutrosophic form of $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2$ of the proposed model can be expressed as: $\sum_{i=1}^{n_{NT}} \left(Y_{NT} - \hat{Y}_{NT}\right)^2 = 128.18 + 137.70 I_N \epsilon\, [0, 0.07]$. It means that the error sum of square under uncertainty can be from 128 to 137 with the measure of indeterminacy is being 0.07. The neutrosophic form $\hat{Y}_N$ for example for country#4

can be given as: $\hat{Y}_{NT} = 6.11 - 6.04I_N\epsilon\,[0, 0.01]$. From this neutrosophic form, the first value 6.11 indicates the trend values for the regression model under classical statistics. The second value $6.04I_N$ indicates the indeterminate part of the neutrosophic form. From this study, it can be noted that the death rate due to dietary fat will be from 6.04 to 6.11 per 100,000. The proposed regression model gives the trended values in intervals rather than the exact values. Therefore, the proposed model is reasonable to apply for the forecasting of the death rate due to dietary fat.

CONCLUSIONS

In this paper, a new trimmed regression model under the neutrosophic was introduced. The mathematical model of the new regression model along with its neutrosophic form was given. The trimmed neutrosophic correlation was also introduced in the paper. The proposed trimmed regression is applied to prostate cancer. The efficiency of the proposed model is discussed with the existing regression model under neutrosophic regression. From the comparisons, it is found that the proposed model provides the minimum error sum of square as compared to the existing model. It is also concluded that the proposed model can be effectively used in forecasting prostate cancer as compared to the existing model. The proposed method can be applied in different areas of applications such as decision-making and multi-level programming. The proposed regression model can be used in medical science, business, and social science as future research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MA and AA-M wrote the paper. Both authors contributed to the article and approved the submitted version.

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Habitual- and Meal-Specific Carbohydrate Quality Index and Their Relation to Metabolic Syndrome in a Sample of Iranian Adults

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Aim: Most studies on diet quality have focused on the habitual and overall intake of foods without considering intakes at specific eating occasions. This study aimed to assess the association between habitual- and meal-specific carbohydrate quality index (CQI) and metabolic syndrome (MetS) in Iranian adults.

Methods: In this cross-sectional study, data from 850 participants were analyzed. Dietary information was obtained from a 3-day nonconsecutive 24 h recall. CQI was calculated from three criteria: dietary fiber, glycemic index, and solid carbohydrate/total carbohydrate ratio. The association between CQI and MetS was assessed by logistic regression.

Results: The prevalences of MetS in the lowest and highest tertile of CQI were 30.1 and 33.7, respectively ($P = 0.6$). In habitual diet and all the three meals, we failed to find any significant association between tertiles of CQI and MetS either before or after adjustment for covariates. However, in the habitual meals [odds ratio (OR): 0.69, 95% CI: 0.47–0.96] and lunch meals (OR: 0.66; 95% CI: 0.47–0.94), the highest CQI in comparison to the lowest one, significantly decreased the low high-density lipoprotein (HDL). In addition, the trend of low-HDL with CQI in habitual meal and lunch meal was statistically significant.

Conclusion: The results of this study showed that CQI was not associated with MetS and its components. Further investigations into the mechanisms underlying the role of carbohydrate quality in developing metabolic disorders are warranted.

Keywords: carbohydrate quality index, metabolic syndrome, meal-specific, habitual meal, cross-sectional study

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INTRODUCTION

Metabolic syndrome (MetS) deputes an interrelated metabolic disorder determined by central obesity, deviant glucose hemostasis, and lipid disorders, namely, elevated serum triglyceride (TG), low high-density lipoprotein cholesterol, and elevated blood pressure (1). The prevalence of the MetS among adults in the United States was estimated between 34.3 and 38.5% (2), and in some European countries, at least 25% (3). Recently published data from Iran show that the prevalence of

MetS is 21.1% (4). Based on the evidence, a combination of genetic and environmental factors has been shown to play a role in developing this syndrome (5, 6). Among environmental factors, meal-timing, meal frequency, and dietary quality may play an essential role in cardiometabolic risk factor management (7). Meal timing can affect circadian rhythm and cardiometabolic risk factors (8–10). Epidemiological studies indicated that eating meals at the inappropriate time of the day increases the risk of obesity, type 2 diabetes, and cardiovascular disease (CVD) (11, 12). Limited studies have been performed on meal frequency (daily eating occasions) and cardiometabolic risk factors with different results. One study showed that meal frequency was inversely related to high TGs, high blood pressure, and obesity (13). Besides, individuals who take three meals/day than individuals who received one meal/day, fewer systolic and diastolic blood pressure, lower cholesterol level, and upper TG concentration have been reported (14).

In recent decades, the effort to preclude MetS has focused on declining risk factors and recommending healthy behaviors, especially healthy eating habits (15). In this context, studies and evidence have shown that improving the quality of dietary carbohydrates, instead of modulating their quantity, may have a more significant impact on modulating cardiac-metabolic risk factors (16). Most of the available evidence has examined the various components of the quality of carbohydrates received, such as fiber intake, glycemic index (GI), and glycemic load, separately (17, 18). Former studies have explored the association between consumption of carbohydrates and MetS and have indicated positive (19–21), contradictory (22), and null (23) effects. But since a single component cannot be an appropriate criteria for evaluating the quality of carbohydrate revived, border criteria that can accommodate several single components are used as the carbohydrate quality index (CQI), which is a convenient indicator of the quality of the carbohydrate intake. The CQI was defined by summing up the following criteria: GI, dietary fiber intake, whole grains to total grains ratio, and solid carbohydrates to total carbohydrates (24). Since whole grains in the Iranian diet are limited, its calculation has been abandoned in this study. Few studies have investigated the association between CQI and pathological conditions. A cross-sectional study in Ghana indicated a reverse association between CQI and abdominal obesity (25). In addition, the reverse association between COI and general obesity/overweight has been demonstrated in one prospective study (24). Researchers assayed the relation between dietary patterns based on macronutrients and blood factors such as lipid profile and fasting blood glucose. Still, there has not been much attention paid to meals (26–28). In addition, a dietary recommendation based on meals can be an effective intervention in changing inappropriate habitual intake (29).

Since carbohydrates as a major nutrient provide an essential part of the energy required of the adult population, it is assumed that carbohydrate intake may play a more prominent role in public health. Hence this study aims to assess the association between CQI of meal-specific dietary patterns and MetS and its components among Tehranian adults.

MATERIALS AND METHODS

Data and Study Participants

This cross-sectional study was done within 25 health houses in the Tehran Metropolis. A total of 850 adult participants aged between 18 and 65 years were included. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All the procedures involving human subjects were approved by the ethical standards of the Tehran University of Medical Sciences (ethic number: IR.TUMS.MEDICINE.REC.1399.797), which approved the protocol and informed consent form. All the participants signed a written informed consent before the start of this study.

Adults with a previous history of any major illness such as myocardial infarction, diabetes, cancer, renal disease, and CVD and who were not desired to contribute to the study were excluded. In addition, those who were experiencing any special diet or diet therapy were also excluded from the study. But, in continuation of this study and review of the questionnaires were a limited number of participants who were not considered acute patients and only had a mild and controlled disease that were not excluded from this study. In this case, to prevent errors in the results of the study, individuals were adjusted in terms of having the underlying disease (**Supplementary Figure 1**). This study included both the genders and living in the study region and willing to participate in this study.

Dietary Assessment, Meal Timing, and CQI Calculation

Dietary data were gathered by using repeated but nonconsecutive (Monday to Sunday) 24-h dietary recall method. The 24-h meal was structured and included breakfast, lunch, and dinner. The first 24-h recall is obtained through interviews, the other two recalls are recorded by telephone during two repeated nonrandom days during the study and information was recorded. The habitual diet was calculated using the mean of three 24-h recalls. Food and the food groups were extracted through these questionnaires. Meals usually include breakfast, lunch, and dinner. More energy content was used to classify meals. Thus the largest meal was considered between 5:00 and 11:00 as breakfast, 11:00 and 16:00 as lunch, and between 16:00 and 23:00 as dinner and the smaller intake between main meals was identified as snacks (30, 31). A habitual diet is the combination of meals and snacks which includes 4 meals and snacks throughout the day, including breakfast, lunch, evening snack, and dinner.

Finally, the total value of nutrients in all the meals and snacks was calculated daily. CQI was calculated based on the energy-adjusted amount of total carbohydrate intake values calculated using the residual method (32). CQI was defined by summing up the following four criteria: (1) ratio of solid carbohydrates to total carbohydrates, (2) dietary fiber intake (g/day), (3) GI, and (4) ratio of whole grains to total grains (whole grains, refined grains, and their products). Subjects were categorized into quintiles and take a value (ranging from 1 to 5) for each quintile according to each of these four criteria; however, the scoring of GI was reversed; thus, those in the fifth quintile received one point,

and those in the first quintile received five points. Finally, an overall CQI was computed by adding all values of the four criteria (ranging from 4 to 20). It was also ranked into quintiles (24). But given that the Iranians are using whole grains in the diet is limited, this component is not calculated, so the final score ranges from 3 to 15.

Glycemic index (GI) values were obtained from international tables (33), the GI of Iranian foods (34), and literature reviews. As if food item was not available in any of the mentioned tables, we used the GI values of chemically and physically similar food items for those foods (34). Glucose was used as the reference (GI for glucose = 100). The mean of the GI values was assigned if more than one eligible GI value was available for a specific food item. The carbohydrate content of each food was determined using standard portion sizes from the United States Department of Agriculture food composition databases (35). All the nuts and vegetables except starchy roots were considered as very low GI (ranging from 10 to 20). Solid carbohydrates were obtained by subtracting the amount of liquid carbohydrate (summing up sweetened beverages and fruit juice) from total carbohydrate intake.

Anthropometric Assessment and Biochemical Tests

Weight was measured using a Seca weighing scale (Seca and Corporation KG; 22 089 Hamburg, Germany; Model: 874 1321009; designed in Germany; made in China) with light clothing (without a coat and raincoat). A wall stadiometer board was used for participants' height without shoes with a sensitivity of 0.1 cm height measurements. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared). Waist circumference was measured according to the guiding protocol of the WHO, at the midpoint between the lower border of the rib cage and the iliac crest, using a nonstretchable fiberglass measuring tape. Eventually, the waist-hip ratio (WHR) was calculated for each person. WHR $\geq$0.5 was adopted for overweight and abdominal obesity for uniformity regarding age differences (36). Blood pressure is measured by a digital barometer (BC 08, Beurer, Germany) after at least 10–15 min of rest and sitting. Blood pressure was measured two times for each person, and the average blood pressure was reported for each person. Of all participants, 10 ml of fasting blood was taken between 7 and 10 a.m. in the acid-washed test tubes without anticoagulant until after room temperature maintenance for 30 min. Minute blood clots and centrifuge at 1,500 g for 20 min. The serums are poured into microclean tubes and stored in the -80°C freezer until the future test. Fasting blood sugar was assayed by the enzymatic (glucose oxidase) colorimetric method using a commercial kit (Pars Azmun, Tehran, Iran) at the sampling day. Serum total cholesterol and high-density lipoprotein cholesterol (HDL-C) were measured using a cholesterol oxidase phenol amino antipyrine method and TG was measured using a glycerol-3 phosphate oxidase phenol amino antipyrine enzymatic method on the same day after collecting all the samples.

Sociodemographic and Lifestyle Variables

General information such as age, marital status, smoking status, living situation (alone or with someone), and disease was recorded by asking participants with a general information questionnaire registered. International Physical Activity Questionnaire is used to examine people's physical activity (37), which records three intensity levels of activity based on the metabolic equivalents (METs). METs were classified as low (<600 MET-min/week), moderate (600–3,000 MET-min/week), and vigorous (>3,000 MET-min/week).

Metabolic Syndrome

Metabolic syndrome (MetS) and its components were defined using the following criterion (1). Individuals who have at least three or more of the following disorders were classified as having MetS: high waist circumference ($\geq$88 for women and $\geq$102 for men); elevated TG levels ($\geq$150 mg/dl); low HDL-C levels ($\leq$50 mg/dl for women and $\leq$40 mg/dl for men); high blood pressures (systolic blood pressure $\geq$ 130 mm Hg and diastolic blood pressure $\geq$ 85 mm Hg) or use of antihypertensive medication; and high fasting glucose levels ($\geq$100 mg/dl) or use of hypoglycemic medication.

Statistical Analysis

Energy-adjusted dietary CQI was used to classify participants into tertiles. According to the type of variables, the comparison of quantitative mean variables between the tertiles of subject characteristics and anthropometric measurement was performed using one-way ANOVA and comparison of qualitative variables distribution between the tertiles with the chi-squared test. Logistic regression was performed to investigate the relationship between CQI as an independent variable and MetS and its components as a dependent variable in an unadjusted and multivariable-adjusted model. In this regard, age, sex, energy intake, physical activity, marital status, smoking status, educated status, underlying disease, and BMI were included as covariates in the modified regression model. All the statistical analyses were done using IBM Statistical Package for Social Sciences (V.22; SPSS Inc.), and $P < 0.05$ was considered as statistically significant.

RESULTS

The mean age of study participants with MetS was 46.1 $\pm$ 10 and the mean BMI with MetS was 29.2 $\pm$ 4.71. The prevalence of MetS among participants in the lowest and highest tertiles of CQI were 30.1 and 33.7, respectively (P = 0.6). The mean CQI in participants with MetS was 9.15 $\pm$ 2.83 (**Supplementary Table 1**).

Among the participants, 30, 24, and 5 participants were removed from breakfast, lunch, and dinner, respectively, due to the lack of enough information for the final analysis. As a result, 820, 826, and 845 participants remained in the study for final analysis at breakfast, lunch, and dinner meals.

General characteristics of study participants according to carbohydrate quality score based on habitual diet and meal is given in **Table 1**. Within lunch meals, those in the top tertiles of CQI were less likely to be current smokers (P = 0.05). In habitual

TABLE 1 | General characteristic of study participants according to tertiles (T) of carbohydrate quality index (CQI).

	CQI (breakfast)				CQI (lunch)				CQI (dinner)				CQI (habitual)			
	T1 (3–7)	**T2 (7–11)**	**T3 (11–15)**	***P*****	**T1 (3–7)**	**T2 (7–11)**	**T3 (11–15)**	***P*****	**T1 (3–7)**	**T2 (7–11)**	**T3 (11–15)**	***P*****	**T1 (3–7)**	**T2 (7–11)**	**T3 (11–15)**	***P*****
Participant	273	274	273		275	276	275		281	282	282		276	277	276	
Sex																
Male%	30	40	30	0.1	37.7	33.3	29	0.3	32.9	32.3	34.8	0.9	27.8	31.9	40.3	0.1
Female%	33.9	31.9	34.2	0.1	32.3	33.7	34	0.3	33.3	33.6	33	0.9	34.5	33.7	31.8	0.1
Age	42.7 ± 11	42.2 ± 10	42 ± 11.6	0.7	42.3 ± 11	42.7 ± 10.8	42 ± 11	0.7	41.5 ± 10.7	43.3 ± 10.9	42.1 ± 11	0.1	41.8 ± 10.7	42.5 ± 10.6	42.4 ± 10.4	0.7
Educated	33.3	33.8	32.9	0.6	33.7	32.5	33.8	0.03	33.4	33.2	33.4	0.9	33.6	33.6	32.8	0.7
Marital status (married %)	32.6	34.9	32.5	0.2	33.2	34.1	32.7	0.8	34.2	33.1	32.7	0.4	32.9	33.8	33.2	0.7
Smoking (smoker %)	23.3	37.2	39.5	0.3	50	30.6	19.4	0.05	32.6	34.9	32.6	0.9	34.1	36.6	29.3	0.8
Underlying disease (yes %)[‡]	35.8	31.3	32.8	0.3	31	34.8	34.2	0.4	31.7	34.8	33.4	0.6	32.5	34.3	33.1	0.8
Activity score																
Low	34.1	32.5	33.4	0.9	34.2	32.4	31.6	0.3	32.1	34.3	33.6	0.5	34.6	33.9	31.6	0.3
Moderate	32.5	34.7	32.8	0.9	32.4	32.4	35.3	0.3	34.8	32.6	32.6	0.5	33.9	32.9	33.2	0.3
High	28.8	34.2	37	0.9	31.6	30.3	38.2	0.3	35.1	32.5	32.5	0.5	24.3	32.4	43.2	0.3
BMI	27.6 ± 7.31	27.2 ± 4.63	27.1 ± 4.63	0.5	27.4 ± 6.84	27 ± 5.27	27.4 ± 5.27	0.6	27.1 ± 4.18	27.7 ± 7.04	27.1 ± 5.3	0.2	27 ± 4.42	27.3 ± 4.52	27.3 ± 4.59	0.6
Weight (kg)	72.4 ± 14.6	72.3 ± 13.6	71.8 ± 13.5	0.8	72.5 ± 13.8	71.6 ± 13.9	72.1 ± 13.9	0.7	71.8 ± 11.6	72.5 ± 14.7	72 ± 15.1	0.8	71.6 ± 13	72.71 ± 4.5	72.8 ± 13.5	0.5
Height (cm)	162.4 ± 9.88	163 ± 8.84	162.5 ± 8.38	0.7	162.9 ± 9.51	162.6 ± 8.13	162.3 ± 9.46	0.7	162.8 ± 8.76	162.1 ± 9.34	162.9 ± 8.89	0.4	162.2 ± 9.13	162.8 ± 8.5	162.9 ± 8.9	0.5
WC (cm)	89.4 ± 12.3	89 ± 12.9	89.1 ± 12.1	0.9	89.5 ± 12.1	88.6 ± 11.4	89.1 ± 12.7	0.6	88.8 ± 11.1	89.2 ± 12.1	89.3 ± 13	0.8	88 ± 11.6	90 ± 12	89.6 ± 11.5	0.09

Values are means ± standard deviations (SD) or percentages.

Chi-square test used for categorical variables, one-way ANOVA for continuous variables.

***P < 0.05.*

[‡]Underlying disease: Including diabetes, hypertension, dyslipidemia, cardiovascular disease, stroke, cancer, respiratory disease, and osteoporosis.

BMI, body mass index; WC, waist circumference; Kg, kilogram; cm, centimeter.

diet and all the three meals, the distribution of participants in terms of other general characteristics across tertiles of CQI was not significantly different.

The evaluation of biochemical biomarkers showed no significant statistical differences in laboratory characteristics across tertiles of CQI in habitual meals and all the three meals (**Table 2**).

The selected dietary intake of study participants across tertiles of CQI is shown in **Supplementary Tables 2**, **3**. In habitual diet, we observed a significant association between tertiles in participants for total sugar, total fiber, GI ($P < 0.001$ for all), and carbohydrate ($P = 0.001$). Within breakfast meal, dietary intake of total sugar ($P = 0.007$), and GI ($P < 0.001$) were significantly different across tertiles of CQI. In lunch meals, dietary intakes of total fiber, total sugar, and GI were significantly different across tertiles of CQI among participants ($P < 0.001$ for all). Moreover, participants' carbohydrate intake ($P = 0.02$) and protein intake ($P = 0.001$) were significant across tertiles of CQI. Within the dinner meal, participants in the top of tertiles of CQI had a higher intake of energy and protein ($P = 0.03$; $P = 0.02$). In contrast, participants in the top of tertiles of CQI had a lower intake of fat and cholesterol ($P = 0.01$; $P = 0.002$). Moreover, dietary intakes of total fiber, total sugar, GI, and saturated fatty acids (SFA) ($P < 0.001$ for all).

The multivariate-adjusted odds ratio for MetS and its component across tertiles of habitual and meal-specific CQI is indicated in **Tables 3**, **4**. In habitual diet and all three meals, we failed to find any significant association between tertiles of CQI and MetS either before or after adjustment for covariates. In habitual meal, before and after adjustment for the covariate, the odds ratio of low-HDL in third tertiles of CQI was significantly lower than the first tertiles [odds ratio (OR): 0.67; 95% CI: 0.47–0.96]. Moreover, within lunch meals, CQI is associated with low-HDL (OR: 0.66; 95% CI: 0.47–0.94) before and after adjustment for potential cofounding. In addition, no overall significant association was observed between CQI and other components of MetS either before or after adjustment for covariates in habitual diet and all the three meals. It should be noted that the trend of low-HDL across tertiles of CQI was marginally significant in habitual diet (OR: 0.67; 95% CI: 0.47–0.96; P-trend = 0.02) and in lunch meal (OR: 0.66; 95% CI: 0.47–0.94; P-trend = 0.01).

DISCUSSION

This study examined the relationship between habitual- and meal-specific CQI and odds of MetS and its components in a sample of Iranian adults. Our findings showed a nonsignificant association between CQI with MetS before and after adjustment for potential confounders in all three meals and habitual diet. But we found a reverse association between CQI and the odds of developing low-HDL in habitual diet and within lunch meal even after adjustment for covariates. No overall significant association was observed between CQI and other components of MetS in habitual diet and all three meals.

Carbohydrates are a heterogeneous class of nutrients and the consumption of refined carbohydrates for enhancing the quality

TABLE 2 | Laboratory results of study participants according to tertiles (T) of CQI.

	CQI (breakfast)				CQI (lunch)				CQI (dinner)				CQI (habitual)			
	T1 (3–7)	T2 (7–11)	T3 (11–15)	P**	T1 (3–7)	T2 (7–11)	T3 (11–15)	P**	T1 (3–7)	T2 (7–11)	T3 (11–15)	P**	T1 (3–7)	T2 (7–11)	T3 (11–15)	P**
Participant	273	274	273		275	276	275		281	282	282		276	277	276	
FBG (mg/dl)	108.1 ± 9.35	107.3 ± 35.5	107.7 ± 35.9	0.96	108.5 ± 35.2	108.6 ± 42.5	106.2 ± 28.4	0.67	103.8 ± 24.7	109.2 ± 44.3	110.5 ± 5.35	0.06	108 ± 38.5	107.6 ± 40.5	107.2 ± 25.1	0.96
TG (mg/dl)	152.8 ± 85	139.5 ± 73.6	141.9 ± 73.7	0.09	147.3 ± 81.8	146.8 ± 75.8	140.7 ± 75.4	0.54	141.8 ± 69.7	147.1 ± 82.5	145.4 ± 1.80	0.7	142.6 ± 75.9	142.7 ± 78.3	149.4 ± 78.9	0.49
TC (mg/dl)	193.7 ± 41.2	199.1 ± 48.3	195.8 ± 45.1	0.35	197.7 ± 44.7	195.5 ± 43.3	194.8 ± 46.9	0.73	196.4 ± 44.7	196 ± 47.4	195.4 ± 5.42	0.9	193.8 ± 43.1	194 ± 45.9	201.1 ± 44.9	0.09
HDL-C (mg/dl)	49.5 ± 10.6	50.5 ± 9.88	49.6 ± 10.1	0.46	49.5 ± 10.4	49.5 ± 10	50.5 ± 10	0.4	49.9 ± 10.1	50.1 ± 10.2	49.5 ± 10.2	0.8	49.4 ± 9.95	49.5 ± 9.99	50.7 ± 10.6	0.23
SBP (mmHg)	118.6 ± 15.1	116.4 ± 14.2	118.8 ± 16.3	0.11	116.5 ± 14.7	119 ± 15.4	118.4 ± 15.4	0.12	117.7 ± 15.4	119.1 ± 14.9	117.3 ± 15.4	0.34	117.9 ± 15.9	116.9 ± 14.4	119.2 ± 15.4	0.19
DBP (mmHg)	79.2 ± 9.23	77.8 ± 9.08	79.2 ± 10.7	0.15	78.4 ± 9.46	78.8 ± 10	78.9 ± 9.6	0.78	78.9 ± 9.35	79.1 ± 10.4	78 ± 9.38	0.35	78.7 ± 9.69	78.5 ± 8.89	79.1 ± 10.5	0.72

Data are presented as mean ± standard deviation (SD).
One-way ANOVA test used for assessment variables.
***$P < 0.05$.*
FBG, fasting blood glucose; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; mg, milligram; dl, deciliter.

TABLE 3 | Odds ratio (OR) and 95% CI for metabolic syndrome and components among the study participants according to tertiles (T) of CQI in habitual diet.

	CQI			
	T1 (3–7)	**T2 (7–11)**	**T3 (11–15)**	***P*-trend**
Participant	276	277	276	
Mets[†]				
Crude	1.00	0.74 (0.51–1.07)	1.05 (0.74–1.5)	0.76
Model 1	1.00	0.77 (0.52–1.13)	1.08 (0.74–1.54)	0.71
Model 2	1.00	0.83 (0.59–1.24)	1.14 (0.78–1.67)	0.52
Model 3	1.00	0.86 (0.57–1.29)	1.13 (0.76–1.69)	0.57
Abdominal obesity				
Crude	1.00	0.79 (0.56–1.1)	1.00 (0.72–1.4)	0.96
Model 1	1.00	0.81 (0.57–1.16)	1.07 (0.75–1.53)	0.7
Model 2	1.00	0.83 (0.57–1.19)	1.1 (0.76–1.58)	0.6
Model 3	1.00	0.79 (0.5–1.23)	1.15 (0.73–1.82)	0.55
Elevated BP[‡]				
Crude	1.00	0.8 (0.49–1.3)	1.05 (0.66–1.67)	0.8
Model 1	1.00	0.88 (0.53–1.46)	1.02 (0.63–1.66)	0.9
Model 2	1.00	0.83 (0.48–1.41)	0.98 (0.59–1.63)	0.98
Model 3	1.00	0.87 (0.51–1.5)	0.98 (0.59–1.65)	0.99
Elevated FBG				
Crude	1.00	0.96 (0.69–1.34)	1.35 (0.97–1.89)	0.07
Model 1	1.00	1.01 (0.72–1.43)	1.37 (0.97–1.93)	0.07
Model 2	1.00	1.02 (0.72–1.45)	1.4 (0.98–1.98)	0.05
Model 3	1.00	1.04 (0.73–1.47)	1.4 (0.98–1.99)	0.05
Low HDL-C				
Crude	1.00	0.95 (0.68–1.32)	0.63 (0.45–0.88)	0.008
Model 1	1.00	0.91 (0.65–1.28)	0.64 (0.46–0.91)	0.01
Model 2	1.00	1.02 (0.72–1.45)	0.67 (0.47–0.96)	0.02
Model 3	1.00	1.02 (0.72–1.45)	0.67 (0.47–0.96)	0.02
Elevated TG				
Crude	1.00	0.86 (0.61–1.22)	1.09 (0.77–1.54)	0.59
Model 1	1.00	0.89 (0.63–1.27)	1.04 (0.74–1.48)	0.79
Model 2	1.00	0.95 (0.66–1.36)	1.08 (0.75–1.54)	0.69
Model 3	1.00	0.97 (0.67–1.39)	1.08 (0.75–1.54)	0.7

Binary logistic regression test used for assessment variables.
Crude: unadjusted model.
Model 1: adjusted for age, gender, and energy intake.
Model 2: In addition, adjusted for marital status, physical activity, education status, smoking, and underlying disease.
Model 3: further adjustment was made for BMI.
[†]*Defined as the presence of ≥3 of the following components: abdominal obesity (waist circumference > 88 for women and > 102 for men); elevated blood pressure (BP ≥ 130/85 mmHg); elevated fasting blood glucose (FBG ≥ 100 mg/dl); low high-density lipoprotein-cholesterol (HDL-C < 50 for women and < 40 for men); elevated triglyceride (TG ≥ 150 mg/dl).*
[‡]*Elevated blood pressure (systolic ≥ 130 and diastolic ≥ 85).*
Mets, metabolic syndrome; BP, blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

of received carbohydrates from a public health perspective has been declared. In this way, the available studies on adults demonstrate that total carbohydrate intake or dietary carbohydrate proportion is not associated with the risk of obesity (38, 39). According to a report from a population, carbohydrate quality is a more important factor compared to the fat quality to determine diet quality (40) as far as, it has been proposed that a decline in fat intake was compensated with an enhancement intake of refined starches and sugars (41). There is convincing evidence that carbohydrate quality has important effects on the advancement and treatment of CVD, MetS, T2D, and obesity (42). Aspects of carbohydrate quality that may be substantial in these components include dietary fiber, whole-grain, GI, and GL, particularly the intake of sugar-sweetened drinks. However, their properties are often highly interrelated, and it may be hard to implicate one over another in any specific condition (42). Therefore, in this context, this seems to be more important in Iranian populations because of the higher intake of carbohydrates (43).

In line with our findings in habitual diets, a cross-sectional study conducted on Korean adults observed no significant associations between CQI and T2DM or MetS. However, the quality of carbohydrates consumed is associated with the risk of obesity and elevated blood pressure (44). Also, in another study, no association was found between GI, GL, and MetS (45). Contrary to our findings, a cross-sectional study from Ghana demonstrates that the diet with high CQI levels is inversely related to general and abdominal obesity (25). Another study identified that consuming refined foods with high carbohydrate content was a direct association with a higher risk of abdominal obesity in Ghanaian University students (46). A cohort study from Spain in university graduates reported an inverted association between dietary CQI and general obesity (24). The result of a systematic review and meta-analysis study focusing on the association between carbohydrate quality and NCDs incidence and metabolic biomarkers demonstrated that daily consumption of dietary fiber was associated with a reduced risk of health-related consequences. These findings are supported by cohort studies, which report an alleviated risk of coronary heart disease, mortality, and occurrence of diabetes (47).

High carbohydrate diets, which are common in developing nations, especially Asian countries, contain a high content of refined sources (such as white rice and white bread), low in fiber. These diets usually reflect poor food quality and mainly have high GI content, which can lead to negative metabolic outcomes (48–50). According to the National Food Consumption Survey, most of the calorie intake in Iranian people, which is about more than 60%, is obtained from carbohydrates. In other words, the amount of carbohydrates in Iranians diet is 450 g per day (rural areas: 413 g/day and urban areas: 518 g/day) (51). In addition, considering the high prevalence of low HDL-c in both sexes of the Iranian people (43%; 95% CI: 33–53) (52), the results of our study can be effective as a strategy in controlling the optimal level of low-HDL. The present observation of a reverse association between CQI and low HDL-c is in consent with previous studies. The study that indicated the relation between carbohydrate quality and the prevalence of MetS showed no association between GI, GL, and MetS. However, these were positively associated with low HDL-c levels in adults and older adults (45). Another study found a positive association between GI, GL, and low HDL-c in women (22).

TABLE 4 | OR and 95%CI for metabolic syndrome and components among the study participants according to tertiles (T) of CQI in meals.

	CQI (breakfast)				CQI (lunch)				CQI (dinner)			
	T1 (3–7)	T2 7–11) (7–11)	T3 (11–15)	P–trend**	T1 (3–7)	T2 (7–11)	T3 (11–15)	P–trend**	T1 (3–7)	T2 (7–11)	T3 (11–15)	P–trend**
Participant	273	274	273		275	276	275		281	282	282	
Mets[†]												
Crude	1.00	0.77 (0.54–1.1)	0.8 (0.56–1.14)	0.21	1.00	26.1 (0.89–1.8)	0.92 (0.64–1.33)	0.69	1.00	0.89 (0.62–1.27)	0.96 (0.68–1.37)	0.85
Model 1	1.00	0.8 (0.55–1.15)	0.8 (0.56–1.16)	0.25	1.00	1.25 (0.86–1.8)	0.92 (0.63–1.34)	0.68	1.00	0.83 (0.57–1.21)	0.86 (0.59–1.25)	0.44
Model 2	1.00	0.86 (0.58–1.26)	0.79 (0.54–1.16)	0.23	1.00	1.32 (0.9–1.94)	0.9 (0.61–1.34)	0.62	1.00	0.85 (0.58–1.25)	0.9 (0.61–1.31)	0.53
Model 3	1.00	0.87 (0.58–1.29)	0.81 (0.55–1.21)	0.32	1.00	1.23 (0.83–1.83)	0.86 (0.58–1.29)	0.48	1.00	0.87 (0.58–1.29)	0.96 (0.65–1.43)	0.8
Abdominal obesity												
Crude	1.00	0.84 (0.6–1.17)	0.87 (0.63–1.22)	0.44	1.00	1.64 (1.17–2.3)	1.31 (0.94–1.84)	0.1	1.00	0.95 (0.68–1.33)	0.88 (0.63–1.23)	0.46
Model 1	1.00	0.88 (0.61–1.25)	0.91 (0.63–1.3)	0.61	1.00	1.63 (1.14–2.34)	1.34 (0.93–1.93)	0.1	1.00	0.87 (0.61–1.25)	0.8 (0.55–1.14)	0.22
Model 2	1.00	0.89 (0.61–1.28)	0.9 (0.62–1.29)	0.57	1.00	1.65 (1.14–2.39)	1.31 (0.91–1.89)	0.1	1.00	0.89 (0.61–1.28)	0.8 (0.56–1.16)	0.25
Model 3	1.00	0.85 (0.54–1.33)	0.94 (0.61–1.47)	0.83	1.00	1.56 (1–2.44)	1.3 (0.83–2.04)	0.2	1.00	0.89 (0.56–1.39)	0.81 (0.51–1.26)	0.34
Elevated BP[‡]												
Crude	1.00	0.7 (0.43–1.15)	1.12 (0.71–1.76)	0.58	1.00	1.19 (0.74–1.9)	1.08 (0.67–1.75)	0.74	1.00	1.04 (0.65–1.65)	0.89 (0.55–1.44)	0.65
Model 1	1.00	0.71 (0.43–1.19)	1.15 (0.72–1.84)	0.54	1.00	1.22 (0.74–1.99)	1.1 (0.66–1.81)	0.7	1.00	0.96 (0.59–1.56)	0.73 (0.44–1.21)	0.22
Model 2	1.00	0.8 (0.47–1.37)	1.1 (0.67–1.8)	0.7	1.00	1.22 (0.73–2.04)	1.12 (0.67–1.88)	0.66	1.00	0.98 (0.59–1.63)	0.69 (0.41–1.17)	0.18
Model 3	1.00	0.85 (0.49–1.45)	1.18 (0.71–1.95)	0.51	1.00	1.17 (0.69–1.98)	1.13 (0.67–1.91)	0.64	1.00	0.99 (0.59–1.66)	0.73 (0.43–1.24)	0.25
Elevated FBG												
Crude	1.00	0.83 (0.59–1.15)	0.83 (0.6–1.16)	0.29	1.00	1.00 (0.72–1.4)	0.93 (0.67–1.3)	0.7	1.00	1.04 (0.74–1.45)	1.23 (0.88–1.72)	0.2
Model 1	1.00	0.84 (0.6–1.18)	0.85 (0.61–1.2)	0.37	1.00	0.99 (0.7–1.39)	0.93 (0.66–1.3)	0.67	1.00	0.97 (0.68–1.36)	1.14 (0.81–1.6)	0.44
Model 2	1.00	0.88 (0.62–1.24)	0.88 (0.62–1.24)	0.47	1.00	0.97 (0.68–1.36)	0.92 (0.65–1.29)	0.66	1.00	0.95 (0.67–1.35)	1.13 (0.8–1.6)	0.42
Model 3	1.00	0.88 (0.62–1.24)	0.88 (0.63–1.25)	0.5	1.00	0.94 (0.66–1.33)	0.91 (0.64–1.29)	0.62	1.00	0.96 (0.68–1.36)	1.16 (0.82–1.64)	0.37
Low HDL-C												
Crude	1.00	0.81 (0.58–1.14)	0.97 (0.7–1.36)	0.9	1.00	0.95 (0.68–1.32)	0.69 (0.5–0.97)	0.03	1.00	0.92 (0.66–1.29)	1.1 (0.79–1.53)	0.55
Model 1	1.00	0.81 (0.58–1.14)	0.97 (0.69–1.35)	0.86	1.00	0.91 (0.65–1.28)	0.68 (0.48–0.95)	0.02	1.00	0.9 (0.64–1.27)	1.13 (0.81–1.58)	0.46
Model 2	1.00	0.8 (0.56–1.13)	0.97 (0.69–1.37)	0.86	1.00	0.98 (0.69–1.38)	0.66 (0.47–0.94)	0.02	1.00	0.97 (0.68–1.37)	1.21 (0.85–1.71)	0.38
Model 3	1.00	0.8 (0.56–1.13)	0.97 (0.69–1.37)	0.86	1.00	0.97 (0.69–1.38)	0.66 (0.47–0.94)	0.01	1.00	0.97 (0.69–1.38)	1.21 (0.86–1.72)	0.37
Elevated TG												
Crude	1.00	0.87 (0.62–1.23)	0.78 (0.55–1.1)	0.16	1.00	0.93 (0.66–1.31)	0.79 (0.56–1.11)	0.18	1.00	0.94 (0.67–1.33)	1.1 (0.78–1.55)	0.55
Model 1	1.00	0.88 (0.62–1.24)	0.79 (0.56–1.12)	0.19	1.00	0.95 (0.67–1.34)	0.8 (0.56–1.13)	0.21	1.00	0.93 (0.65–1.32)	1.03 (0.72–1.45)	0.86
Model 2	1.00	0.94 (0.66–1.34)	0.81 (0.57–1.16)	0.26	1.00	0.96 (0.68–1.37)	0.78 (0.55–1.12)	0.18	1.00	0.92 (0.64–1.31)	1.04 (0.73–1.48)	0.83
Model 3	1.00	0.94 (0.66–1.34)	0.82 (0.58–1.18)	0.29	1.00	0.93 (0.65–1.33)	0.77 (0.54–1.11)	0.16	1.00	0.93 (0.65–1.33)	1.07 (0.75–1.52)	0.73

Binary logistic regression test used for assessment variables.

Crude: Unadjusted model.

Model 1: Adjusted for age, gender, and energy intake.

Model 2: In addition, adjusted for marital status, physical activity, education status, smoking, and underlying disease.

Model 3: further adjustment was made for BMI.

***P < 0.05.*

[†] *Defined as the presence of ≥3 of the following components: abdominal obesity (waist circumference > 88 for women and > 102 for men); elevated blood pressure (BP ≥ 130/85 mmHg); elevated fasting blood glucose (FBG ≥ 100 mg/dl); low high-density lipoprotein-cholesterol (HDL-C < 50 for women and < 40 for men); elevated triglyceride (TG ≥ 150 mg/dl).*

[‡] *Elevated blood pressure (systolic ≥ 130 and diastolic ≥85).*

Mets, metabolic syndrome; BP, blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

Meanwhile, Data from NHANES III (1988–1994), the Cooper Center Longitudinal Study, reported a positive link between GL and low HDL-c in males and females (53, 54). The exact mechanism of this association is not known yet. When dietary GI and GL content is high, digestion and absorption of food are done at high speed, resulting in high blood sugar and consequent hyperinsulinemia release. These adjustments are made by regulatory hormones (cortisol, glucagon, and growth hormone), reducing reactive hypoglycemia and enhancing the secretion of free fatty acid (55). The metabolic response by increasing satiety and decreasing fat storage can lead to obesity. Excess body fat and the increased release of free fatty acids could contribute to the development of dyslipidemia, containing low HDL-c levels. In addition, diets with high GI/GL could cause insulin resistance, oxidative stress, and chronic inflammation aggravating dyslipidemia (56, 57).

Insulin resistance as a predictor factor of MetS, one of the main complications of chronic inflammation and stress oxidative can be considered. Particular attention has been paid to the quantitative characteristics of dose-response relationships and the underlying mechanisms that can investigate the nature of biphasic hormonal responses after exposure to redox-active agents, such as free radical oxygen species and identify their effect on inflammatory and anti-inflammatory pathways (58). Understanding that hormesis may prolong life and reduce the incidence of chronic diseases involves the optimal challenge of cells and entire organisms by any of the broader stressors, including pharmacological, physical, dietary, exercise, and ischemic (59). Antiaging and neuroprotective effects of hormetic have been reported using experimental protocols in a wide range of *in vitro* and *in vivo* models (60, 61). Among foods, several bioactive compounds, including polyphenols, have been considered as health promoters. Polyphenols include four main classes of flavonoids, phenolic acids, acetylbones, and lignans, each of which has different effects (62). Polyphenols with anti-inflammatory and antioxidant effects are a great choice to improve the diet quality (63). Polyphenols regulate cellular and enzymatic activities involved in inflammatory pathways by preventing the overproduction of reactive oxygen species and inhibiting free radicals (64). In addition to reducing apoptosis, promoting pancreatic β-cell proliferation, are involved in glucose homeostasis (65).

