DIABETES IN THE MIDDLE EAST

EDITED BY: Mohamed Abu-Farha, Jehad Ahmed Abubaker and Jaakko Tuomilehto

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DIABETES IN THE MIDDLE EAST

Topic Editors:
Mohamed Abu-Farha, Dasman Diabetes Institute, Kuwait
Jehad Ahmed Abubaker, Dasman Diabetes Institute, Kuwait
Jaakko Tuomilehto, National Institute for Health and Welfare, Finland

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Table of Contents

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EDITORIAL
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Editorial: Diabetes in the Middle East

Mohamed Abu-Farha[1*], *Jaakko Tuomilehto*[2,3] *and Jehad Abubaker*[1]

[1] Biochemistry and Molecular Biology Department, Dasman Diabetes Institute, Dasman, Kuwait, [2] Department of Public Health, University of Helsinki, Helsinki, Finland, [3] Diabetes Research Group, King Abdulaziz University, Jeddah, Saudi Arabia

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Editorial on the Research Topic

Diabetes in the Middle East

INTRODUCTION

Diabetes is a rapidly growing disease that is affecting people worldwide, particularly due to the rise in unhealthy lifestyles and increased risk factor levels. Although countries and regions are affected differently, the Middle East in general has witnessed a spike in the occurrence of diabetes. The Gulf region has experienced an especially marked and sudden increase in rates of diabetes where Kuwait, Saudi Arabia and Bahrain now rank among the top 10 countries with highest prevalence of type 2 diabetes worldwide (1). Additionally, children have also experienced a dramatic increase in rates of type 1 as well as type 2 diabetes in this region. Urgent action has been called for in order to tackle this problem in the Middle East, with the aim of reducing and preventing new cases of diabetes and complications due to diabetes.

This Research Topic has broadly focused on diabetes research in the Middle East, highlighting the increasing burden of the disease through recent epidemiological data. Weiderpass et al. conducted a cross-sectional study on Kuwaiti adults using the STEP-wise approach to surveillance of non-communicable disease risk factors. They updated the statistics for the prevalence of overweight and obesity in the population and found them to be 37% and 40.3%, respectively. Similarly, Djalalinia et al. conducted a national cross-sectional study of non-communicable disease risk factor surveillance in Iran. They found a significant difference between the prevalence of obesity in men vs. women (15.3 vs. 29.8%). They also report a considerable variation in the geographical pattern of the prevalence of obesity and overweight where BMI increased from the southeastern to the northwestern regions of the country. These findings show striking differences among the Middle East countries and the high burden of obesity affecting people in the Arabian Gulf region.

Our Research Topic also discussed childhood obesity where Goodson et al. conducted a longitudinal study on Kuwaiti children and performed salivary metabolomic analysis. They report that the level of salivary N1-Methyl-2-pyridone-5-carboxamide (2PY), a biomarker for uranium uptake, has the highest direct association with obesity. Elkum et al. performed a cross-sectional analysis on schoolchildren in Kuwait with Arab ethnicity, the prevalence of overweight and obesity was 17.7% and 33.7%, respectively. They also identified several predictors of childhood obesity including high birth weight, advanced maternal age at index pregnancy and small family size. Additionally, Saraswathi et al. presented a systematic literature review of childhood diabetes research in the Middle East Region. They reported that while many studies focused on the incidence/prevalence of different types of diabetes in childhood, there is a lack of consolidated studies focusing on national

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***Correspondence:**
Mohamed Abu-Farha
mohamed.abufarha@dasmaninstitute.org

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epidemiological data of childhood diabetes and also no studies reporting clinical trials in children with diabetes in the Middle East Region. Furthermore, Farran et al. performed a retrospective cohort study of health data from Kuwait to evaluate the use of non-invasive parameters and machine-learning algorithms for predicting future risk of type 2 diabetes. They found that machine-learning techniques such as k-nearest neighbors (k-NN) and support vector machines (SVM) outperformed the most used logistic regression methods.