However, previous studies did not examine the relationship between meals. Based on our knowledge, there has been no observational study examining the relationship between carbohydrate quality in meals and metabolic disorders. Dietary approaches derived from meal timing are hopeful for modulating circadian rhythms and clock-controlled metabolic functions in humans. Besides, studies have proposed that specific times are more suitable for consuming carbohydrate-rich or fat-rich food to maintain metabolic health. A crossover trial investigated that consumption of high-carb meals in the evening undesirable influences blood glucose level and glycemic control in individuals with impaired glucose metabolism (66). In agreement with this finding, other studies in humans suggested that a carbohydrate-rich diet at the beginning of the day could be safe vs. the development of diabetes and MetS (67, 68). In addition, the inclusion of lunch as the main meal, compared to dinner, seems to have beneficial effects on reducing MetS risk factors (69).

Western main meals usually end with a sweet dessert. In addition, drinking beverages during the main meal was considered part of modern lifestyle; also, the food eaten, especially at breakfast, is mainly different from the foods eaten at other meals (70). On the other hand, skipping breakfast is very common in modern societies. In a study of eight young men, participants ate three main meals (breakfast, lunch, and dinner), while in the other condition, the same amount of energy was consumed at lunch and dinner times only. They found that skipping breakfast enhanced the blood glucose concentration during the afternoon and sleep and increased 24-h average blood glucose concentration (71). Another study assessed the glucose metabolism of healthy adults in two conditions of breakfast and dinner skipping. They showed breakfast skipping conduced higher glucose concentrations and insulin resistance after lunch (72). Similarly, our study highlights the importance of meals, especially lunch meals. In this way, if the lunch meal has a good CQI (for example, it is prepared from whole grains and fruits and vegetables with low GI and GL), it can play a role in controlling and managing the normal level of HDL.

The content of dietary fiber and vitamin minerals in whole grains is higher than in refined carbohydrates. The protective effects of these nutrients vs. the risk of chronic diseases are well-known (73, 74). Due to their physical structure and dietary fiber content, whole grains are categorized as low GI foods (34). The use of whole grains is a useful way to increase fiber in the diet and reduce noncommunicable diseases (NCDs). In addition, fruits and vegetables are important factors in fiber intake in the diet. According to the above, our calculated CQI has low quality. Still, due to the consumption of medium to the high fiber content in this study, it can be concluded that the intake of fruits and vegetables and solid carbohydrates in the diet of individuals are higher. Based on the considerable role of carbohydrates in Iranian diets and their low-quality diets, focusing on improving carbohydrate quality would be a beneficial strategy to make better food choices among Iranians (43).

In prospective studies, liquid carbohydrates intake was associated with weight gain, while there was a reverse association between consumption of solid carbohydrates and high weight gain (75–78). Examination of the systematic evidence presented for the effect of long-term intervention with low GI and GL on fasting insulin level and proinflammatory markers showed that it could effectively prevent obesity-related disease (79).

Dietary fiber and whole grains are more related to health outcomes than the GI or GL content of foods. Although the GI provides a measure of the glycemic potential of the carbohydrate content of foods, some low GI foods might have other attributes that are not health promoting. Foods with added fructose or sucrose and mixed foods high in SFA and carbohydrate (i.e., confectionery products) may have a low GI (33). Findings from a dose-response meta-analysis showed that diets identified by low dietary fiber contribute to NCDs, and therefore, quantitative recommendations for dietary fiber intake will be beneficial. While consumption in the range of 25–29 g/day is sufficient,

dose-responses data showed that amounts >30 g/day have more advantages (47).

Given the effect of low GI in the development of obesity, there is evidence that low GI diets enhance satiety by reducing voluntary food intake, thus reducing total energy intake. It can be effective for body-weight maintenance. It may prevent obesity (80–82). In contrast, the intake of a high GI diet motivates increases in hunger and leads to increased food intake, thus affecting energy balance and body composition (83). Fiber-containing foods should be chewed before passing through the stomach and into the small bowel, affecting satiety, glucose and insulin responses, and lipid absorption. Whole foods that require chewing and retain much of their structure in the gut are more likely to cause a feeling of satiety, leading to weight loss and balance of carbohydrate and lipid metabolism. In the large bowel, fiber is almost completely broken down by the resident microflora under a set of anaerobic reactions known as fermentation. The gut microbiota plays a substantial role in human health (84).

This study has important strengths. To the authors' knowledge, this study was the first to investigate CQI in meals and its association with MetS and its components. We had a sufficient sample size in this study that was done within 25 health houses in the Tehran Metropolis. Despite these strengths, the study has some limitations. First, this study had a cross-sectional design, and the findings do not establish causality between CQI and MetS; therefore, the results should be interpreted with caution. Second, using the questionnaire retrospectively may reduce information recall. There was also under-reporting and over-reporting of food items received. Third, even though the data were controlled for some potential confounders, the effects of eating behavior, menopausal status, and residual confounding cannot be discounted.

CONCLUSION

In conclusion, in this study, CQI was not associated with MetS. However, CQI may contribute to the nutritional therapy of improving low-HDL. Although, findings should be treated with caution, considering several conflicting results between studies. Further investigations into the mechanisms underlying the role of carbohydrate quality in the development of metabolic disorders are warranted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Tehran University of Medical Sciences (Ethic Number: IR.TUMS.MEDICINE.REC.1399.797). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS-B and KD contributed to the conception/design of the research and critically revised the manuscript. MM and ZA contributed to the acquisition of data. MM, FH, and EB participated in the analysis and interpretation of the data. MM, AL, and HI drafted the manuscript. SS-B agreed to be fully accountable for ensuring the integrity and accuracy of the work. All authors contributed to manuscript revision, read, and approved the submitted version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.763345/full#supplementary-material

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Added Sugar and Oral Health: A Position Paper of the Brazilian Academy of Dentistry

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Excessive sugar consumption is the main cause of dental caries. Dental caries is highly prevalent and negatively impacts the quality of life at all stages. Furthermore, sugar consumption is associated with other noncommunicable conditions and diseases, such as obesity, diabetes, and cardiovascular diseases. The aim of this paper is to propose recommendations at the individual and population levels for health professionals, families, educators, stakeholders, and public officials to reduce the burden of dental caries and other noncommunicable diseases that are caused by the excessive sugar intake. A systematic search was performed in PubMed and Cochrane databases to investigate the effectiveness of strategies and policies aiming to reduce sugar consumption as well as the impact of different patterns of sugar consumption on the occurrence of dental caries. Reference list of the identified papers and practice guidelines were manually reviewed as well. Based on the best evidence available, the Brazilian Academy of Dentistry recommends not to offer sugars to children younger than 2 years of age, and to limit total sugar consumption to <25 g per day after 2 years of age. Furthermore, families should be informed to limit sugar exposure, sugar-free areas should be available, content of food labels and advertisement should be regulated, taxation of products with sugar should be introduced, and reformulation of foods and drinks to reduce concentrations of sugars should be considered.

Keywords: dental caries, sugars, diet, policy, oral health

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INTRODUCTION

Untreated dental caries in the permanent dentition is the most prevalent condition among all human diseases, affecting more than 2.5 billion people [1, 2]. A regional evaluation of dental caries showed marked social disparity, with the poor and disenfranchised significantly more affected [3]. These disparities are established very early in life, prior to and at the time children start elementary school [4, 5]. In the primary dentition, untreated dental caries is the single most common chronic childhood disease, affecting 621 million people worldwide, which corresponds to more than 50% of children under 6 years of age in the majority of countries, reaching nearing 100% in some countries [6].

Studies published in the last two decades showed the negative impact of dental caries in all aspects of life related to oral health in the infancy, school age, adolescence, adulthood, and among the elderly. Dental caries causes pain, impairs function, and has an impact in the emotional and social wellbeing [7, 8]. Dental caries affects all functions that comprise the current definition of oral health—the ability to speak, smile, smell, taste, swallow, and express emotions with confidence, without pain, discomfort or disease [9]. Further, dental caries impacts academic performance, leads to loss of working hours, and direct and indirect costs for the individual and society, including loss of productivity [2]. Untreated dental caries can also potentially lead to more dramatic outcomes, such as documented instances of blindness [10] and death [11].

Due to its complexity, dental caries requires different strategies to promote health and reduce the burden of the disease in the population. Dental caries occurrence depends on educational interventions, community fluoridation policies, and individual factors such as socioeconomic status, behavior, and biological factors [12]. The literature consistently shows that diet, with excessive sugar consumption, particularly sucrose (the sugar originated from sugar cane or beets), is the main cause of dental caries in all ages [13]. Hence, sugar continues to be highly consumed worldwide from the first year of life. The Foreign Agricultural Service of the US Department of Agriculture states that Brazil has been, for decades, the world's largest sugar producer and exporter, exerting major influence on global sugar supply and prices. Between May 2020 and May 2021, Brazilian sugar production achieved 42 million tons, of which 24.1% was destined to domestic consumption, which means an average sugar consumption of ∼47.6Kg/inhabitant/year or 130.4g/inhabitant/day at the period [14].

In Brazil, foods and drinks high in sugar are consumed by almost all children at the age of 6 months, with long-term implications for general and oral health [15–17]. In this context, individual and collective strategies to reduce sugar consumption and the burden of non-communicable diseases have been tested in the last decade in different countries. Some promising results point to the need to gather this knowledge in order to propose policies to be implemented according to the context of each country.

Since dental caries is a sugar intake-based disease, the aim of this report is to propose recommendations at the individual and population levels, to promote action with the goal of reducing the burden of dental caries and other noncommunicable diseases that are caused by the excessive sugar intake.

METHODS

A PICO question (Population, Intervention, Comparator/s, Outcomes) strategy was carried out in PubMed and Cochrane to identify intervention studies and systematic reviews of intervention studies on the effectiveness of educational strategies to reduce sugar consumption (Intervention), compared to a control group (Comparator/s) in any age (Population) in the occurrence of dental caries (Outcome) [18]. In addition, a search on results (impact) of programs and implementation of protocols to reduce sugar consumption was done. Finally, another strategy using the PEO question (Population, Exposure, Outcome) to identify longitudinal studies, randomized clinical trials, and systematic reviews that ascertained in any age, the impact of different patterns of sugar consumption (exposure) on the occurrence of dental caries (outcome) [18]. A manual search on the reference list of the identified papers and practice guidelines was also done.

TABLE 1 | Terms and definitions used for sugars [19–21].

Term	Definition
Sugar	A sweet, crystalline substance, C12H22O11, obtained chiefly from the juice of the sugarcane and the sugar beet.
Total sugars	The term is used conventionally to describe the monosaccharides glucose, galactose, and fructose, as well as the disaccharides sucrose, lactose, maltose, and trehalose. Total sugars include all sugars in a food or beverage from any source, including those naturally occurring (such as fructose in fruit and lactose in milk, starch in vegetables) and those added to foods.
Naturally occurring sugars	Include those that are an innate component of foods (e.g., fructose in fruits and vegetables and lactose in milk and other dairy products).
Added sugars	Include all sugars used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table. Sucrose and high-fructose corn syrups are the most commonly added sugars.
Intrinsic sugars	Sugars that are present within the cell walls of plants (e.g., naturally occurring sugars) and are always accompanied by other nutrients.
Extrinsic sugars	Those sugars not located within the cellular structure of a food and are found in fruit juice, honey, and syrups and added to processed foods.
Free sugars	Include monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates.

Sugar as a Risk Factor to Dental Caries

Sugars as risk factor for dental caries may be described by different terminology depending on the context (scientific literature, protocols and recommendations, labels and regulations). The lack of consistency on how to describe sugars, even in the scientific literature, makes the understanding of the issue more difficult. **Table 1** lists terms and definitions found in the literature.

The World Health Organization (WHO) usually uses and recommends "free sugars," since noncommunicable diseases associate with this contextual meaning of sugars [19]. On the other hand, the American Heart Association uses the terminology "added sugars" to refer to the risk factor for cardiovascular diseases. Many studies investigated the effect of drinks with added sugars, since these drinks are an important portion of individual energy intake. Calories from drinks with added sugars have low nutritional content and high energy, giving a similar satiation perception than solid foods. As a result,

energy consumption may increase leading to excessive weight gain [19].

The term "early sugar consumption" refers to the intake of added sugars by a child very early in life. The hazardous potential to health of diets with added sugars for infants may be bigger, since these diets are not preconized for this age group. Natural carbohydrates, such as the lactose found in milk and starch found in fruits and vegetables, are the best energy source to complement the diet of a child. There is some variation in the literature of the meaning of early diet, which can mean the first months of life (usually the first 6 months), the first year of life, or the first 2 years of life.

Added sugars, particularly sucrose, appears to be the best terminology in regards to oral health, since it directly relates to the establishment and progression of dental caries lesions. Some data, however, are still unclear, such as the reference to fructose as the added sugar in several products for human consumption in the United States [20]. We, in this paper, are using "sugar" with the meaning of added sucrose to foods and beverages. In practice, it is impossible to avoid that different nutrients are represented and distinct individual behaviors are present in different epidemiological studies, due to the limitation of questionnaires and interviews used, which capture a behavior that can constantly and quickly change.

Dental Caries and the Etiological Role of Sugars From the Diet

Two aspects related to sugar consumption make dental caries worse and should be the focus of future designed interventions: early life sugar exposure and the high frequency of sugars in all ages. There is evidence of sugar intake during the first year of life and early childhood caries [22–24]. On the other hand, sugar consumption right after the eruption of the first teeth facilitates the establishment of a cariogenic microbiota, which is a predictor for future early childhood caries experience [25, 26]. The early exposure of a child to sucrose influences the child's preference for sweets, leading the child to favor foods and drinks with added sugars instead of healthier foods [27], which in turn contributes to the dental caries experience in the future.

Investigations with different populations over the decades have shown that diet rich in sugars have a role in the occurrence and severity of dental caries. The readily available sugars allows for the repetitive production of acids from the bacterial metabolism keeping the pH low and leading to the imbalance between the demineralization and remineralization of the enamel surface. The demineralization will overcome remineralization, leading to the formation and progression of dental caries, and this is further worsened by the continuous exposure to sugars from the diet [28, 29]. Data show a dose-response relationship between sugar consumption and dental caries; the higher the sugar intake, higher the caries experience and severity of the disease [30, 31].

The association between high dietary sugar intake and dental caries is independent from socioeconomic and other demographic factors. This association is the basis for recommending intervals between meals. Although unclear what can be considered safe, it is reasonable to assume that sugar consumption once or twice a day (desserts) is not associated with an important increase in risk of dental caries [32].

Sugar Consumption: Common Risk Factor of Noncommunicable Diseases

Noncommunicable diseases are the main causes of death, and in many instances, these occur prior to 70 years of age [33, 34]. There is an association between sugar consumption and diabetes, cardiovascular diseases, nonalcoholic fatty liver disease, and cancer [13, 21, 33]. Due to these data, the Brazilian Ministry of Health, the American Heart Association (AHA), the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and the International Association of Pediatric Dentistry recommend that children should not eat foods and drinks with added sugars (sucrose, fructose, and glucose) before the age of two [20, 21, 35, 36].

The World Health Organization (WHO) emphasizes the role of free sugars (sugars added to food and drinks and naturally present in foods, such as fruit juices and honey) in the incidence of noncommunicable diseases. The WHO recommends limiting the consumption of free sugars to up to 10% of the total energy intake, suggesting that limiting this consumption to 5% of the total food consumption brings an additional benefit to children and adults alike [13, 33]. This corresponds to ∼25 g (six tea spoons or 100 kilocalories) of sugar per day per individual with a healthy BMI body mass index) and has the support of the AHA and ESPGHAN [20, 21]. Although these recommendations are based on the amount of sugars and dental caries depends on the frequency of consumption, it is reasonable to assume that the reduction of sugar amounts will lead to the reduction of frequency of sugar consumption and vice-versa.

Since knowing that added sugar consumption is growing, it is already at levels much higher than the ones recommended, and is a risk factor for dental caries and other noncommunicable diseases, intervention for the reduction of sugar consumption is the responsibility of all health professionals, including the dentist, foundations promoting the wellbeing, opinion leaders, and public decision makers.

Individual and Community Interventions

Articles retrieved through the literature search included randomized clinical trials, mostly focused on behavior change strategies, and before and after studies, which investigated the impact of different policies and programs to reduce sugar consumption in a country or community. Overall, five strategies have been proposed to reduce sugar consumption and to promote oral health of the individual and communities.

Educational Strategies for Families

At the individual level, there is moderate evidence from randomized clinical trials showing the benefit of delaying sugar intake and reducing its consumption in the first years of life [15, 37]. These educational strategies reduced the occurrence of relevant outcomes in Brazil, such as respiratory disease and dental caries, but depend on the frequency and vigor of these educational interventions, and the link between the family and health centers of health professionals [37–39]. In Hong Kong,

incorporating diet counseling through motivational interview into dental care for adolescents improved oral health behaviors and prevented new dental caries lesions [40].

Although educating mothers and families regarding healthy eating may reduce disease risks, broader approaches not depending on "behavior changes" may be more effective in reducing sugar consumption, and ultimately health disparities [41]. The experience in several countries include promising interventions for reducing obesity and the burden of noncommunicable diseases, including dental caries [42].

Taxation of Sugary Drinks and Foods

Taxing products with added sugars has been tested and tried in more than 40 countries, with evidence of reduction in the consumption of these products [43]. This intervention potentially may contribute to the reduction of sugar consumption and improving the health of the population by (a) increasing retail prices and reducing sales and purchases of taxes drinks and foods; (b) raising public awareness on negative health effects of sugars; (c) encouraging no added costs industry responses, such as product reformulation; and (d) generating government revenue, which can be directed toward services that improve population health [42, 44, 45].

France increased taxes of sugary beverages in 2012, which resulted in immediate reduction of the consumption of these products. United Kingdom started increasing taxes according to the excess of concentration of sugar in drinks and foods [42]. With super-taxation of sugary drinks in Ireland, which increased prices to the consumer by 10% caused a reduction of 11% in the consumption of these products. In Mexico, price increases of 10% of sugary drinks in 2014 caused an immediate reduction of 6% in the consumption of these products and a 12% reduction after 1 year. This reduction was even more pronounced (17%) in poor communities [42]. The increase of taxation of sugary drinks in Thailand had similar effects, particularly among individuals with lower socioeconomic status [46]. Another before and after study in South Africa showed a large reduction in taxed beverage intake one year after the implementation of a sugar-content-based tax. Among taxed beverages, sugar intake decreased from 8.8 g/capita/day pre-tax to 19.8 post-tax [45]. In Brazil, the federal government implemented a tax on soft drinks in 2013, with a rate of 27% for juice drinks, nectars, and other sugar sweetened beverages. However, the federal government decreased the tax rate in 2016 and 2018, contradicting global trends [17].

These experiences showed that taxation requires engaging stakeholders, such as politicians, the public and private sectors, the media, and health professionals. These strategies should include a broader agenda, beyond the health sector, and engage partners that can influence public decision making and involve the media [47].

Reformulation of Foods With High Sugar Content to Reduce Sugar Concentration

The reformulation of foods by reducing sugar content during production is an attractive idea since it may reduce sugar consumption without behavior change [42]. This approach would be ideal for food products marketed to infants, which typically have sugar levels way above the recommended [48]. In the United Kingdom, a call for reformulation of foods that contributed the most for sugar consumption by children was made by the government. Between 2016 and 2019, a significant reduction in sugar consumption could be seen, particularly due to the reformulation of yogurts by reducing the concentration of sugars [49]. In Brazil, the Ministry of Health and the food industry signed an agreement in 2018 to reduce the amount of sugar in products to diminish the population-wide sugar consumption by 144,000 tons by 2025. However, there are no legal means in the country to regulate the execution of these agreements and the promised results are not likely to be achieved [17].

Regulation of Advertisement and Labeling of Foods With Sugars

Advertisement and sales of products with sugars, as well as their labeling, influence the consumer behavior of families and the dietary habits of children [42]. The WHO and Pan American Health Organization made a series of recommendations regarding advertisement and commercialization of foods and nonalcoholic drinks for children. In Chile, it was implemented in 2016 the "Law of Foods," which regulated packages of foods and drinks, disallowing the use of cartoons, and the sales of sugary drinks in schools. Furthermore, language for food and drink label warnings was normalized to improve the ability of the consumer to differentiate less healthy from healthier foods and drinks. Those measures not only aim to contribute with the reduction of sugar, salt, and fat consumption, but also to motivate the industry to reformulate their products. The result was a reduction of 24% in the consumption of sugars in Chile [50]. Ecuador and Peru followed by adopting new rules regulating packages of foods.

The addition of health warnings in the labels of sugary drinks in Peru, Uruguay, Mexico, Israel, Chile and some states of the United States resulted in a reduction of the sales of sugary drinks and positive changes in behavior, such as a different perception of risks to diseases causes by high sugar consumption and a reduction in the intent of consuming such products [51]. Brazil recently decreed a new food labeling legislation to help consumers better understand the nutritional information on labels, including the presence of added sugars, hopefully allowing for more informed food choices [17].

Promoting Sugar Free Areas

Promoting areas that are sugar free, particularly in and around schools and pre-schools, has been proposed. In Australia, it is public policy that schools are not allowed to sell foods or drinks with excessive amounts of sugars or salt. In Brazil, it is law since 2009 that food planning in schools should be done by trained nutritionists, who should emphasize the restriction of sugars and fat. In addition, regulations included prohibition of sales of sugar sweetened beverages in schools. However, there are differences in compliance with these rules across Brazil [17]. Hungary, through the HAPPY (*Hungarian Promoting Programme in the Young*) program, put emphasis on drinking water rather than soda associated with increased taxation of sugary beverages. HAPPY reduced the consumption of soda among Hungarian children

[44]. The Chilean law of Food Labeling and Advertising, which caused significant reduction of sugar consumption, included banning the sale of foods and beverages containing added sugar in schools and nurseries [50].

Simply recognizing a risk factor is not enough to change risk behaviors associated with disease. For decades, it is known that smoking is associated with a four-fold increase in heart attacks ands stroke, 20-fold increase in lung cancer, and a ten-fold increase in emphysema and chronic obstructive pulmonary disease. However, a marked reduction in smoking, particularly in Brazil, happened after implementation of broader interventions, such as advertisement and labeling restrictions, and increase in taxation. Data from 500 million male smokers showed that the biggest impact in smoking occurred when taxation was of 100% over price value [52].

On the other hand, policies and programs to reduce sugar consumption must be integrated and take into consideration the reality in each country and community. This includes not only the specification of these measures (e.g., definitions of products to be taxed; type of tax to implement; uses of the tax revenue; which products should be banned in schools), but also the participation of the actors involved [17, 42]. In this sense, dental professionals and research organizations should disseminate the evidence, through advocacy and direct actions. The measures should be supported by civil society engagement, based on the recognition that high sugar consumption is a public issue that has severe impacts on society. Governments need to develop strategies to ensure that these interventions are implemented and monitored, which includes capturing the impacts of sugar reductions [42].

The present analysis has limitations. In particular, the recommendations are based on a few randomized clinical trials, restricted to behavior change strategies to reduce sugar consumption. Thus, the reported benefits of upstream policies are based on observational studies, without a control group. However, it is not plausible to randomize people or clusters to some of the measures, such as increasing taxes on sugary products or regulating advertising. Furthermore, the mandatory implementation of these policies by governments is very new and the results are still short-term, and the current available evidence in limited.

CONCLUSION

Dental caries is prevalent, unevenly distributed, and has an impact in quality of life of individuals and societies. However, the disease is highly preventable, but requires interventions that can lead to reduction of its primary cause: the excessive consumption of added sugars to the diet. Such interventions have the potential to reduce dental caries and other noncommunicable diseases that are linked to sugar consumption, such as diabetes and cardiovascular diseases.

Future Directions and Recommendations

Based on what was exposed above, The Brazilian Academy of Dentistry recommends that foods and drinks with added sugars are not offered to children before they are 2 years of age, and no more than 25 g per day should be consumed, preferably with or right after meals. To achieve this goal, the following actions are proposed:

(a) To implement educational family interventions targeting the individual and communities, preferably at primary health centers.
(b) To promote sugar-free environments, prioritizing schools, pre-schools and the work environment.
(c) To regulate the content of labels of foods and beverages with added sugars.
(d) To restrict advertisement of products with sugars.
(e) To increase taxation of foods and beverages with sugars.
(f) To reformulate foods and beverages in regards to sugar content, to reduce sugar concentrations.

Future investigations should measure the long-term impact of different programs and policies, in order to assess whether the expected results in reducing sugar consumption are sustained. Furthermore, policy evaluation should not be restricted to the assessment of reducing sugar consumption, and also include measures of impact on clinically relevant outcomes such as dental caries and other non-communicable diseases.

AUTHOR CONTRIBUTIONS

CF wrote the first draft of the manuscript in Portuguese and critically revised the final submitted version. LP, JC, FM, MG, RC, and HP critically revised the manuscript and approved the final version that was submitted. AV critically revised the original version of the manuscript in Portuguese and generated the version in english and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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Effectiveness of the Lorodent Probiotic Lozenge in Reducing Plaque and *Streptococcus mutans* Levels in Orthodontic Patients: A Double-Blind Randomized Control Trial

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Orthodontic patients are at a significant risk for oral diseases due to increased plaque accumulation and oral bacterial dysbiosis. We aimed to determine the efficacy of the commercially available Lorodent Probiotic Complex at reducing plaque accumulation and *Streptococcus mutans* bacterial levels in adolescent orthodontic patients. Sixty adolescents undergoing fixed orthodontic treatment for a minimum of 6 months were recruited in a randomized, double-blind, parallel-group, placebo-controlled trial. They received either Lorodent probiotic lozenge (intervention, $n = 30$) or placebo lozenge (control, $n = 30$) orally every day for a 28-day administration period. Participants were assessed at four appointments (T1–T4) over a total of 56 days. Compliance and lozenge satisfaction were monitored. Saliva samples and supragingival plaques were collected for evaluation of *S. mutans* levels. Clinical assessment using a Plaque Index (PI) was used. Compliance with lozenge intake of all participants was over 90%. There was no significant change in the PI and composite PI scores in both placebo and probiotic groups at each time frame (all $p > 0.05$) or the relative *S. mutans* DNA levels in the saliva and plaque between the probiotic and placebo groups. The findings of high compliance and satisfaction with the probiotic lozenges combined with the study's rigorous design offer a baseline for subsequent testing of further potential probiotics (of varying formulations, concentrations), especially in adolescents.

Keywords: plaque indices, lozenges, *Streptococcus mutans*, saliva, supragingival plaque, compliance

INTRODUCTION

A major negative effect of having fixed orthodontic appliances is a potential increase in oral diseases such as caries and periodontal diseases [1, 2]. Orthodontic patients can develop gingivitis [3] that may progress to periodontal disease [4]. Indeed, in children, gingivitis is the most commonly occurring periodontal disease [5] and approximately one third of North Americans suffer from either gingivitis or periodontal disease [6].

The formation of a dental biofilm, generally known as dental plaque, is fundamental to the disease processes observed in the oral cavity [7]. Dental plaque comprises an aggregation of bacteria, salivary components and their exopolymer matrix [8]. Substantial epidemiologic evidence has shown that the presence in plaque of aciduric and acidogenic bacteria such as *Streptococcus mutans* and lactobacilli plays an important role in the formation of caries [9]. Various preventive approaches against dental caries and periodontal diseases have been thoroughly researched (e.g., good oral hygiene practices, use of fluoride, sugar substitutes, remineralizing agents, and antimicrobial chemical rinses); in spite of this, the incidence of gingivitis and caries remains high in orthodontic patients [10]. There remains a need for a simple, adjunctive aid that can be used to reduce plaque accumulation and cariogenic oral pathogens, especially for at-risk patients such as the orthodontic population. Recent evidence suggests that probiotic therapy might be applied to the maintenance of oral health [11–13].

The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [14]. The favorable effects of probiotic therapy are mainly achieved through the modulation of existing microbial flora associated with the host, thus attaining a balanced and healthy microbe-host relationship. Classic probiotic strains, such as those that belong to the genus *Lactobacillus*, have been tested for their ability to confer a probiotic effect in the oral cavity [15–19]. Indeed, probiotics have been used in orthodontics, with conflicting results [20–22]. The Lorodent Probiotic Complex (Integra Medical LLC) is a commercially available probiotic lozenge. It is a blend of six probiotic bacteria with *Streptococcus salivarius* BLIS K12 and five probiotic strains of the genus *Lactobacillus* (new nomenclature according to [23]): *Lacticaseibacillus paracasei* (previously *L. paracasei), Lactiplantibacillus plantarum* (previously *L. plantarum), Ligilactobacillus salivarius* (previously *L. salivarius*) *and Limosilactobacillus reuteri* (previously *L. reuteri*), and *Lactobacillus acidophilus,* being the key ingredients. The aim of the current study was to investigate the effectiveness of the Lorodent Probiotic Complex with a 3×10^5 CFU/lozenge in reducing plaque and salivary/plaque *S. mutans* levels in adolescent participants undergoing fixed orthodontic appliance therapy. We hypothesized that the Lorodent Probiotic Complex lozenges could improve gingival health and reducing the level of *S. mutans* in plaque and saliva.

METHODS AND MATERIALS

Trial Design

This randomized, double-blind, parallel-group, placebo-controlled trial was conducted at the Graduate Orthodontic Clinic at the University of Toronto, Faculty of Dentistry (**Supplementary Material - CONSORT Checklist**) and subjects were recruited between August 2014 to October 2014. The clinical trial was registered and conducted in compliance with Health Canada (#185428). The study was approved by the Research Ethics Board at the University of Toronto (protocol #30148). The study was registered at the University of Toronto Faculty of Dentistry Center for Clinical Research.

Participants

Patients undergoing orthodontic treatment at the Graduate Orthodontic clinic, University of Toronto, Faculty of Dentistry, were screened by two orthodontic residents (FE and SH) under the supervision of an orthodontist (SGG) for eligibility by combining review of the medical and dental histories with a dental examination.

Eligibility criteria for the study included male and female subjects between 11 and 18 years of age, in healthy medical condition, who were not pregnant, not past or current users of alcohol or tobacco, and who had not used antimicrobial mouth rinses, probiotics, antibiotics or anti-inflammatory drugs within 1 month prior to the study.

Inclusion Criteria

- Fully erupted teeth #16, 21, 23, 36, 41, 43;
- No active caries;
- Mild to moderate plaque accumulation (Plaque Index [24] score of at least 1);
- Mild to moderate crowding;
- Undergoing fixed orthodontic treatment on both arches with edgewise metal orthodontic brackets on at least 20 teeth and 1st molars bonded for at least 6 months and submitted to a standardized orthodontic archwire sequence of $0.016''$ NiTi, $0.016'' \times 0.022''$ NiTi, $0.019'' \times 0.025''$ NiTi, and $0.019'' \times 0.025''$ stainless steel.

Exclusion Criteria

- Allergies or sensitivity to milk or milk products, gluten, soy or any other ingredient present in the Lorodent Probiotic Complex;
- Existing dental caries or xerostomia;
- Any systemic condition that could directly affect gingival condition;
- Recent (within the past 45 days) or planned (within the next 90 days) surgery of any kind (major or minor);
- Participated in another clinical trial within 30 days prior to randomization;
- Experienced any nausea, fever, vomiting, bloody diarrhea or severe abdominal pain within the past 30 days;
- Patients with orthodontic bands.

Participants were randomly assigned to two groups, using a randomization protocol (details included in **Supplementary Material**—Methods and Materials). All participants received professional tooth cleaning at baseline, i.e., just before being enrolled in the clinical trial. The appliance was bonded in all participants using a standard protocol, e.g., standard bonding procedure using 37% phosphoric acid, Transbond™ Plus Self Etching primer (3M Unitek), and Transbond™ light cure adhesive (3M Unitek) (light cured Bis-GMA composite resin). Participants were withdrawn from the study for the following reasons: (1) personal reasons; (2) reports of fever, nausea, vomiting, diarrhea or severe abdominal pain after having used the probiotic; and (3) pregnancy,

antibiotic use, or a severe medical condition. The clinical data and samples collected from such subjects were withdrawn from the study analysis.

Interventions

The blueberry flavored Lorodent probiotic and placebo lozenges (Integra Medical Inc.) were chosen for this study because of its commercial availability and prior *in vitro* testing of its effectiveness against cariogenic bacteria by the company (Integra Medical, Inc.; data not shown). The probiotic complex was formulated to contain active probiotics (*S. salivarius* K12, and five probiotic strains of the genus *Lactobacillus*: *Lacticaseibacillus paracasei, Lactiplantibacillus plantarum, Lactobacillus acidophilus, Ligilactobacillus salivarius and Limosilactobacillus reuteri*) at a total probiotic concentration of $\sim 3 \times 10^5$ CFU/lozenge. In addition, both probiotic and placebo contained lactitol, inulin, dicalcium phosphate, blueberry flavor (natural), dextrose, fructose, stearic acid, citric acid, vanilla flavor (natural), and stevia rebaudiosidea (97%) as excipients. Stored in a -80°C freezer until distributed to participants, all subjects were instructed to store the lozenges in their fridge at home for the duration of the trial. All lozenges had expiration dates that exceeded the end of the trial study by a minimum of 6 months.

The lozenges were administered for 28 consecutive days, followed by another 28-day follow-up without lozenge administration, for a total trial length of 56 days (**Figure 1**). Previous studies on probiotic lozenges have examined their use with an ∼1-month period of intervention [25, 26]. An additional time point midway through the 28-day intervention was added to the present study in attempt to gain a better understanding of any progression of changes that may occur. A fourth time point was added 28 days after cessation of lozenge administration to evaluate if any potential changes would persist after discontinuation of lozenge administration. An initial loading dose of two lozenges two times per day (between 7:00–9:00 a.m. and 7:00–9:00 p.m.) for the first 7 days, followed by a maintenance dose of two lozenges once a day (between 7:00 and 9:00 a.m.) for the next 21 days was prescribed. The total administration period was 28 days, in line with other similar studies [25, 26] and previous safety assessments with *S. salivarius* K12 (1×10^{10} CFU) [27]. Participants of both groups were given standardized oral hygiene instructions and information on how and when to take the lozenges, based on a written script used by both the examiners (FE and SH who were not involved in the orthodontic treatment of the participants and who met to calibrate each other prior to the start of patient contact). Specifically, subjects were instructed to take the lozenges after their tooth brushing and to slowly dissolve the lozenges on their tongue for 5 min without chewing or swallowing and not to brush or rinse their mouth for 1 h following administration of the lozenges. Subjects were also told, based on the written script, to maintain the current standard of care regarding oral hygiene, i.e., to brush two times per day and floss once per day at a minimum. They were also instructed to not brush their teeth before the appointment or upon arrival to the clinic or to use any antimicrobial mouth rinse during the 56-day trial period. Any adverse events at each appointment or immediately after ingestion, e.g., fever or gastrointestinal discomfort including nausea, vomiting, bloody diarrhea or severe abdominal pain, were noted. Changes to their medical history were also noted throughout course of study.

Data and sample collection were taken at the following four time points (**Figure 1**):

- T1—baseline examination and sample collection at day 0 and initiation of lozenge administration.
- T2—examination and sample collection at day 14.
- T3—examination and sample collection at day 28. Lozenge administration ceased and all remaining lozenges returned to investigators.
- T4—follow up examination and sample collection at day 56.

Sample collection and clinical measurements at T1, T3, and T4 occurred and coincided with the subject's regular orthodontic visits at the Graduate Orthodontic Clinic. One additional appointment (T2), 14 days after the initial data collection, did not coincide with orthodontic visits. Patients were compensated financially in the form of gift cards for their participation in this clinical trial.

Outcome Measures

Clinical Evaluation of Plaque

The Plaque Index (PI) was used to clinically grade the extent and severity of plaque accumulation [24]. PI scores range from 0 (no plaque in gingival area), 1 (a film of plaque), 2 (moderate) to 3 (abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface) (**Supplementary Table S1**). A modification was made in the study to the Ramfjord teeth to replace the first premolars with the canines, since the canines have one of the highest incidences of white spot lesion formation during orthodontic treatment [28, 29]. Also, by including subjects with extracted first premolars (a common orthodontic treatment plan and found in over a third of the patients treated in the orthodontic clinic), subjects were recruited from a larger pool. Scores of 0–3 (PI) were assigned for the buccal, lingual, mesial and distal surfaces of teeth # 16, 21, 23, 36, 41, and 43 at four time points (T1, T2, T3, and T4) (**Figure 1**). A total of 24 (6 teeth with 4 surfaces) PI scores each were documented for each subject at each time point. Each participant's overall plaque status at each time point was represented by composite PI (cPI) scores, obtained by adding all 24 PI scores. PI was assessed by two calibrated examiners (FE and SH). Alignment and assessment of examiner scoring were performed at the start of the study (**Supplementary Material**—Methods and Materials).

S. mutans DNA Quantitation and Real Time Quantitative PCR

Plaque and salivary samples were collected at each time point and analyzed for the levels of *S. mutans* DNA levels. Well established and validated protocols in DNA extraction and real time qPCR using *S. mutans* specific primers (details provided in **Supplementary Material**—Methods and Materials) were used to quantify the levels of *S. mutans* DNA levels in saliva and plaque.

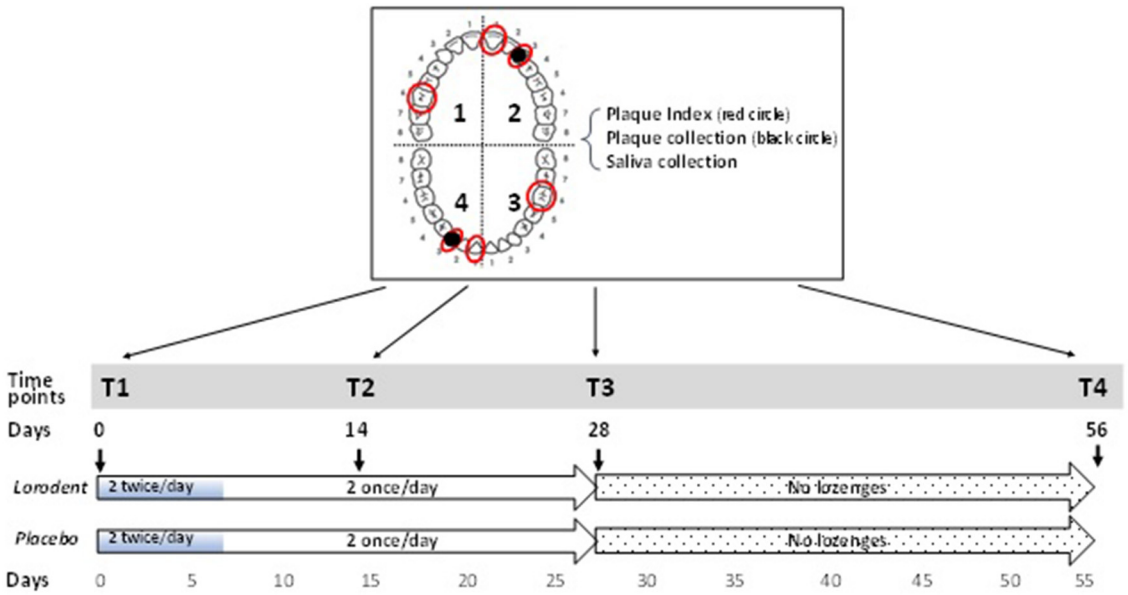

FIGURE 1 | Experimental design and time points of clinical measurements and sample collection. Two lozenges (Lorodent or placebo) were taken twice a day for 7 days (total of 4/day for 7 days = 28 lozenges), followed by 2 lozenges a day for the next 21 days (total of 2/day for 21 days = 42 lozenges); total number of lozenges in trial = 28 + 42. Plaque Index was measured and plaque and saliva samples collected at T1 (0 days), T2 (14 days), T3 (28 days), and T4 (56 days) of the study. The plaque index was measured on teeth # 16, 21, 23, 36, 41, and 43 (red circles) and plaque were collected teeth # 23 and 43 (black circles in tooth arch diagram on top left).

TABLE 1 | Changes in *S. mutans* DNA in plaque and saliva.

Sample	T1 median (IQR)	T3 median (IQR)	§*P*-value
Probiotic			
% *S. mutans* DNA in supragingival plaque ($n = 18$)	0.0501 (0.224)	0.039 (0.561)	0.372
% *S. mutans* DNA in saliva ($n = 14$)	0.116 (0.965)	0.259 (2.039)	0.875
Placebo			
% *S. mutans* DNA in supragingival plaque ($n = 20$)	0.208 (1.900)	0.584 (2.585)	0.247
% *S. mutans* DNA in saliva ($n = 12$)	0.774 (1.303)	0.0773 (0.132)	0.117

§ *Wilcoxon Signed Ranked test.*

Measurement of Compliance and Lozenge Satisfaction

At each appointment, subjects were queried as to their ability to follow the oral hygiene, use of mouth rinses, etc. Participants recorded days of lozenge intake on a compliance paper calendar. Compliance was assessed based on the percentage of boxes/lozenges from the total number of lozenges of 70. Monitoring of lozenge safety was conducted through verbal questioning at each appointment. At the conclusion of the study, each participant was asked to complete a 10-item "End of Study Questionnaire", adapted from a similar questionnaire used by the Xylitol for Adult Caries Trial [30] (**Supplementary Table S2**). The questions in the questionnaire included their satisfaction with taste of lozenges, success at taking 2 lozenges per day for 28 days, difficulty in taking 2 lozenges per day, difficulty in remembering to take the lozenges every day, whether the study length was too long, whether participants feel the need to prevent white spots, decay or gum disease, whether they lost interest in the study, what they think of the effectiveness of the lozenges, the type of lozenges participants believed they were taking and the likelihood of participants using lozenges if they were shown to be effective at reducing white spots, decay or gum disease.

Statistical Methods

Inter- and intra-rater reliabilities for PI assessments for both FE and SH were computed using weighted kappa statistics and outcomes were interpreted according to Landis and Koch [31]. An independent *T*-test was used to test age differences between groups. Compliance with lozenge intake was appraised at T2 and T3 using a 2-sided chi-square test. The sex distribution in both groups was tested with a chi-square test.

PI scores were considered scalar values as in previous studies (e.g., [32]). A mixed-effect model was used to test between group and within group differences in PI scores using the study group, the timepoint, and the interaction Group-by-timepoint as fixed factors. Wilcoxon Signed Rank tests were used to test within group changes in microbial DNA (from T1 to T3). Mann-Whitney *U*-tests were used to test between-group differences the microbial DNA at each time point. *Post-hoc* comparisons were adjusted using the Bonferroni method. The operator involved

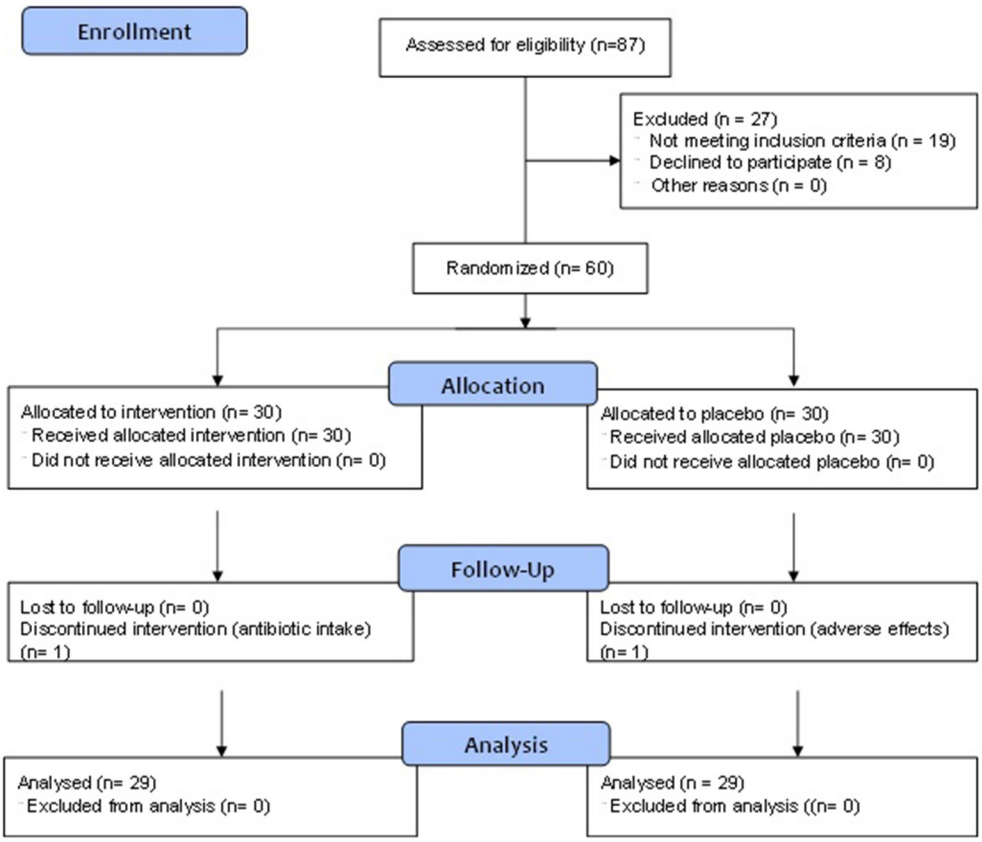

FIGURE 2 | CONSORT flow diagram.

in the statistical analysis (IC) was blinded to the allocation of participants to the two groups.

An a priori power analysis was conducted using G*Power (Heinrich-Heine-Universität Düsseldorf, Germany) [33]. As this study was not designed to test sex differences, sex was not considered while computing the sample size. A total sample of 49 participants was required to achieve a power of 0.80 using a medium effect size ($d = 0.5$) and an alpha of 0.05 (considering two study groups, 4 timepoints, and interactions). The level of significance was set at $p < 0.05$. SPSS ver. 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.) was used for the statistical analyses.

RESULTS

Subject Recruitment Demographics

Out of the 87 subjects screened, 60 met the eligibility criteria and were randomized into two equal groups of 30 each in the probiotic/placebo lozenge groups (**Figure 2**). A final number of 29 subjects in each in the probiotic/placebo groups was obtained—one participant from the probiotic group withdrew due to antibiotic intake following an accident and a participant from the placebo group was withdrawn due to reports of adverse events (gastrointestinal discomfort and diarrhea) after initiation of study.

Demographic characteristics did not significantly differ between the two groups. Overall, more females were enrolled in the study compared to males (56.9% females, 43.1% males)—in the probiotic group, 16/29 (55.2%) were males and 13/29 (44.8%) were females compared to 9/29 (31.0%) males, and 20/29 (69.0%) females in the placebo groups. The distribution of male and female participants did not differ significantly across groups [$(X^2 = 1, N = 58) = 3.445$, $p = 0.063$]. The mean ± SD participants' ages were 15.7 ± 1.7 years; the mean ± SD age in the probiotic group was 15.75 ± 1.67 years compared to 15.64 ± 1.75 years in the placebo group. The groups were similar in age ($p = 0.807$).

Rater Reliability

Examiner alignment before the study resulted in "substantial" agreement between the examiners for Samples B (intra-oral photos: kappa = 0.72 for PI, p-values for both of < 0.001) and C (live clinical patients: kappa = 0.74 for PI; p-values for both of < 0.001). After the study, the inter-rater agreement improved to "almost perfect" (kappa = 0.82 for PI; p-values for both of < 0.001) when the examiners re-scored Sample B. Intra-rater reliability of the PI scores assigned to Sample B (intraoral photos) showed that both examiners independently had "almost perfect"

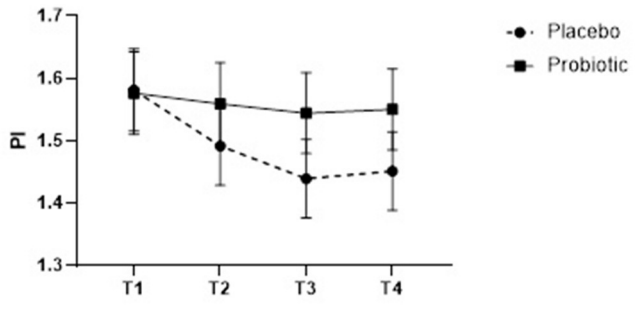

FIGURE 3 | Plaque Index (PI) scores of participants taking control and probiotic lozenges at the 4 time points of the study. Note that the PI scores range from 1 to 4 (**Supplementary Table S1**) and PI scores shown in both groups ranged between 1.4 and 1.6.

agreement (PI- examiner 1: kappa = 0.83, examiner 2: kappa = 0.84; p-values < 0.001). The intra-rater reliability was not significantly different between examiners ($p < 0.001$).

Compliance With Intervention, Patients' Satisfaction, and Adverse Events

Analysis of compliance with lozenge intake at T2 and T3 revealed that all participants reported compliance of over 90%. The mean values between groups were also very similar: At T2 and T3, 89.7% ($p = 1.00$) and 72.4% ($p = 0.56$) of subjects in both groups reported a perfect compliance, respectively, with no significant difference between the two groups.

The lozenges were well received by subjects, with 89.6% of participants reportedly very satisfied or satisfied with the lozenge taste and 81% responded they would be fairly likely to use them if they were shown to be effective at reducing white spots, decay or gum disease.

One participant, later identified to be in the placebo group, discontinued use of lozenges 2 weeks after the initial intake of lozenges due to reports of gastrointestinal pain and diarrhea that continued for a couple of days after discontinuation of lozenge administration. The subject was monitored for another 6 weeks, with no recurrence of symptoms. None of the participants in the probiotic group reported any adverse events.

Effects of the Interventions on Plaque Index and Bacterial Levels

PI scores were not different between groups ($F = 0.866$, $p = 0.347$) or timepoints (Group-by-timepoint interaction $F = 0.629$, $p = 0.596$). No significant improvements in both PI and cPI scores from baseline were seen throughout the intervention period for the probiotic group at any time frame ($p > 0.05$) (**Figure 3**, show plots depicting temporal changes of PI in both groups).

Supragingival plaque and salivary samples were analyzed from 58 (29 probiotic; 29 placebo) and 29 (15 probiotic; 14 placebo) at T1 and T3 for the relative levels of *S. mutans* DNA. The average DNA yields from the plaque and saliva samples were 1.2 and 5.5 ng/μL, respectively. In general, the relative proportions of *S. mutans* DNA in the plaque and saliva samples were relatively low and undetectable in 20 plaque and 3 saliva samples (**Figure 4**). Of the remaining samples with detectable values, no significant differences in the amount of change in the relative proportions of *S. mutans* were found between the two groups (**Table 1**). A trend, however, for the *S. mutans* levels to decrease ($p = 0.372$), and increase ($p = 247$), was noticed in the supragingival plaques in the probiotic and placebo groups, respectively.

DISCUSSION

This current study joins the many previous studies designed to better understand the efficacy of a probiotic strategy in the improvement of oral health. The vulnerability of orthodontic patients to caries and poor gingival health and the built-in nature of recurrent orthodontic visits over an extended period make them especially suitable as study subjects for a probiotic study. Additionally, any findings generated in this population group are applicable to the understanding of the effectiveness of oral probiotics in improving oral health in all individuals, whether under orthodontic treatment or not.

Our findings of a lack of statistical differences in PI and salivary and plaque *S. mutans* levels between the probiotic to control groups were corroborated by other studies targeting the orthodontic population. For example, Benic et al. [11] and Kohar et al. [34] found no statistically significant differences in PI measures in orthodontic subjects given *Limosilactobacillus reuteri* (formerly *L. reuteri*) and *S. salivarius* M18 lozenges, respectively. In addition, Gizani et al. [17] did not find a statistical significant difference in salivary MS counts in subjects consuming lozenges containing two strains of *Limosilactobacillus reuteri* once daily for 17 months. In contrast, a study found that the proportion of streptococci was significantly reduced, compared to the administered probiotic strains containing combinations of *Enterococcus* and *Lactobacillus* strains [35]. Also, the authors of a systematic review concluded that, of the 9 included randomized controlled trials, 8 provided evidence that probiotics "improves oral health in patients undergoing fixed orthodontic therapy" [22]. Interestingly, several of these studies used yogurt as a delivery vehicle, suggesting that dairy products might be more effective carrier vehicles for probiotics for oral health, rather than lozenges, a point reinforced by a systematic review and meta-analysis that concluded the effectiveness of dairy products in reducing *S. mutans* levels [36]. Indeed, bacteria such as *Lacticaseibacillus rhamnosus* (formerly *L. rhamnosus*) [37], *Lacticaseibacillus rhamnosus* with *Bifidobacterium animalis* subsp. *lactis* [38], and *S. salivarius* M18 [1] appeared to be effective in reducing dental caries, especially if milk is used as a delivery vehicle (reviewed in [36]). Because dairy products contain calcium phosphate and casein phosphopeptides, enamel remineralization of the carious tooth can be enhanced [39], especially when these probiotics are supplemented with fluoride, another product known to improve remineralization [40]. Although the Lorodent Probiotic Complex was formulated with the premise that a combination of bacterial strains might have a synergistic effect that together would be

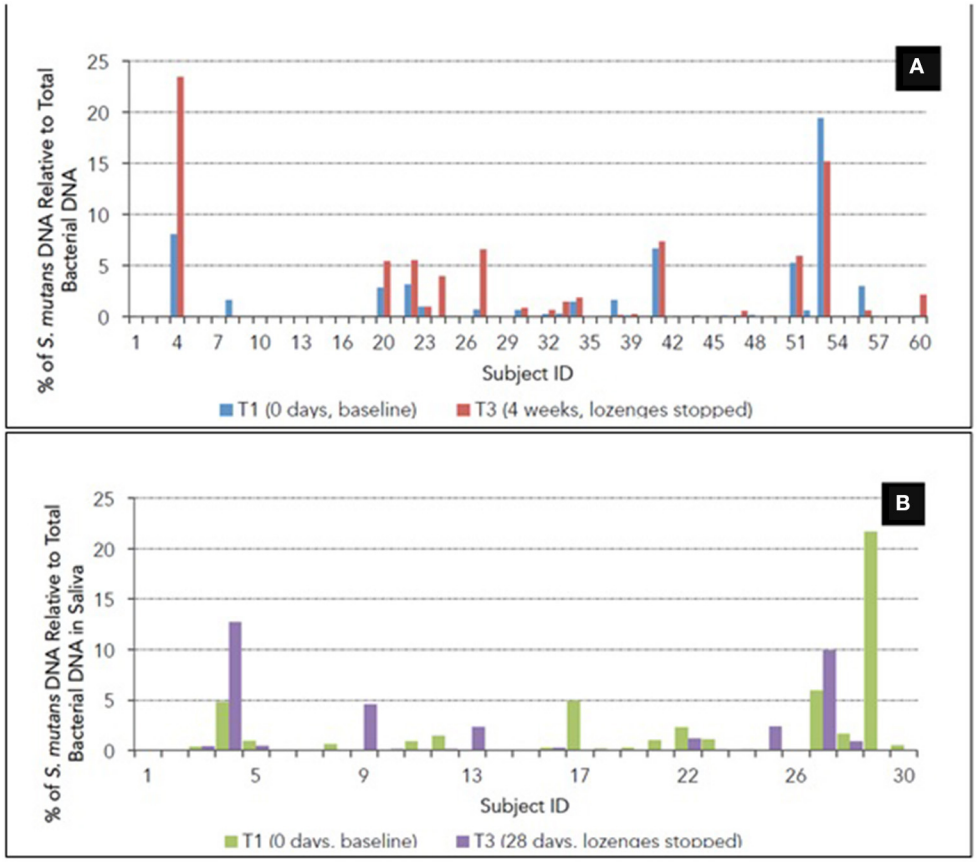

FIGURE 4 | Percentage of *S. mutans* DNA relative to total bacterial DNA from **(A)** supragingival plaque and **(B)** saliva for each participant at T1 and T3. In the majority of samples, *S. mutans* DNA levels were not detectable.

effective in combating oral diseases, similar to some probiotics used for gastrointestinal health [41, 42], that expectation of synergistic effect with combined probiotic strains was not achieved in the current study. One potential reason for the lack of any clinically measurable effects of the probiotic complex on plaque accumulation could be due to the fact that the active CFUs/lozenge was $\sim 10^5$ CFUs/lozenge, an amount below the 10^9 active CFUs/lozenge usually used for probiotic therapy in the gastrointestinal literature [1, 38, 40, 43]. Future studies could be dedicated toward using dairy products to carry the Lorodent Complex, a higher dose of the probiotic complex in addition to the adoption of evaluation of its anti-caries properties, e.g., measurement of the presence of white spot lesions, and changes in microbial composition of the plaque and/or saliva during administration of the probiotic (e.g., by next-generation sequencing).

Although no differences were detected in the PI scores or microbiological measurements of *S. mutans* in supragingival plaque or saliva in subjects taking the probiotic complex, one finding from the study stood out clearly. That is, the current study showed a strikingly high compliance of 90% with the blueberry-flavored lozenge use, even when it involved the necessity of taking the lozenges twice a day for 7 days. Many past studies of prescribed drug regimens in adolescence showed compliance rates around 50% (reviewed in [44]). The high compliance and acceptability of these lozenges suggest that commercialization marketing of lozenges as a delivery vehicle for anti-caries probiotics or any therapeutic modality will be well received by adolescents, a major target group of anti-caries efforts. Additionally, no adverse events were experienced in the probiotic group in this study, corroborating the safety shown in other preclinical studies of the probiotic species included in the Lorodent lozenge [27], and thereby establishing the safety of the Lorodent Probiotic Complex in humans at the dosage tested.

Dental crowding has been directly associated with plaque accumulation [45]. In this study, only subjects that had been treated orthodontically for longer than 6 months, with the majority of the subjects being treated longer than 9 months, where little or no crowding were observed. The lack of crowding and the regular monitoring of their oral hygiene suggest that factors related to the probiotics might be at play in the outcome of the study. For example, it is also possible that a longer probiotic treatment may be needed to establish the Lorodent probiotic strains in the oral cavity—oral hygiene in adolescent orthodontic patients is usually poor and mechanical debridement around the orthodontic bracket more arduous, resulting in oral biofilms that are thicker, denser and generally more pathogenic. Indeed, previous studies have also shown that *S. mutans*

levels increased about four times in patients undergoing active orthodontic treatment compared to controls [46]. In this regard, perhaps it may help to have complete mouth disinfection with chlorhexidine prior to probiotic usage to increase the ability of the probiotic strains to compete and establish themselves, as had been previously reported [47].

In the current study, we measured the levels of *S. mutans* DNA in the saliva and plaque in addition to the PI scores, both of which are indicators of oral health. Although plaque accumulation itself is not a risk factor for caries, the dental biofilm is a critical component involved in the development of caries and periodontal diseases, both of which are the leading causes of tooth loss [48]. We observed that there was no significant difference in the PI scores in both probiotic and control groups. The Silness and Löe's Plaque Index, as used in the current study, may suffer from lack of accuracy and reliability for the current study. The index is used in periodontology to assess plaque in marginal gingival areas; in contrast, plaque in orthodontic patients usually accumulates in the direct vicinity of the bracket. Furthermore, the scale may not be precise enough to detect less noticeable changes in plaque levels produced by the probiotic. Although alternative plaque indexing scales exist, e.g., the Orthodontic Plaque Index (OPI) [49], or the use of a digital plaque image analysis (DIPA; using a digital camera, UV flash units and software evaluation) [50], they suffer from lack of validation and/or are expensive and technically demanding. In the microbial analysis, although we used a highly sensitive and specific technique (real-time quantitative PCR), the vast number of samples in the current study necessitated the use of a multiplex DNA extraction kit that, although convenient, tends to result in lower yields of DNA that in turn might have resulted in lack of detection of *S. mutans* in many of the plaque samples. Finally, it must be noted that this study was based on an *a priori* sample size calculation for which we considered a medium effect size, and not on data retrieved from other studies or pilot investigations using a similar research design or methods. Computing the sample size using data from previous studies or pilot investigations could have improved the quality of our sample size calculation. Also, our study was not designed and sufficiently powered to test the effect of age and sex on the outcome measures. However, the study groups in the current study had similar age and sex distributions.

Our study illustrated that probiotic therapy in the forms of lozenges at the specific formulation and bacterial count did not influence the plaque accumulation and *S. mutans* levels in saliva and supragingival plaque over the 2-month period in patients undergoing orthodontic treatment. Our study also showed that probiotic lozenges can be practically implemented in the clinical orthodontic setting, in terms of its acceptability by adolescents. Future probiotic studies should focus on bacterial strains, e.g., that of the *S. salivarius* M18 [1], with strong initial *in vitro* evidence to have anti-caries activities, the most effective dosage and frequency of administration for any strains or combination of strains and delivery vehicle. Future probiotic studies could also investigate combination therapy with chlorhexidine or fluoride to increase the probiotic's effectiveness and the possibility of incorporating a pretreatment phase of oral disinfection using chlorhexidine to reduce the oral bacterial load prior to probiotic administration favor probiotic colonization of the oral microbiome.

CONCLUSIONS

The results from this randomized, double-blind, parallel-group, placebo-controlled trial suggested that, although the Lorodent Probiotic Complex was not effective at improving the plaque index or the salivary and plaque levels of *S. mutans* among adolescents undergoing fixed orthodontic appliance therapy, there was high compliance and acceptability of the product. Future studies utilizing probiotics against oral diseases will need to focus on variables such as efficacious and higher dosages of bacterial strains and delivery vehicles, different dosages and frequencies of administration, and possible combinations with chlorhexidine and/or fluoride, and high bacterial enumeration.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Toronto Human Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Informed consent was obtained from all individual participants included in the study.

AUTHOR CONTRIBUTIONS

FE, SM, S-GG, PC, and JB conceived and designed the experiment. FE, SM, KJ, and KM performed the experiment. FE, SM, S-GG, IC, and CL analyzed the data. IC statistical analyses. FE, SM, and S-GG wrote the draft manuscript. All authors reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/froh.2022.884683/full#supplementary-material

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Oolong Tea Consumption and the Risk of Oral Squamous Cell Carcinoma: A Propensity Score-Based Analysis in Southeast China

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Oolong tea is one of the world's most popular non-alcoholic beverages, particularly in coastal Southeast China. Hitherto, epidemiological studies on the association between oolong tea consumption and the risk of patients with oral squamous cell carcinoma (OSCC) are very limited. This study aimed to evaluate the potential effect of oolong tea consumption on OSCC risk in Southeast China. From January 2010 to October 2020, face-to-face interviews were conducted for 744 newly diagnosed OSCC patients and 1,029 healthy controls to collect information on demographics, oolong tea consumption behaviors, and other lifestyle factors. Propensity score matching (PSM), inverse probability of treatment weight (IPTW), and stabilized inverse probability of treatment weight (SIPTW) were utilized to minimize confounding effects. Multivariate, conditional, and weighted logistic regression was used to evaluate the associations of oolong tea consumption behaviors with OSCC risk. Participants who drank oolong tea showed a lower risk of OSCC when compared to their non-drink counterparts [PSM population, OR (95%CI): 0.69 (0.49–0.97); SIPTW population, OR (95%CI): 0.74 (0.58–0.94)]. Moreover, the reduced risk was found to be significantly associated with certain tea-drinking habits (consumed amount over 500 mL per day, a duration of <20 years, age at initiation older than 30 years, and warm and moderately concentrated tea). Similar results were yielded in the sensitivity analyses (Multivariate adjustment and the IPTW analysis). Furthermore, subgroup analysis revealed that the negative association of oolong tea drinking with OSCC risk was more evident among those with poor oral hygiene. This study provides supportive evidence that oolong tea consumption may have a potentially beneficial effect in preventing OSCC, especially for those with poor oral hygiene.

Keywords: oolong tea consumption, OSCC, propensity score analyses, oral hygiene, risk assessment

INTRODUCTION

Oral cancer is the most typical tumor in the head and neck, with oral squamous cell carcinoma (OSCC) accounting for nearly 90% of its pathological classifications (1). The incidence of oral cancer has shown a gradual upward trend in recent years, which makes it a growing concern for the global public, particularly in developing nations (2, 3). It was estimated that the worldwide number of new oral cancer will reach 421,907 by 2025 (4).

Tea has been recognized as one of the world's most renowned beverages, with common varieties including green tea, black tea, and oolong tea (5). Among which, oolong tea is a traditional type of tea origin in southeast China, very popular throughout Asia (6). Accumulating evidence has indicated that oolong tea is rich in polyphenols, flavonoids, and other chemical compounds, which has been posing an increasing interest worldwide (7). Previous studies revealed that oolong tea consumption was associated with a lower risk of numerous chronic diseases, such as hypertension (8), dyslipidemia (9), ischemic stroke (10), and cardiovascular diseases (11). There are also some reports on the effect of oolong tea consumption on several cancers including esophageal cancer (12), ovarian cancer (13), and nasopharyngeal cancer (14). However, to date, research on the potential association between oolong tea consumption and OSCC risk is very limited. Although our previous study suggests a negative association between drinking tea and oral cancer risk (15, 16), continued research into the role of oolong tea consumption is worthy of further exploration. Therefore, the purpose of this study was to investigate the relationship between oolong tea drinking behaviors and oral cancer risk using propensity score analyses (including propensity score matching, PSM; inverse probability of treatment weight, IPTW; and stabilized inverse probability of treatment weight, SIPTW) to minimize the potential confounding effects.

MATERIALS AND METHODS

Study Population

The study was conducted on a hospital-based case-control design which was conducted from January 2010 to October 2020 in Fujian Province, China. A total of 744 patients with newly diagnosed cases of OSCC were recruited from the First Affiliated Hospital of Fujian Medical University, whereas 1,029 healthy controls were registered from the hospital's physical examination center during the same period. Potential research participants who satisfied all inclusion and exclusion criteria and provided written informed consent to participate were enrolled. Furthermore, the inclusion and exclusion criteria were previously described in detail (15). Briefly, all the participants were (1) aged 18–90 years old; and (2) capable of answering questions effectively. Cases were defined as histologically confirmed primary OSCC with no history of chemotherapy or radiation. Controls were classified as healthy people with no history of ophthalmic, cutaneous, respiratory, gastrointestinal, or oncological disorders. This study was approved by the Institutional Review Board of Fujian Medical University (Fuzhou, China) and carried out in conformity with the ethical criteria outlined in the Helsinki Declaration in 1964.

Data Collection

After obtaining written informed consent from research respondents, data were collected by trained professional investigators using an interview-based structured questionnaire. The information obtained included (1) demographics: age, gender, occupation, education level, residence, body height, and weight, etc.; (2) self-reported lifestyle habits: alcohol consumption (yes or no), tobacco smoking (yes or no), and oolong tea drinking-related habits; (3) common dietary intake frequency (Red meat, vegetable intake and fruits intake); and (4) oral hygiene indicators: tooth brushing/day, the numbers of missing teeth, duration of wearing dentures (years), regular dental visits (no/yes), and whether they had oral ulcers (no/ yes). The age was divided into non-elderly (<60 years) and elderly groups (≥60 years) according to the definition of elderly of the United Nations (1). And the dietary intake frequency (red meat, vegetable intake, and fruit intake) was grouped into two groups according to the median of the control group in the overall population. Red meat intake: <3 and ≥3 times/week. Vegetable intake: <2 and ≥2 times/day. Fruit intake: <3 and ≥3 times/week. We constructed a comprehensive index for assessing oral health conditions that are based on the five indicators above. The details have been published in a previous article (17) and were summarized in **Supplementary Table 1**.

Those who smoked more than 100 cigarettes throughout their lifetime were defined as smokers (18). And those that drank alcohol had at least one drink every week for at least 6 months (15). Oolong tea drinkers were defined as those who consume at least one cup of tea every week for at least 6 months. In this study, there were only 119 oolong tea drinkers who did not smoke or drink alcohol (90 in the control group and 29 in the OSCC group). The detailed data and interaction between tea consumption and smoking or alcohol drinking were presented in **Supplementary Table 2**.

The following are details on oolong tea drinking habits: (1) Oolong tea drinking history (yes or no); (2) Years of tea-drinking (years); (3) Average daily tea consumption (ml/day); (4) age reported started tea-drinking (in years); (5) Tea temperature (non-drinker/warm/hot); and (6) Tea concentration (light/moderate/strong). It relied on the subjects' assessment of the temperature of the tea. To minimize miss classification, we set a judgment criterion based on the average time from mixing the tea leaves with boiling water to the time of tea drinking. The temperature of tea drinking was classified as very hot (<1 min), hot (1–5 min), warm (5–10 min), and cold tea (more than 10 min) (19). The number of individuals who drink cool tea or very hot was too small to include in this analysis; and the concentration of tea was evaluated based on the volume filled by the brewed tea leaves in the cup (light, <25% of the cup; moderate, 25–50% of the cup; and strong, >50% of the cup) (15). Additionally, other oolong tea drinking habits (such as amount, duration, and age at initiation) were set as categorical variables according to the median of the control group who had a history of drinking oolong tea in overall population. The details are listed

TABLE 1 | Baseline characteristics of case and control groups after propensity score analyses.

Variables		PSM population			SIPTW population		
		Control (%)	Case (%)	SMD	Control (%)	Case (%)	SMD
N		487	487		1,038.0	748.0	
Gender	Male	265 (54.4)	261 (53.6)	0.016	572.7 (55.2)	416.1 (55.6)	0.009
	Female	222 (45.6)	226 (46.4)		465.3 (44.8)	331.9 (44.4)	
Age (years)	<60	274 (56.3)	280 (57.5)	0.025	622.6 (60.0)	456.3 (61.0)	0.021
	≥60	213 (43.7)	207 (42.5)		415.4 (40.0)	291.7 (39.0)	
Occupation	Farmer	125 (25.7)	140 (28.7)	0.088	266.0 (25.6)	184.7 (24.7)	0.034
	Worker	78 (16.0)	84 (17.2)		166.7 (16.1)	114.7 (15.3)	
	Office worker and others	284 (58.3)	263 (54.1)		605.3 (58.3)	448.6 (60.0)	
Education level	Illiteracy	55 (11.3)	66 (13.6)	0.070	118.7 (11.4)	89.4 (11.9)	0.034
	Primary-middle school	282 (57.9)	278 (57.0)		577.3 (55.6)	403.6 (54.0)	
	High school and above	150 (30.8)	143 (29.4)		342.0 (33.0)	255.0 (34.1)	
BMI	18.5–23.9	291 (59.8)	300 (61.6)	0.038	620.8 (59.8)	451.9 (60.4)	0.013
	<18.5 or ≥24	196 (40.2)	187 (38.4)		417.2 (40.2)	296.1 (39.6)	
Residence	Rural	221 (45.4)	233 (47.8)	0.049	464.9 (44.8)	334.8 (44.8)	<0.001
	Urban	266 (54.6)	254 (52.2)		573.1 (55.2)	413.2 (55.2)	
Smoking status	No	325 (66.7)	324 (66.5)	0.004	701.2 (67.6)	512.3 (68.5)	0.020
	Yes	162 (33.3)	163 (33.5)		336.8 (32.4)	235.7 (31.5)	
Drinking status	No	369 (75.8)	362 (74.3)	0.033	784.6 (75.6)	578.2 (77.3)	0.041
	Yes	118 (24.2)	125 (25.7)		253.5 (24.4)	169.8 (22.7)	
Red meat intake	<3 times	280 (57.5)	281 (57.7)	0.004	577.0 (55.6)	394.8 (52.8)	0.056
(per week)	≥3 times	207 (42.5)	206 (42.3)		461.0 (44.4)	353.2 (47.2)	
Vegetable intake	<2 times	185 (38.0)	172 (35.3)	0.055	358.0 (34.5)	257.4 (34.4)	0.002
(per day)	≥2 times	302 (62.0)	315 (64.7)		680.0 (65.5)	490.6 (65.6)	
Fruit intake	<3 times	290 (59.5)	281 (57.7)	0.038	536.2 (51.7)	383.3 (51.2)	0.008
(per week)	≥3 times	197 (40.5)	206 (42.3)		501.8 (48.3)	364.7 (48.8)	

PSM, propensity score matching; SIPTW, stabilized inverse probability of treatment weight; SMD, standardized mean differences.

as followed: average daily intake amount was grouped into three groups (never drinking, <500 ml/d, ≥500 ml/d); duration of tea consumption was grouped into three groups (never drinking, <20 years, ≥20 years); age at onset of regular drinking was also classified into three groups (never drinking, <30 years, ≥30 years).

Statistical Analysis

In the overall data, Chi-square or Fisher's exact tests were used to compare baseline features between OSCC patients and healthy controls. Propensity score analyses, including propensity score matching (PSM), inverse probability of treatment weight (IPTW), and stabilized inverse probability of treatment weight (SIPTW), were used to minimize selection bias and balance baseline differences and other confounding factors. The matching ratio in PSM was 1:1, and the caliper was 0.02, with 487 patients correctly matched with 487 healthy controls. Based on the PSM, IPTW is then calculated with the estimated propensity score. SPTW was also used to minimize sample size inflation and ensure accurate variance estimates (20, 21). Group differences were measured using standardized mean differences (SMD), with an SMD value of 0.1 considered balanced after the matching. To determine the associations between oolong tea-drinking habits and OSCC risk, we utilized multivariate logistic regression (in unmatched data), conditional logistic regression (in PSM data), and weighted logistic regression (in IPTW and SIPTW data). A two-tailed *P*-value of 0.05 was deemed statistically significant. R software version 4.0.3 was used for all analyses. For propensity scoring and matching analysis, the "MatchIt" package was used, and the "forestploter" package was used to visualize the stratified analysis results.

RESULTS

This observational case-control research comprised 744 OSCC patients and 1,029 healthy controls. A baseline summary of pre-and post-matching participant demographics (age, gender, occupation, education level, BMI, and residence), lifestyle habits (smoking and alcohol consumption), and consumption frequency of red meat, vegetables, and fruits are presented in **Table 1** (population after PSM and SIPTW) and **Supplementary Table 3** (overall population and population after IPTW). Before matching analysis, imbalances were noted between the case and control subjects in the collected 11 variables except for gender ($P < 0.05$). After propensity

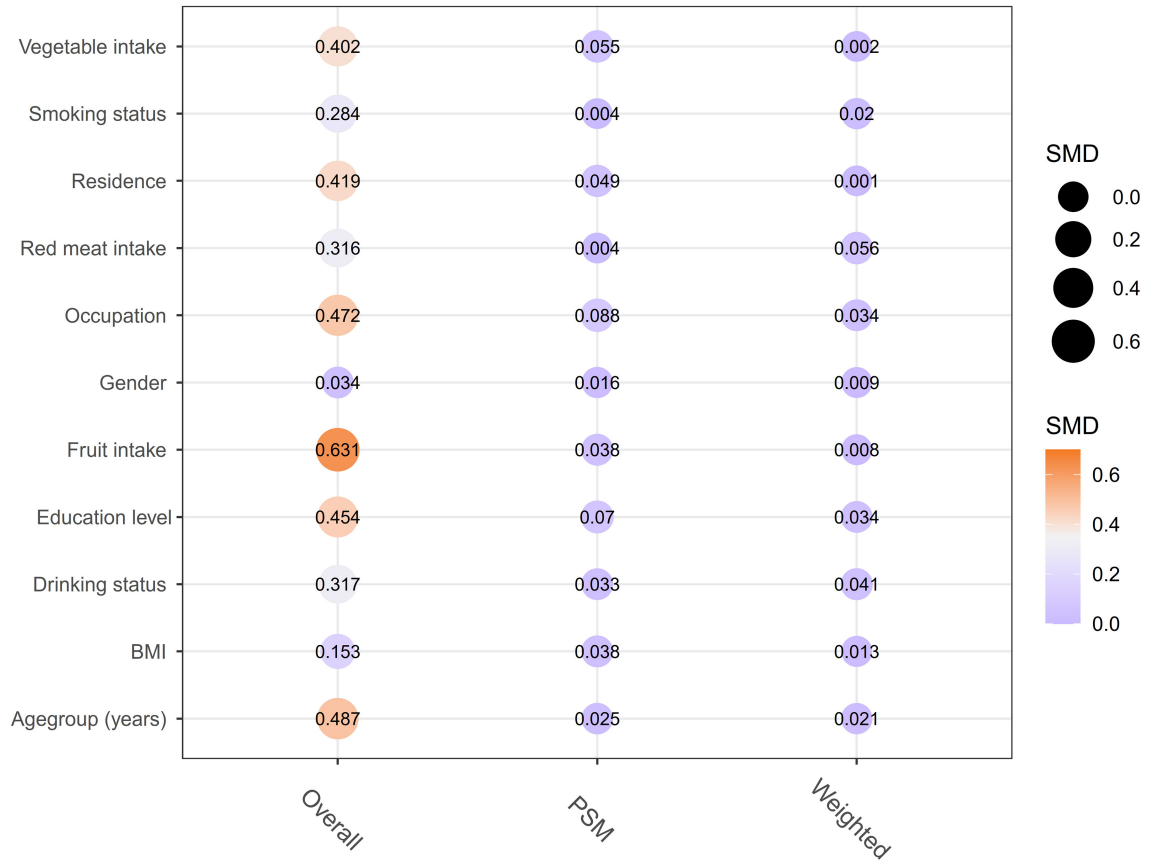

FIGURE 1 | Comparison of the distribution of matching factors between cases and controls before and after matching, Group differences were assessed using standardized mean differences (SMD), with an SMD value of 0.1 considered balanced.

matching adjustment (PSM, IPTW, and SIPTW analysis), all the distributions of observed covariates were comparable (SMD < 0.1, **Table 1** and **Supplementary Table 3** and **Figure 1**).

Table 2 presents the relationships between oolong tea-drinking habits and the risk of OSCC in PSM and SIPTW populations. Reduced risk of OSCC was observed among participants who had the consumption of oolong tea as compared to non-drinkers [PSM population, OR (95%CI): 0.69 (0.49–0.97); SIPTW population, OR (95%CI): 0.74 (0.58–0.94)]. Moreover, oolong tea drinkers who consumed over 500 mL per day had a statistically significant decrease in OSCC risk with the OR (95%CI) being 0.59 (0.38–0.92) in PSM analysis and 0.71 (0.57–0.96) in SIPTW analysis. Additionally, those who had a duration of oolong tea consumption of <20 years and those who started drinking tea at an age older than 30 years were less likely to develop OSCC than non-tea drinkers. Of note, both temperature and concentration of oolong tea were correlated with the risk of OSCC. In PSM data, there is a lower risk of OSCC for oolong tea drinkers who prefer warm and moderately concentrated tea over non-tea drinkers [Tea temperature: warm vs. never drinking, OR (95% CI): 0.56 (0.33–0.93); Tea concentration: moderate vs. never drinking, OR (95% CI): 0.56 (0.33–0.93)]. Similar relationship patterns were also identified in SIPTW data [Tea temperature: warm vs. never drinking, OR (95% CI): 0.70 (0.50–0.99); Tea concentration: moderate vs. never drinking, OR (95% CI): 0.64 (0.46–0.90)]. Subsequently, we performed sensitivity analyses on the overall population and the IPTW population to assess the stability of the results, and comparable results were obtained (**Supplementary Table 4**).

Based on the oral hygiene scores, the subjects were divided into subgroups for further analysis. A greater reduction in OSCC risk with oolong tea drinking was observed among those with poor oral hygiene compared to non-drinkers with good oral hygiene. Of note, as the sample size increases (PSM population to IPTW population), the observed inverse association with OSCC seems to be more pronounced (**Figure 2**).