This Research Topic also covered research conducted on diabetes-related pathways involving genomics, obesity, insulin resistance, inflammation dyslipidemia as well as diabetes complications. Qaddoumi et al. performed a retrospective study to evaluate metabolic control in patients with type 2 diabetes at Dasman Diabetes Institute, a specialist diabetes clinic and research center. They concluded that the therapeutic management of type 2 diabetes in Kuwait is suboptimal. They provide some recommendations such as better adherence to American Diabetes Association guidelines, evaluating the high obesity rates, as well as promoting diabetes education and self-empowerment. Azzam et al. investigated genetic variations associated with diabetic retinopathy and coronary artery disease in a case-control study conducted in UAE. They reported two SNPs to be associated with diabetic retinopathy (rs9362054 near *CEP162* and rs4462262 near *UBE2D1*) and rs12219125 near *PLXDC2* to be associated with coronary artery disease. They also reported rs9362054 near *CEP162* to be significantly associated with both diabetic retinopathy and coronary artery disease. Hebbar et al. present an extensive perspective on the possible causes for observed differences in the metabolic trait loci profiles between Europeans and Arabs. They also suggested analysis strategies and study designs that can be integrated for identifying genetic risk variants associated with diabetes and related traits in Arab populations. A cross-sectional study by Alghanim et al. investigated the expression levels of circulating ANGPTL5 protein in the circulation in people with obesity and type 2 diabetes and found that higher levels of ANGPTL5 in the circulation were associated with insulin resistance.

Al-Ozairi et al. investigated the feasibility of intermittent fasting (in the form of Ramadan fasting) in people with type 1 diabetes and concluded that intermittent fasting can be safe for patients with uncomplicated diabetes when combined with structured education and advanced glucose monitoring systems. Barda et al. investigated the impact of carbohydrate restriction and insulin treatment on placental maternal and fetal vascular circulation in obese and non-obese women with gestational diabetes mellitus. They reported that the combination of obesity and gestational diabetes increased the risk of fetal thrombo-occlusive disease and the occurrence of gestational hypertension. They also observed that carbohydrate restriction diet plus insulin treatment was associated with improved fetal placental vascular circulation. The study of Alajmani et al. assessing depression among people with type 2 diabetes in UAE found that the overall depression prevalence using a cutoff of 16 points in the Beck Depression Inventory was 17%. They concluded that the intensive service in a diabetes mini clinic compared to primary health care centers appears to benefit psychological aspects in diabetic patients.

In conclusion, our Research Topic covered multiple topics relating to obesity and diabetes in the Middle East. It highlighted the increased burden of these metabolic disorders especially in the Arabian Gulf region. It also showed the importance of enhancing diabetes research in the region, and the lack of structured early diabetes prevention and management programs that can aim at reducing the burden of diabetes and its associated complications needs to be developed urgently.

AUTHOR CONTRIBUTIONS

All authors have contributed equally to this work. All authors contributed to the article and approved the submitted version.

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PERSPECTIVE
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A Perception on Genome-Wide Genetic Analysis of Metabolic Traits in Arab Populations

Prashantha Hebbar[1,2], Jehad Ahmed Abubaker[1], Mohamed Abu-Farha[1], Jaakko Tuomilehto[1†], Fahd Al-Mulla[1] and Thangavel Alphonse Thanaraj[1*]*

[1] Genetics and Bioinformatics Unit, Dasman Diabetes Institute, Kuwait City, Kuwait, [2] Doctoral Program in Population Health, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Despite dedicated nation-wide efforts to raise awareness against the harmful effects of fast-food consumption and sedentary lifestyle, the Arab population continues to struggle with an increased risk for metabolic disorders. Unlike the European population, the Arab population lacks well-established genetic risk determinants for metabolic disorders, and the transferability of established risk loci to this population has not been satisfactorily demonstrated. The most recent findings have identified over 240 genetic risk loci (with ∼400 independent association signals) for type 2 diabetes, but thus far only 25 risk loci (*ADAMTS9,* ALX4, *BCL11A, CDKAL1, CDKN2A/B,* COL8A1, *DUSP9, FTO, GCK, GNPDA2, HMG20A, HNF1A*, *HNF1B, HNF4A, IGF2BP2, JAZF1, KCNJ11, KCNQ1, MC4R, PPARγ, SLC30A8, TCF7L2, TFAP2B, TP53INP1,* and *WFS1*) have been replicated in Arab populations. To our knowledge, large-scale population- or family-based association studies are non-existent in this region. Recently, we conducted genome-wide association studies on Arab individuals from Kuwait to delineate the genetic determinants for quantitative traits associated with anthropometry, lipid profile, insulin resistance, and blood pressure levels. Although these studies led to the identification of novel recessive variants, they failed to reproduce the established loci. However, they provided insights into the genetic architecture of the population, the applicability of genetic models based on recessive mode of inheritance, the presence of genetic signatures of inbreeding due to the practice of consanguinity, and the pleiotropic effects of rare disorders on complex metabolic disorders. This perspective presents analysis strategies and study designs for identifying genetic risk variants associated with diabetes and related traits in Arab populations.

Keywords: Arab population, type 2 diabetes, genome-wide association studies, risk loci, Kuwait, Euro-centric risk variants, genetics, epigenetics

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Ondřej Šeda,
Charles University, Czechia

Reviewed by:
Zhichao Feng,
Albert Einstein College of Medicine, United States
Nirav Dhanesha,
The University of Iowa, United States

***Correspondence:**
Fahd Al-Mulla
fahd.almulla@dasmaninstitute.org
Thangavel Alphonse Thanaraj
alphonse.thangavel@dasmaninstitute.org

†Present Address:
Jaakko Tuomilehto,
Department of Public Health, University of Helsinki, Helsinki, Finland

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BACKGROUND

Discovery of oil reserves in the Arabian Gulf since the 1930s increased overall wealth in these countries. Rapid socioeconomic transitions in Arab countries in the rich post-oil era marked changes in the nutritional patterns and food habits, including a shift from locally grown natural products to a Western diet and change from nomadic way to urbanized life. These resulted in an increasingly sedentary lifestyle and wide-spread obesity (1–3) and an increased prevalence of

diabetes (4, 5), and metabolic syndrome (6) in the last few decades. Diabetes is sweeping through Middle East; as per the International Diabetes Federation Atlas 8th Edition for 2017, the age-adjusted comparative prevalence of diabetes (18–99 years) in the Middle East and North Africa region is 10.5%, which is the second highest after the North America and Caribbean region. Up to 30% of native adult hospital visitors in Kuwait are afflicted with T2DM (4). The prevalence among adults in the countries from the Peninsula (Kingdom of Saudi Arabia 31.6%, Oman 29%, Kuwait 25.4%, Bahrain 25.0%, and United Arab Emirates 25.0%) are significantly associated with high per capita GDP (gross domestic product) and energy consumption (7). T2DM results from a complex interplay of adverse lifestyle exposures and genetic predisposition. Despite the high prevalence of T2DM, the Arab population lacks convincingly determined T2DM genetic risk variants and reports that sufficiently demonstrate the replication of established risk variants.

Identified T2DM Risk Loci in Arab Population Compared With Established Gene Loci

Since the advent of genome-wide association (GWA) studies, many T2DM risk loci have been globally identified, which concentrated mainly on the European and Asian populations. A recent study by Mahajan et al. (8), that combined data from 32 European-descent GWAS imputed to high-density reference panels, identified 243 genome-wide significant T2D-risk loci with 403 independent association signals. Therefore, it is important to understand the relevance and performance of these established loci in the Arab population and how often such novel risk loci are identified in this population. Published reports on T2DM genetics in the Arab population originate from Kuwait, Lebanon, Saudi Arabia, Qatar, UAE, Oman, and Tunisia (**Table 1**). Some of these studies illustrate certain overlaps with established T2DM risk loci, some illustrate the complete absence of any overlap (**Table 1**), and some identify novel risk loci. Approximately 25 established T2DM loci (*ADAMTS9,* ALX4, *BCL11A, CDKAL1, CDKN2A/B,* COL8A1, *DUSP9, FTO, GCK, GNPDA2, HMG20A, HNF1A*, *HNF1B, HNF4A, IGF2BP2, JAZF1, KCNJ11, KCNQ1, MC4R, PPARγ, SLC30A8, TCF7L2, TFAP2B, TP53INP1, and WFS1*) and few established obesity loci (*ADIPOQ, FTO, RFX7, and USP37*) are observed to replicate in the Arab population (**Table 1**). The replicated T2DM risk loci fall into three categories (**Table S1**): (i) those that impact the T2DM risk through impaired *β*-cell function (*ALX4, BCL11A, CDKAL1, CDKN2A/B, GCK, HNF1A, HNF1B, HNF4A, IGF2BP2, JAZF1, KCNJ11, KCNQ1, SLC30A8, TCF7L2, WFS1*); (ii) those that act through modulating insulin action *(ADAMTS9, DUSP9, PPARγ);* and (iii) those that were primarily associated with BMI, obesity and adiposity but subsequently identified also as affecting T2DM risk through insulin action (*FTO, GNPDA2, MC4R*, *TFAP2B*).