DISCUSSION

This hospital-based case-control study sought to shed light on the relationship between oolong tea consumption and OSCC risk using three powerful propensity score analyses. Overall, our results support a beneficial effect of oolong tea consumption on the reduced risk of OSCC. Furthermore, OSCC risk was found to be significantly associated with certain tea-drinking

TABLE 2 | The relationship between oolong tea-drinking habits and OSCC risk after propensity score analyses.

Variables	PSM population			*P* for trend	SIPTW population			*P* for trend
	Control	Case	OR (95%CI)		Control	Case	OR (95%CI)	
Oolong tea consumption								
No	386 (79.26)	411 (84.40)	1.00		817.5 (78.75)	623.5 (83.36)	1.00	
Yes	101 (20.74)	76 (15.60)	**0.69 (0.49–0.97)**		220.6 (21.25)	124.4 (16.64)	**0.74 (0.58–0.94)**	
Average daily intake (ml/d)				0.025				0.001
Never drinking	386 (79.26)	411 (84.40)	1.00		817.5 (78.75)	623.5 (83.36)	1.00	
<500	36 (7.39)	32 (6.57)	0.84 (0.51–1.36)		86.3 (8.31)	51.7 (6.91)	0.79 (0.55–1.13)	
≥500	65 (13.35)	44 (9.03)	**0.59 (0.38–0.92)**		134.3 (12.94)	72.8 (9.73)	**0.71 (0.52–0.96)**	
Duration of tea consumption (years)				0.275				0.180
Never drinking	386 (79.26)	411 (84.40)	1.00		817.5 (78.75)	623.5 (83.36)	1.00	
<20	51 (10.47)	23 (4.72)	**0.41 (0.24–0.70)**		103.7 (9.99)	42.4 (5.67)	**0.54 (0.37–0.78)**	
≥20	50 (10.27)	53 (10.88)	0.96 (0.63–1.47)		116.9 (11.26)	82.0 (10.97)	0.92 (0.68–1.24)	
Age at onset of regular drinking (years)				0.002				<0.001
Never drinking	386 (79.26)	411 (84.40)	1.00		817.5 (78.75)	623.5 (83.36)	1.00	
<30	38 (7.80)	48 (9.85)	1.12 (0.71–1.77)		87.6 (8.44)	78.7 (10.52)	1.18 (0.85–1.63)	
≥30	63 (12.94)	28 (5.75)	**0.42 (0.26–0.68)**		133.0 (12.81)	45.7 (6.12)	**0.45 (0.32–0.64)**	
Tea temperature				0.103				0.010
Never drinking	386 (79.26)	411 (84.40)	1.00		817.5 (78.75)	623.5 (83.36)	1.00	
Warm	45 (9.24)	28 (5.75)	**0.56 (0.33–0.93)**		102.3 (9.85)	54.8 (7.32)	**0.70 (0.50–0.99)**	
Hot	56 (11.50)	48 (9.85)	0.78 (0.52–1.18)		118.3 (11.40)	69.7 (9.32)	0.77 (0.56–1.06)	
Tea concentration				0.156				0.020
Never drinking	386 (79.26)	411 (84.40)	1.00		817.5 (78.75)	623.5 (83.36)	1.00	
Light	29 (5.95)	20 (4.10)	0.64 (0.36–1.16)		50.8 (4.89)	31.7 (4.24)	0.82 (0.52–1.29)	
Moderate	54 (11.09)	33 (6.78)	**0.56 (0.35–0.90)**		119.5 (11.52)	58.7 (7.85)	**0.64 (0.46–0.90)**	
Strong	18 (3.70)	23 (4.72)	1.20 (0.61–2.37)		50.2 (4.84)	34.0 (4.55)	0.89 (0.57–1.39)	

OSCC, oral squamous cell carcinoma; PSM, propensity score matching; SIPTW, stabilized inverse probability of treatment weight; OR (95% CI), the odds ratio and its 95% confidence interval. The significant ORs (95% CI) was bolded for ease of viewing.

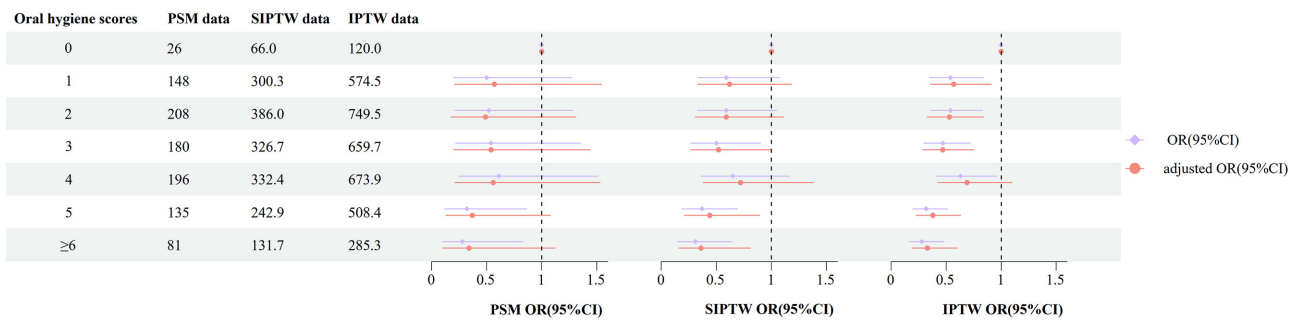

FIGURE 2 | Associations of oolong tea consumption and the risk of OSCC, stratified by different oral hygiene scores. Adjusted OR (95% CI) was calculated by adjusting for age, gender, occupation, education level, BMI, residence, smoking, and alcohol consumption, consumption frequency of red meat, vegetables, and fruits.

habits (amount, duration, age at initiation, temperature, and concentration). Of note, the possible preventive benefits of oolong tea consumption against OSCC were more prominent in individuals with poor oral hygiene.

In the present study, we found the consumption of oolong tea reduced the risk of OSCC by 25–30%, which corresponded to the results of our previous studies (15, 16). In addition, the risk can be further reduced by a higher dose of tea exposure (more than 500 ml/day). Zhou et al. (22). recently published a meta-analysis and systematic review also showing that increasing one cup of tea per day reduces the incidence of oral cancer by 6.2%. More than 100 chemical components have been isolated and identified from oolong tea, among which polyphenols are the most significant ones (23, 24). The polyphenols contain multiple functional components, such as (-)-epigallocatechin gallate (EGCG) and theaflavin (TF) (25, 26), which were reported to have anti-inflammatory and antioxidant effects, and other biological properties (5, 27). Also, multiple *in vitro and in vivo*

studies have indicated that several oolong tea polyphenol extracts could induce apoptosis or proliferation of cancer cells, including oral cancer cells, leading to tumor growth inhibition (28–31).

In the present study, individuals who had consumed oolong tea for <20 years and those who began drinking tea at an age ≥30 years were less likely to develop OSCC, indicating the long-term benefits of oolong tea, when compared to non-oolong-tea drinkers. It is hypothesized that the possible explanation is that the incidence of OSCC grows with increasing age, and young individuals accounted for more in the group with <20 years of tea drinking, while the elderly accounted for more in the group with ≥20 years of tea drinking. Certainly, this hypothesis will require further investigation in future studies.

In the present study, we also found a significantly lower risk of oral cancer among warm tea drinkers compared to non-tea drinkers, while this protective effect tended to diminish among hot tea drinkers (not reaching statistical significance). Recently, the International Agency for Research on Cancer (IARC) classified drinking very hot beverages above 65°C as "probably carcinogenic to humans" (32). According to the most recent study by Ernst et al. (33), hot beverages would increase the cell division rate in the oral mucosa at temperatures above 60°C, leading to cytotoxic effects and increased risk of cancer. Drinking moderately concentrated tea would be most beneficial to reducing OSCC risk in our study (**Table 2** and **Supplementary Table 4**). However, the literature is controversial concerning the optimal tea-drinking concentration. Dose-response analyses of one meta-analysis suggested that with the concentration of tea consumption increased, the risk of oral cancer decreased (22). However, there have been other studies showing that fluoride found in oolong tea helps to prevent dental caries and promote healthy bone growth, but excess fluoride could lead to detrimental health problems in humans, especially fluorosis of the teeth and skeletal fluorosis (7).

It is crucial to highlight that the preventative advantages of oolong tea drinking may vary on oral hygiene status, with individuals with poor oral hygiene experiencing stronger protective benefits. According to Yoo et al. (34), oolong tea extract has an antibacterial effect on oral streptococci such as *Streptococcus mutans* and *Streptococcus sobrinus*. In polyphenols, notably catechin, epigallocatechin-3-gallate selectively inhibits the development and adhesion of periodontopathogens (35). These findings imply that long-term consumption of oolong tea may be effective in mitigating the negative effects of poor oral hygiene on OSCC, which has significant public health implications.

There are certain merits to this study. Our findings represent the first comprehensive analysis of oolong tea consumption and related habits (including amount, duration, age at initiation, temperature, and concentration) and OSCC risk by utilizing traditional multivariate logistic regression (**Supplementary Table 4**) and advanced propensity score analyses (PSM, IPTW, and SIPTW analyses, **Table 2** and **Supplementary Table 4**). However, several limitations of our investigation were unavoidable. Firstly, despite the adjustment of potential influencing factors to minimize this effect when performing the stratified analysis of oral hygiene scores, the balance between groups may still be disrupted, and future studies using other statistical methods (such as propensity score stratification) are needed. Secondly, it's difficult to accurately correlate each subject with the true situation since some subjects consumed more than one type of tea, while only the main tea consumed was recorded in the survey, and differences in composition between oolong teas of different origins may further obscure the true association between tea drinking and the risk of OSCC. Third, considering the relatively small sample size of oolong tea drinkers who neither smoked nor consumed alcohol in this study, we are unable to further analyze the associations of oolong tea consumption habits (amount, duration, age at initiation, and concentration) with OSCC risk among this population. the results must be validated in larger cohorts. Further research with larger sample size is warranted to confirm these results.

CONCLUSION

In conclusion, this study suggests an inverse association between oolong tea consumption and the risk of OSCC, especially for those with poor oral hygiene conditions. These findings may provide an additional understanding of the beneficial role of oolong tea consumption in decreasing the risk of OSCC, which has public health implications for oral cancer prevention.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Fujian Medical University (Fuzhou, China) and carried out in conformity with the ethical criteria outlined in the Helsinki Declaration in 1964. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FC, BH, and JW participated in the design of the study. BH, LL, and YQ were responsible for recruiting participants. MG, YL, MY, LL, WH, and YuxW were responsible for interviewing participants. QD, YuyW, and HW analyzed the data. QD, YuyW, and XH wrote the manuscript, which was revised by all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.928840/full#supplementary-material

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Association between levels of blood trace minerals and periodontitis among United States adults

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Aim: Evidence linking trace minerals and periodontitis is limited. To investigate the relationship between trace minerals (selenium, manganese, lead, cadmium, and mercury) and periodontitis, data from the National Health and Nutrition Examination Survey (NHANES) were analyzed after accounting for potential confounding factors. No known studies have explored the relationship between these five trace minerals and periodontitis.

Materials and methods: A total of 4,964 participants who had undergone a full-mouth periodontal examination and laboratory tests for five trace minerals were studied in a cross-sectional study. Clinical attachment loss (CAL) and periodontitis grading were used to measure periodontitis severity. Linear and logistic regression models were used to evaluate the association between trace minerals and periodontitis. Further subgroup analyses were performed.

Results: Blood lead and cadmium levels were positively associated with mean CAL, and blood selenium was negatively associated with mean CAL; however, blood mercury, blood manganese, and mean CAL were not significantly associated. The association between trace minerals and mean CAL was more significant in males, the elderly, and patients with diabetes. There was a threshold effect between blood cadmium levels and mean CAL. Among the Black population, the relationship between blood cadmium levels and mean CAL followed an inverted U-shaped curve. There was a saturation effect in the study of blood lead in people aged 45–59 years old.

Conclusion: Our study highlighted that blood selenium, lead, and cadmium levels were significantly associated with periodontitis in a nationally representative sample of United States adults.

KEYWORDS

trace minerals, periodontitis, selenium, manganese, lead, cadmium, mercury, NHANES

Introduction

Periodontitis is a common, microbially generated, chronic inflammatory illness defined by the loss of alveolar bone and supporting periodontal ligament. Approximately 10.8% (743 million) of people worldwide had severe periodontitis in 2010, making it the sixth most prevalent health condition (1). A study showed that between 2009 and 2012, 46% of American adults had periodontitis, and 8.9% had severe periodontitis (2). Periodontitis can cause periodontal abscesses and tooth loss. Several systemic diseases are known to be strongly associated with periodontitis. Patients with periodontitis are at higher risk of developing and/or exacerbating diabetes, Alzheimer's disease, chronic obstructive pulmonary disease, and cardiovascular diseases (3, 4). The finding of comorbidity between periodontitis and various medical disorders has raised periodontitis to a new level of importance and has become a worldwide health issue.

Periodontitis is primarily driven by an exacerbated host immune-inflammatory response, which has many drivers (5). Trace mineral imbalances may contribute to the causal pathway in some patients. Many trace minerals are related to antioxidant properties and oxidative stress, which are associated with periodontitis. Selenium and manganese are essential minerals in the body and are found mainly in foods such as nuts and fish. Periodontal tissue benefits from selenium primarily because of its antioxidant action. Selenium and selenoproteins participate in immune regulation and prevent overreactions that can cause autoimmunity or chronic inflammation (6). In patients with diabetes and low selenium levels, chronic periodontitis progresses (7). Thus, selenium has a protective effect on periodontal tissues. Manganese participates in various biochemical functions, such as immune function, growth, and development, and serves as a component of reactive oxygen species (ROS) detoxification (8). Manganese is a cofactor for several enzymes, including oxidoreductase and glutamine synthetase, and protects organisms from oxidative stress (9).

Lead, cadmium, and mercury are considered potentially harmful trace elements but may have beneficial effects at low concentrations. Lead exposure can adversely affect bone metabolism and the immune system, which are potential risk factors for periodontitis. Long-term lead exposure may damage the periodontal ligaments, gums, and alveolar bones (10). Higher blood lead levels have been found to be significantly associated with a decline in adult periodontal health (11). Cadmium exposure has numerous effects (12). Cadmium increases bone resorption, inhibits osteoclast activity, reduces calcium absorption, and impairs kidney function, thereby promoting osteoporosis (13). Moreover, cadmium exposure induces the production of ROS, which may be a potential cause of periodontitis progression (14, 15). In a rodent model of long-term cadmium poisoning, cadmium has been shown to cause loss of the dental alveolar bone (16). Apart from food and fish, dental amalgam is a particular source of mercury, which was popular in the past; however, it is now being reduced and eliminated (17). Despite the low levels of Hg in the environment, its bioaccumulation, toxicity, and persistence may still threaten human health (18). Mercury exposure increases the production of ROS, free radicals, and superoxide anions (19). Wildemann et al. (20) reported that the critical role of mercury-induced toxicity is to inhibit the antioxidant defense system, change the oxidation-antioxidant balance, and increase ROS, which may aggravate damage to periodontal tissue.

To our knowledge, there have only been two early studies published, both ten years ago, that used NHANES III data (1988–1994), focused on the relationship between single trace minerals (lead and cadmium) and periodontitis among adults in the United States of America (21, 22). There is limited evidence linking trace minerals to periodontitis. Thus, this study investigated whether the blood levels of the five trace minerals were associated with periodontitis and whether the blood trace mineral level showed a dose-response relationship. To this end, we conducted a cross-sectional study using data from the United States NHANES dataset.

Materials and methods

Ethics statement

This study was exempt from Institutional Review Board review, for we used data that were deidentified from the NHANES database. Additionally, the protocols for NHANES 2011–2012 and NHANES 2013–2014 were approved by the National Center for Health Statistics Ethics Review Board, a part of the Centers for Disease Control and Prevention (CDC). Prior to the commencement of the survey, all participants were required to provide written informed consent. The NHANES assesses the health and nutritional status of adults and children in the United States, which is a cross-sectional, stratified, multi-stage study. In our analysis, we combined data from two NHANES cycles (2011–2012 and 2013–2014).

Study population

An analysis of NHANES 2011–2012 and 2013–2014 data was conducted to evaluate the health and nutritional status of a representative sample of non-institutionalized people in the United States. Survey participants were asked to complete household interviews and physical examinations in mobile examination centers (MEC). NHANES is a publicly available dataset, and more details of methods and other information are available at www.cdc.gov/nchs/nhanes/. In the

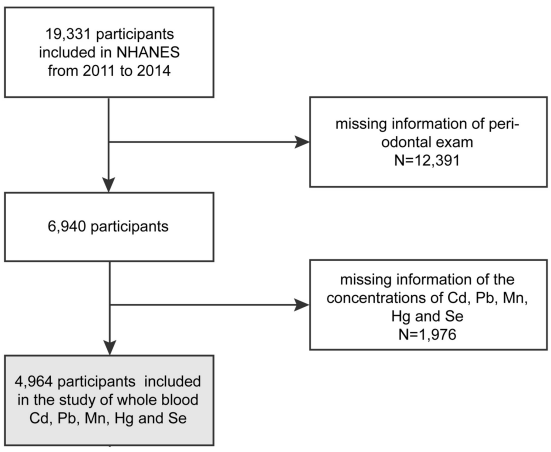

FIGURE 1
Flow diagram of selection of the study participants, from NHANE 2011–2012 and 2013–2014.

NHANES cycles included in this study, 19,331 participants were interviewed. A total of 6,940 adults aged 30–80 with periodontal exams constituted the study sample. We further excluded those who had not yet completed blood laboratory tests for mercury, cadmium, selenium, manganese and lead, leaving 4,964 individuals in the analysis cohort (Figure 1). The missing values of continuous covariates (vitamin D and Ca intake) were less than 0.3%; these were replaced by the average value, and the missing values of classified covariates were stratified based on missing-data status.

Periodontal examination

If a participant aged over 30 had at least 1 natural tooth, a full-mouth periodontal examination was conducted. All dental examiners were trained and calibrated by the survey reference examiner. The pocket depth and gingival recession at 6 sites per tooth were measured with a color-coded periodontal probe which graduated in 2-mm increments. Professional inspectors examined all four quadrants and the measurements were rounded to the full millimeter. In the data entry program, the clinical attachment loss (CAL; distance between the cementoenamel junction and the bottom of probing pocket) was automatically calculated by the algorithm. The result variables of this analysis were mean CAL and the severity of periodontitis. The grade according to the severity of periodontitis is shown in Table 1 based on Eke et al.'s research (23).

Blood sampling

During the laboratory exam, whole blood specimens were collected, shipped on dry ice to the National Center for Environmental Health, Atlanta, GA, and stored frozen at 20°C until analysis. Whole blood concentrations of essential (selenium and manganese) and potentially toxic elements (lead, cadmium and mercury) were measured using inductively coupled plasma mass spectrometry (ICP-MS) after a simple dilution sample preparation step. Detailed analysis methods can be found on the NHANES website. Following all quality control procedures recommended by the manufacturer, the results of all measurement reports met the Division of Laboratory Science's quality control and quality assurance performance standards for accuracy and precision, similar to the Westgard rules. For all biomarkers, according to the NHANES protocol, the concentration below the detection limit was divided by square root 2.

Other covariates

We also collected other covariates. The gender was male or female. The age of the participants was from birth to the date of interview. Race/ethnicity was classified as: non-Hispanic White, non-Hispanic Black, Mexican American, and other race/ethnicity. The educational level of the participants was classified as below high school, high school or above. The poverty-to-income ratio was calculated by dividing family income by the yearly poverty threshold published by the Census Bureau. The poverty ratio was categorized into three levels: ≤ 1; 1–3; >3. Smoking status was categorized as never smoking (lifetime smoking less than 100 cigarettes), current smoking (lifetime smoking more than 100 cigarettes and smoking during the survey period) and former smoking (lifetime smoking more than 100 cigarettes but not smoking during the survey period). Participants with diabetes were recorded if they met one of the following criteria: fasting blood glucose ≥ 7.0 mmol/L, 2-h blood glucose ≥ 11.1 mmol/L, glycated hemoglobin $\geq 6.5\%$

TABLE 1 Definitions of periodontal status according to the severity of periodontitis.

Periodontal status	Definition
Severe periodontitis	≥2 interproximal sites with AL ≥6 mm (not on same tooth) **and** ≥1 interproximal site with PD ≥5 mm
Moderate periodontitis	≥2 interproximal sites with AL ≥4 mm (not on same tooth), **or** ≥2 interproximal sites with PD ≥5 mm (not on same tooth)
Mild periodontitis	≥2 interproximal sites with AL ≥3 mm, **and** ≥2 interproximal sites with PD ≥4 mm (not on same tooth) or one site with PD ≥5 mm
Noperiodontitis	No evidence of mild, moderate, or severe periodontitis

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TABLE 2 Characteristics of the 4,964 participants with and without periodontitis.

Characteristics	All	No periodontitis (n = 2455)	Periodontitis (n = 2509)	*P-value*
Age (years)	51.04 ± 13.52	48.53 ± 12.84	54.57 ± 13.67	<0.01
Male (%)	48.99	42.19	58.54	<0.01
Race (%)				<0.01
Non-Hispanic White	68.59	74.71	59.97	
Non-Hispanic Black	10.14	7.56	13.76	
Mexican American	8.04	6.08	10.8	
Other race	13.23	11.64	15.47	
Education (%)				<0.01
<High school	14.4	8.75	22.35	
High school	20.83	16.46	26.96	
>High school	64.76	74.78	50.67	
Poverty-income ratio (%)				<0.01
<1	11.49	7.77	16.72	
1–3	32.1	26.12	40.5	
>3	50.51	60.86	35.96	
Not recorded	5.9	5.25	6.82	
BMI (%)				0.22
Underweight	0.34	0.27	0.43	
Normal	26.25	27.23	24.86	
Overweight	36.18	36.85	35.24	
Obese	36.75	35.27	38.83	
Not recorded	0.49	0.37	0.64	
BMD (%)				<0.01
Quartile1	15.47	16.36	14.23	
Quartile 2	15.36	15.68	14.91	
Quartile 3	15.74	18.26	12.21	
Quartile4	15.89	18.33	12.46	
Not recorded	37.53	31.37	46.19	
Vitamin D (nmol/L)	72.31 ± 29.02	75.01 ± 28.78	68.53 ± 28.93	<0.01
Smoking status (%)				<0.01
Never	55.58	63.54	44.4	
Former	27.09	25.97	28.68	
Current	17.29	10.49	26.85	
Diabetes (%)	13.74	9.38	19.87	<0.01
Periodontal treatment (%)	23.38	17.96	30.98	<0.01
Days per week using floss (%)				<0.01
0	27.53	22.47	34.64	
1–2	17.47	18.77	15.65	
3–4	14.89	15.74	13.7	
5–7	40.09	43.02	35.96	
Days per week using mouthwash (%)				<0.01
0	44.75	46.55	42.22	
1–2	10.52	12.17	8.2	
3–4	10.73	10.63	10.86	
5–7	33.99	30.65	38.68	
Calcium intake (mg)	970.96 ± 549.61	978.95 ± 540.26	959.73 ± 562.29	<0.01

(Continued)

TABLE 2 (Continued)

Characteristics	All	No periodontitis (n = 2455)	Periodontitis (n = 2509)	*P-value*
Hypertension (%)	34.68	30.04	41.21	<0.01
Hyperlipidemia (%)	38.91	37.69	40.62	0.11
Congestive heart failure (%)	2.39	1.04	3.48	<0.01
Heart attack (%)	2.63	1.62	4.06	<0.01
Coronary heart disease (%)	2.39	1.39	3.8	<0.01
Angina pectoris (%)	1.71	0.87	2.89	<0.01
Stroke (%)	2.04	1.35	3.02	<0.01
Physical activity (%)				<0.01
0	21.87	19.7	24.93	
1	14.77	15.73	13.42	
2	11.64	12.55	10.37	
3	51.71	52.02	51.28	

BMI, body mass index; BMD, bone mineral density.

TABLE 3 Association between trace mineral concentration (μmol/L) and CAL (mm).

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Blood lead	2.3426 (2.0183, 2.6669)***	1.4298 (1.1158, 1.7438)***	0.6081 (0.3211, 0.8950)***
Blood cadmium	0.0512 (0.0464, 0.0561)***	0.0535 (0.0490, 0.0580)***	0.0266 (0.0213, 0.0318)***
Blood mercury	−0.0038 (−0.0059, −0.0017)***	−0.0057 (−0.0077, −0.0038)***	−0.0015 (−0.0033, 0.0003)
Blood selenium	−0.1714 (−0.2420, −0.1008)***	−0.1948 (−0.2607, −0.1290)***	−0.1056 (−0.1647, −0.0465)***
Blood manganese	−0.0005 (−0.0009, −0.0001)**	0.0002 (−0.0002, 0.0006)	0.0002 (−0.0001, 0.0006)

Model 1, unadjusted.
Model 2, age, sex, race/ethnicity were adjusted.
Model 3, age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. **$P < 0.01$; ***$P < 0.001$.

and self-reported diabetes diagnosis. Body mass index (BMI) was the data obtained by dividing weight (kg) by the square of height (m). BMI was classified as underweight, normal weight, overweight, or obese based on BMI <18.5, 18.5–25, 25–30, or >30, respectively. In MEC, blood samples were collected through venipuncture and tested for serum 25(OH)D levels using the standard protocol. Bone mineral density (BMD) was measured at the lumbar vertebrae. The levels of BMD were categorized using quartiles (quartile 1: <25th percentile, quartile 2: 25th–50th percentile, quartile 3: 50th–75th percentile, quartile 4: >75th percentile). We based on the response to the questions in NHANES: In the past week, how many days have you/SP used dental floss or other devices to clean your teeth besides brushing your teeth with a toothbrush? (OHQ.870); How many days have you/SP used mouthwash or other mouthwash products to treat dental diseases or problems in the past week? (OHQ.875). Four categories were created from the responses: from 0 day, 1–2 days, 3–4 days, and 5 or more days in the last week. Periodontal treatment was divided into "yes" or "no" according to the participants' response to the question, "Ever had treatment for gum disease?". The presence of hypertension and hyperlipidemia was assessed by the questions of the interview, "Ever told you had high blood pressure?", "Doctor told you high cholesterol level?". Dietary recall interviews were collected in person in the MEC. Daily calcium intake was then calculated. Based on the 2008 Physical Activity Guidelines for Americans,[1] four levels of physical activity were created: (1) sufficiently active: engaging in ≥300 min/week of moderate activity, or ≥150 min/week of vigorous activity, or ≥300 min/week of an equivalent combination; (2) active: engaging in ≥150 min/week of moderate activity, or ≥75 min/week of vigorous activity, or ≥150 min/week of an equivalent combination; (3) insufficiently active: reporting some physical activity, but not enough to meet the active definition; (4) inactive: no physical activity. Responses to questions of the interviews were used to assess the presence of congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, and physical activity. Detailed information regarding each particular covariate is available at www.cdc.gov/nchs/nhanes/. If a given covariate resulted in

1 http://www.health.gov/paguidelines/

TABLE 4 Association between blood lead concentration (μmol/L) and CAL (mm), and stratified by sex, race/ethnicity, age and diabetes history.

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Blood lead	2.3426 (2.0183, 2.6669)***	1.4298 (1.1158, 1.7438)***	0.6081 (0.3211, 0.8950)***
Lowest quartiles	Reference	Reference	Reference
2nd	0.1526 (0.0793, 0.2260)***	0.0017 (−0.0701, 0.0734)	−0.0779 (−0.1427, −0.0131)*
3rd	0.3935 (0.3178, 0.4691)***	0.1551 (0.0785, 0.2317)***	0.0158 (−0.0550, 0.0866)
4th	0.7043 (0.6283, 0.7803)***	0.3987 (0.3190, 0.4783)***	0.1517 (0.0767, 0.2268)***
P for trend	<0.001	<0.001	<0.001
Stratified by sex			
Male	1.8883 (1.4720, 2.3046)***	1.3609 (0.9599, 1.7619)***	0.4966 (0.1361, 0.8572)**
Female	2.7857 (2.1833, 3.3880)***	1.5989 (0.9964, 2.2015)***	0.6120 (0.0398, 1.1842)*
Stratified by race/ethnicity			
Non-Hispanic White	2.8884 (2.3317, 3.4451)***	1.8592 (1.2973, 2.4210)***	0.8259 (0.3181, 1.3337)**
Non-Hispanic Black	3.0393 (2.3438, 3.7347)***	1.8568 (1.1799, 2.5337)***	0.8896 (0.2289, 1.5503)**
Mexican American	0.7008 (0.1253, 1.2762)*	0.5346 (0.0064, 1.0627)*	0.2545 (−0.2502, 0.7592)
Other	2.4036 (1.6128, 3.1943)***	1.3249 (0.5675, 2.0823)***	0.3983 (−0.3225, 1.1191)
Stratified by age			
30–44 years	1.2188 (0.8313, 1.6062)***	0.9279 (0.5501, 1.3056)***	0.3966 (0.0426, 0.7506)*
45–59 years	3.0993 (2.4414, 3.7572)***	2.4614 (1.8203, 3.1025)***	1.0921 (0.5260, 1.6582)***
60–74 years	1.3067 (0.6360, 1.9773)***	0.9105 (0.2606, 1.5605)**	0.2472 (−0.3635, 0.8578)
≥75 years	2.9180 (1.0676, 4.7684)**	2.6220 (0.8521, 4.3920)**	2.0299 (0.2003, 3.8595)*
Stratified by diabetes history			
Diabetic	4.1482 (3.0791, 5.2173)***	3.2662 (2.2254, 4.3071)***	2.0253 (1.0549, 2.9956)***
Non-diabetic	2.1271 (1.7971, 2.4570)***	1.2641 (0.9415, 1.5866)***	0.4134 (0.1185, 0.7083)**

Model 1, unadjusted.
Model 2, age, sex, race/ethnicity were adjusted.
Model 3, age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. In the subgroup analysis stratified, the model is not adjusted for the stratification variable itself. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

a change in effect estimate of more than 10% (24), or was significantly associated with mean CAL, the variable was chosen as a confounder. The above covariates were selected *a priori* based on established associations and/or reasonable biological relationships and tested.

Statistical analyses

All analyses were conducted using sampling weights based on the recommendation to interpret the complex NHANES survey design. Continuous variables were expressed as the means ± standard deviations and compared using the t-test. Categorical variables were expressed as percentages and compared using the chi-square test. Linear regression models were used to evaluate the association between the concentrations of the five trace minerals and the mean CAL. Continuous variables of trace mineral concentrations were converted to quartile variables to perform trend tests for a robust analysis in the models. Subgroup analyses were also conducted. A generalized additive model and smooth curve fitting method were used to identify the non-linear relationship. When non-linearity was detected, the turning point was calculated using the recursive algorithm, and then a piecewise linear regression model was constructed to calculate the threshold effect and saturation effect of trace minerals on mean CAL. Multinomial logistic regression models were used to evaluate the association between trace mineral concentrations and periodontal status when data did not pass the test of parallel lines. As recommended by the STROBE statement, we examined the results of the unadjusted or minimum adjustment analysis in parallel with those of the fully adjusted analysis. Model 1, unadjusted; Model 2, age, sex, and race/ethnicity were adjusted; Model 3, age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. All of these analyses were performed using the statistical software packages R[2] (The R

2 http://www.R-project.org

TABLE 5 Association between blood cadmium concentration (μmol/L) and CAL (mm), and stratified by sex, race/ethnicity, age, and diabetes history.

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Blood cadmium	0.0512 (0.0464, 0.0561)***	0.0535 (0.0490, 0.0580)***	0.0266 (0.0213, 0.0318)***
Lowest quartiles	Reference	Reference	Reference
2nd	0.0420 (−0.0295, 0.1136)	0.0211 (−0.0461, 0.0882)	−0.0046 (−0.0676, 0.0584)
3rd	0.1890 (0.1149, 0.2632)***	0.1367 (0.0650, 0.2084)***	0.0255 (−0.0437, 0.0946)
4th	0.7677 (0.6918, 0.8436)***	0.7468 (0.6751, 0.8184)***	0.3168 (0.2286, 0.4049)***
P for trend	<0.001	<0.001	<0.001
Stratified by sex			
Male	0.0796 (0.0713, 0.0879)***	0.0769 (0.0690, 0.0847)***	0.0418 (0.0324, 0.0511)***
Female	0.0344 (0.0292, 0.0396)***	0.0356 (0.0307, 0.0405)***	0.0181 (0.0123, 0.0239)***
Stratified by race/ethnicity			
Non-Hispanic White	0.0515 (0.0450, 0.0580)***	0.0546 (0.0485, 0.0606)***	0.0258 (0.0186, 0.0329)***
Non-Hispanic Black	0.0493 (0.0349, 0.0638)***	0.0493 (0.0361, 0.0625)***	0.0201 (0.0032, 0.0370)*
Mexican American	0.0745 (0.0446, 0.1045)***	0.0733 (0.0456, 0.1011)***	0.0570 (0.0243, 0.0898)***
Other	0.0426 (0.0314, 0.0537)***	0.0391 (0.0287, 0.0495)***	0.0230 (0.0112, 0.0348)***
Stratified by age			
30–44 years	0.0291 (0.0234, 0.0349)***	0.0311 (0.0255, 0.0366)***	0.0130 (0.0064, 0.0196)***
45–59 years	0.0539 (0.0462, 0.0617)***	0.0584 (0.0511, 0.0657)***	0.0294 (0.0210, 0.0377)***
60–74 years	0.1030 (0.0881, 0.1179)***	0.0989 (0.0847, 0.1132)***	0.0558 (0.0379, 0.0738)***
≥75 years	0.0777 (0.0342, 0.1212)***	0.0820 (0.0413, 0.1227)***	0.0350 (−0.0088, 0.0789)
Stratified by diabetes history			
Diabetic	0.0771 (0.0603, 0.0940)***	0.0761 (0.0601, 0.0922)***	0.0538 (0.0359, 0.0716)***
Non-diabetic	0.0495 (0.0446, 0.0544)***	0.0514 (0.0469, 0.0559)***	0.0235 (0.0181, 0.0289)***

Model 1, unadjusted.
Model 2, age, sex, race/ethnicity were adjusted.
Model 3, age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. In the subgroup analysis stratified, the model is not adjusted for the stratification variable itself. *$P < 0.05$; ***$P < 0.001$.

Foundation), SPSS (Version 22.0 for Windows; SPSS Inc.), and EmpowerStates[3] (X & Y Solutions, Inc., Boston, MA, United States). *P* values less than 0.05 (two-sided) were considered statistically significant.

Results

Baseline characteristics of participants

A total of 4,964 subjects were enrolled in our study (Figure 1), of whom 2,509 met the criteria for periodontitis. Table 2 showed the weighted sociodemographic and medical characteristics of the participants with and without periodontitis. Of all participants, the mean age was 51.04 ± 13.52 years, 48.99% were males, 68.59% were White, 10.14% were Black and 8.04% were Mexican-American. Among the different groups with and without periodontitis, age, sex, race/ethnicity, education, income-poverty ratio, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, calcium intake, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity and BMD were all significantly different.

Association between blood trace minerals and mean clinical attachment loss

Three weighted univariate and multivariate linear regression models were constructed: Model 1, unadjusted; Model 2, adjusted for age, sex, race/ethnicity; Model 3, adjusted for the covariables in Table 1. In the fully adjusted model, we observed a significant positive association between blood lead and cadmium and mean CAL [0.6081 (0.3211, 0.8950); 0.0266 (0.0213, 0.0318)], especially blood lead, and a negative association between blood selenium and mean CAL [−0.1056 (−0.1647, −0.0465)]. However, the associations between blood mercury, blood manganese and mean CAL were

3 http://www.empowerstates.com

TABLE 6 Association between blood selenium concentration (μmol/L) and CAL (mm), and stratified by sex, race/ethnicity, age, and diabetes history.

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Blood selenium	−0.1714 (−0.2420, −0.1008)***	−0.1948 (−0.2607, −0.1290)***	−0.1056 (−0.1647, −0.0465)***
Lowest quartiles	Reference	Reference	Reference
2nd	−0.1900 (−0.2700, −0.1100)***	−0.1921 (−0.2665, −0.1177)***	−0.1057 (−0.1720, −0.0395)**
3rd	−0.1870 (−0.2653, −0.1086)***	−0.2014 (−0.2744, −0.1284)***	−0.0869 (−0.1524, −0.0215)**
4th	−0.2319 (−0.3113, −0.1525)***	−0.2597 (−0.3339, −0.1856)***	−0.1454 (−0.2118, −0.0789)***
P for trend	<0.001	<0.001	<0.001
Stratified by sex			
Male	−0.3919 (−0.5153, −0.2686)***	−0.3659 (−0.4825, −0.2493)***	−0.1974 (−0.3007, −0.0942)***
Female	−0.0787 (−0.1534, −0.0040)*	−0.0686 (−0.1392, 0.0020)	−0.0346 (−0.0995, 0.0303)
Stratified by race/ethnicity			
Non−Hispanic White	−0.1427 (−0.2386, −0.0468)**	−0.1794 (−0.2705, −0.0882)***	−0.1195 (−0.1985, −0.0405)**
Non-Hispanic Black	−0.1638 (−0.4045, 0.0769)	−0.2661 (−0.4877, −0.0446)*	−0.0858 (−0.2944, 0.1227)
Mexican American	0.0040 (−0.3188, 0.3268)	−0.1024 (−0.3991, 0.1943)	0.1235 (−0.1672, 0.4143)
Other	−0.1990 (−0.3484, −0.0496)**	−0.2783 (−0.4173, −0.1393)***	−0.1629 (−0.2941, −0.0317)*
Stratified by age			
30–44 years	−0.1716 (−0.2747, −0.0684)**	−0.2035 (−0.3034, −0.1037)***	−0.1581 (−0.2516, −0.0646)***
45–59 years	−0.3407 (−0.4976, −0.1838)***	−0.3245 (−0.4743, −0.1746)***	−0.1233 (−0.2525, 0.0060)
60–74 years	−0.0989 (−0.2097, 0.0119)	−0.1050 (−0.2105, 0.0004)	−0.0662 (−0.1582, 0.0259)
≥75 years	−0.1482 (−0.4747, 0.1783)	−0.1818 (−0.4893, 0.1257)	−0.1573 (−0.4602, 0.1456)
Stratified by diabetes history			
Diabetic	−0.3435 (−0.5703, −0.1166)**	−0.3064 (−0.5248, −0.0881)**	−0.0854 (−0.2871, 0.1164)
Non-diabetic	−0.1557 (−0.2278, −0.0835)***	−0.1828 (−0.2504, −0.1153)***	−0.1130 (−0.1733, −0.0527)***

Model 1, unadjusted.
Model 2, age, sex, race/ethnicity were adjusted.
Model 3, age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. In the subgroup analysis stratified, the model is not adjusted for the stratification variable itself. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

not significant [−0.0015 (−0.0035 0.0003); 0.0002 (−0.0001, 0.0006)] (Table 3). In addition, blood lead, cadmium and selenium levels were transformed into quartiles. And the mean CAL of the highest quartiles of blood lead increased by 0.15 mm compared with the lowest quartiles [0.1517 (0.0767, 0.2268)] (Table 4). The mean CAL of the highest quartiles of blood cadmium increased by 0.32 mm compared with the lowest quartiles [0.3168 (0.2286, 0.4049)] (Table 5), while for blood selenium, the mean CAL decreased by 0.15 mm [−0.1454 (−0.2118, −0.0789)] (Table 6). The overall results appeared to be roughly the same, indicating that the conclusion was relatively stable. Furthermore, all *P* for trend < 0.001 indicated that the variation trends were significant.

Subgroup analyses of blood cadmium, blood lead, blood selenium, and mean clinical attachment loss

Further subgroup analysis of blood lead showed that the β value in females was slightly higher than males (Table 4). The association was significant in White and Black people [0.8259 (0.3181, 1.3337); 0.8896 (0.2289, 1.5503)], but not in other race/ethnicity. The strongest association was observed in the elderly with ≥5 years [2.0299 (0.2003, 3.8595)]. The β value in the diabetic group was almost five times higher than that in the non-diabetic group [2.0253 (1.0549, 2.9956); 0.4134 (0.1185, 0.7083)]. All subgroups were statistically different in blood cadmium (Table 5). The β value of males was significantly higher than that of females [0.0418 (0.0324, 0.0511); 0.0181 (0.0123, 0.0239)], and the association was strongest in Mexican-Americans [0.0570 (0.0243, 0.0898)]. Among the different age groups, the β value of the 60–74 years old was the highest [0.0558 (0.0379, 0.0738)]. The β value of the diabetic group was almost twice that of the non-diabetic group [0.0538 (0.0359, 0.0716); 0.0235 (0.0181, 0.0289)]. For blood selenium (Table 6), we observed a negative association between blood selenium and mean CAL in males [−0.1974 (−0.3007, −0.0942)], while it was not significant in females [−0.0346 (−0.0995, 0.0303)]. For people aged 30–44 [−0.1581 (−0.2516, −0.0646)] and without diabetes [−0.1130 (−0.1733, −0.0527)], the negative association was statistically significant.

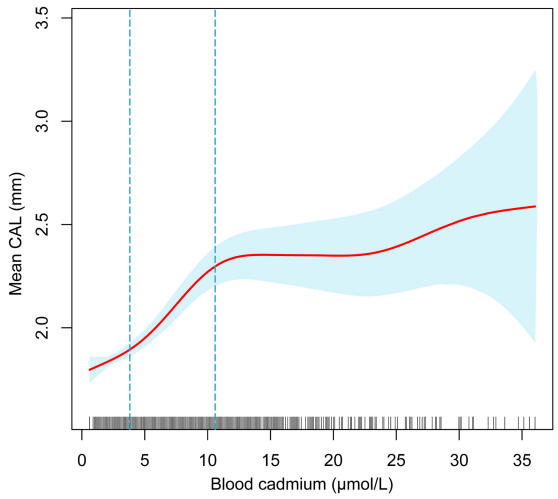

FIGURE 2
The non-linear relationship between blood cadmium and mean CAL. The blue area represents the 95% confidence interval. The red line represents point estimates. Age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted.

Curve fit analysis

The adjusted smoothing curve showed a non-linear relationship between blood cadmium and mean CAL (Figure 2). Based on the piecewise linear regression model, the turning points were calculated to be 4.03 and 10.32 μmol/L, respectively (Table 7). When the blood cadmium level was <4.03 μmol/L, the β value was very small and the association was not significant [0.0152 (−0.0143, 0.0447)], until beyond 4.03 μmol/L, a threshold effect occurred, which was positively associated with mean CAL [0.0889 (0.0550, 0.1228)]. After the blood cadmium level reached 10.32 μmol/L, the β value decreased greatly and became saturated [0.0178 (0.0073, 0.0284)]. Stratified by age, sex, race/ethnicity, and diabetes history, and further using smooth curve fitting analysis to determine the non-linear relationship between blood lead, blood cadmium, blood selenium and mean CAL, we discovered that there was an obvious turning point in blood lead among people aged 45–59 and in blood cadmium among Black people (Table 7). The mean CAL increased with the increase in blood lead among people aged 45–59 [6.6634 (3.4448, 9.8820)], until the turning point (0.065 μmol/L) (Table 7; Figure 3). Similarly, the relationship between blood cadmium and mean CAL in Black people followed an inverted U-shaped curve with a turning point of 12.99 μmol/L (Table 7; Figure 4). There was an almost linear relationship between the blood selenium subgroups and mean CAL (Supplementary Figure 1).

TABLE 7 Threshold effect and saturation effect analysis of blood cadmium and blood lead on mean CAL.

Mean CAL (mm)	Adjusted ß (95% CI), *P*-value
Blood cadmium < 4.03 (μmol/L)	0.0152 (−0.0143, 0.0447), 0.3115
4.03 (μmol/L) < Blood cadmium < 10.32 (μmol/L)	0.0889 (0.0550, 0.1228), <0.0001
Blood cadmium > 10.32 (μmol/L)	0.0178 (0.0073, 0.0284), 0.001
Non-Hispanic Black	
Blood cadmium < 12.99 (μmol/L)	0.0508 (0.0222, 0.0794), 0.0005
Blood cadmium > 12.99 (μmol/L)	−0.0130 (−0.0431, 0.0171), 0.3980
45–59 years	
Blood lead < 0.065 (μmol/L)	6.6634 (3.4448, 9.8820), <0.0001
Blood lead > 0.065 (μmol/L)	0.6459 (0.0354, 1.2564), 0.0383

Age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. In the subgroup analysis stratified, the model is not adjusted for the stratification variable itself.

Association between blood trace minerals and periodontal status

The relationship between blood trace mineral levels and periodontal status was determined using multinomial logistic regression (Table 8). Blood lead and cadmium levels showed significant differences in different periodontal statuses. Compared with people without periodontitis, for every unit increase in blood lead, the risk of moderate periodontitis increased by 3.14 times [4.143 (1.233, 13.921)], and the risk of severe periodontitis increased by 27.15 times [28.145 (7.112, 111.376)]. For every unit increase in blood cadmium, the incidence of severe periodontitis was 1.06 times higher than that of non-periodontitis [1.058 (1.036, 1.08)].

Discussion

Heavy metals are known to have long-term adverse health effects, but exposure continues, especially in less developed countries and communities (25). Lead exposure mainly comes from informal battery recycling and manufacturinge, electronic waste, metal mining, and food adulteration, especially in spices. A study compiled background values of blood lead levels of children in 34 countries to estimate a background distribution of 1,300 million of them. It was estimated that 48.5% of the children (632 million) had blood lead levels exceeding the 5 μg/dL reference value of CDC (26). Agricultural and industrial sources contribute to the presence of cadmium in the environment as a pollutant. Cadmium accumulates in

plants and animals, with a long half-life of approximately 25–30 years. Cigarette smoke is the primary source of exposure to cadmium in smokers because tobacco leaves accumulate high levels of cadmium in the soil (27). In non-smokers, cadmium is most commonly ingested through food and water contaminated with cadmium (28). Selenium is an essential trace element. Currently, the recommended daily selenium allowance is 55 μg/day (29). Most people are exposed to selenium through food, water and air. Selenium enters the food chain through plants, and low dietary selenium intake in humans is related to crops grown in selenium-deficient soils. Therefore, animal food can be used as an alternative source because animals can accumulate selenium from feed supplemented with selenium or pastures with selenium fertilizers (30). The general population is mainly exposed to mercury through food, fish, and dental amalgams. Manganese is primarily consumed in the diet; however, occupational exposure to manganese may also occur.

By analyzing a nationally representative sample of adults in the United States, this study investigated the relationship between blood trace mineral concentrations and periodontitis (mean CAL and periodontal status). Linear regression analyses showed a positive association between blood lead, blood cadmium, and mean CAL, and a negative association between blood selenium, blood mercury, blood manganese, and mean CAL. Even after adjusting for all other factors to minimize potential confounding, blood lead, blood cadmium, and blood selenium levels were still significantly associated with mean CAL, but there was no significant association between blood mercury, blood manganese, and mean CAL (**Table 3**). The results of regression analyses of quartiles of blood lead, blood cadmium, and blood selenium, as well as the results of trend tests (P for trend < 0.001) both confirmed their association with mean CAL (**Tables 4–6**). There was a significant positive association between blood lead and cadmium levels and periodontitis severity in the fully adjusted model by multinomial logistic regression. Therefore, the next research focus was on the association between blood lead, cadmium, selenium, and periodontitis.

Periodontitis leads to the loss of alveolar bone and support of the periodontal ligament (31). Clinical attachment loss is the direct result of periodontal supporting tissue destruction, a vital sign to distinguish gingivitis from periodontitis, and an essential index for the clinical diagnosis and grading of periodontitis. Periodontal pocket depth may cause errors owing to gingival inflammation, resulting in false-positive results. In contrast, clinical attachment loss can reflect the degree of periodontal tissue destruction more objectively and genuinely.

Periodontitis is a complex infectious disease that is affected by many factors such as diabetes history, smoking status, age, stroke and inadequate oral hygiene (32, 33). The imbalance of trace minerals may be part of the pathogenic pathway and play a unique role in the complex etiology and mechanism networks. Various subgroup analyses were performed after controlling for confounding variables. Surprisingly, the threshold effect between blood cadmium and mean CAL was determined by curve fitting and piecewise linear regression. When blood cadmium reached 4.03 μmol/L, the mean CAL began to increase with the increase in blood cadmium concentration. This suggests that people should maintain blood cadmium below 4.03 μmol/L as much as possible, which is beneficial to periodontal health. Subgroup analyses showed that the β values of blood cadmium and blood selenium in males were significantly higher than females. There was a significant positive association between blood lead and mean CAL in the elderly, with a β value of 2.03, indicating that the elderly should pay special attention to the control of blood lead levels, which is helpful in maintaining periodontal health. For White and Black people, there was a significant positive association between blood lead and mean CAL. Similarly, the association between blood cadmium levels and mean CAL was stronger in Mexican Americans than in other populations. Interestingly, the β values of blood lead, and blood cadmium in participants with diabetes were much higher than those in participants without diabetes. In contrast, the association between blood selenium and mean CAL was not affected in those people, indicating that patients with diabetes had a higher risk of periodontitis, even from the perspective of trace minerals. There was a non-linear relationship between blood cadmium and mean CAL. Among the Black population, the relationship between blood cadmium levels and mean CAL followed an inverted U-shaped curve. There was a saturation effect in the study of blood lead in people aged 45–59 years old.

Lead exposure can cause dysbacteriosis in dental plaques (34). Among children exposed to severe lead pollution, the prevalence rate of *A. actinomycetemcomitans* infection is as high as 17%, which is the primary pathogen associated with juvenile periodontitis (11, 35). Lead can also damage various biochemical processes, including inhibition of calcium and reaction with proteins. Even at low blood lead levels, lead may inhibit the activity of various enzymes by competing with the necessary cations of binding sites, causing structural changes, such as glucose 6-phosphate dehydrogenase, catalase superoxide dismutase, glutathione peroxidase, and antioxidants such as glutathione (19). Additionally, lead can induce the production of ROS and oxidative stress, which will further lead to the deterioration of dental health. Nattaporn et al. (11) investigated the periodontal status of children in a lead-polluted environment, finding that the high-lead group showed more deep pockets at tooth 46 and tooth 16 than the low-lead group.

The level of cadmium in human cadaver mandibles was measured, and it was found that there may be an association

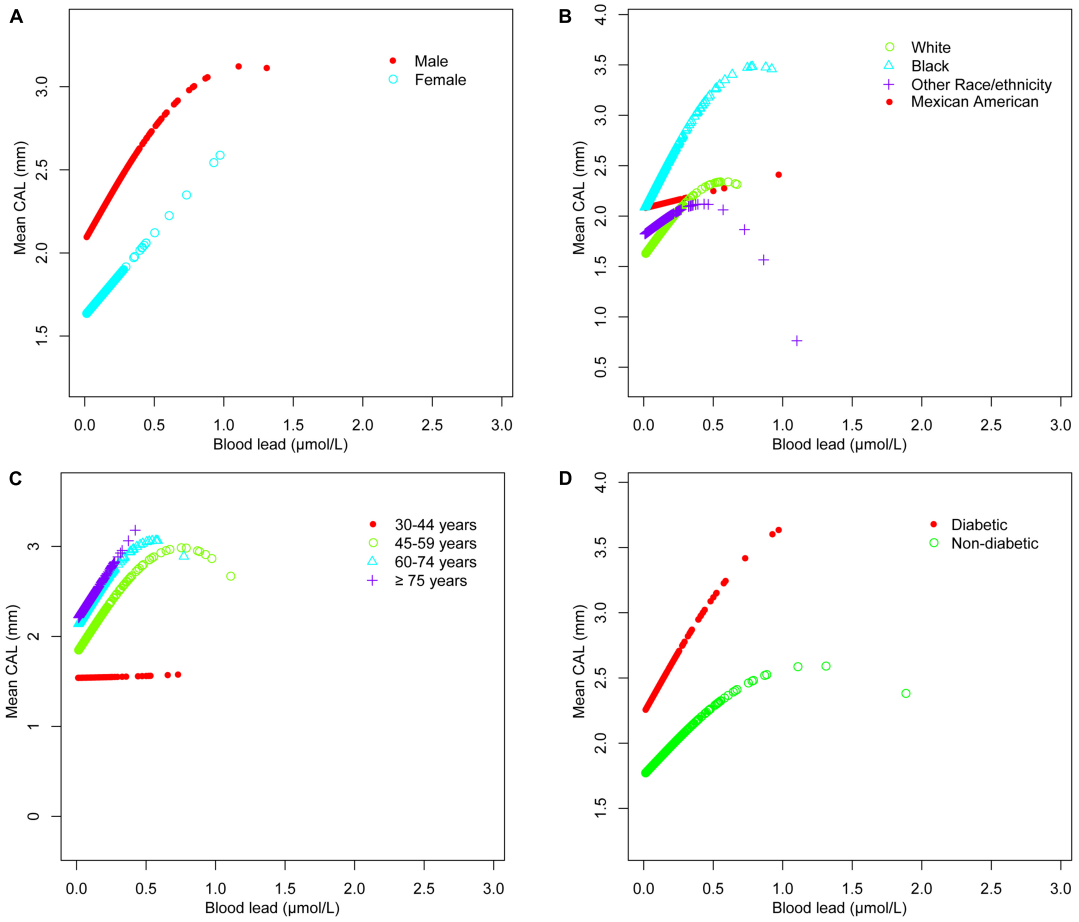

FIGURE 3
Blood lead and mean CAL dose-response relationship. **(A)** Stratified by sex. **(B)** Stratified by race/ethnicity. **(C)** Stratified by age. **(D)** Stratified by diabetes history. Age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. In the subgroup analysis stratified, the stratification variable itself was not adjusted.

TABLE 8 Association between trace mineral concentration (μmol/L) and periodontal status (mm).

	No periodontitis	Mild periodontitis	Moderate periodontitis	Severe periodontitis
Blood lead	1.0	1.077 (0.024, 49.176)	4.143 (1.233, 13.921)	28.145 (7.112, 111.376)
Blood cadmium	1.0	0.924 (0.853, 1.002)	1.013 (0.995, 1.031)	1.058 (1.036, 1.08)
Blood mercury	1.0	0.987 (0.967, 1.007)	1.0 (0.995, 1.005)	0.993 (0.984, 1.002)
Blood selenium	1.0	1.29 (0.812, 2.049)	0.908 (0.748, 1.101)	0.782 (0.570, 1.072)
Blood manganese	1.0	0.999 (0.996, 1.002)	1.0 (0.999,1.001)	1.0 (0.98, 1.001)

No periodontitis was considered as reference group. Age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted.

between the level of cadmium in basal bone and the presence of periodontitis. In addition, alveolar bone in the mandible accumulated higher amounts of cadmium than the basal bone, which might impact the progression of skeletal changes associated with periodontitis (36). Cadmium induces oxidative stress and ROS production, causing the oxidation and damage of biologically important macromolecules, such as lipids, proteins, DNA, and cellular membrane phospholipids. Cadmium may interfere with the activity of antioxidant enzymes, such as manganese superoxide dismutase, catalase, and copper-zinc superoxide dismutase which is a zinc-oncentrating protein that can be used as a free-radical scavenger (37), further

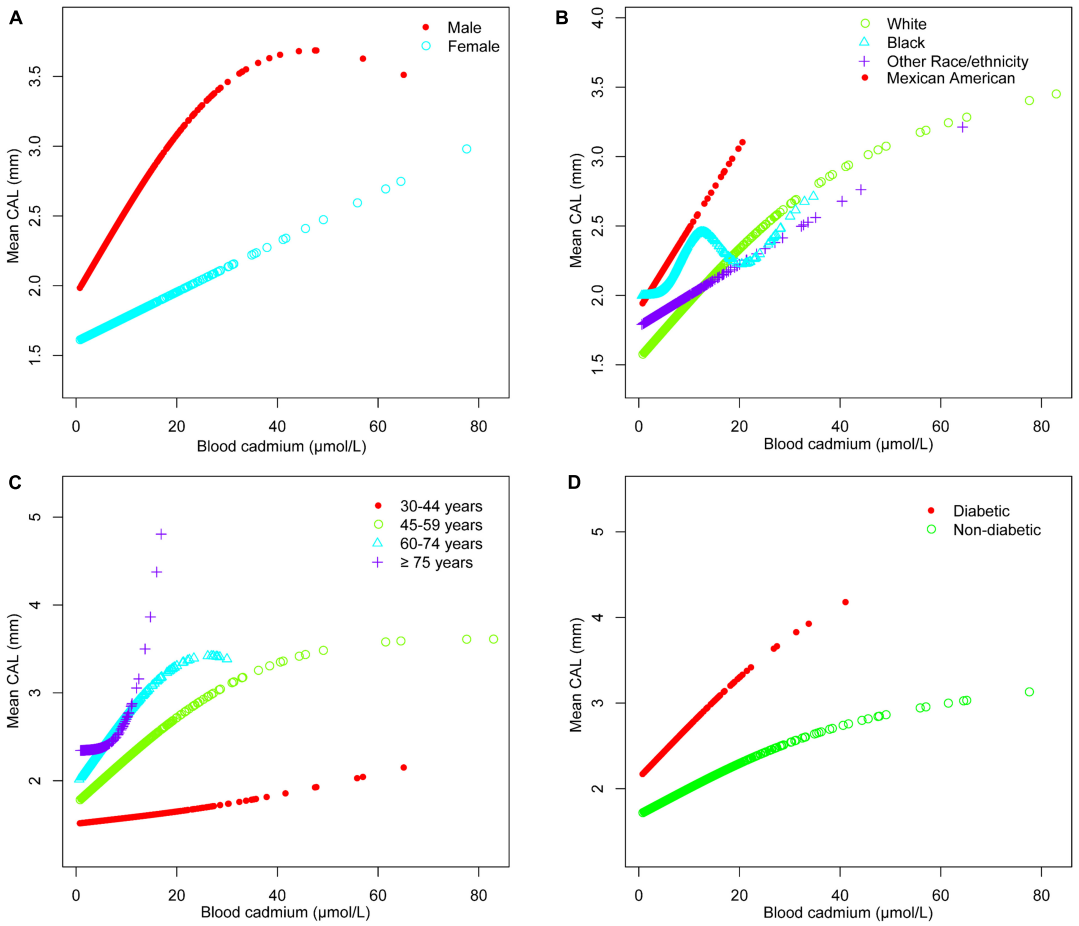

FIGURE 4
Blood cadmium and mean CAL dose-response relationship. **(A)** Stratified by sex. **(B)** Stratified by race/ethnicity. **(C)** Stratified by age. **(D)** Stratified by diabetes history. Age, sex, race/ethnicity, BMI, income-poverty ratio, education, vitamin D, smoking status, diabetes, frequency per week using floss and mouthwash, periodontal treatment, hypertension, hyperlipidemia, BMD, congestive heart failure, coronary heart disease, angina pectoris, heart attack, stroke, physical activity, and calcium intake were adjusted. In the subgroup analysis stratified, the stratification variable itself was not adjusted.

aggravating the damage of oxidative stress. In addition, cadmium can destroy signaling cascades and cause a variety of toxic effects, mainly because of its physicochemical similarity to the calcium ion. It can destroy calcium-mediated signaling pathways, which may be achieved by significantly changing the activation of calmodulin and calmodulin-dependent protein kinase II in cell death pathways, such as apoptosis, necrosis or autophagy (38).

Selenium may be essential to periodontal tissue. It had been found that, in *in vitro* and animal experiments, adding selenium to α-Tocopherol could accelerate cell proliferation and wound healing. his might be due to selenium promoting the synthesis of basic fibroblast growth factor and type I collagen by gingival and periodontal ligament fibroblasts (39). Selenium can prevent the aggravation of the immune response in chronic inflammation. At the cellular level, selenium may affect the function of various leukocyte effectors, including adhesion, migration, phagocytosis and secretion of cytokines (40). The glutathione peroxidase system is the most widely studied, which uses the selenium of its active site to detoxify ROS, such as hydrogen peroxide and phospholipid hydrogen peroxide. Similarly, selenium regulates the activity of transcription factors (nuclear factor-kappa B and activator protein-1) and the expression of related genes (41). Selenium even reduces levels of tumor necrosis factor-α and cyclooxygenase-2 produced by macrophages in response to endotoxins and down-regulates the expression of adhesion molecules. In addition, it also contributes to the metabolism of arachidonic acid and eicosanes (42).

In the present study, we analyzed a representative sample of multiracial populations for better generalizability to the United States population. In addition, participants were evaluated and operated by trained staff, and interviews were conducted following standardized procedures and strict quality

control to obtain examination data and laboratory data, which improved the accuracy and validity of the data. In the data analysis, we adjusted for a considerable number of potential confounding variables. Such a large sample size enabled us to carry out further subgroup and piecewise model analyses. This is the main advantage of this study. In addition, the potential association between trace elements and periodontitis found in this study may help scholars to carry out more randomized controlled trials in related aspects because, at present, only a few clinical trials have investigated their relationship. However, the present study had several limitations. First, it had a cross-sectional design, and the direction of the association between trace minerals and periodontitis was difficult to explain as causality. We could not infer long-term trends of periodontal status because all measurements of an object were taken at the same time. Second, although we adjusted for more than a dozen major covariates in the analysis, we might have omitted some possible residual confounders that were not taken into account when the model was designed or for which there was not enough information in the database to allow proper adjustments. For example, NHANES 2011–2012 and 2013–2014 did not collect information such as plaque index, dental calculus index, bad oral habits, probing bleeding, and parents' periodontal history. Third, the data source of NHANES database is the United States population. Therefore, the findings should be extrapolated cautiously to other populations in different countries. The mechanism underlying the relationship between trace minerals and periodontitis requires further study. Nevertheless, the role of trace minerals in periodontitis should be emphasized because it is relatively easy to control trace minerals in the general population. Individuals with periodontitis require special attention to diet and environment to improve their trace mineral levels, which may help alleviate periodontitis.

Conclusion

Blood lead and cadmium levels were positively associated with mean CAL, and blood selenium was negatively associated with mean CAL; however, blood mercury, blood manganese, and mean CAL were not significantly associated. The association between trace minerals and mean CAL was more significant in males, the elderly, and patients with diabetes. There was a threshold effect between blood cadmium levels and mean CAL. Among the Black population, the relationship between blood cadmium levels and mean CAL followed an inverted U-shaped curve. There was a saturation effect in the study of blood lead in people aged 45–59 years old. Blood selenium, lead, and cadmium levels were significantly associated with periodontitis. Due to the limitations of this study, further research is needed, including mechanistic experiments and large-sample multicenter prospective studies, to explore the pathogenesis behind this special relationship.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: www.cdc.gov/nchs/nhanes/.

Author contributions

HH, JYa, and NY contributed to the conception and design of the study. HH, LY, LT, JYu, and YG contributed to drafting the article. ZL contributed to revising the article critically. All the authors have read and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.999836/full#supplementary-material

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A cross-sectional study on dietary assessment, oral hygiene behavior, and oral health status of adolescent girls

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Adolescents are a population group that is vulnerable to nutritional problems other than toddlers, especially young women. Special attention to the dietary issues of adolescent girls needs to be obtained along with the increase in the adolescent population in Indonesia because this affects the growth and development of the body and will impact adult nutrition problems. The purpose of the study was to analyze the relationship between diet assessment and oral health status of adolescent girls, the relationship between oral hygiene behavior and oral health status of adolescent girls, and the simultaneous relationship between dietary assessment and oral hygiene behavior with the oral health status of adolescent girls. Analytical research using the survey method was conducted on 96 young women in two junior high schools in Bandung. Assessment of diet seen from eating behavior and anthropometric examination. Eating behavior using the Adolescent Food Habit Checklist Index questionnaire and anthropometric investigations were carried out by looking at body height, body weight, and Mid Upper Arm Circumference using the standards from the Indonesian Minister of Health Regulation 2020. Oral hygiene behavior using the Oral Hygiene Behavior Index questionnaire. Oral health status using the Dental Health Status Assessment. The results were statistically analyzed with Spearman's Rank Correlation, and Multiple Linear Regression tests showed no significant relationship between dietary assessment and oral health status (eating behavior with a p-value = 0.429 and anthropometric examination with a p-value = 0.262). A significant association between oral hygiene behavior and oral health status, with a p-value of 0.003, while there is no simultaneous relationship between diet assessment and oral hygiene behavior with oral health status, with multiple r^2 = 13.2%.

KEYWORDS

adolescent school girls, oral health status, oral health behavior, diet assessment, stunting and skinniness

Introduction

In Indonesia, the prevalence of small infants from the small mother group (body height =150 cm) is currently 34.8% (1). The study of Demographic Health Surveys in 54 countries found that for every 1 cm less in mother's height, the risk of underweight and stunting in children under five increased. In an analysis of 52 of 54 countries (96%), the association between maternal height and stunting was statistically significant (2). However, the association between maternal and infant length may reflect both genetic background and the environmental determinants and developmental experiences mothers experience during childhood, maturation, and subsequent offspring growth. For example, women with short stature have a higher risk of having babies with lower birth weight (LBW), and teenage pregnancy may also increase the risk of LBW events. In addition, stunting seems to be interpreted as malnutrition themselves, but especially in Indonesia, it was shown that stunting is more influenced by education and social structure than nutrition (3–6). There is a new concept of the modern view of growth regulation that involves social-economic-political-emotional (SEPE), which was explained by Bogin (7). This modern view will be explained why stunting prevention needs to involved not only health parameters.

Prevention of stunting has become a national priority so that the younger generation in Indonesia can grow and develop in the best possible way. The government has issued the National Strategy to Accelerate Stunting Prevention guide for central and local governments to implement stunting prevention efforts. The National Stunting Strategy includes specific and sensitive nutritional interventions to improve nutrition. Specific nutrition interventions aim to address nutrition-related issues directly through the health sector. At the same time, sensitive nutritional interventions aim to address non-health issues contributing to stunting, such as providing clean water, food security, and health insurance (8).

A group at high risk for nutritional problems is adolescents (9). With the increasing adolescent population in Indonesia, adolescent nutrition must be given special attention as it affects physical growth and development and will impact adult nutrition. Nutritional problems in adolescents, whether under- or over-nutrition, can adversely affect public health. Adolescent malnutrition will have a negative impact on public health, such as reduced physical fitness, reduced productivity, and even the fertility of adolescents themselves, especially young women (10).

One of the methods used to assess a person's nutritional status is dietary assessment. Direct assessment of diet is by looking at eating behavior and anthropometry. Adolescent eating behavior has received increasing attention in recent years amid claims that many adolescents have a poor diet, including high levels of fatty foods and low intakes of fruit and vegetables. Variations in adolescent dietary intake are likely to reflect available diets, values and circumstances of parents, school and peers, and their motivation. If adolescents' eating habits/behavior are poor, it will affect the clinical condition that indicates a nutritional deficiency, thus affecting their anthropometry (11).

Oral health plays a fundamental role in overall health and wellbeing. Oral health has a significant impact, significantly on the quality of life (12). Many factors affect a person's oral health: socioeconomic status, education, living and school environment, and individual behavior. Eating behavior and oral health have a synergistic and dynamic relationship (13). Diet, nutritional composition, and poor oral and dental conditions will interact and have a role in the formation play a role in caries' formation motion of sweet foods contained in individual snack patterns are the main factors of a cariogenic diet. Oral health is closely related to oral hygiene because oral hygiene is a primary factor in creating oral health. Oral hygiene can determine a person's level of oral health. Young women need to consider maintaining nutritional status and oral hygiene, starting from pre-wedding preparations, especially brides-to-be, to preconception, gestation, and postpartum, which may directly impact the fetus later on; what does it contain (14). Therefore, this study aimed to analyze the association between nutritional assessment, oral hygiene behaviors, and oral health status in adolescent girls. It is hypothesized that there is a simultaneous relationship between the evaluation of diet and oral hygiene behavior with the oral health status of adolescent girls.

Materials and methods

Study design and participant

Analytical observational school-based studies with a cross-sectional design were used, and survey data were collected through questionnaires and anthropometric surveys. Two schools were selected due to being located at a stunting locus [based on the Decree of the Mayor of Bandung regarding stunting loci in Bandung City (15)]. Participants were recruited by simple random sampling. The subjects of this study were middle school girls who met the following criteria: (i) were in the age range of 10–15 years, (ii) had no chronic systemic disease, and (iii) were willing to participate in the study (proofed by parental signed informed consent).

Sample size

In this study, correlation analysis was used. The formula used to determine the sample size was the formula used to determine the sample size using correlation analysis, (i) type I

error (Zα) = set at 5% with a one-way hypothesis, so Zα = 1.96; (ii) type II error (Zβ) = set at 10% with a one-way hypothesis, then Zβ = 0.84; and (iii) research correlation coefficient (r) = 0.3. Based on the calculation of the sample size formula above, a minimum of 84 people is required (16).

$$n = \left[\frac{(Z\alpha + Z\beta)}{0,5\ \ln[\frac{1+r}{1-r}]}\right]^2 + 3$$

Dietary assessment

Dietary assessments will be assessed using adolescent girls' dietary habits and anthropometric surveys. Eating behavior is a student's repetitive pattern of eating habits, which can be evaluated using a questionnaire. At the same time, anthropometry is assessed by examining the student's height, including height, weight, and mean upper arm circumference (MUAC). The dietary assessment tool consisted of the Adolescent Eating Habits Checklist (AFHC) questionnaire (7), adapted into Indonesian for this study and tested on 64 adolescents, which is not included in this study. The reliability of the tested questionnaire leads to a Cronbach's alpha = 0.86.

The AFHC questionnaire contains 23 "correct" or "incorrect" or "not applicable to me" questions. Responses indicating healthy behaviors are scored as 1. Final scores were adjusted for "not applicable" and missing responses using the following formula: AFHC score = number of "healthy" responses × (18/number of items answered). The measurement scale uses the Guttman scale, and the last level multiplied by 100 becomes the interval scale. The examples of the questions for instance, if I am having lunch away from home, I often choose a low-fat option (11).

Anthropometric examination by calculating the formula body-mass-index (BMI) per age and MUAC. BMI category (kg/m2): thin underweight, weight level < 17.00; Mild weight loss 17.00–18.49; Normal 18.50–24.99; Overweight/ Mildly overweight 25.00–26.99; Excess weight level > 27.00, then the BMI results are entered in the Z-Score formula, while the MUAC threshold used is 23.5 cm. Anthropometric examination measuring scale using a ratio scale. The procedure measuring MUAC was to determine the mid-point between the elbow and the shoulder (acromion and olecranon). Placing the colored tape measure around the left arm (the arm should be relaxed and hang down the side of the body). Measure the MUAC while ensuring that the tape neither pinches the arm nor is left loose. Read the measurement from the window of the tape or from the tape. Record the MUAC to the nearest 0.1 cm or 1 mm. If a measurement in the green zone means the child is properly nourished; a measurement in the yellow zone means that the child is at risk of malnutrition; a measurement in the red zone means that the child is acutely malnourished (17).

Oral hygiene behavior

Oral hygiene behaviors were participants' verbal care behaviors assessed using a questionnaire. The Oral Hygiene Behavior Questionnaire was developed based on the theory of planned behavior (18) and tested on 50 adolescents that are not included in the main study (Cronbach's alpha = 0.842). The questionnaire contains 17 items assessing attitudes, subjective norms, and perceived behavioral control. The response score is 0–5. Total scores range from 0 to 85, with higher scores indicating positive oral hygiene behaviors. The measurement scale adopts the Likert scale, and the final result is multiplied by 100 to become the interval scale.

Oral health status

Oral health status is an oral health condition felt by the participant assessed from the questionnaire. Because it was still a pandemic, oral health status was measured using a self-reported dental health status assessment questionnaire (19) trans-adapted into Indonesian (Cronbach's alpha = 0.758). The original questionnaire contained 10 statement items to assess caries risk, but only seven items remained after undergoing trans-adaptation and pre-test. Responses were scored from 0 to 1. The total score ranged from 0 to 7, with higher scores indicating individuals with better health status. The measurement scale uses a Likert scale. The final score times 100 becomes an interval scale.

Data collection

Data collection took place in April 2022. Prior to the day of data collection, informed consent was given to the parents of the students who explained the course of the research. In addition, parents are asked to fill in the child's health data, whether the child has a particular disease or disability. It is necessary to determine the confounding factors in this study. Adolescents with a history of chronic systemic disease were excluded from participation. Aside from the child's health history, the parents also asked questions regarding social-economic status.

Participants previously received instructions on how to complete the questionnaire. After participants understood, they simultaneously met all questionnaires (AFHC, Oral Hygiene Behavior Questionnaire, Oral Health Status Questionnaire) in two separate rooms. A research assistant accompanied each. After completing the questionnaires, the researchers (principal investigators) administered anthropometric measurements to the participants, whom research assistants assisted. Anthropometric measurements include weighing with a digital scale to measure body weight. All participant were weight with their school uniform clothes on and barefooted.

Body height was measured using microtoise stature meter with an accuracy of 0.1 cm (20).

Data analysis

The collected data were processed descriptively and analytically. For descriptive data, statistical measures of counts and percentages for categorical and numerical data, by plotting mean, standard deviation, median, and range. When the data are not normally distributed, analyze the data using statistical tests with Pearson's correlation analysis or Spearman's rank correlation. Normality tests used Kolmogorov-Smirnov data, while multiple linear regression analysis was used to analyze simultaneous associations between dietary ratings, oral hygiene behaviors, and oral health status. Use a p-value < 0.05 to determine the significance of test results.

Research ethical aspects

This study has been ethically reviewed by the Research Ethics Committee of Padjadjaran University No: 463/UN6.KEP/EC/2022.

Results

Ninety-six students completed questionnaires and anthropometric measurements. Table 1 shows the characteristics of the participants. As seen from the characteristics table, the average age of adolescent girls in both schools is 13.8 years old, with a range of 12–15 years. Family socioeconomic status was predominantly middle class (47.9%); most educational qualifications were classified as low 43.8% (secondary school and below), and anthropometric findings were described as normal (85.4%) based on BMI/age and based on MUAC was normal (52.1%).

A description of the variables studied; the oral hygiene behavior score, the eating behavior score, and the oral health status score are presented in Table 2. As seen from the table, the data for the three variables based on the normality test yielded a p-value < 0.05; the data are not normally distributed, leading to non-parametric statistics being used for further analysis.

The statistical analysis by Spearman rank correlation in Table 3 shows that the correlation between oral health status score and oral hygiene behavior score was $r = 0.300$; $p = 0.003$, which means that the higher the oral hygiene behavior score, consequently increase the oral health condition score. While the relationship between eating behavior and oral health status in adolescent girls showed a non-significant correlation ($r = 0.082$; $p = 0.429$).

Table 4 presents the comparative analysis of oral health status scores of various characteristics of adolescent girls. From the table, it shows that there is a difference in oral health status scores; the socioeconomic status of the family ($p = 0.033$), the most significant oral health status score, in the middle socioeconomic status with a median = 4, while the lower and upper socioeconomic status with a median = 3. For parental education, there seems to be a tendency that the higher the

TABLE 1 Characteristics of participants ($n = 96$).

Characteristics	Statistics value
1. Age (year)	
• Mean (SD)	13.8 (0.78)
• Median	14
• Range	12–15
2. Parental social-economic status	
• Low	42 (43.8%)
• Middle	46 (47.9%)
• High	8 (8.3%)
3. Parental education	
• Low	41 (42.7%)
• Middle	35 (36.5%)
• Tinggi	20 (20.8%)
4. BMI/Age	
• Thinness	10 (10.4%)
• Normal	82 (85.4%)
• Overweight	4 (4.2%)
5. Mid Upper Arm Circumference	
• Thinness	39 (40.6%)
• Normal	50 (52.1%)
• Overweight	7 (7.3%)

TABLE 2 Statistical description of nutritional (diet) behavior and oral hygiene behavior scores by adolescent oral health status.

Variable	Mean	SD	Median	Range	Normality test data (p-value*)
1. Oral hygiene behavior	75.2	7.20	75	58–85	0.041
2. Diet behavior	9.69	4.00	10	2–22	0.017
3. Oral health status	3.11	1.44	3	0–6	<0.001

*Based on the Kolmogorov-Smirnov test. SD, standard deviation. Significant if p-value < 0.05.

TABLE 3 Correlation of nutritional assessments (eating behaviors) and oral hygiene behaviors with oral health status in adolescents.

Correlation of oral health status scores with	Correlation coefficient (r_s)	P-value
Oral hygiene behavior score	0.300	0.003
Eating behavior score	0.082	0.429

r_s, Spearman rank correlation. Significant if p-value < 0.05.

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TABLE 4 Comparison of oral health status scores based on characteristics.

Characteristics	Oral health score			*P*-value*
	Subjects (n)	Median	Range	
1. Parental social-economic				
• Low	42	3	1–6	0.033
• Middle	46	4	0–6	
• High	8	3	1–5	
2. Parental education:				
• Low	41	3	0–6	0.088
• Middle	35	3	0–6	
• High	20	3.5	1–6	
3. Dietary assessment BMI/ age				
• Thinness	10	2	1–5	0.262
• Normal	82	3	0–6	
• Overweight	4	3.5	1–5	
4. Dietary assessment MUAC				
• Thinness	39	3	1–6	0.262
• Normal	50	3	0–6	
• Overweight	7	3	3–6	

*Based on Kruskal-Wallis's test.

parental education, the higher the oral health status, although statistically not significant (p = 0.088/p > 0.05). Comparison of oral health status scores based on dietary assessment from anthropometric examinations (BMI/Age and MUAC) obtained a p-value = 0.262, which showed no significant difference or in other words anthropometric examination is not significantly related to the oral health status of adolescent girls.

Furthermore, to analyze the relationship between dietary assessment and oral hygiene behavior from various variables (because the data is not homogeneous) with oral health status scores using multiple linear regression analysis. The variables involved in this analysis have a p-value < 0.25 from the results of the bivariable study; parents' education, socioeconomic, eating behavior scores, and oral hygiene scores. The results of multiple linear regression analysis are presented in Table 5.

Table 5 presents the analysis of the relationship between oral health status scores based on multiple linear regression of various variables. The first step is to estimate the model or any variables studied because the variables studied are dietary assessments (eating behavior and anthropometric examination) with oral health status with data that varies on research subjects or is not homogeneous, so that confounding variables such as status socioeconomic and education are also included in the initial model in this multiple linear regression analysis. The second step is to carry out classical assumptions, namely to obtain a model estimated to be feasible or not in interpreting the influence of the independent variable on the dependent variable. The variables involved in the final model have a

TABLE 5 Relationship of oral health status scores with various variables based on multiple linear regression.

Variable	B-Coefficient	SE (B)	t	*P*-value
Initial models				
Parent's education	0.315	0.217	1.449	0.151
Socio-economic status	0.292	0.267	1.093	0.277
Eating behavior score	0.022	0.038	0.585	0.560
Oral hygiene behavior score	0.058	0.020	2.820	0.006
Final models				
Parent's education	0.436	0.181	2.411	0.018
Oral hygiene behavior score	0.060	0.019	3.105	0.003
Constanta	−2.181			

For the final model multiple r = 0.363 (r^2 = 13.2%). B-Coefficient, regression coefficient; SE(B), Standard of Error in coefficient B; t, B-coefficient /SE(B).

p-value < 0.25. From the four initial models that have been analyzed, the final model that relates to oral health status scores is parental education and oral hygiene behavior scores with a positive B coefficient, meaning that the more parents' education and oral hygiene scores increase the oral health status score. The magnitude of the coefficient of determination (r2 multiple) = 13.2%; it means that 13.2% of the variation of the oral health status score is influenced by parental education and oral hygiene behavior scores, and the remaining 86.8% is influenced by other factors not studied. This analysis concludes that the hypothesis is rejected, which states that there is a simultaneous correlation between the evaluation of diet and oral hygiene behavior with the oral health status of adolescent girls.