Studies illustrating a replication of established risk loci in Arab population are generally based on targeted genotyping, whereas those failing to observe the established loci are GWA-based. GWA studies for quantitative traits in the Arab population in Kuwait were unsuccessful to demonstrate established loci at genome-wide significance but instead led to identification of novel risk loci (**Table 2**); these identified novel loci are often reported in literature as associated with biological processes relating to the T2D traits (**Table S2**). Moreover, even at nominal *p*-values, very few established markers are identified in the data sets in the Kuwaiti population (**Table 2**—Items V–VI). The exemplary susceptibility gene loci namely, *PPARγ, KCNJ11, TCF7L2, SLC30A, ABCC8, HHEX, CDKN2A, IGF2BP2, CDKAL1,* and *FTO*, known to be well-replicated in other ethnic population groups were not identified in our studies on the Kuwaiti population. Similarly, only two established T2DM risk gene loci namely (*CDKAL1* and *TCF7L2*) were identified using an imputed data set of 5,000,000 single nucleotide polymorphisms (SNP) in the Lebanese population, which has a higher affinity to European populations compared with other Middle Eastern populations (21). It was also observed that the established markers do not necessarily replicate among inter-Arabic population groups. For example, Mtiraoui et al. (17) (**Table 1**) illustrated differences between the Levant Arabs (from Lebanon) and north African Arabs (from Tunisia) by demonstrating the association of *TCF7L2* in both groups, *IGF2BP2* and *PPARγ* exclusively in the Lebanese group, and *KCNJ11* and *SLC30A8* exclusively in the Tunisian group.

The partial overlap of established markers and differential replication of established markers in inter-Arabic populations along with the identification of novel loci are also observed in case of other complex disorders, such as rheumatoid arthritis, myocardial infarction/coronary artery disease, prostate cancer, and breast cancer (37–40). It is highly interesting to perform genetic association studies in ethnic populations such as Arabs, not only to re-confirm findings from other ethnic groups but since they may also lead to identification of novel T2DM risk loci.

POSSIBLE CAUSES FOR OBSERVED DIFFERENCES IN THE RISK LOCI PROFILES

Study Cohorts

The above-mentioned observations in Arab populations are associated with the size of study cohorts used in the studies, which is considerably lower than those used conventionally in global GWA studies (**Table 1**). This deficit affects the strength of the studies and leads to issues such as non-consideration of markers that have become rare in Arab populations. Other shortcomings of the study designs are inconsistencies (such as in age) between case and control cohorts and the failure to include in the set of markers tested for replication those that are in LD with established markers.

Differences in Phenotype Profiles Between Arab and Global Populations

While most of the well-characterized T2DM genes appear to be associated with β-cell dysfunction, diabetes observed in the Arab population is supposedly associated with obesity. This is underlined by the following observations in the Arab population: the prevalence of obesity in T2DM patients is high (41), Arab

TABLE 1 | Studies on T2DM risk loci from the region and comparison with established T2DM loci.

Study	Testing of established loci or general GWAS study; Study population	Sample size	Replicates known markers or identifies novel markers?	Conclusions
TARGETED GENOTYPING—REPLICATION STUDIES				
Li-Gao et al. (9)	Tested 122 T2DM risk variants (from 84 loci) in Saudi Arabian population	659 T2DM cases and 919 controls	**Absence of any overlap:** None of the tested markers was replicating at *p*-value corrected for Bonferroni threshold (0.05/122). At $p < 0.05$, 11 were seen replicating	Failure to replicate any of the tested 122 risk variants was attributed to low sample size and also to study design—controls were not age-matched. The authors recommend large-scale GWAS
Osman et al. (10)	Tested established loci (BMI:87; WC:58; obesity with T2DM:145) in UAE Arab population	880 BMI cases; 455 WC cases; 464 T2DM cases and 415 contols	**Partial overlap and novel loci:** Replicates *FTO, USP37,* and *RFX7 (*for obesity*)* and *TCF7L2* and *MC4R (*for T2DM). Also reports novel associations *KCNK3* and *RARB* for T2DM	Could replicate very few established obesity and T2DM loci; the study could identify few novel associations for T2DM in Arabs
Cauchi et al. (11)	Tested 44 variants from 37 established loci for T2DM in North African Arabs (Morocco and Tunisia)	1,193 T2DM cases and 1,055 controls from Morocco. Associations were then assessed in 1,446 T2DM cases and 942 controls from Tunisia	**Partial overlap:** 13 of 37 established gene loci confirmed in Moroccans and Tunisians. *BCL11A, ADAMTS9, IGF2BP2, WFS1, CDKAL1, TP53INP1, CDKN2A/B, TCF7L2, KCNQ1, HNF1A, FTO, MC4R,* and *GCK.*	Concludes sharing of T2DM risk loci between Europeans and North African Arabs
O'Beirne et al. (12)	Tested 37 variants from 29 established T2DM gene loci, and an additional 27 tag SNPs in Qatari population	1,124 T2DM cases and 590 controls	**Partial overlap:** Only *TCF7L2* of the tested 29 loci was seen replicating in Qatari population	Concludes that the genetic risks for T2DM are likely different in Qataris compared to Europeans and Asians
Al-Daghri et al. (13)	Tested 28 established T2DM loci in Saudi Arabian Population	1,166 T2DM cases and 1,235 controls	**Partial overlap:** Replicates 9 of the 28 tested established T2DM loci of *TCF7L2, WFS1, JAZF1, SLC30A8, CDKN2A/B, KCNQ1, HMG20A, HNF4A,* and *DUSP9*	Concludes overlap in T2DM risk loci across ethnicities irrespective of prevalence
Tomei et al. (14)	Tested 23 established obesity-related loci in Qatari population	804 Qatari individuals	**Partial overlap:** Could identify only two (*TFAP2B* and *GNPDA2*) of the tested 23 obesity loci	Concludes a different genetic profile associated with obesity in the Qatari population compared to Western populations
Almawi et al. (15)	Tested 19 SNPs in/near 15 established T2DM loci in Lebanese Levant population	995 T2DM cases and 1,076 controls	**Partial overlap:** 4 (*COL8A1, KCNQ1, ALX4, HNF1B*) of the 15 established loci were seen associated with T2DM in Levants. The authors have shown in their previous works replicability of IGF2BP2, CDKAL1, TCF7L2.	Concludes that insufficient power as the reason for the inability to detect all the tested loci in Levants
Al-Sinani et al. (16)	Tested 10 variants from 9 established T2DM loci—*KCNJ11, TCF7L2, CDKAL1, CDKN2A/B, FTO, IGF2BP2, SLC30A8, CAPN10, HHEX* in Omani Arabs	992 T2DM cases and 294 controls	**Partial overlap:** Only four of the 9 tested loci could be replicated—*KCNJ11, TCF7L2, CDKAL1, CDKN2A/B*	Suggests large-scale studies, other than case-control design, to detect rare variants that might explain the missing heritability
Mtiraoui et al. (17)	Tested 7 established T2DM loci - *ENNP1, IGF2BP2, KCNJ11, MLXIPL, PPARγ, SLC30A8*, and *TCF7L2* in Levant Arabs from Lebanese and North African Arabs from Tunisia	Lebanese: 751 T2DM cases and 918 controls Tunisia: 1,470 T2DM cases and 838 controls	**Partial overlap with established markers and differences in overlap between the two Arab popualtions:** *TCF7L2* was replicating in both; *IGF2BP2* and *PPARγ* were replicating in Lebanese and not in Tunisia; *KCNJ11*, and *SLC30A8* were replicating in Tunisia but not in Lebanese. Neither *ENNP1* nor *MLXIPL* was seen replicating in Lebanese or Tunisians.	Concludes differences in replicability of T2DM loci between Lebanese and Tunisians as well as between Europe and these two Arab groups.
El Hajj Chehadeh et al. (18)	Investigated the association between the *MTHFR* SNPs (C677T and A1298C) and T2DM in Emirati Arabs	169 T2DM cases and 209 controls	**Absence of any overlap:** *MTHFR* gene polymorphisms are not related to T2DM in the Emirati population	Could not establish MTHFR variants as risk variants for T2DM in Emirati population