Discussion

The characteristics of the research subjects obtained based on the inclusion and exclusion criteria were 96 people with an average age of 13.8 years with a range of 12–15 years. According to WHO (21), the age of 10–15 is the early adolescent stage. At this stage, it can be used as a basis for nutritional counseling and planning for youth education programs (21). Some things need to be considered in young women, which is maintaining nutritional status and oral hygiene, starting with preparing themselves from pre-wedding, especially the bride-to-be, until the pre-pregnancy, pregnancy, and postnatal period (22). Based on the 2018 Indonesian Research Data report, 57.6% of Indonesians experience dental and oral health problems in the form of dental caries and periodontal disease. A significant increase in dental and oral issues occurs in adolescents aged 12–18 (23). In addition, the Ministry of Health also released the Effective Medical Demand (EMD) value in adolescent girls, which was higher (9.1) than in teenage boys (7.1) (24). Therefore, adolescent girls are more prone to dental and oral problems than boys.

The results of the correlation analysis of eating behavior scores in this study showed a non-significant relationship; This means that good eating behavior does not necessarily indicate good health status. Assessment of diet (eating behavior) is related to a person's nutritional status. The better a person's nutritional status, the better the dental health status. This is in line with Budisuari's study which states that a person's eating habits or patterns can affect the occurrence of caries, especially if a person tends to consume sugar which is the leading cause of caries. The level of sugar consumption has increased overall in developing countries, and the increase in the prevalence of dental caries in developing countries has been ascribed to the rise in sugar consumption (25). Adolescents begin to decide what to eat without relying on their parents anymore. They also tend to be craving between meals and are very interested in consuming sugary snacks, such as chocolate, candy, and carbonated drinks, which triggers an increase in caries (26). The existence of sweet foods, snacks, and carbonated beverages inside and outside the school complex has reached students to buy and consume them, the interest of teenagers also supports this at this age who are more interested in the taste and appearance of food than its nutritional value (27), but in this study, the eating behavior of adolescent girls was not significantly associated with oral health status. This is understandable because eating behavior is not the only one of the factors that can affect oral health status. Oral health status is influenced by the interaction of four factors: behavior, environment, health services, and genetics. In developing countries such as Indonesia, behavior is indeed the most dominant factor in influencing the status of dental and oral health (28).

In this study, anthropometric examination was not significantly associated with oral health status in adolescent girls. The correlation of anthropometric examination and oral health can be explained as follows; the anthropometric examination is related to a person's nutritional status, and this is in line with the study by Busman and Atigah which states that nutritional status is a sign of the body's appearance caused by a balance between nutrient intake and health expenditure as seen through the variables of height, weight, and growth. Lack of nutrition is caused by various factors, including infectious diseases and food intake (29). Another study from Ratnasari and Junaidi (30), the effect of dental caries can cause disturbances in the digestive process and eating difficulties that generate growth and development disorders, and vice versa, meaning that the better a person's nutritional status, the better the dental health status. Nutrition is essential in developing and defending oral health, especially teeth and gingiva. Healthy or unhealthy conditions of teeth and gingiva can affect food intake. In adolescents with dental caries, there is often a disturbance in the information about food substances which is a factor causing lack of nutrition, and it can cause a decrease in the body's biological function or malnutrition (29). The oral health is part of the health of the body that affects each other. In this study, anthropometric assessments of adolescent girls were not significantly associated with oral health status. This is consistent with Nurlaila and Herwati a survey conducted in Karangantu District, Banten Province, that found no significant association between nutritional status and dental caries in school children aged 9–14. This situation is more because cariogenic food can have a direct impact as the cause of dental caries if it is supported by a state of low tooth resistance, a form of saliva that is less than normal and dense (31).

Adolescents are not only physically mature but also cognitively and emotionally. They seek identity, strive to be independent and acceptable, and pay a great interest to their self-appearance. These changes significantly impact eating behavior and skipping between meals, snacking, and café's dine in. These habits are further influenced by families, groups, and the media. In addition, the influence of the physical environment, such as air that has experienced air pollution and extreme temperatures, as well as physical activity, although generally considered positive, can give stress to the body, affecting a person's nutritional needs. In addition, the influence of the non-physical environment, such as the family and community environment, socio-cultural factors, and socioeconomic factors, also affect the nutritional status of adolescents (26).

The results of the correlation analysis of oral health status scores with oral hygiene behavior scores showed that there was a significant relationship (rs = 0.300; p = 0.003). This means that the higher the oral hygiene score, the higher the oral health status score. Good oral hygiene behavior will affect oral health status. Study by Budisuari stated that tooth brushing behavior affects the occurrence of caries. This is related to the process of caries itself, where if sucrose stays in the mouth for a long time and is not cleaned immediately, it will lead to the possibility of caries. This follows Gustafson's opinion, stating that sugar consumption increases caries attack activity. The most significant risk is if sugar is eaten in a form that is easily attached and not cleaned immediately (32). Another study that supports this opinion conducted by Stephen, stated that after consuming carbohydrates, the pH of the mouth will drop in 5–10 min to 5.5, which is acidic, but will return to normal within 30–45 min, with the addition of sucrose in the form of drinks, bread, chocolate, caramel and candy between meals can cause an increase in caries activity. Still, if consumed at mealtimes, the formation rate will be reduced, or in other word, no caries will form (33). Two approaches can be taken to improve one's oral hygiene. First, the individual approach through accelerating the increase in the ability to help oneself behave in a healthy life. For individuals who suffer from systemic disorders and periodontal disease, the behavior of maintaining oral hygiene should be intensified. The individual approach includes age, knowledge, children's dependents, type of work, expenses, sources of costs, distance to the dentist, smoking habits, attitudes, and actions. Meanwhile, another study stated that individual factors include

oral hygiene, frequency of brushing teeth, and eating acidic foods (PH < 7) (34).

The second approach, the contextual approach, includes the ratio of dentists to the population, the ratio of hospitals to residents, the ratio of dental clinics to residents, and the ratio of health centers to residents. Other contextual factors are the source of drinking water and the acidity of the water. Piped drinking water in all areas and distribution is under the supervision of the local PDAM, as well as water acidity tests by the health center are carried out periodically. In addition, other contextual factors also include the availability of dental nurses, dentists, per capita health budget, and other environmental factors that significantly influence the prevalence of oral dental disease (34). From the results of multiple linear regression analysis, the final model that relates to the oral health status score is the oral hygiene behavior score, with a positive B coefficient, meaning that the higher the oral hygiene score, the higher the oral health status score. From the magnitude of the coefficient of determination (r2 multiple) = 13.2%, it means that 13.2% variation of the score of oral health status is influenced by parental education and oral hygiene behavior scores, and the remaining 86.8% is influenced by other factors not studied. H L Blum stated that the degree of health of a person or society is influenced by four factors, namely: environment, behavior, heredity, and health services. According to Laurence Green, three factors influence a person's behavior, namely predisposing factors, supporting factors, and reinforcing factors. Health behavior is divided into knowledge, attitude, and action. Knowledge is a very important domain for the formation of one's actions. Factors that affect a person's knowledge are education, occupation, age, interests, experience, and ease of getting information (35).

Adolescents' dental and oral health maintenance is often neglected, while adolescents are also vulnerable to oral health problems during puberty. In addition, many bad habits of teenagers can cause damage to the teeth and mouth. These bad habits include laziness of night time tooth-brushing, the practice of consuming sweet foods, and the pattern of drinking sweet or soda beverage (36). Contextual factors that influence oral health status are environmental factors and followed by behavioral factors. Environmental factors affect the oral health status of about 40%, and behavioral factors affect about 30%, so it can be said that environmental and behavioral factors will affect more than two-thirds of oral health status in the community (34). Personal factors that play a role in improving oral health status include age and gender. In addition, education, occupation, economic status, knowledge, attitudes, and actions also play a role in improving oral health status. Patterns of healthy living behavior of individuals and families, non-smoking behavior followed by the ability to help oneself also affect oral health. Research in the UK states that social factors, on individual factors, are the main determinants of dental and oral health status. Individual factors were obtained from the frequency of tooth brushing, oral hygiene, and eating habits of vinegar/acidic food (PH < 7) (5, 34).

Nutrition and oral health have a two-way relationship; proper nutrition is essential in maintaining oral health. Otherwise, oral health is also crucial for maintaining adequate nutritional intake. Untreated dental caries can cause pain, causing not only eating problems but also speech and sleep problems. Furthermore, these eating disorders can have long-term impacts such as iron deficiency, anemia, and malnutrition (37–39). In a study by Morenike et al. (40) in Nigeria, it was stated that malnutrition could lead to enamel hypoplasia, which creates a niche environment for plaque retention. Furthermore, malnutrition results in hypofunction of the salivary glands, changing saliva composition and reducing its buffering capacity, thereby increasing caries formation. In addition, the study found that nutritional deficiencies were associated with oral hygiene. Previous studies also conducted in Nigeria have identified that oral hygiene is associated with a higher prevalence of caries. In contrast, other studies have highlighted the increased prevalence of caries due to poor oral hygiene (40). Although nutrition and oral hygiene can affect the oral health status of young women, which will have an impact on their quality of life (one of which is malnutrition which later as a mother-to-be can cause the risk of stunting in her child), many other factors that have been described previously, can also affect oral health status in adolescent girls. Therefore, the limitations of this study are the existence of variables or other factors related to oral health, both individually and family dimensions, as well as overall, as well as the use of self-assessment that is not supported by clinical examinations due to the COVID-19 pandemic.

Conclusion

There is a relationship between oral hygiene behavior and adolescent girls' oral health status. However, the simultaneous relationship between dietary assessment and oral hygiene behavior with the oral health status of adolescent girls was not proven—similarly, the relationship between dietary assessment and oral health status of adolescent girls.

The above indicates the need for further research on other factors that can be related to oral health so that it can complement the research that has been done so that it can become a study in stunting prevention, as well as dental and oral examinations on research subjects.

Oral health and nutrition education with health promotion and nutrition counseling. It is hoped that there will be a collaboration with other professions, including doctors, parents, schools, nutritionists, health educators, and the youth health community who can help regulate adolescent nutrition. In addition, it is recommended to visit a dental polyclinic, a dentist's private practice or a health center that must be done at least every 6 months.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Research Ethics Committee of Padjadjaran University No: 463/UN6.KEP/EC/2022. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AS and RI conceptualized the study design. YM and AS collected the data. YM analyzed the data. IM, AS, and YM conceptualized the first draft. RI reviewed the first draft. AS finalized the writing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Nutritional factors influencing microbiota-mediated colonization resistance of the oral cavity: A literature review

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The oral cavity is a key biocenosis for many distinct microbial communities that interact with both the external environment and internal body systems. The oral microbiota is a vital part of the human microbiome. It has been developed through mutual interactions among the environment, host physiological state, and microbial community composition. Indigenious microbiota of the oral cavity is one of the factors that prevent adhesion and invasion of pathogens on the mucous membrane, i.e., the development of the infectious process and thereby participating in the implementation of one of the mechanisms of local immunity–colonization resistance. The balance between bacterial symbiosis, microbial virulence, and host resistance ensures the integrity of the oral cavity. In this review we have tried to address how nutritional factors influence integrity of the oral indigenous microbiota and its involvement in colonization resistance.

KEYWORDS

oral microbiota, biofilm, colonization resistance, nutrition, microbial composition, competition, synergism

Introduction

The oral cavity is considered a unique ecological system, creating favorable conditions for the vital activity of manifold commensal microorganisms that may reside either as planktonic cells or inhabit the biofilms (1). These microbial communities contribute to oral and systemic health by maintaining homeostasis and modulating the immune system (2). The oral cavity becomes colonized with a microbiota, the composition and characteristics of which reflect the local aspects, including potential nutrients, receptors for adhesion, oxygen levels, microbial competitors/collaborators, and local innate and adaptive immune factors. The presence of certain nutrients

can lead to a defined spatial architecture within the oral biofilms and contribute to colonization resistance.

Colonization resistance of the oral cavity is a complex, multifaceted phenomenon and characterizes the ability of the resident microbial community to oppose the invasion by exogenous microorganisms. The colonization level, in general, depends on how well the host oral cavity is suitable for growth and on the physiological requirements of the microorganisms. Several principal factors known as "colonization barriers" control the microbial background in direct or indirect pathways (3, 4). Qualitative and quantitative changes in microbiome accompanying the oral cavity's diseases have been characterized and studied in sufficient detail over the past decade (5, 6).

Especially prominent is the "mucous barrier," which consists of mechanical, humoral, and other factors protecting the mucous membrane from the colonization of harmful microorganisms (7). In general, the mucous membranes of the lips, palate, cheeks, tongue, gums, teeth, and saliva provide a favorable environment for the growth and reproduction of a wide range of microorganisms. These surfaces are typically densely colonized by complex microbial communities interacting through sophisticated biochemical and biophysical mechanisms (8). Many published studies mention antagonistic activity and adhesiveness of the resident microbiome as the main factors in maintaining intestinal colonization resistance (9). However, the colonization resistance of the oral cavity mediated by nutritional factors remains poorly understood in many aspects. Therefore, the present review furnishes a brief overview the main mechanisms and factors responsible for nutrient-related colonization resistance of the oral microbiota.

Materials and methods

A comprehensive literature search was carried out in the online version of the Science Citation Index Expanded (SCI-EXPANDED) from the Web of Science (WoS) database. WoS was chosen as it covers multidisciplinary areas being the oldest citation database. No time restrictions were placed on these searches, and only articles published in English were retrieved. The date when all searches were last performed was September 23, 2022. The search strategy combined three search strings: #1 "oral microbiome" OR "oral microbiota" OR "oral microbiocenosis" OR "oral microbial communities"; #2 "colonization resistance" OR "bacterial interference"; #3 "nutrition" OR "diet" and combining these by "AND" to obtain only the intersection. Results were imported into a bibliographic referencing tool (EndNote 20) and assessed for relevance and quality, removing articles that have no relation to the review topic. Finally, the query results were manually checked before excluding duplicates (**Figure 1**).

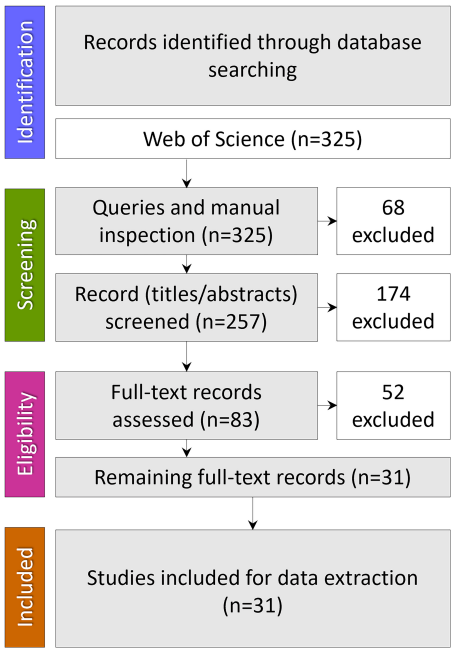

FIGURE 1
Data identification, screening, eligibility, and inclusion.

Results

Our set of queries identified three hundred twenty-five records; after manual inspection and excluding the duplicates, 257 remained. Of these, 174 articles were excluded at the title/abstract level and 52 at the full-text assessment level. Thirty-one were found to meet inclusion criteria to describe the nutritional factors influencing microbiota-mediated colonization resistance of the oral cavity and were used in the analysis.

The evaluation of the keywords in the included studies is valuable to provide a detailed picture of the review topic, reflecting the research hotspots in the current discipline. Here, the publication keyword analysis to word cloud visualization (Biblioshiny app from the Bibliometrix-R package) revealed that the most common keywords of the thirty-one studies were oral, microbiota, biofilm, formation, saliva, colonization, and resistance (**Figure 2**). This emphasizes that most studies have focused on biofilm formation and colonization resistance in the oral cavity, as well as saliva's role in determining the oral microbiota composition.

Colonization and principal composition of the oral microbiome

The mean total surface area of the mouth is $214.7 \pm 12.9\ cm^2$ (10), and the mean surface area of the oral mucosa is

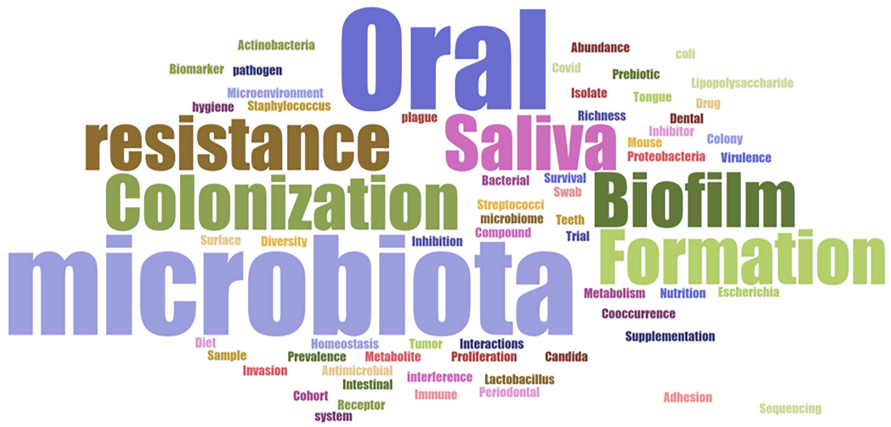

FIGURE 2
Word cloud based on the main keywords related to the review topic.

196.96 ± 24.20 cm^2 (11). Each of the anatomical surfaces of the oral cavity, including tooth, gingival sulcus, tongue, hard and soft palates, tonsils, and saliva, is covered with a conglomerate of microorganisms (12, 13). In addition, a large number of microbes are located on the back of the tongue, in the cracks, crevices, and fissures of the tonsils, and in gingival pockets (14).

Intestinal colonization gets its origin from the oral cavity. As the oral cavity is in constant contact with the external environment, it is populated by microorganisms representing a complex biocenosis. In other words, microorganisms making up the microbiocenosis of the oral cavity are intrinsically diverse in their abundance and properties (15). Various microbial taxonomic groups colonize the oral cavity as a kind of ecological niche involving biochemical, immunological, and other interactions with the host. The evolutionary complex and symbiotic communities of microorganisms are therefore specific for a particular area of the mucosal surfaces.

Approximately 99% of all bacteria live together as a biofilm, forming spatially and functionally complex communities (16). Biofilms act as protective shells, making inhabitants more resistant to physical, chemical, and biological factors in comparison with planktonic (free-floating) bacteria (17, 18). In addition to that the biofilm polymers provide adhesion, stabilization, and nutrient flows within the biofilm (19).

Microbial populations in the oral cavity can be divided into resident and transient groups. The resident (indigenous) microbiome includes relatively constant species characteristic of a certain oral biotope and the age of the host. An indigenous microbiome can be divided into core (shared by all host organisms) and variable (due to physiological and biochemical differences between individuals) categories. The transient (exogenous) microbiome consists of non-pathogenic or opportunistic microorganisms that populate the oral cavity for a limited period without causing disease (20). In case of violations or loss of the indigenous microflora, members of the transient can replace the "vacant" niche of a specific biotope that subsequently can contribute to the development of pathology.

The Human Oral Microbiome Database (HOMD[1]) has been created to systematize the bacteria in the human oral cavity, which includes both members of normal microflora and pathogens. HOMD collected 16S rRNA gene sequences from oral prokaryote species into a curated phylogeny-based database. The HOMD contains approximately 772 microbial species, where 70% are culturable, and 57% of which are officially named. Most of the HOMD-listed bacterial species belong to transient microflora since they are not capable of long-term survival under special conditions of the oral cavity. The 16S rDNA profiling of the healthy cavity categorized the inhabitant bacteria into six broad phyla, namely, *Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Bacteroidetes,* and *Spirochaetes* constituting 96% of total oral bacteria (21).

Streptococcus is the most abundant genus in the oral cavity (8). In the HOMD, the *Streptococcus* genus is represented by 37 species, of which 29 are named, four are not named, and four are lost. The species of *Streptococcus* occupy a specific niche in the oral cavity and thus play a key role in establishing and shaping the oral microbiota (22). *S. gordonii* and *S. oralis* are among the first microorganisms that colonize the oral cavity, followed by cryogenic *S. sanguinis*, *S. mutans,* and *S. sobrinus*, initiating biofilm formation (21). Other pioneer organisms include *Actinomyces* spp. *Granulicatella adiacens*, *Abiotrophia defectiva*, *Gemella* spp., and *Rothia* (23). Diverse molecular forces, including hydrogen bonds, hydrophobic interactions, calcium bridges, van der Waals forces, acid-base interactions, and electrostatic interactions, contribute to the attachment of pioneer bacteria to the salivary acquired pellicle (a layer of proteins and glycoproteins of salivary origin that tightly coat the tooth surface) (24). The early colonizers are predominantly

1 www.homd.org

members of the normal microbiota, and just a few are known to be directly responsible for disease development (20). Species of *Streptococcus* initiate numerous cooperative and antagonistic bacterial interactions within the dental microbial community. Thus, mainly streptococci determine and shape the composition of later colonizers in the oral biofilm and greatly impact the health or disease status of the host (22, 25). Polymicrobial colonization and biofilm development have been well-described and depicted by D. Verma et al. (21).

The role of the normal oral microbiota

Normal microbiota performs protective functions due to indirect antagonism toward pathogenic and opportunistic microorganisms, particularly by preventing colonization of mucous membranes and diminishing the penetration of microbes, microbial toxins, and xenobiotics into the host organism (26, 27). Additionally, the functions of normal oral human microbiota include:

- the regulation of the gas composition of the intestine and other cavities of the host;
- morphokinetic effect;
- the production of enzymes involved in the metabolism of proteins, carbohydrates, lipids, and nucleic acids;
- production of biologically active compounds (vitamins, antibiotics, toxins, hormones, etc.); immunogenic role;
- participation in the recirculation of bile acids, cholesterol, and other macromolecules; mutagenic/antimutagenic role;
- detoxification of exogenous and endogenous substrates and metabolites;
- source of endogenous infection and,

storage of microbial plasmid and chromosomal genes (28–30).

Nutritional factors influencing the oral microbiota

Saliva is the medium by which the host "supplies" its resident microorganisms with nutrients, including amino acids, proteins, glycoproteins, peptides, and vitamins. In addition, a host-derived nutrient, gingival crevicular fluid (GCF), favors the growth and activity of microorganisms in the oral cavity. In a smaller proportion, the gingival crevice, through GCF secretion, contributes with additional nutrients such as albumin and heme-containing molecules as a source of vital iron (31). Host hormones, such as sex steroid hormones, cholesterol, and catecholamines, delivered through saliva can also be utilized by resident bacteria (32). Many studies suggest that these hormones have the potential to modulate the composition of the oral microbiome (33, 34).

Despite the obvious impact of diet on the oral microbiome, relatively scant information is available regarding this. This can partly be explained by the fact that the primary substrates for oral microbial growth are endogenous nutrients provided by saliva, tissue excludes, GCF, degenerating host cells, or other bacterial metabolites (35).

Studies by Hatakka et al. and Jiang et al. have shown no difference in the growth rates of oral bacteria in the presence or absence of food, indicating no relationship between diet and the composition of oral bacterial communities (36, 37). In contrast, another very recent study by W. G. Wade revealed differences in salivary metabolomic profiles in relation to diet type (omnivorous, ovo-lacto-vegetarian, or vegetarians) (38).

Reduced food intake and fasting periods may affect microbiome-based colonization resistance. The salivary flow and secretion stasis due to dehydration or decreased oral water intake retrograde bacterial migration and colonization (39, 40). Fasting has also been found to be associated with oral cytokine levels caused by resident and transient microbiome (41).

Oral colonization resistance

The composition of microbial communities in different biotopes of the oral cavity is determined by environmental and biological factors, giving rise to synergistic or antagonistic relationships (**Figure 3**). Especially antagonistic relationships between different groups of microbes can be induced by various factors [such as lack of saliva, its bactericidal substances, stimulants (e.g., smoking), increased sugar content, acidic microenvironment] that alter the microbial community structure subsequently impacting colonization resistance (8).

Colonization resistance (also known as bacterial interference) refers to a set of mechanisms providing individual specificity and stability to the microbial community and preventing the host surfaces colonization by pathogens. The term "colonization resistance" was coined by D. van der Waaij (42) who also pointed out that the normal microbiota being a combination of many microbiocenoses characterized by a certain composition and occupying a particular biotope in the human body, plays deciding role in such resistance (43).

In the case of weakened colonization resistance, the fraction of "core" bacteria resident for the surfaces of the human body reduces, while the number and spectrum of potentially pathogenic microorganisms increase. This can lead to their translocation to internal organs and even to the development of purulent-inflammatory processes (44, 45).

Germ-free animals are the primary models showing the pivotal role of resident microflora in colonization resistance and overall health. For example, P. D. Marsh has shown that the absence of resident microbiome has a negative impact on its

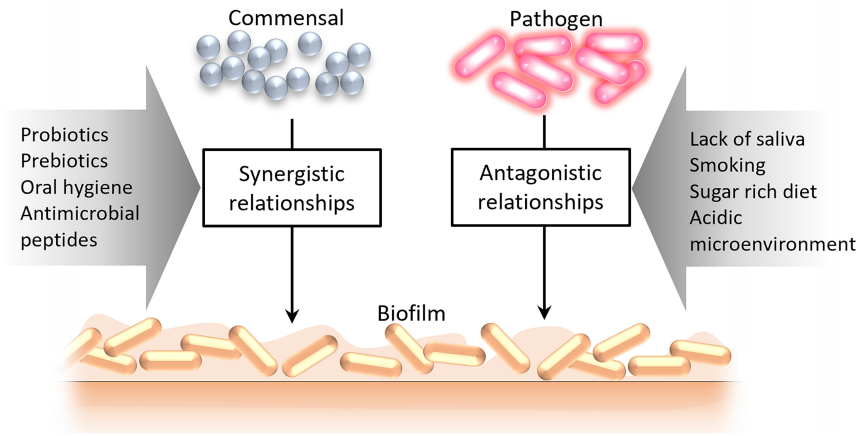

FIGURE 3
Factors leading to the formation of synergistic and antagonistic relationships between oral microorganisms.

host, leading to thinning of the intestinal walls, ill-developed villi, poor nutrient absorption, vitamin deficiencies, caecum enlargement, etc., (46). Later, further experimental validations of the role of normal microflora in preventing infections for different microbial models have reported (47–49).

According to Marsh and Percival (50) the mechanisms mediating colonization resistance can be divided into (a) competition for nutrients, (b) competition for attachment sites, (c)production of antagonistic compounds, and (d) creation of adverse environmental conditions for exogenous microorganisms (**Figure 4**). Here, we would like to update and address the abovementioned factors in more detail.

Nutrient-related factors of colonization resistance

Temperature, pH, atmospheric conditions, salinity, redox potential, sheer and mechanical forces, chemical exposure from hygiene practices, and water of saliva affect the formation of biofilms in the oral cavity (51). However, overall microbial biomass and its composition become considerably controlled by competition for nutritional substances that can be utilized at a low redox potential conditions and in the presence of metabolic inhibitors synthesized by oral microorganisms (52). Salivary amino acids, peptides, GCF, and glycoproteins (such as mucin) are the main limiting nutrient sources for bacteria inhabiting dental plaques. Saliva is the primary nutrient source for bacteria that reside in supragingival biofilm, while GCF provides nutrients for bacteria of the subgingival biofilms (53).

The exact composition of saliva, and therefore the availability of particular nutrients, displays significant interindividual differences as well as high temporary variability (54). In general, resident bacteria outcompete periodontopathogens in the uptake of these nutrients (55–57). However, periodontitis-associated microorganisms destruct tissue through degradation of the extracellular matrix, which leads to additional release of specific nutrients (heme-containing compounds, sources of amino acids, and iron). These nutrients are carried into the gingival crevice through GCF, which favors the atypical growth of asaccharolytic and proteolytic microorganisms with iron-acquisition capacity in the subgingival region (15).

"Food sharing" through bacterial metabolic products also strongly shapes the microbial composition, by encouraging the growth of some species while averting others. For instance, lactic acid, produced by *Streptococcus* and *Actinomyces* in the mouth as a result of carbohydrate fermentation can be utilized by *Veillonella,* allowing for menadione production that is, in turn, important for the growth of *Porphyromonas* and *Prevotella*. *Fusobacterium* produces fatty acids that are used by *Treponema*. *Porphyromonas* cal also cooperate with *Treponema* to generate end metabolites that are utilized by *Mogibacterium timidum* (58).

As shown by Van Hoogmoed et al., some conventional oral commensals, such as *S. sanguinis, S. cristatus, S. salivarius, S mitis*, and *A. naeslundii*, decrease the ability of a pathogen *Porphyromonas gingivalis* to adhere to the substrate and retrieve essential nutrients (59). Under optimal conditions, *L. lactis,* a member of the normal oral microbiota, produces nisin, a bacteriocin that mitigates pathogen-mediated oral tumorigenesis (36, 60). Numerous mutualistic nutritional behaviors have also been observed for bacteria growing in saliva as their sole nutrient source (37, 61).

One of the emerging therapeutic approaches could be the introduction of probiotic bacteria which may prevent pathogen colonization in the oral cavity by limiting their adhesion and producing antimicrobials that selectively target disease-associated bacteria (62, 63). For instance, *Streptococcus salivarius* displayed properties compatible with their potential use as probiotics antagonizing *Streptococcus pyogenes* (64–66). However, the main disadvantage here is that the presence of

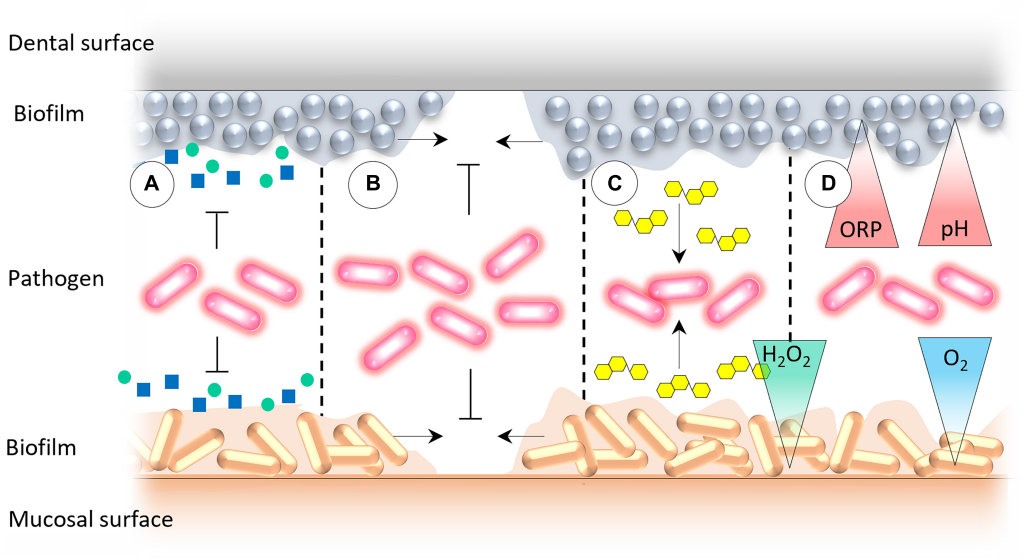

FIGURE 4
The mechanisms that underpin the oral colonization resistance: **(A)** Competition for essential nutrients and co-factors for microbial growth, **(B)** competition for binding sites for microbial attachment on mucosal and dental surfaces, **(C)** production of antagonistic compounds by the resident oral microbiota, and **(D)** creation of adverse environmental conditions that prevent the growth of exogenous microorganisms.

probiotic bacteria can be only transient since they are not indigenous to the oral cavity. Therefore, one more attractive therapeutic strategy could be the nutritional stimulation of indigenous bacteria, promoting oral health.

Indeed, prebiotic substances often induce desirable microbial composition and activity changes, thus delivering local health benefits (67–69). For example, Slomka et al. (69) observed that potential oral prebiotic compounds, such as beta-methyl-D-galactoside and N-acetyl-D-mannosamine selectively stimulate beneficial commensal bacteria of the resident oral microbiome while suppressing the growth of pathogenic bacteria.

Colonization resistance affects the oral ecology in health and disease by preventing or modulating the prevalence of specific microbial groups. Many clinical studies revealed that nutritional compounds are the key factors that alter the oral microbial composition by colonizing microbial biofilms, co-aggregating, and competing with pathogenic bacteria, subsequently reducing/replacing their numbers (**Table 1**).

Other types of colonization resistance

Competition for microbial attachment sites on mucosal and dental surfaces

A large number of bacterial species appear to exhibit specific tropism in relation to various anatomical surfaces of the oral cavity. By examining 40 bacterial species, Mager et al. (80) have shown that bacteria that inhabit numerous oral cavity surfaces use very many different receptors and adhesion molecules that define the formation of biofilms (29). Resident oral bacteria form a robust and tight biofilm on the surface of mucous membranes, hindering the adhesion of foreign microorganisms. The most prominent mechanism inhibiting biofilm formation and inducing detachment of extrinsic bacteria from the native biofilm is known as biosurfactant action. Several *in vitro* studies indicated that many bacterial species, especially *Streptococcus*, rely predominantly on this method to prevent foreign colonization of the oral cavity (59, 81, 82). This and other attachment-related strategies are briefly summarized in **Table 2**.

Production of antagonistic compounds (inhibitory metabolites)

End products of metabolism of resident microflora are also used for effective protection against extraneous colonization. The antagonism of microorganisms that make up the normal microbiota concerning potentially pathogenic bacteria is due to the production of bacteriocins, lysozyme, and other substances (**Table 3**).

Various bacteriophages represent very abundant and interesting group of oral antimicrobial agents. Oral phages are able to invade many other bacteria besides their putative bacterial hosts. Therefore, phages strongly shape the ecology of oral bacterial communities, accelerate their molecular diversity and help to acquire new gene functions (115–117).

In addition to the metabolic antimicrobials of the microbiota listed in the **Table 2**, various organic acids should be mentioned, such as short-chain fatty acids (SCFAs), that may act as inhibitory factors (119, 120). Though SCFAs are mainly produced in the intestines, they also contribute to preventing colonization by pathogenic microorganisms in the oral cavity (55).

TABLE 1 Clinical studies to assess the significance of colonization resistance of the oral cavity.

Study type	Patients	Evolved microorganisms	Results	Ref.
Assessment of the microbial prevalence in the oral flora of patients with advanced cancer	Patients with advanced cancer	Yeasts, coliforms, and coagulase-positive staphylococci	A loss of colonization resistance of the oral mucosa during advanced cancer	(70)
The development of new non-invasive differential diagnostic criteria for severity of bronchitis	Children with acute bronchitis	Oral streptococci, *Candida albicans*	Children with bronchitis had significantly lower colonization index and anti-adhesive saliva activity than the control group	(71)
The role of colonization resistance of the oral mucosa in the influence of individual-typological characteristics	Individuals susceptible to caries and inflammatory periodontal diseases	Oral streptococci	Reducing the oral colonization resistance diagnosed in emotionally unstable introverts correlated with their low resistance to periodontal diseases	(72)
The colonization resistance state of the oral mucosa of patients and their dependence on the intensity of the teeth carious	Young patients with different body mass indices	Oral streptococci	In patients with 1st and 2nd degree obesity, in 70% of patients, suppression of oral colonization resistance was observed, compared with patients with average body mass index	(73)
The early diagnosis of the oral cavity's microecological disorders that assess colonization resistance	Patients with caries and catarrhal gingivitis	Oral streptococci	The development of dental caries and catarrhal gingivitis is accompanied by a decrease in the level of colonization resistance of the oral cavity	(74)
The study of *S. salivarius* to produce a variety of bacteriocin-like inhibitory substances	Healthy patients	*Streptococcus salivarius*	Prevention of streptococcal pharyngitis by anti-*S. pyogenes* inhibitory substances produced by *S. salivarius*	(66)
Application of probiotics Bifidumbacterin in the therapy of periodontal inflammations	Patients with gingivitis and different degrees of periodontitis	*Bifidobacterium bifidum*	Probiotics had a positive effect on the normalization of oral colonization resistance	(75)
Bacillus subtilis, as an effective probiotic for prevention of periodontitis	Patients with periodontitis	*Bacillus subtilis*	Mouth rinsing with *B. subtilis* significantly reduced periodontal pathogens	(76)
Reducing the prevalence of oral *Candida* by probiotic-containing cheese	Elderly people	*Lactobacillus rhamnosus, L. rhamnosus, Propionibacterium freudenreichii* ssp. *shermanii*	The probiotic intervention reduced the risk of high *Candida* counts by 75%, and the risk of hyposalivation by 56%	(62)
Assessment of the probiotics to treat gingivitis and evaluation of its influence on plaque	Patients with moderate to severe gingivitis	*Lactobacillus reuteri*	*L. reuteri* was efficacious in reducing gingivitis and plaque through colonization	(77)
Examination of possible effects of *Bifidobacterium* in yogurt on caries-associated microorganisms	Healthy young adults	*Bifidobacterium*	Probiotic bifidobacteria may reduce the levels of selected caries-associated microorganisms in saliva	(78)
Assessment of the beneficial effects *L. rhamnosus* in the oral cavity for long-term caries prevention	Children with the risk of caries	*Lactobacillus rhamnosus*	*L. rhamnosus* was found to reduce the risk of caries significantly, showing antagonism to *Streptococcus mutans*	(79)

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TABLE 2 The mechanisms involved in the competition for attachment sites of colonization resistance.

Mechanisms	Examples	Ref.
Interruption of biofilm formation	*S. cristatus* inhibits the expression of FimA, a gene encoding the major protein subunit of *P. gingivalis* fimbriae	(83–86)
	S. intermedius produces arginine deaminase that can repress the expression of FimA and Mfa1 (minor fimbria) in *P. gingivalis*	(87)
Detachment of microorganisms from the biofilm	The transcriptional regulator Nrg1p controls *Candida albicans* biofilm dispersion	(88)
	Modification of the protein composition of the binding site, which is necessary for adhesion of *S. mutans*	(89)
Production of biosurfactants that prevent adhesion	A biosurfactant generated by S. *mitis* decreases the adhesion of *S. mutans* and several periodontopathogens.	(90)

TABLE 3 Production of inhibitory factors by the resident oral microflora that contribute to "colonization resistance".

Antagonistic agent	Produced by	Against	Ref.
Mutacin, nisin, etc., (lantibiotics and non-lantibiotics)	*S. mutans, Lactococcus lactis*	Gram-positive bacteria, in particular, other streptococci	(91–95)
Sanguicin	*S. sanguinis*	*S. agalactiae* and *S. uberis*	(96)
Salivaricin	*S. salivarius*	a range of streptococci	(96–98)
Reuterin	*Lactobacillus reuteri*	many members of Gram-positive and Gram-negative bacteria	(99)
A bacteriocin	*Lactobacillus paracasei*	*P. gingivalis, Prevotella intermedia, Tannerella forsythensis, S. salivarius, and S. sanguinis*	(100, 101)
Nigrescin	*Prevotella nigrescens*	*P. gingivalis, T. forsythia,* and *Actinomyces* species	(102)
A bacteriocin	*Fusobacterium nucleatum*	a wide range of Gram-negative and Gram-positive bacteria	(103, 104)
Hydrogen peroxide	*S. gordonii*	*S. mutans*	(92, 105)
	S. sanguinis	a range of Gram-positive species	(106–112)
	S. saprophyticus, S. infantis, and S. sanguinis	non-oral *Escherichia coli*	(113)
	S. oligofermentans	*S. mutans*	(114)
Lytic phages	numerous species	numerous species	(115–117)
Nitrite	*S. parasanguinis, S. sanguinis, S. gordonii*	*P. aeruginosa*	(118)

Creation of microenvironments that inhibit the growth of exogenous bacterial species

Here the competition is enabled due to altered environmental conditions, such as pH, oxygen pressure, redox potential, etc., in oral biofilms. The members of *Lactobacillus* and *Streptococcus* are the powerful acid producers, making the local pH drop as low as 4.5, thus dramatically suppressing the growth of all acid-sensitive bacteria (121). Suppression of *S. sanguinis* by mixture of organic acids produced by *S. mutans* has been mentioned in many studies as well (112, 122, 123).