(Continued)

TABLE 1 | Continued

Study	Testing of established loci or general GWAS study; Study population	Sample size	Replicates known markers or identifies novel markers?	Conclusions
Al Safar et al. (19)	Tested the T2DM risk loci—*TCF7L2 (*rs10885409*)* and *PPARγ2 (*rs1801282*)* in Arab Emirati population	272 T2DM cases and 216 controls	**Partial overlap:** Confirms the association of the *TCF7L2* variant but not *PPARγ2* as a T2DM loci in UAE Arabs	Confirms one of the two established markers as T2DM loci; and the other established marker as NOT a T2DM loci
Khan et al. (20)	Tested the established obesity loci—*FTO* (rs9939609) and *VDR* (rs1544410), in UAE population	201 obese, 115 overweight, and 98 normal subjects	**Partial overlap:** Replicates the *FTO* marker as adult obesity risk loci in UAE. *VDR* could not be replicated	No significant association is seen in UAE population for the established marker from *VDR*
POPULATION-BASED GWA AND FAMILY-BASED STUDIES				
Ghassibe-Sabbagh et al. (21)	Performed GWAS (on genotyped and imputed markers) in Lebanese population	3,286 Lebanese participants	**Partial overlap and Novel loci:** Only two established loci (*CDKAL1* and *TCF7L2*) surfaced	Concludes that the replication of established markers in Lebanese population is less than expected
Al Safar et al. (22)	Performed family-based GWAS for T2DM in UAE Arabs	Discovery cohort: $N = 178$ (66 cases, 112 controls); Replication cohort: $N = 315$ (116 cases and 199 controls)	**Novel loci:** Identified novel associations (*KCTD8*, *PRKD1*, *GABRA2*, *GABRA4*, and *GABRB1*)	Did not identify any of the established gene loci
Zadjali et al. (23)	Family-based study ("Oman Family Study") to investigate the association of SNPs (rs17300539 and rs266729) from adiponectin gene *ADIPOQ* with obesity traits in Oman	328 Arabs in one large extended family from Oman	Showed family-based evidence for association of one (rs266729) of the two tested SNPs from *ADIPOQ* defining obesity in Arab population	Concludes that *ADIPOQ* as obesity loci in Arabs from Oman
Our own studies (24–26)	Performed GWAS for quantitative metabolic traits on Arabs from Kuwait	Discovery cohort: 1,353; Replication cohort: 1,176 from Kuwait	Identified novel associations for metabolic traits	Only three established markers from *CETP* and *STARD3* are seen at borderline *p*-values

studies often find obesity-related genetic loci (e.g., *ADIPOQ* gene) to contribute to the genetic risk of T2DM in Arab populations (10, 23, 42, 43), and established T2DM-related SNPs are seen associated with obesity phenotypes in Arabs (44).

High Rate of Consanguinity and Prevalence of Rare, Mendelian, and Familial Disorders in Arab Populations

Marriages in Arab populations are traditionally often consanguineous (45, 46). An increased risk of T2DM has been observed among the offspring of such consanguineous marriages in Saudi Arabia and Qatar (47, 48). The familial clustering of T2DM has been reported in Arab populations from Morocco (49), Tunisia (50), Oman (51), and Qatar (52). Additionally, Arab populations exhibit many rare, Mendelian, and familial genetic disorders. Blair et al. (53) identified thousands of associations between Mendelian and complex diseases in the medical records of over 110 million patients from USA and revealed a non-degenerate, phenotypic code that links a complex disorder to a unique collection of Mendelian loci. Using GWA studies, they further demonstrated that common variants associated with complex diseases are enriched in the genes indicated by this "Mendelian code." Thalassemia, cystic fibrosis, Huntington's disease, and Friedreich's ataxia are examples of rare disorders that increase patient's pre-disposition to diabetes (54–58). Examples of T2DM risk genetic loci, which are also associated with rare recessive disorders, are *LIPC* (Hepatic lipase deficiency), *PDX1* (Pancreatic agedness 1), *ENPP1* (Hypophosphatemic rickets, also associated with obesity), *WFS1* (Wolfram syndrome 1), and *SLC2A2* (Fanconi-Bickel syndrome). **Table 2** summarizes OMIM disease annotations, from the OMIM (Online Mendelian Inheritance in Man) catalog of human genes and genetic disorders (59), for risk loci that were identified for metabolic traits in Kuwaiti Arab populations. The genetic locus *OTX2-AS1* associated with hypogonadism is particularly interesting as literature reports have provided hints regarding the connection between hypogonadism and T2DM in Arab consanguineous families (60, 61). Al Hayek et al. reported that 36.5% men with T2DM from Jordan had low serum testosterone levels; 17% of such T2DM patients with low serum testosterone levels had primary hypogonadism, whereas the remaining had secondary hypogonadism (62). A study based on Saudi Arabian population revealed a significantly higher positive family history of schizophrenia in patients with first or second cousin parents (63). An increased prevalence of T2DM has been observed in patients with schizophrenia (SCZ); the co-occurrence of SCZ and T2DM may partly be driven by shared genetic factors (64) such as cell adhesion molecules (65, 66). Furthermore, the QDiabetes-2018 model (used within the UK National Health Service) (67) now includes schizophrenia, learning disabilities, use of atypical antipsychotics, treated