The factors affecting/influencing colonization patterns

In healthy people, the microbial composition of the oral cavity depends on the physiological and ecological aspects of the host, such as age, nutrition preferences, oral hygiene, anatomical features of the oral cavity, hormonal status, general somatic state, etc., (124). The richness and composition of the oral microbiome are relatively stable due to moisture availability, the constant presence of antimicrobial substances (nisin, diplococcin, acidophilus, lactocidin, lactolin lysozyme, amylases, immunoglobulins A, G, M), organic acids (lactic, acetic, ketoglutaric and succinic) and the state of general cellular and humoral immunity (12).

As described in the previous parts, the colonization resistance is determined by factors of microbial, exogenous, and host origin (125, 126).

Microbial factors

Each human individual is characterized by a specific genetically determined spectrum of microorganisms. As we already saw, the normal microflora plays a vital role in the antimicrobial defense system of the oral cavity. The term "normal" indicates a microbial population that colonizes various ecological niches of the healthy oral cavity and takes part in the metabolism of nutrition, protects against highly virulent bacteria by blocking receptors of epithelial cells from adhesion of pathogens, stimulates the immune response, and produces biologically active substances which regulate metabolic processes (127).

Exogenous factors

A person's normal microflora should also be always considered in the context of the whole organism and its environment. The oral cavity is the very beginning of the digestive tract and serves as the "primary portal" for chemical substances and foreign microorganisms. Therefore, numerous external factors affecting the body also affect the microflora. The most pronounced and well-characterized phenomena depend on colonization resistance from smoking status, alcohol consumption, diet (quality and quantity), socioeconomic status, and antibiotic use (125). Smoking is a major environmental factor associated with the pathophysiology of oral diseases. Toxic components in cigarette impact oral microbiota directly or indirectly through oxygen deprivation, immunosuppression, biofilm formation, or other potential mechanisms, leading to loss of colonization resistance (128). Despite different sampling sites, numerous studies have shown the predominance of *Fusobacterium nucleatum* and *F. naviforme* in oral from smokers compared with non-smokers (129). Alcohol consumption may also affect oral microbiota composition affecting functional microbial pathways. Thomas et al. observed the reduced bacterial richness in the oral biofilm of alcohol drinkers (130). Liao et al. found that the genus *Prevotella* and *Moryella* were significantly enriched in drinkers; meanwhile, the genus *Lautropia*, *Haemophilus,* and *Porphyromonas* were depleted significantly (131). Studies indicate that socioeconomic status may alter oral microbiota community structure and higher diversity (132, 133). The oral microbiota is a major reservoir of antibiotic-resistant bacteria; many studies have demonstrated that using amoxicillin, erythromycin, and tetracycline changes oral microbiota composition and enriches bacteria resistant to antibiotics (134, 135).

Host factors

Host mechanisms involved in the colonization resistance phenomenon include mucosal desquamation, the antimicrobial effect of secrets, the composition and quantity of mucin, oxygen tension along with the thickness of the biofilm, the pH of the medium, the rate of renewal, maturation and metabolism of mucosal epithelium, innate, and adaptive immune mechanisms, etc., (136). The immune factors, in turn, can involve macrophage activity, lysozyme, lactoferrin, other bactericidal substances of leukocytes, as well as a variety of immunoglobulins, primarily IgA, which prevent microbial adhesion and thus promote the removal of extraneous microorganisms to the external environment (137). The antibacterial potential of saliva on the one side and the number of microorganisms in the oral cavity on the other side exist in dynamic balance. Any infringement of the former leads to disturbances of the normal microflora and the emergence of pathogenic microorganisms by developing various types of pathology in the oral cavity. However, the main functional properties of the host antimicrobial system of saliva not only include suppression of microflora but also effectively control its qualitative and quantitative composition at a level sufficient to maintain microbiocenosis (31, 138).

Global health relevance

Dental infection and antibiotic resistance remain important global health concerns with significant morbidities. There are convincing scientific pieces of evidence that impaired oral health potentiates the severity of numerous systemic diseases, such as endocarditis, diabetes mellitus, osteoporosis, and tumors (139–142). Severe microbial oral infection and subsequent inflammation, along with meningitis and endocarditis, are reported to be associated with cerebral infractions among male patients (143).

As mentioned above, microbial populations in the oral cavity are of two major types: the resident and transient microbiome; their delicate balance is essential for normal oral functions. Poor oral hygiene, smoking or chewing tobacco, inadequate nutrition, and overuse of antibiotics, not only can disrupt such homeostatic balance between oral resident and transient microbiome, but can also induce antimicrobial resistance (144, 145). Since normal oral microbiota exerts defensive functions against opportunistic harmful microorganisms, developing an approach to restore normal oral microbiota in infectious and inflammatory diseases would likely reduce the oral burden of diseases. The administration of healthy fecal microbiota to restore colonization resistance and displace multi-drug resistant (MDR) bacteria is already a commonly used therapeutic practice (146–148). Whether a similar approach could be employed to restore normal oral microbiota in oral diseases is an area that requires further experimental, theoretical and ethical validation.

A better understanding of microbiota-mediated colonization resistance of the oral cavity would promote rational dental care, and minimize oral-infection related chronic debilitating pathologies, which is also a global health concern. As frequently mentioned, the mouth is the gateway to total body wellness; consequently, the oral microbiome is likely to influence the overall health of an individual. Therapeutic manipulation of the oral microbiome in a patient, by targeting harmful species, to maintain healthier oral status in a community will further assist in the maintenance of good health and well-being, in general.

Conclusion

The abundance and composition of the oral microbial communities are characterized by the constancy and integrity of the relationships along with the antagonistic and stimulatory effects between microorganisms and their hosts. Colonization

resistance is one of the phenomena of local immunity, which depends on a combination of factors that prevent the adhesion and reproduction of exogenous bacteria on dental and mucous surfaces. A certain role in this belongs to resident microflora, which is a potent inhibitor of pathogens and synergist for commensals of the same ecological niche. The antagonistic effect of normal oral microflora is due to the significant adhesive and colonizing ability of resident microbial species, as well as the production of specific substances that inhibit the growth of transient pathogens. Nutritional factors may also modulate microbiota-mediated colonization resistance. So far, the available evidence to assess the real impact of different nutrients on the colonization resistance of the oral microbiome is still insufficient, and more studies are needed.

Author contributions

All authors contributed to the planning, writing, scientific content, reviewing, editing of this document, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Brick tea consumption and its relationship with fluorosis in Tibetan areas

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Brick tea-type fluorosis (BTF) due to a high intake of brick tea is possible in Tibetan populations, and dental fluorosis (DF) and skeletal fluorosis (SF) are its primary manifestations. To determine the prevalence of DF and SF and their relationships with brick tea intake in Tibetan populations, a literature review was conducted for studies published between 1994 and 2021. The available evidence revealed that brick tea may be produced from older stems and leaves of the tea plant and that the fluoride content of brick tea exceeds the national standard. The harsh environment of the plateau has led to limited food sources for the local Tibetan people who form the habit of drinking tea leaves as a satiation solution to digest greasy food and replenish vitamins, and regular consumption of brick tea leads to excessive exposure of Tibetan residents to fluoride. Studies in Tibet showed that the prevalence of DF in children was 14.06–75.93% in different districts, and the overall pooled prevalence of DF was 26.08%. The prevalence of SF in adults was 19.90–74.77% in different Tibetan districts, and the overall pooled prevalence of SF was 33.84%. The analysis of risk factors showed that the prevalence of BTF may be related to high-altitude and different working and living conditions, and BTF in children may be associated with fluoride intake during mothers' pregnancy and lactation. With the development of bioinformatics research, gene polymorphisms were suspected to be related to susceptibility to fluorosis in Tibetan populations. The study of BTF in Tibetan people needs to be further investigated and standardized, and additional studies evaluating the pathogenesis and preventive measures of BTF are warranted.

KEYWORDS

brick tea-type fluorosis, brick tea, dental fluorosis, skeletal fluorosis, Tibetan, food security

Introduction

Fluorosis is an endemic disease that is closely associated with the excessive intake of fluoride. Fluorine ranks 24th among all elements in universal abundance and 13th in terrestrial abundance, accounting for 0.08% of the Earth's crust (1). An appropriate intake of fluoride is beneficial to health (2). Fluoride participates in the metabolism of calcium and phosphorus and improves the strength of teeth and bones (3, 4). However, fluoride overdose may cause detrimental health effects, and fluoride can be ingested through drinking water, fluoride supplementation, or fluoridated toothpaste. According to the World Health Organization, long-term fluoride intoxication is associated with damage to the teeth and bones as well as the endocrine, gastrointestinal, renal, neurological, and reproductive systems (5, 6). Dental fluorosis (DF) and skeletal fluorosis (SF) are the most common manifestations of excessive fluoride intake (7–9). Symptoms of DF include cloudy white or chalky lines, spots, or marks on teeth, yellow or brown discoloration, surface irregularities, and visible pits in tooth enamel. SF is a metabolic bone disease, and it is the most common and severe manifestation of fluorosis in adults. The main clinical presentations of SF include lumbar and leg joint pain, joint rigidity, bone deformation, and spinal cord compression.

Endemic fluorosis is prevalent in China because of the high-fluoride content of water sources and coal combustion in some areas (10). The fourth National Oral Health Survey conducted in mainland China in 2016 (11) revealed that DF had a prevalence of 40.3% in Tibet, which was the third highest after those of Guizhou Province and Tianjin City. The fluoride intake of Tibetan people is associated with certain local factors, and the excessive intake of brick tea may be one of them.

The tea plant is a perennial plant that can maintain a steady yield for up to 100 years. It prefers to grow in acidic soil (pH 3.5–5.6) where it can obtain the element fluorine. After the decomposition of the aluminum fluoride complex in the soil, fluoride in ionic form (F^-) is absorbed by the roots, and excessive amounts of F^- form complexes with Al^{3+} and are transferred to old leaves (12, 13). Tea beverages are popular in many regions worldwide, and the fluoride enrichment effect of the tea plant carries a risk of excessive fluoride intake for tea drinkers (14–19). The diversity of tea beverages, various production techniques, and soil conditions of tea-producing areas may result in varying differences in the fluoride content of tea (14, 16–18, 20–26).

Since ancient times, Chinese people have had the tradition of drinking tea. The types of tea and drinking habits differ across regions. Tibetans are primarily distributed in the Qinghai–Tibet Plateau, which has an average altitude of 3,000–5,000 m. Owing to the cold weather, high-altitude, and higher radiation exposure, the residents of this region are accustomed to eating high-fat and high-sugar foods to adapt to the harsh environment. Tea consumption is important for digesting greasy food and replenishing vitamins. However, Tibetan regions with high-altitude mountain landforms do not produce tea; thus, the supply of tea is primarily from low-altitude Han-inhabited areas (27). The ancient Tea Horse Road (28, 29), a famous passage for economic and cultural exchanges in ancient China that rose from the Tang Dynasty (approximately 8th century A.D.), was derived from the complementary trade of tea and horses between the Tibetan and Han people.

As tea plants selectively absorb fluoride from the soil, the concentration of fluoride increases with maturity (30, 31). The materials used to make brick tea include old leaves and stems from the tea plant, and as the name suggests, this tea is molded into a brick shape for better preservation (**Figure 1**). Therefore, drinking brick tea in large quantities may increase fluoride intake compared to consuming other drinks, potentially resulting in brick tea-type fluorosis (BTF).

Existing studies (27, 32–36) have reported a relationship between brick tea consumption and fluorosis in Tibet. These studies were conducted in isolated cities, towns, or villages, and the information was relatively limited. To comprehensively compare fluoride intake from brick tea, prevent the occurrence of fluorosis, and confirm the relationship between brick tea consumption and fluorosis, the factors associated with excessive fluoride intake need further examination. A review is warranted to assess the prevalence of BTF and evaluate the effectiveness of the prevention and control of endemic BTF in recent years. In this study, the pooled prevalence rates of DF and SF and their existing risk factors were assessed to minimize bias and ensure objectivity, authenticity, and reliability of the research results, and a literature review was conducted to identify possible risk factors.

Materials and methods

Search strategy and identification criteria

Reasonable inclusion and exclusion criteria were established by evaluating the research content and scope of previous reports on BTF. Although many reports about brick tea are reported in Chinese, to ensure the objectivity, openness, and repeatability of this study, only articles written in English were included. The electronic search databases of PubMed and Cochrane Library were searched for studies published between January 1994 and September 2021. The search terms were "("brick tea" OR "tea" OR "brick-tea") AND ("fluorosis" OR "dental fluorosis" OR "skeletal fluorosis" OR "fluoride")." The reference lists of the included articles were added to the screening list to identify eligible studies. Two authors independently screened the titles and abstracts of these studies and retrieved the full texts of potentially eligible articles if they met the inclusion criteria.

FIGURE 1
A piece of brick tea produced in Yunnan province. Brick tea looks like a brick, and is a representative type of pressed tea, which is made of tea leaves, tea stems, and sometimes tea dust.

Any conflicts regarding the inclusion or exclusion of a study were discussed, and a third researcher was invited to make the final judgment.

Inclusion and exclusion criteria

To meet the requirements of the analysis and reduce bias, studies were required to meet the following criteria: (1) The study was conducted in Tibetan residential areas, i.e., the Tibet Autonomous Region, the Qinghai Province, and portions of the Sichuan and Yunnan provinces; (2) the included participants were local individuals from the field sites rather than selected volunteers; (3) clear diagnostic criteria for fluorosis and accurate fluoride concentration measurement methods were reported; and (4) the time of the survey was reported. In cases of multiple reports of the same samples in the same area during the same period of time, the report with the most detailed information was included.

The exclusion criteria included duplicate articles; articles that discussed fluorosis of animals or laboratory research; articles focusing on fluoride intoxication that was not caused by brick tea; articles focusing on the association of fluoride with other health-related issues, such as cancer, intelligence, or physical development; studies not conducted on Tibetan individuals or in Tibetan-inhabited areas; and studies with unclear age, sex, and other demographic data of the surveyed participants or data that could not be obtained from the authors.

Literature screening, data extraction, and compilation

Relevant data were subsequently extracted and used to compile a database. The data from all included studies were clearly tabulated. Deviations were considered and identified during the quality assessment stage. The following data were collected from each study using specifically designed tables: Author; year of publication; time and place of investigation; altitude of the investigated place; sample size; demographic information; sex; occupation; volume of daily brick tea consumption; prevalence of DF and SF of the investigated participants; fluoride level of brick tea; and fluoride level of brick tea infusions. The altitude was based on the average height of the urban area where the study was located according to publicly available information, like Baidu Baike, if the data was not given in article.

The fluoride level of brick tea was the enumeration of the fluoride content of dry brick tea obtained from different studies, and the volume of daily brick tea consumption was based on the amount of brick tea consumed or the amount of liquid applied. Due to the different methods reported in various studies, the fluoride and tea consumption levels may use different units of measurement; this study enumerated them without changing the units. The prevalence of DF and SF were based on the ratio of the number of patients with fluorosis in the study to the total number of enrolled people in the study. The DF index was reported by the authors of the study if available. Based on these

TABLE 1 Included studies on brick tea-type fluorosis.

References	Districts	Regions	Participants	Diseases
Cao et al. (35)	Daofu	Tibet AR	Children and adults	DF and SF
Cao et al. (36)	Xiege'er	Tibet AR	Children	DF
	Zhangmu	Tibet AR		
Cao et al. (27)	Naqu	Tibet AR	Adults	SF
Fan et al. (45)	Lhasa	Tibet AR	Children and adults	DF and SF
	Ali	Tibet AR		
	Nagqu	Tibet AR		
	Shigatse	Tibet AR		
	Shannan	Tibet AR		
	Linzhi	Tibet AR		
	Changdu	Tibet AR		
Zhang et al. (46)	Ganzi	Sichuan	Children and mothers	DF
Li and Zhao (47)	Haidong	Qinghai	Children	DF
	Hainan			
	Yushu			

AR, Autonomous Region; DF, dental fluorosis; SF, skeletal fluorosis.

data, the overall pooled prevalence of DF and SF were calculated. Pearson's correlation coefficients of the relationship between the prevalence of DF and SF and the altitudes of the investigated areas were also calculated.

Results

General database statistics

The initial electronic and manual searches yielded 297 articles. After screening the titles and abstracts, 274 articles were excluded because they were not human studies, were not conducted in Tibetan areas, did not focus on brick tea consumption, did not relate to DF and SF, or were not available in English. Then, 23 articles were read and reviewed in full. Among them, eight articles (37–44) were excluded because they had unclear demographic data or unclear SF or DF results, or the results were not consistent with the theme. Finally, 15 studies were included in the research: 6 studies (27, 35, 36, 45–47) that focused on BTF in Tibetan-inhabited areas were included in group 1; 4 studies (27, 45, 47, 48) that discussed the fluoride content of brick tea were included in group 2 (three articles were included in both group 1 and 2); and 8 articles (38, 43, 49–54) were included in group 3 because they primarily discussed the genetic susceptibility of Tibetans to fluorosis.

The PRISMA flowchart of the literature screening process was constructed (**Supplementary material**). **Table 1** provides a summary of articles related to BTF prevalence. DF was investigated only in children in most included studies; however, the study by Fan et al. (45) studied DF in both children and their mothers. SF was only studied in adults in all included literature.

Fluoride level of brick tea

The Chinese national standard requires the fluoride concentration of dry tea to be within 300 mg/kg (GB 19,965-2005). Four articles disclosed the fluoride content of dry brick tea. **Table 2** provides a summary of the fluoride content analysis. Three studies (27, 47, 48) compared the fluoride content of dry tea leaves in different sampling districts, whereas the researchers of one article (45) disclosed the fluoride content of tea infusions. Among the dry brick tea samples from Tibet, all samples had fluoride levels exceeding the national standard (i.e., 348.34–1085.70 mg/kg), except for one sample from Shigatse, which had a fluoride level of 96.53 mg/kg. The lowest reported fluoride content occurred in Shigatse (3.74 ± 5.09 mg/L), while the highest reported fluoride content occurred in Nagqu (10.90 ± 1.90 mg/L). Because the fluoride content of tea infusions is directly related to the method of mixing and there is no national standard for fluoride in beverages, it was impossible to conclude whether the fluoride content in tea beverage was significantly over the limit or not for studies that only evaluated tea solutions.

Prevalence of brick tea-type DF among children

In the five studies that included the prevalence of DF, 13,127 children were examined and 2,404 children were thought to have developed some degree of DF. The reported prevalence rate of DF was 14.06–75.93%, and the pooled prevalence rate

TABLE 2 Included studies on the fluoride concentration of brick tea.

References	Districts[1]	Region	Tea-type	Content
Cao et al. (48)	Bianxiao	Sichuan and Hunan	Dry brick tea	491.8 mg/kg
Cao et al. (27)	Naqu	Tibet AR	Dry brick tea	739 ± 27 mg/kg
Fan et al. (45)	Lhasa	Tibet AR	Brick tea infusion	8.15 ± 2.33 mg/L
	Ali	Tibet AR		7.38 ± 6.20 mg/L
	Nagqu	Tibet AR		10.90 ± 1.90 mg/L
	Shigatse	Tibet AR		3.74 ± 5.09 mg/L
	Shannan	Tibet AR		8.65 ± 2.84 mg/L
	Linzhi	Tibet AR		5.34 ± 4.06 mg/L
	Changdu	Tibet AR		4.66 ± 0.67 mg/L
Li and Zhao (47)	Haidong	Qinghai	Dry brick tea	724.74 ± 322.25 mg/kg
	Hainan	Qinghai		882.37 ± 404.71 mg/kg
	Yushu	Qinghai		1467.00 ± 279.20 mg/kg

AR, Autonomous Region.

[1] With the exception of Bianxiao, the other districts in this table refer to the places where the tea samples were obtained rather than where the tea was produced.

TABLE 3 Prevalence of dental fluorosis in different studies.

District	Altitude (m)	Index for measurement of DF	*N*	Prevalence of DF (%)	DF index
Zhangmu (36)	2,000	Dean's	116	25.86	0.67
Xiege'er (36)	4,300	Dean's	403	75.93	3.11
Nagqu (45)	4,500	National criteria (WS/T 208-2011)[2]	63	50.79	1.75
Lahsa (45)	3,650	National criteria (WS/T 208-2011)[2]	87	44.83	1.79
Shigatse (45)	4,000	National criteria (WS/T 208-2011)[2]	118	27.97	1.61
Shannan (45)	3,700	National criteria (WS/T 208-2011)[2]	109	25.69	2.07
Linzhi (45)	3,100	National criteria (WS/T 208-2011)[2]	13	23.08	1.17
Changdu (45)	3,500	National criteria (WS/T 208-2011)[2]	33	21.21	2.57
Daofu (35)	3,000	Dean's	375	51.2	1.33
Ganzi (46)	2,600	Dean's	368	62.23	2.30
Haidong (47)	N/A 2,000[1]	Dean's	2,677	15.09	0.26
Hainan (47)	N/A 3,000[1]	Dean's	3,975	14.06	0.24
Yushu (47)	N/A 4,000[1]	Dean's	4,790	32.61	0.55
Sum			13,127	26.08%	

DF, dental fluorosis; N/A, not available.
[1]The altitudes of these areas are not available from the literature, but they are available from publicly available information.
[2]Study by Fan et al. (45) used the national criteria in the text instead of Dean's index, but the two have very similar presentation and scoring criteria.

of DF was 26.08%. The areas with the highest DF prevalence rates were Xiege'er (75.93%) and Ganzi (62.23%). Dean's DF index was used in most studies (36, 46, 47), and the Chinese national standard of DF was used in one study (45) (**Table 3**). According to our detailed examination of the statements of the two standards, the classification and statements of both are the same. The highest DF index was reported in Xiege'er (3.11), while the lowest index was reported in Hainan Autonomous Prefecture (0.24). However, it is important to note that differences may be due to the possible detection heterogeneity of different studies.

Prevalence of brick tea-type SF among adults

Three articles (27, 35, 45) discussed the prevalence rate of SF. A total of 2,089 adults were examined in these studies, and 923 adults were detected to have some degree of SF. The reported prevalence of SF was 19.90–74.77%, and the pooled prevalence rate of SF was 33.84%. The districts with the highest SF prevalence were Ali (74.77%) and Nagqu (68.25%). SF had no well-recognized index; therefore, the index of SF was not calculated (**Table 4**). Since there is no internationally recognized evaluation index for SF, no indexing score was given, but the authors classified them according to severity.

Fluoride intake per day for included participants

Fluoride intake among Tibetans may come from various sources, such as brick tea, zanba (a type of food made from cooked barley), butter tea, and water. **Table 3** shows the average total daily fluoride intake per person from brick tea in different districts for the included participants. Fluoride intake differed significantly between districts and was much higher than the national standard in most areas (National standard GB 17,018-2011: The daily fluoride intake should be <3.5 mg per person). Among the four studies that reported daily fluoride intake, only Cao et al. (36) reported a fluoride intake of 2.905 mg/day in Zhangmu, which did not exceed the standard. Fan et al. (45) reported that the highest fluoride intakes were in Naqu (55.630 mg/day) and Ali (35.60 mg/day) in their study (**Table 5**).

Correlations of DF and SF with altitude

Pearson's correlation coefficients of the relationship between the prevalence of DF and SF and the altitudes of the surveyed areas were 0.389 and 0.803, respectively. Moreover, Pearson's correlation coefficient of the DF index with the altitude was 0.5157, and that of the daily fluoride intake from brick tea with the altitude was 0.5186. A significant difference in Pearson's

TABLE 4 Prevalence of skeletal fluorosis in different studies.

District	Altitude (m)	Index for measurement of SF	*N*	Prevalence of SF (%)
Nagqu (45)	4,500	National criteria (WS/T 192-2008)	315	68.25
Lahsa (45)	3,650	National criteria (WS/T 192-2008)	115	59.13
Shigatse (45)	4,000	National criteria (WS/T 192-2008)	124	35.48
Shannan (45)	3,700	National criteria (WS/T 192-2008)	322	27.64
Linzhi (45)	3,100	National criteria (WS/T 192-2008)	201	19.90
Changdu (45)	3,500	National criteria (WS/T 192-2008)	136	52.94
Ali (45)	4,500	National criteria (WS/T 192-2008)	107	74.77
Daofu (35)	3,000	<Manual of preventing endemic fluorosis>	658	32.83
Naqu (27)	4,500	<Standards of endemic fluorosis>	111	89.0
Sum			2,089	33.84%

SF, skeletal fluorosis.

correlation coefficient was only detected between the prevalence of SF and the altitude (Table 6).

Relationship between fluorosis susceptibility and genotype

In this study, eight articles (38, 49–55) related to the genetic susceptibility to fluorosis were obtained (Table 7). Wu et al. (55) investigated the role of *GSTP1* rs1695 polymorphisms in the susceptibility to fluorosis. Pei et al. (51) reported a significant correlation between the *MMP2* rs2287074 genotype and SF severity, and the A allele of *MMP2* rs2287074 was a protective factor in Tibetans. Yang et al. (50) indicated that Tibetans were more likely to develop moderate and severe brick tea-type SF, and CT/TT genotypes of vitamin D receptor-*Fok*I may be a protective factor. Li et al. (54) indicated that a polymorphism in the extrapituitary prolactin promoter may decrease the risk of brick tea-induced SF in the Kazakh people. Chu et al. (38) suggested an association between the *AlOX15* gene polymorphism and SF risk in Han participants. The CC/CC diplotype had a protective effect on SF risk in Han participants, whereas the CA/CC diplotype influenced SF risk in participants aged ≥65 years. Liu et al. (53) indicated that serum Klotho may be a potential mediator of SF in BTF-endemic areas. Yang et al. (49) suggested there might be differential genetic influence on SF risk in Kazakh and Tibetan participants and that this difference might be modified by tea fluoride intake. However, a study by Lou et al. (52) did not find an association between *BMP2* single nucleotide polymorphisms and SF in their cross-sectional case-control study.

TABLE 5 Fluoride intake through brick tea in different studies.

District	Region	Altitude (m)	Daily fluoride intake from brick tea (mg/day)
Zhangmu (36)	Tibet AR	2,000	2.905[1]
Xiege'er (36)	Tibet AR	4,300	8.045[1]
Nagqu (45)	Tibet AR	4,500	55.630
Lahsa (45)	Tibet AR	3,650	25.026
Shigatse (45)	Tibet AR	4,000	9.800
Shannan (45)	Tibet AR	3,700	22.350
Linzhi (45)	Tibet AR	3,100	12.010
Changdu (45)	Tibet AR	3,500	23.430
Ali (45)	Tibet AR	4,500	35.600
Daofu (35)	Tibet AR	3,000	7.80[2]
			3.89[3]
Naqu (27)	Tibet AR	4,500	8.03[4]

AR, Autonomous Region.
[1]Data for children.
[2]Data for individuals aged >15 years.
[3]Data for individuals aged 8–15 years.
[4]Data for adults.

A schematic diagram (Figure 2) shows the fluoride accumulation effect of brick tea in old leaves and stems, the result of BTF in Tibetan populations, and possible influencing factors.

Discussion

Relationship between brick tea and fluoride

Because of the accumulation effect of fluoride in tea plants, drinking tea has become a source of excessive fluoride in the human body. The influences of different tea-producing areas, production methods, drinking habits, and dosages on the development of fluorosis should be noted.

Chandrajith et al. (16) reported that in Sri Lanka, the traditional habits of locally blended black tea consumption can cause an additional intake of fluoride and promote adverse health conditions that may also be related to chronic kidney disease. Whyte et al. (17) reported that the popular instant

tea powder contributes to approximately 80% of F^- exposure, and SF from the habitual consumption of large volumes of extra-strength instant tea calls for recognition. Apart from these, several studies (14, 56–60) have also raised health concerns regarding the excessive intake of fluoride from tea in certain regions.

Brick tea is relatively low-end tea, especially that sold in remote mountainous areas. Because of the long distances traveled, tea needed to be preserved for a long time and easily transported, so it was often pressed into the brick form that gave it its name. The ethnic people in the mountainous areas consume more meat and milk and less vegetables, and drinking tea not only helps digest greasy food, but it also supplements essential vitamins and trace elements. Therefore, it is said that "it is better to go without food for a day than without tea for a day." Brick tea has become a necessity of Tibetan life.

Previous studies have reported that the fluoride content of brick tea was higher than that of ordinary tea (44, 48, 61). The Chinese national standard requires that the fluoride concentration of dry tea should be within 300 mg/kg. Studies (27, 47, 48) on BTF in Tibet have reported that the fluoride content of dry brick tea samples greatly exceeded the national standard, some of which reached three to five times the limit. In addition, animal experiments (47, 48) have also shown that rats exposed to brick tea infusions present with DF. Therefore, the association between high-fluoride brick tea consumption and endemic fluorosis in Tibet can be verified.

To distinguish the link between brick tea and fluorosis, we also need to know if the source of fluoride comes from other substances used in tea brewing, such as water and fuel. However, the fluoride content of drinking water in nearly all Tibetan residential areas is within the national drinking water range (≤1.2 mg/L, GB 5,749-2006), and the possibility of water-initiated fluorosis is low (27, 36, 39, 45). Among the four studies that reported fluoride concentrations in local drinking water samples, only the concentration of one water sample exceeded the national standard (in Ali, 2.01 mg/L). The fluoride concentrations of other water samples were lower than the national standard (0.03–0.96 mg/L). Moreover, the use of coal is often a cause of fluorosis, but Tibetan-inhabited areas are not coal-producing areas and do not have the tradition of using coal. Tibetan individuals generally use wood and cow dung as fuel in the countryside. Therefore, the possibilities of fluoride intake caused by coal pollution and drinking water can be eliminated.

TABLE 6 Pearson correlation coefficients of DF- and SF-related variables with altitude.

	r	95% confidence interval	*P* (two-tailed)
Prevalence of DF%	0.3894	−0.2057 to 0.7743	0.1884
Prevalence of SF%	0.8030	0.2976 to 0.9568	0.0092*
Dean's index of DF	0.5157	−0.04924 to 0.8307	0.0712
Daily fluoride intake from brick tea[1]	0.5186	−0.2220 to 0.8797	0.1526

[1]Daily fluoride intake data from children in Zhangmu, Xiege'er, and Daofu were excluded. *Indicates a statistically significant difference.

Relationship between DF and brick tea

Dental fluorosis is characterized by chalky-white to brown patches on the enamel and, in severe cases, defects of the enamel. Fluorosis can be divided into three types based on severity: chalky type (mild), discoloration type (moderate), and defect type (severe) (62). DF is more common in permanent teeth, and affected teeth are less resistant to friction but more resistant to acid etching (63, 64). Excessive fluoride intake can delay the mineralization of the enamel and increase its surface roughness, thus promoting plaque accumulation (65). Excessive fluoride binds more enamel amelogenin and increases enamel organic substances in the secretory phase of enamel formation, thereby leading to hypomineralization and DF (66, 67). The typical histological feature of DF is the low mineralization rate of the enamel, which becomes fragile, easily eroded, and exfoliated.

Dental fluorosis is rare and mild in deciduous teeth due to their development in the embryonic and lactation stages, and the placenta has a certain barrier effect on fluoride. However, if fluoride intake exceeds the limit of its screening function, it can also result in irregularities on the deciduous teeth (68).

In the literature included in this study, most studies only examined children for DF. The reported prevalence of DF was quite different between different studies, indicating that the heterogeneity of these studies may be strong. Although the prevalence of DF varied, all included studies (35, 36, 45–47) identified DF caused by brick tea as severe and of great concern.

Relationship between SF and brick tea

Skeletal fluorosis is one of the most common and severe manifestations of fluorosis in adults, and it is also an important index to evaluate the severity of endemic fluorosis. Radiographic examination of SF may show increased bone matrix density and fibrous ossification, tendon attachment calcification, joint degeneration, degenerative hyperplasia, and ossification changes in the fibrous tissues (27, 69, 70). Such patients may also have DF, whereas those exposed to excess fluoride after 12 years of age may not have DF since the development of ameloblasts in the dental germ is finished by that age.

Cao et al. (27) indicated that the detection of SF positively correlated with long-term fluoride exposure from brick tea, and SF was increasing in individuals aged >50 years, while its severity was positively correlated with age.

Skeletal fluorosis needs to be differentiated from diseases such as osteoblastic metastatic carcinoma and renal bone

TABLE 7 Genetic studies associated with brick tea-type fluorosis susceptibility.

References	Country	Gene	Population	District
Wu et al. (55)	China	Glutathione S-Transferase pi-1	Tibetan, Mongolian, Han, Kazakh, and Russian	Qinghai, XUAR, Inner Mongolia
Yang et al. (50)	China	Vitamin D Receptor Gene FokI	Tibetan, Kazakh, Mongolian, and Han	Qinghai, XUAR, Inner Mongolia
Li et al. (54)	China	Prolactin Promoter at -1,149	Tibetan, Kazakh, and Mongolian	Qinghai, XUAR, Inner Mongolia
Pei et al. (51)	China	Matrix Metallopeptidase-2	Tibetan and Kazakh	Qinghai, XUAR
Yang et al. (49)	China	Frizzled Related Protein 1	Tibetan and Kazakh	Qinghai, XUAR
Liu et al. (53)	China	Serum Soluble Klotho	Tibetan and Kazakh	Qinghai, XUAR
Lou et al. (52)	China	Bone Morphogenetic Protein 2	Tibetan and Kazakh	Qinghai, XUAR
Chu et al. (38)	China	Arachidonate-15-Lipoxygenase	Tibetan, Kazakh, and Han	Qinghai, Inner Mongolia, XUAR

XUAR, Xinjiang Uyghur Autonomous Region.

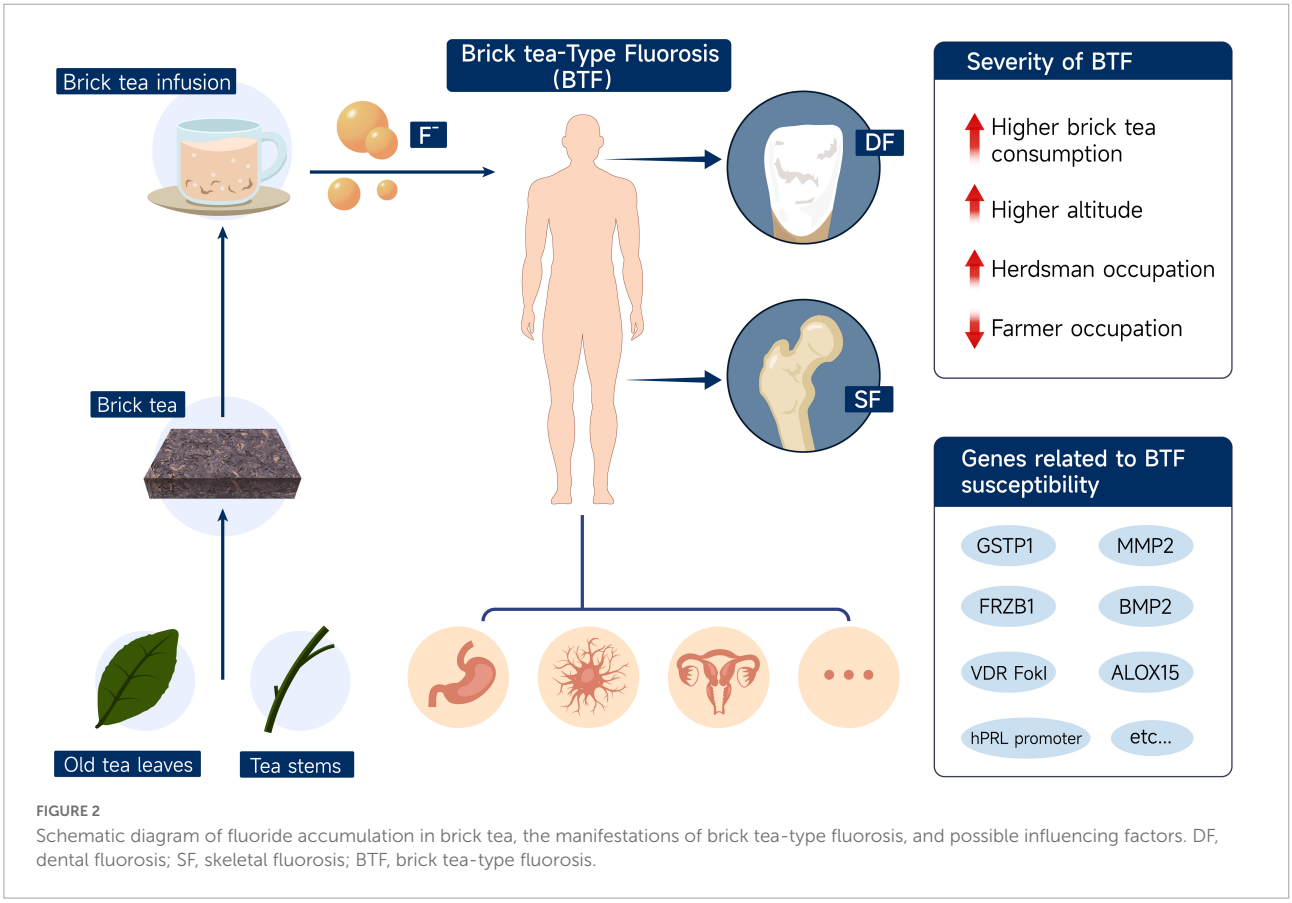

FIGURE 2
Schematic diagram of fluoride accumulation in brick tea, the manifestations of brick tea-type fluorosis, and possible influencing factors. DF, dental fluorosis; SF, skeletal fluorosis; BTF, brick tea-type fluorosis.

disease. Patients with SF who experience pain should be given an appropriate dose of non-steroidal analgesics. Those with skeletal deformities should have local fixed or orthopedic surgery to prevent deformity exacerbation.

Relationship between fluorosis and altitude

Previous studies (36, 45, 46) have shown that high-altitude is a risk factor for fluorosis. Animal experiments have shown that under low oxygen conditions, mice ingested more fluoride, and their enamel formation was affected. Higher altitudes result in a lower oxygen concentration of the air and lower pH levels of the urine, and they affect the metabolism of fluoride and calcium and increase the reabsorption of fluoride by the kidneys (71–73). Changes in acid–base balance caused by high-altitude hypoxia may increase fluoride levels in organisms' internal environments by increasing the reabsorption of fluoride and reducing fluoride excretion in the urine.

The effects of altitude on the prevalence and severity of fluorosis were first reported (73) in Kenya. Rwenyonyi et al. (74) indicated that altitude was a risk indicator of fluorosis after

controlling for confounding variables, such as water source and vegetarianism. An animal study (75) also indicated that rats in the high-altitude group had more severe cartilage damage, coagulative kidney necrosis, and hydropic liver degeneration than those in the low-altitude group.

Zhang et al. (46) reported that a higher altitude was a risk factor for DF, and the prevalence of DF at higher altitudes (2,560–3,300 m) was nearly three times higher than that at lower altitudes (1,400 m). When a study included districts with varying altitudes, the prevalence of DF in low-altitude areas tended to be lower than that in high-altitude areas (**Table 3** and **Figure 3**). Similarly, this trend also existed for SF (**Table 4** and **Figure 4**). However, these trends may not be obvious in the surveys of small samples, and they may be affected by various factors, such as the economic and health statuses of individuals living in the surveyed areas. In low-altitude areas, residents may have a more diverse diet, relatively better economic and hygiene conditions, and easier access to clean water sources. These confounding factors should be noted in future studies. Thus, large data with less heterogeneous results are needed to illustrate this issue.

Relationship between BTF and occupation

Some researchers have proposed the possibility of correlations between BTF and residents' occupation, economic status, and health. Fan et al. (45) analyzed the prevalence of fluorosis across different occupational populations in Tibet. For the rural population, occupations can be briefly divided into agriculture and animal husbandry. Interestingly, they indicated that the likelihood of BTF was significantly associated with occupational factors; it was higher in villages where animal husbandry was the main occupation, whereas it was lower in agricultural villages (45). They explained that, owing to harsh living conditions, herdsmen consumed less vegetables but more brick tea for vitamins and minerals, which might have led to a higher prevalence of fluorosis among them.