TABLE 2 | Risk variants identified in our previous studies on Arab individuals from Kuwait.

Metabolic traits	Gene loci /variant	Model; *p*-value; Beta value for associations	OMIM annotation for the gene (where available)
I. OBESITY TRAITS (25)			
Waist Circumference (WC)	*TCN2*/rs9606756	Additive; 1.46E-07 (9.46E-08 upon correction for medication); 4.815	Transcobalamin II deficiency (AR) (PMIM: 275350)
II. BLOOD PRESSURE TRAITS (27)			
Systolic Blood Pressure (SBP)	*MC3R*/rs3827103 [Val81Ile]	Additive; Sequencing the genes. 0.01; 4.9	Mycobacterium tuberculosis, protection against; (PMIM: 607948); Obesity, severe, susceptibility to, BMIQ9; (PMIM: 602025)
III. METABOLIC TRAITS (26)			
Glycated hemoglobin (HbA1c)	*ZNF106* (W > R)/rs12440118	Recessive; 7.07E-08; 2.006	
Fasting Plasma Glucose (FPG)	*OTX2-AS1*/rs7144734	Recessive; 2.82E-07; 1.465	OTX2 with Microphthalmia, syndromic 5 (AD) (PMIM: 610125); Pituitary hormone deficiency, combined, 6 (AD) (PMIM: 613986); Retinal dystrophy, early-onset, with or without pituitary dysfunction (AD) (PMIM: 610125). AS1 with susceptibility to Asthma (PMIM: 607277)
Triglyceride (TG)	*PLGRKT*/rs17501809	Recessive; 1.04E-07; 1.807	
Triglyceride (TG)	*LOC105376072*/ rs11143005	Recessive; 4.03E-07; 0.419	
Triglyceride (TG)	*IGF1*/rs10860880	Recessive; 2.07E-07; 1.596	Growth retardation with deafness and mental retardation due to IGF1 deficiency (AR) (PMIM: 608747). Decreased IGF-1 secretion occurs in the majority of the thalassemia patients particularly those with growth and pubertal delay (28). Thalassemia is recessively inherited
Triglyceride (TG)	[*THSD4, NR2E3*]/ rs900543	Recessive; 1.27E-07; 1.625	NR2E3 with Enhanced S-cone syndrome (AR) (PMIM: 268100); Retinitis pigmentosa 37 (AD,AR) (PMIM: 611131)
IV. LIPID TRAITS (24)			
Triglyceride/Fasting Plasma Glucose/Glycated hemoglobin—TG/FPG/HbA1c	*RPS6KA1*/rs1002487	Recessive; 7.17E-11; 6.517/1.64E-08; 8.315 (for FPG)	
Triglyceride (TG)	*LAD1*/rs11805972	Recessive; 2.16E-17; 8.485	Leukocyte adhesion deficiency (AR) (PMIM: 116920)
Triglyceride (TG)	*OR5V1*/rs7761746	Recessive; 1.31E-09; 6.006	
Triglyceride (TG)	[*CTTNBP2, LSM8*]/rs39745	Recessive; 1.57E-08; 5.643	
Triglyceride (TG)	*PGAP3*/rs2934952	Recessive; 1.16E-09; 6.086	Hyperphosphatasia with mental retardation syndrome 4 (AR) (PMIM: 615716)
Triglyceride (TG)	[*RP11-191L9,CERK*]/rs9626773	Recessive; 7.47E-15/7.776	
Triglyceride (TG)	*ST6GALNAC5*/rs10873925	Recessive; 4.11E-08/0.633	
Triglyceride (TG)	*SPP2_ARL4C*/rs4663379	Recessive; 8.38E-09/1.841	
Triglyceride (TG)	*NPY1R*/rs10033119	Recessive; 8.79E-09/2.698	
Triglyceride (TG)	*LINC00911_FLRT2*/rs17709449	Recessive; 5.12E-08/1.173	
Triglyceride (TG)	*CDK12-NEUROD2*/ rs11654954	Recessive; 2.18E-08, 0.9881	
Triglyceride (TG)	*STARD3*/rs9972882	Recessive; 1.81E-08, 0.7284	