Similarly, the correlation between BTF and occupation may be associated with living conditions and economic factors. The correlation between BTF and occupation, like the correlation between BTF and altitude, may be merely an external manifestation of the correlations between BTF and socioeconomic and living conditions. More detailed studies need to be conducted to identify confounding factors for this issue.

Considering the influence of occupation on diet and ultimately the likelihood of fluorosis, the large number of part-time or full-time religious workers in Tibet cannot be ignored. Tibetan Buddhist religious people may choose to become a monk or nun for life or a period of time, and the food sources for them are limited owing to their religious beliefs. Zanba, butter tea, and brick tea constitute a high proportion of their diet; however, to date, no data on the prevalence of fluorosis exist for this group of people.

Fluorosis and maternal brick tea consumption

During pregnancy and lactation, although the transfer of fluoride from mother to fetus is regulated by the placenta and the transfer of plasma to breast milk is inhibited (76), fluoride concentrations in breast milk and amniotic fluid increase significantly after the intake of fluorinated foods (77–81). Excessive fluoride exposure results in increased organic material in the secretory stage of enamel formation. Later, in the maturation period, the regulation of ameloblast modulation is disrupted by superfluous fluoride, and the mineralization of the enamel is disrupted, leading to hypomineralization and DF (66, 82, 83). Zhang et al. (46) reported an association between regular excessive maternal consumption of brick tea and DF in children. They indicated that the excessive consumption of brick tea by mothers during pregnancy and lactation could disrupt the development of children's tooth germs.

In addition to the effect of fluoride levels on children's teeth, existing research also suggests that higher levels of fluoride exposure in women during pregnancy may be associated with diseases or adverse reactions in their children. A report on fluoride intake in pregnant women indicated that those with a high intake produced offspring with lower IQs, and the effect was greater in boys (84). More attention should be paid to fluoride exposure in pregnant women.

Relationship between genetic polymorphisms and BTF

Recent advancements in bioinformatics have revealed many diseases to be associated with a genetic predisposition (85). Fluorosis may result from excessive fluoride intake as well as from prolonged exposure and the body's response, but epidemiological and animal studies (86–88) have also indicated individual differences in the severity of fluorosis.

It is speculated that fluorosis may be caused by the complex interaction between candidate genes of fluorosis and specific environmental exposure, including dietary fluoride intake, among others. The severity of fluorosis does not always depend on the consumption of fluoride. Genetic polymorphisms may lead to individual differences in sensitivity or resistance to fluoride exposure.

Existing studies have been conducted with different laboratory mouse strains to identify possible genetic determinants of DF. Everett et al. (89) showed that different genotypes of mice with the same fluoride levels could produce different degrees of DF severity when controlling

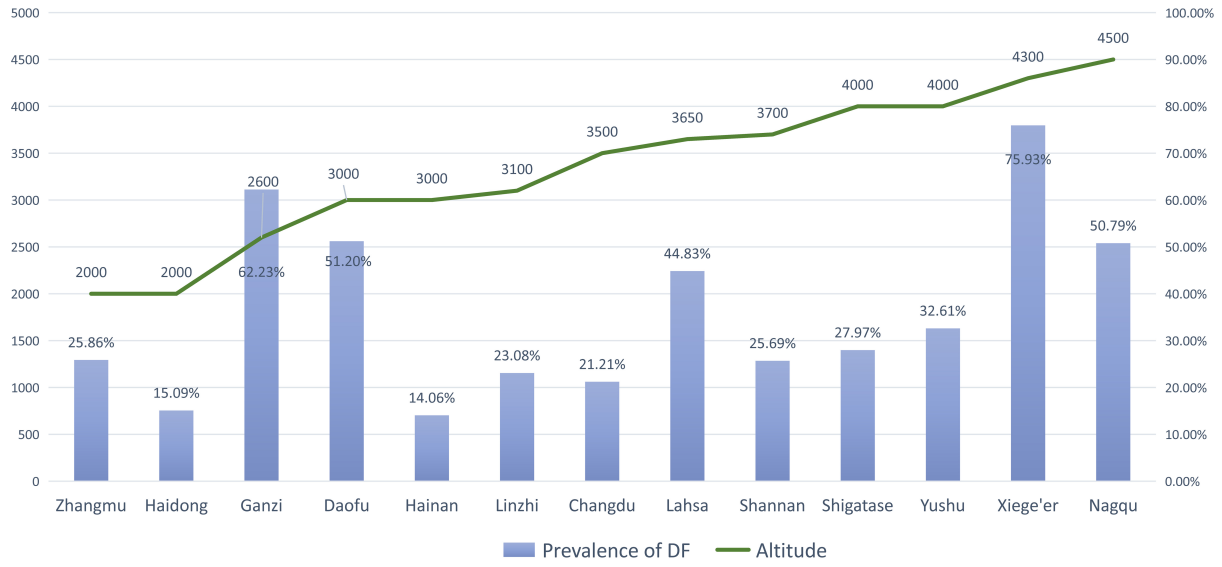

FIGURE 3
The prevalence of dental fluorosis (DF) and the altitude of surveyed areas (The line graph shows the altitude of the research site, and the bar graph shows the reported prevalence of DF at the research site).

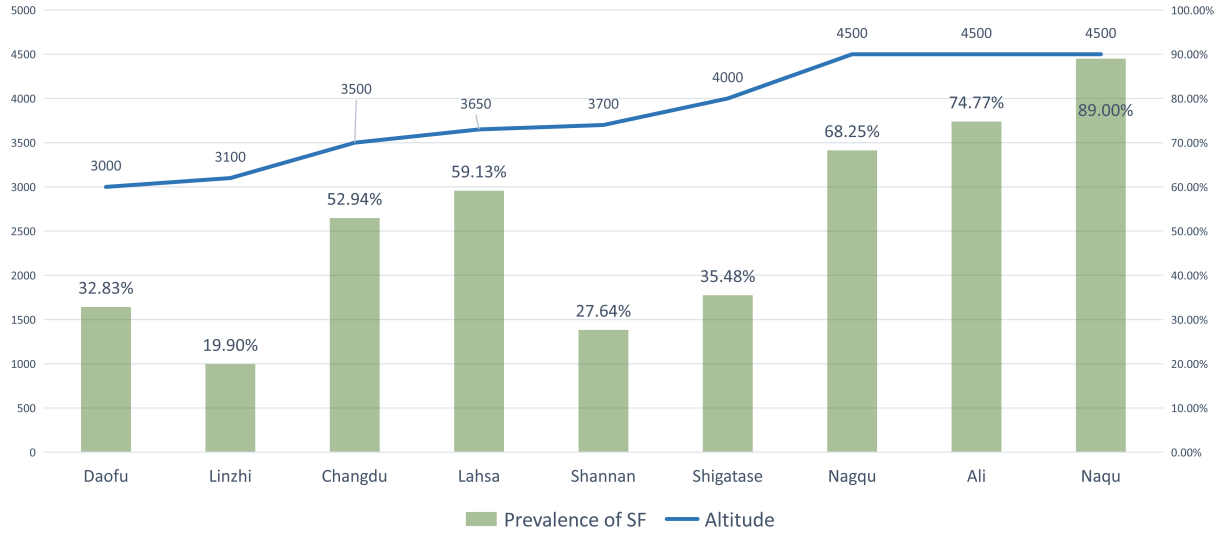

FIGURE 4
The prevalence of skeletal fluorosis (SF) and the altitude of surveyed areas (The line graph shows the altitude of the research site, and the bar graph shows the reported prevalence of SF at the research site).

for variables such as sex, food, age, housing, and drinking water fluoride concentration. Mousny et al. (90) analyzed the role of genetic factors on the effects of fluoride on bone metabolism in an animal study. Their study evaluated the effect of increasing fluoride doses in three inbred strains of mice (susceptible, resistant, and intermediate). Significant changes in bone quality were observed in susceptible strains, moderate changes were seen in intermediate strains, and no changes were noted in resistant strains. Thus, the findings suggest that genetic factors may contribute to variations in bone quality.

Some human studies have also confirmed the role of genetic factors in fluorosis. Researchers found that genes related to signal transduction (G-protein, ERK, MEK1, and MEK2) (91–93), immune lymphokines (interleukin 6, interleukin 8) (94, 95), and estrogen receptors (96) may influence the occurrence and development of fluorosis. Case-control studies (97–99) carried out in fluorosis-endemic areas have analyzed the

relationship between gene polymorphisms of candidate genes and fluorosis susceptibility.

We found that many studies in China have analyzed the genetic diversity of BTF in Tibetan populations and evaluated the susceptibility to BTF caused by different genes in Tibetan, Kazak, Mongolian, and Han populations. GSTP1, vitamin D receptor-*Fok*I, prolactin promoter at -1,149, MMP2, FRZB1, serum soluble Klotho, BMP2, and ALOX15 and their related genes are thought to be involved (38, 49–55). However, the exact correlation between BTF and genetics needs further research.

Preventive measures and effects of brick tea consumption

The prevention and control of BTF have been underway since the discovery of a link between brick tea consumption and fluoride intake in Tibetan areas. To reduce the effect of BTF, the government and disease control authorities have acted to rectify the production of brick tea containing high-fluoride concentrations. The standard in China (GB 19,965-2005) stipulates that the fluoride content of brick tea should not exceed 300 mg/kg. According to the classification standard of endemic fluorosis (GB 17,018-2011), an endemic fluorosis area can be determined if the average daily tea fluoride intake of people over 16 years of age is more than 3.5 mg and there are patients with SF confirmed by X-ray examination.

Strengthening health education allows residents to understand the harm of high-fluoride brick tea and consciously develop healthier tea drinking habits, such as changing the single diet structure, balancing nutrition, advocating for the reduced dependence on brick tea, not drinking high-fluoride tea, and eating more fresh vegetables and fruits.

In addition, the tea preparation method should be changed; before adding water to boil, the tea should first be mashed and washed once with 80°C water. Strong tea, tea that has been boiled for a long time, and tea that has been soaked for too long should be avoided. Tea drinkers should avoid adding alkali when boiling brick tea since the dissolution efficiency of fluoride in tea is higher under alkaline conditions.

Jin et al. (39) reported their findings from a 3-year (2008–2011) observation study on the prevention of brick tea-induced intoxication. By reducing the availability of brick tea in school canteens and switching to low-fluoride brick tea, the total daily fluoride intake of children decreased to a relatively safe level, although it was still at the level of chronic intoxication. Though the short-term use of low-fluoride brick tea reduced urinary fluoride levels, this difference was not significant, and the serum fluoride levels remained unchanged. This finding may have been attributed to the maintenance of internal homeostasis, and the bones may have become fluoride buffers in the body. Fluoride accumulation in the body may take a long time to be eliminated, and the effect of preventive measures on brick tea needs to be verified by controlled experiments with a larger sample over a longer period of time.

Study limitations

To our knowledge, this is the first review of BTF in Tibetan residents, but limitations still exist. To date, the research on BTF is not comprehensive and has not been taken seriously enough. The literature that could be included in this study was limited, and most researches were conducted by the few limited scholars. In addition, the research methods are not unified and have a certain heterogeneity. The detection of fluoride in brick tea and the identification and rating of DF and SF should use widely recognized international standards. And no data are available on fluorosis among certain groups of Tibetans, especially Tibetan Buddhist monks and nuns. Lastly, the correlation between genotypes and the susceptibility to fluorosis caused by brick tea intake requires further investigation.

Conclusion

At present, the high-fluoride content of brick tea is an important pathogenic factor for fluorosis in Tibetan regions. To our knowledge, this is the first review on the issue of BTF. DF had prevalence rates of 14.06–75.93% and an overall pooled rate of 26.08% in children, whereas SF had prevalence rates of 19.90–74.77% and an overall pooled rate of 33.84% in adults. BTF susceptibility may be associated with occupation and the altitude at which individuals reside. Genetic polymorphisms were suggested risk factors. BTF among Tibetan residents needs more attention, and further studies evaluating its pathogenesis and preventive measures are warranted.

Author contributions

CW designed the study, applied for grant support, and conducted the literature search and screening, data collection, manuscript writing, and revision. QZ conducted the literature screening and data collection. FX participated in literature screening. JJ participated in data analysis. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1030344/full#supplementary-material

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Dietary vitamin D and calcium and periodontitis: A population-based study

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Aim: This study aimed to explore the relationship between dietary vitamin D and calcium intake and periodontitis among adults and whether it differs from males to females.

Methods: Cross-sectional analysis of a population-based cohort study with adults aged 20 to 60 from Southern Brazil. Intake of vitamin D and calcium were gathered in 2012 using two 24h-dietary recalls. Clinical examination assessed the clinical attachment level and bleeding on probing. Confounders included sex, age, family income, smoking, and obesity. The controlled direct effect of vitamin D and calcium on periodontitis was examined using marginal structural modeling. Analyses were also stratified by sex.

Results: Of the 1,066 investigated adults (mean age 35 ± 11.7 years; 49% females), 12.3% (95%CI 10.2;14.7) had periodontitis. Calcium intake had a direct protective effect on periodontitis (risk ratio (RR) 0.61; 95%CI 0.45;0.83), whereas no association between vitamin D and periodontitis was observed (RR 1.13; 95%CI 0.82;1.56). Stratified analyses revealed a null association between both vitamin D and calcium intake and periodontitis among men, but a protective association between calcium and intake and periodontitis among women (RR 0.56; 95%CI 0.38;0.79), while vitamin D remained without any association (RR 1.07; 95%CI 0.72;1.61).

Conclusion: Our findings suggest a protective association between dietary calcium intake and periodontitis among women.

KEYWORDS

periodontal disease, diet, nutrition, micronutrients, epidemiology

1 Introduction

Nutrition has been associated with a longer life expectancy and the prevention of several non-communicable systemic diseases, including type 2 diabetes and cardiovascular disease (1). In regards to oral health, the relationship between diet, especially rich in fermentable carbohydrates, and dental caries has been thoroughly investigated (2). Even though the association between nutrition and periodontitis, a chronic inflammatory disease affecting the supporting tissue of the teeth, has been explored in the literature, it seems this discussion can be further substantiated (3–6).

Although the local biofilm may influence the onset and progression of periodontal tissue destruction, environmental and genetic factors related to the host inflammatory response and their ability to solve it appear to account for approximately 80% of the periodontitis risk (7–9). As nutrition influences the ability of the immune system to mount and modulate inflammatory responses properly, it is possible to speculate a relationship between diet and periodontitis (1). While vitamin C seems to be the most investigated micronutrient possibly associated with periodontitis, considerable attention has also been given to the role of vitamin D and calcium (6). Nevertheless, the topic has not been fully clarified in the literature yet.

This interest relies on the potential mechanisms underlying the relationship between vitamin D, calcium, and periodontitis, which involve immune and hormonal effects that vary with sex. Vitamin D affects the inflammatory response by promoting macrophage shifting phenotype from their primary pro-inflammatory response (M1) toward an anti-inflammatory (M2) pattern (10). A similar effect of vitamin D is also observed among B- and T-cells, as the release of pro-inflammatory cytokines is inhibited, concomitant to an enhanced expression of anti-inflammatory cytokines (11). Additionally, calcium absorption is highly dependent on vitamin D levels. Low vitamin D levels reduce calcium absorption, which in turn upregulates parathyroid hormone release, osteoclastogenesis, and bone resorption to prevent hypocalcemia, thus, increasing bone loss (12). On a related note, low calcium intake has been associated with a greater risk of alveolar bone loss related with periodontitis (13). This mechanism, however, may differ from man to woman, as osteoclasts possess estrogen receptors but no androgen receptors (14). It is of utmost importance to explore the role of sex in this relationship.

Studies investigating the association between vitamin D and calcium intake with periodontitis have reached conflicting results. A systematic review found three observational studies exploring the association between dietary vitamin D intake and periodontitis, one of which indicated a null association, while the other two suggested a protective effect (6). Although more studies on the association between serum vitamin D levels and periodontitis are available, they yielded inconsistent results. Antonoglou et al. demonstrated that dietary vitamin D deficiency was associated with a higher prevalence of periodontitis (15), whereas Lee and colleagues found no association (16). Hence, to date, the role of vitamin D, if any, in periodontitis is still unclear. Similar findings were also observed in clinical trials. The available evidence about vitamin D supplementation (alone or combined with calcium), in part, revealed small effects among short-term studies (17–19).

On a similar note, the association between calcium intake and periodontitis is not yet evident. While some studies have indicated a detrimental effect of a calcium-deficient diet on periodontitis (20–22), others failed to identify any association (23). Despite the growing literature on the topic (5, 6, 24), few studies in periodontology have evaluated the dynamic association involving vitamin D and calcium intake. Thus far, most studies have focused on exploring the isolated effect of either vitamin D or calcium intake, neglecting their correlation (25). Either way, the use of conventional regression analysis fails to account for this complex relationship, and this might explain the controversial results found in the literature. In this case, an analytical approach that considers this framework may elucidate this matter further.

Thus, it becomes evident from the above the need for population-based studies with a large sample and the use of proper statistical methods to clarify the relationship between dietary vitamin D and calcium and their association with periodontitis. Accordingly, this study aimed to evaluate the direct and indirect relationship between dietary intake of vitamin D and calcium with periodontitis in adults from a population-based cohort study in Southern Brazil and assess whether these relationships differ between males and females.

2 Materials and methods

2.1 Participants and sampling procedures

This study used data from the EpiFloripa Cohort Study, a population-based prospective study conducted in Florianópolis, a state capital in Southern Brazil. The baseline sample size (n = 1,720) was estimated considering the reference population between 20 and 59 years of age living in the urban area of the city in 2009. Sample size calculation accounted for a cluster sampling selection. Initially, 1/7 of all 420 census tracts of the city were selected in each household income decile; then, the households (1,134/16,755) were systematically chosen within the nominated census tracts. Adult residents in each house were considered eligible if aged between 20 and 59 years. Exclusion criteria comprised the presence of a severe physical or neurological impairment. In 2012, all participants were re-contacted for a follow-up examination (n = 1,066) (**Figure 1**). Further details

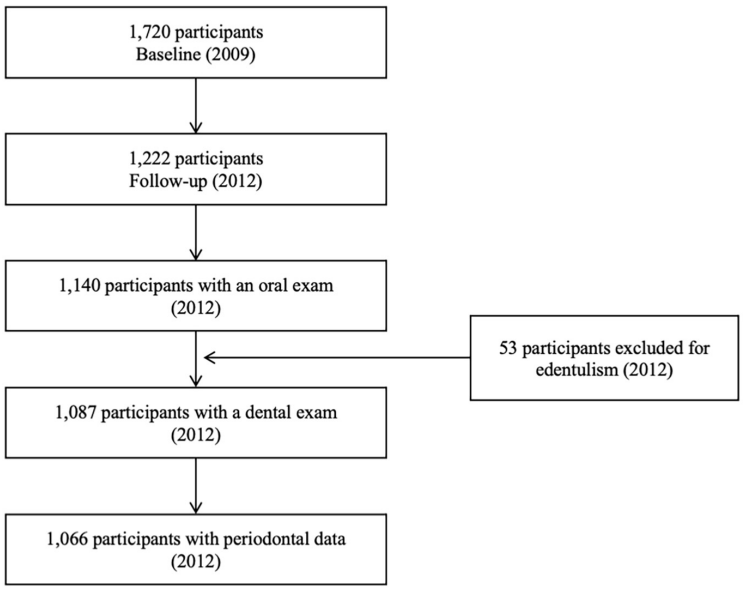

FIGURE 1
Flowchart of the EpiFloripa cohort study.

on the study methodology (sampling and eligibility criteria) are available elsewhere (26).

The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. The Ethics Committee in Human Research of the Federal University of Santa Catarina (351/2008 and 1772/2011), Brazil, approved the study. All participants signed a written consent form. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed to report the study.

2.2 Periodontitis: Outcome

In 2012, eight dentists performed dental examinations on all cohort members to assess for dental caries and periodontal outcomes at participants' homes. Headlamps were used to improve visualization. In addition, dental examinations were standardized before fieldwork by comparing the results of the oral examinations performed on 20 adults (not cohort members) against a gold standard (27).

The periodontal examination of the cohort members included assessing the clinical attachment level (CAL) and bleeding on probing (BOP). Two diagonal quadrants were randomly selected according to the participant identification number. Six sites per tooth were examined using a ball-point periodontal probe (WHO probe) in the chosen quadrants. CAL was measured in mm and later dichotomized as absent (0-3 mm) or present $\geq$ 4 mm, while BOP after 15 s of probing was recorded as present. The outcome of the present study (periodontitis) was defined as the presence of CAL ($\geq$ 4mm) and BOP in the same tooth (28).

2.3 Dietary vitamin D and calcium intake: Exposure

Information on dietary intake was elicited in 2012 using two 24h-dietary recalls. Data were collected following the "Multiple Pass" method (29), consisting of three stages: a "quick list," a detailed description of food and beverage items consumed, and a review. After all cohort members completed the first 24 h-dietary recall, 40% of the participants were randomly selected for a second 24 h-dietary recall. The second recall was structured so that participants would report a week and a weekend day to capture the diversity of food consumed.

Dietary information gathered from the food recalls was entered into the Nutrition Data System for Research (NDSR) software from the University of Minnesota Nutrition Coordinating Center, USA, following the validation proposed by Fisberg and coworkers (30) for the Brazilian context. At this stage, all food sources were converted into grams, milliliters, or liters according to Brazilian standards. As the NDSR software uses information from the United States Department of Agriculture, typical Brazilian aliments not found in the software database had their nutritional values estimated and inserted in the NDSR software following Brazilian guidelines. Nutritional values related to total energy intake, vitamin D, and calcium were calculated for all participants. Subsequently, data from the two 24-hour recalls were used to adjust for the intra-

and interindividual variability to reflect the usual intake. The Iowa State University (ISU) method was used for symmetrical food/nutrients variables without zeros in their distribution (TEI and TCVUPP), and the National Cancer Institute method (NCI) (31) was used for variables with a non-normal distribution and/or occasionally consumed nutrients. Both nutrient intake variables considered adjustment for the total energy intake – nutrient residual (energy-adjusted model) – recommended by Willet et al. (32). For analytical purposes, vitamin D and calcium intake were included as continuous variables in the model.

2.4 Covariates

Sex, age group (20/39 or 40/59 years), household income (in tertiles), smoking status (never, former, or current smoker), and waist circumference, an indicator of central obesity, (all collected in 2009) were considered potential confounders and included in the analytical models accordingly. While the former information was elicited from questionnaires, waist circumference (in cm) was measured in the narrower trunk region or at the midpoint between the last rib and the upper border of the iliac crest when the narrower trunk region was not apparent, using an inelastic tape measure (Sanny®, São Bernardo do Campo, São Paulo, Brazil) of 160 cm in length and a precision of 1 mm. Waist circumference was categorized into quartiles, and individuals in the last quartile were considered obese.

2.5 Theoretical framework

Based on the literature, a directed acyclic graph was drawn to depict the relationship between micronutrients intake and periodontitis, given a set of potential confounders supported by previous evidence (**Figure 2**). We considered the intake of both micronutrients as potential exposures and established interaction between them, as indicated in the literature (25).

2.6 Analytical approach

Descriptive analysis of all variables used in the study is provided as absolute and relative frequencies for categorical variables and means with their respective standard deviations for continuous variables. Analyses were conducted using sampling weights clustered to the census sector, accounting for the inverse of the selection probability in 2009 and the probability of participating in 2012.

Given our complex analytical scenario comprising two highly correlated exposures, vitamin D and calcium intake, marginal structural modeling (MSM) appears as a valuable asset to performing multivariable analyses that model complex relations among a set of variables. It also allows the estimation of marginal risk ratios considering the counterfactual scenario. For this study, we calculated the controlled direct effect of vitamin D and calcium intake on periodontitis by estimating the inverse probability weight of both variables independently given the confounders and later multiplying them to obtain the final stabilized weight. In order to check the consistency of our final stabilized weight, a mean value of approximately 1.00 was expected.

Furthermore, we conducted a sensitivity analysis for unmeasured confounding by calculating the E-value, as proposed by VanderWeele and Ding (33). Briefly, a large E-value indicates that considerable unmeasured confounding would be required to eliminate the association between the exposures and outcome. All analyses were conducted using the Stata 16.1 (StataCorp., College Station, TX) software.

3 Results

Of the 1,222 participants evaluated in 2012, periodontal data were available for 1,066 adults (**Table 1**). At baseline, nearly 49% of the participants were females with a mean age of 35 years (SD ± 11.7), 58% were never-smokers, and 14% were classified as having central obesity. The total energy intake was 2,341.2 Kcal in males (SD ± 266.8) and 1,818.1 in females (SD ± 240.7), which was higher than the basal metabolic rate (1,676.1 Kcal SD ± 10.3 and 1,313.3 Kcal SD ± 7.1, respectively). The median difference between the total energy intake and the basal metabolic rate was 541 Kcal (interquartile range 381 – 726 Kcal).

The overall prevalence of periodontitis was 12.3% (95%CI 10.2;14.7), and the average vitamin D and calcium intake were 4.4 μg (SD ± 0.4) and 744.1 mg (SD ± 109.1), respectively. Correlations between vitamin D and calcium intake were 0.31 for the whole sample, 0.42 among men, and 0.30 among females. While vitamin D intake was similar among participants periodontally healthy (4.4 μg) or with periodontitis (4.5 μg), the latter had lower levels of calcium intake (748.7 and 711.6 mg, respectively). A higher prevalence of periodontitis was also observed among males, older adults, current smokers, centrally obese, and the poorest (lowest income tertile). Regarding sex, men had an average intake of 4.5 μg of vitamin D and 714.9 of calcium, while women had 4.3 μg and 773.2, respectively.

Table 2 displays the risk ratios (RR) with their respective 95% confidence intervals (95% CI) for the total sample and separately for men and women. Calcium intake protected from periodontitis in the whole sample (RR 0.61; 0.45;0.83), whereas no association between vitamin D and periodontitis was observed (RR 1.13; 95%CI 0.82;1.56). The same null association between vitamin D intake and periodontitis was observed among males (RR 1.13; 95%CI 0.65;1.97) and females (RR 1.07;

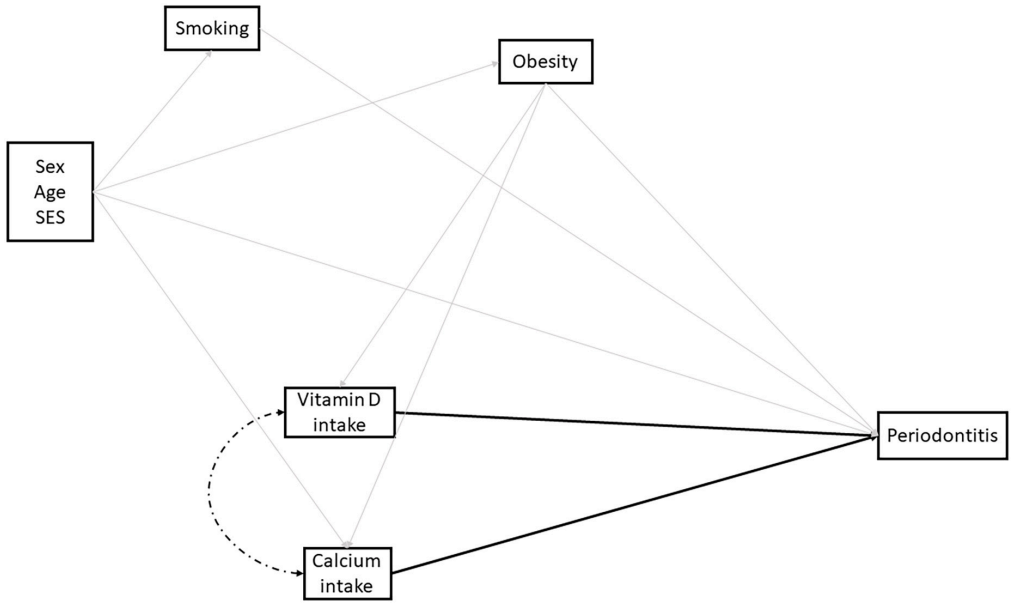

FIGURE 2
Directed acyclic graph depicting the relationship between dietary vitamin D and calcium and periodontitis among Brazilian adults. Solid light gray lines indicate potential confounders included in the analysis, whereas solid black lines indicate the direct effect of dietary intake of vitamin D and calcium on periodontitis. Finally, the dashed black line indicates the influence of vitamin D and calcium intake on each other.

TABLE 1 Sample characteristics and distribution according to periodontitis.

	n (%)[1] or mean (SD)[2]	Periodontitis %[1] or mean[2] (95%CI)	
		No	Yes
Sex[1]			
Male	463 (49.3)	85.4 (81.8; 88.4)	14.6 (11.6; 18.2)
Female	603 (50.7)	90.0 (87.6; 92.0)	10.0 (8.0; 12.4)
Age[1]			
20-39	562 (63.9)	93.6 (90.7; 95.6)	6.4 (4.4; 9.3)
40-60	504 (36.1)	77.4 (72.3; 81.4)	22.6 (18.6; 27.1)
Equalized income in Brazilian reais[1,2]			
3rd tertile (highest income)	662.7 (270.4)	91.2 (87.0; 94.2)	8.8 (5.8; 13.0)
2nd tertile	1,585.7 (355.8)	87.6 (83.8; 90.6)	12.4 (9.4; 16.2)
1st tertile (lowest income)	4,775.5 (4,154.0)	83.8 (79.4; 87.4)	16.2 (12.6; 20.5)
Smoking status[1]			
Never-smoker	584 (58.8)	90.5 (87.6; 92.7)	9.5 (7.3; 12.4)
Former smoker	285 (23.9)	86.2 (81.4; 89.9)	13.8 (10.1; 18.6)
Current smoker	191 (17.3)	80.5 (75.3; 84.8)	19.5 (15.1; 24.7)
Central obesity[1]			
Eutrophic	866 (86.1)	89.2 (86.7; 91.4)	10.7 (8.6; 13.3)
Obese	169 (13.8)	76.7 (69.6; 82.3)	23.0 (17.0; 30.4)
Vitamin D intake (μg)[2]	4.4 (4.4; 4.5)	4.4 (4.4; 4.5)	4.5 (4.5; 4.6)
Calcium intake (mg)[2]	744.1 (729.0; 759.2)	748.7 (733.1; 764.2)	711.6 (692.3; 730.9)
Periodontitis[1]			
No	910 (87.7)	–	–
Yes	156 (12.3)	–	–

[1]n (%); [2]Mean (SD).

TABLE 2 Association between vitamin D, calcium, and periodontitis estimated using marginal structural modeling.

	Total sample	Men	Women
	Risk ratio (95%CI)	Risk ratio (95%CI)	Risk ratio (95%CI)
Vitamin D intake	1.13 (0.82; 1.56)	1.13 (0.65; 1.97)	1.07 (0.72; 1.61)
Calcium intake	0.61 (0.45; 0.83)	0.69 (0.40; 1.14)	0.55 (0.38; 0.79)
E-value for calcium intake[1]	2.66 (1.70)	–	3.04 (1.84)

Estimates given are risk ratios and respective 95% confidence intervals. Analyses are shown for the total sample, as well as stratified by sex.
[1]The *E*-value was calculated only for positive associations. Thus, there is no values for vitamin D intake. The *E*-value provides the point estimate and lower limit of the confidence interval (between brackets).

0.72;1.61). However, there was evidence of heterogeneity in the association between calcium intake and periodontitis (*P*-value for interaction 0.018), as the protective association between calcium intake and periodontitis was observed in women (RR 0.55; 95%CI 0.38;0.79) but not among men (RR 0.69; 95%CI 0.40;1.14).

Finally, our sensitivity analysis for unmeasured confounding shows that the strength required for an unmeasured confounder to eliminate the effect of calcium intake on periodontitis would be 3.0 (**Table 2**).

4 Discussion

Our findings suggest that a high vitamin D intake was not associated with periodontitis, while high dietary calcium intake had a protective, though weak, effect on periodontitis, especially in women. These results could be noticed by using an analytical approach able to deal with the complex relationship between vitamin D and calcium intake and periodontitis. Thus, it is possible to speculate that the conflicting results found in the literature regarding this association could be partially attributed to analytical methods used thus far, as conventional regressions do not support exploring intertwined exposure variables, as in our study.

Prior to discussing our findings, we should carefully examine the limitations inherent to our study. Firstly, although we used data from a cohort study, our analysis is cross-sectional, as information on dietary intake and periodontitis was elicited in 2012. However, we do not believe that periodontitis might have impacted calcium and vitamin D intake, as even severe cases of periodontitis would not prevent individuals from eating foods rich in these nutrients. Thus, the chance of reverse causality, if any, is trivial. Secondly, our periodontal data did not allow the employment of the classification system recommended by the two major periodontal organizations, i.e., the American Academy of Periodontology and the European Federation of Periodontology, nor an approach that would account for the multidimensional aspect of periodontitis (latent). While this might have underestimated our results, other studies using a similar classification, including the Global Burden of Diseases, observed a prevalence analogous to ours (34). Moreover, we found comparable results from other classification systems when examining the association between periodontitis and obesity using the current periodontal classification (28).

Additionally, the intake of vitamin D and calcium was assessed using a 24h-dietary recall. It is possible to speculate that eliciting this information only twice (once in the total sample and replicated in a random subsample) might not have captured the habitual food consumption variation. However, replication rates of >40%, as in our case, may not lead to a loss of dietary assessment precision (35). In addition, one might speculate about the possibility of underreporting the total energy intake by the participants, which was identified in only 2.3% of the cohort. Thus, it is unlikely that underreporting has affected our results, as the use of the multiple pass method to assist participants in remembering their food intake aimed to minimize this source of bias.

Furthermore, although one might assume that vitamin D and calcium supplementation might have biased our results, in 2009, only 2.4% of our sample had used supplements containing vitamin D and calcium (data not shown), hence, not impacting our findings. Moreover, while calcium levels highly depend on vitamin D, we did not assess calcium and vitamin D serum levels. In the EpiFloripa study, for instance, a correlation of 0.06 was observed between intake (assessed in 2012) and serum levels of vitamin D (collected in 2014) (data not shown). Given the weak, if any, correlation between vitamin D intake and serum levels [25(OH)D] (36), it is possible to speculate that the null association between vitamin D and periodontitis might have resulted from the lack of data on 25(OH)D. Another potential explanation for this null association might relate to the age of our sample, as an inverse association between vitamin D and periodontitis was observed only among Americans over 50 years (37). Therefore, future studies with a broader age sample are encouraged to clarify this relationship.

It is also relevant to mention that few individuals in this study had a vitamin D and calcium intake considered appropriate following the current recommendations (38). Although the prevalence of inadequate intake of calcium and

vitamin D is high in Brazil, calcium and vitamin D intake in our cohort is higher than the national as well as the regional average (Southern Brazil) (39). Finally, we did not have data on sun exposure, an important factor influencing the serum levels of vitamin D. Still, a recent study also conducted in Southern Brazil, in a geographical region where sun exposure is similar to ours, indicated a positive effect of sun exposure on serum levels of vitamin D, among men, but not among women, whose crucial factor was vitamin D intake (40). Therefore, it is possible to speculate that our results would not have been extensively impacted by adding this information to our analytical models, but future studies should investigate this topic. On a similar note, our sensitivity analysis for unmeasured confounding revealed that only variables with an approximately 3-fold association with the outcome would eliminate our results, hereafter supporting the robustness of our findings.

The nutritional assessment and analysis and the representativeness of the sample concerning the target population are among the strengths of our study. Furthermore, the thoughtful analytical approach should be pointed out. Most, if not all, studies investigating the relationship between vitamin D, calcium, and periodontitis did not account for the complex dynamic between these variables by neglecting the relationship between vitamin D and calcium. This might explain the conflicting results regarding this relationship in the literature, as evident in the systematic reviews conducted on the topic, where the results vary from negative to null and positive (4–6).

Other factors that may explain the lack of consistent results regarding the association between vitamin D, calcium, and periodontitis relate to the assessment of the micronutrients. In a study using Mendelian randomization, the authors did not find an association between 25(OH)D serum levels and periodontitis in a sample of approximately 45,000 Europeans (41). While that study corroborates our findings regarding the lack of association between vitamin D intake and periodontitis, the authors did not use the information on calcium. The potential lack of direct association between vitamin D intake and periodontitis may be explained by studies using calcium tracers. Even though appropriate 25(OH)D levels increase the calcium absorption efficiency, a serum concentration of at least 50 nmol/L is necessary (42). Therefore, vitamin D supplementation does not enhance calcium absorption efficiency in most healthy humans. However, future studies should focus on the interplay among the serum levels of vitamin D, calcium, parathyroid hormone, metabolic dysfunction, and periodontitis.

From a biological perspective, a protective, though weak, association between calcium but not vitamin D and periodontitis was found in women. However, low vitamin D levels reduce calcium absorption, upregulating parathyroid hormone release, osteoclastogenesis, and bone resorption to prevent hypocalcemia (12). Systematic reviews (43, 44) have not identified a significant impact of vitamin D supplementation alone on the risk of fractures, whereas calcium supplementation, mainly in combination with vitamin D, had a protective effect on fractures. Thus, it is possible to speculate a similar pattern in periodontitis. While a positive effect of vitamin D and calcium on bone mineral density is noticed, it appears that this combination also normalizes Parathyroid Hormone Intact (PTHi) and 25(OH)D levels, which, in turn, regulate serum calcium concentration and bone remodeling, especially among women (45). Moreover, studies argue that women tend to store more fat than men and that fat tissue sequester vitamin D from serum (46). Reduction in vitamin D levels upregulates intracellular calcium accumulation in adipocytes, resulting in lipogenesis and weight gain (47). In addition, as osteoclasts possess estrogen receptors but no androgen receptors (14), factors influencing the bone remodeling process become more evident among women, thus, explaining our findings.

5 Conclusion

Our findings suggest a small protective association between dietary calcium intake and periodontitis among women. However, further studies accounting for the dynamic relationship between vitamin D and calcium (intake and serum levels) are needed to further elucidate this association.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee in Human Research of the Federal University of Santa Catarina (351/2008 and 1772/2011). The patients/participants provided their written informed consent to participate in this study.

Author contributions

GN contributed to conception, data analysis and interpretation, and drafted and critically revised the manuscript. FL contributed to the data analysis and interpretation and critically revised the manuscript. DG-C and KP contributed to the conception and design and critically revised the manuscript. MP contributed to the conception and design, data interpretation, and critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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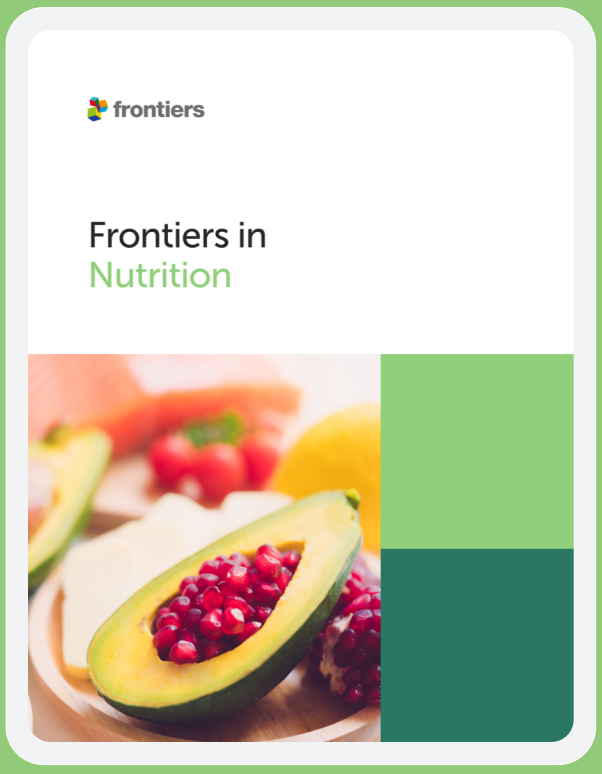

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