(Continued)

TABLE 2 | Continued

V. ESTABLISHED MARKERS (AT GENOME-WIDE SIGNIFICANCE IN GWAS CATALOG) APPEARING IN KUWAITI DATA SET AT NOMINAL *p*-VALUES FOR ASSOCIATION		
Triglyceride (TG)	rs9326246/*BUD13*	Additive; 5.19E-06; 0.24 (KWT) ≤1.27E-229; 0.22 [European population (29)]
High-Density Lipoprotein (HDL)	rs3764261/*CETP*	Additive; 1.10E-05 (KWT)—reached 4.64E-08 under joint analysis. 1E-769 [European, East Asian, South Asian and African ancestry (30)]
High-Density Lipoprotein (HDL)	rs1864163/*CETP*	Additive; 4.64E-06 (KWT)—reached 1.15E-08 under joint analysis. 7E-39 [FUSION, SardINIA, Diabetes Genetics Initiative studies; also, in East Asian, and European populations (30, 31)]
High-Density Lipoprotein (HDL)	rs1800775/CETP	Additive; 4.99E-06 (KWT)—reached 5.51E-08 under joint analysis. 4E-93 [Europeans and Filipinos (32)]
Triglyceride (TG)	rs9972882/STARD3	Additive; 4.07E-07 (KWT) An LD marker rs1877031/ STARD3 is an established marker in East Asians for the related trait of HDL at 1E-21 [East Asians (30)]
VI. ESTABLISHED MARKERS (AT SUGGESTIVE *p*-VALUES IN GWAS CATALOG) APPEARING IN KUWAITI DATA SET ALSO AT NOMINAL *p*-VALUES FOR ASSOCIATION		
Triglyceride (TG)	rs900543/[*THSD4,NR2E3*]	Recessive; 2.26E-07; 1.625 (KWT). 9.40E-05; 0.036 [Europeans (33)] Fasting insulin
Triglyceride (TG)	rs11143005/*LOC105376072*	Recessive; 3.218E-07; 0.420 (KWT). 4.47E-05; 0.11 [Europeans (34)] 2 h fasting glucose
Triglyceride (TG)	rs17569297/[*LOC105369738, LOC105369739*]	Recessive; 6.963E-06; 0.773 (KWT) 1.51E-06; NA [Europeans (35)] HDL
Total Cholesterol (TC)	rs10935794/[*RPL32P9, LINC01213*]	Additive; 3.65E-06; 0.2037 (KWT) 9.80E-05; NA [Europeans (36)] Serum ratio of Arabinose fructose

hypertension, and polycystic ovary syndromes as additional risk factors.

ANALYSIS STRATEGIES FOR GWA STUDIES IN ARAB POPULATIONS

The current analysis strategies for GWA studies in Arab populations and suggested extensions are presented in **Figure 1** and are discussed below.

Participant Recruitment and Sample Size

The sample size has a linear relationship with the number of identified risk loci; a plateau has not been observed for any trait to date (68). Recruiting the required large number of participants from the intended sample population continues to be challenging in Arab countries (69, 70). Clinical research in Arab countries experiences a lack of public outreach capabilities and coordination between research institutes and hospitals or Ministries of Health. This challenge can be circumvented by understanding public perception and attitude toward medical research and by seeking out means to increase public trust and awareness of clinical research in the Arab population.

The optimum sample size is determined by various factors including homogeneity seen in the population, prevalence of the disorder, variance in the trait measurements, genetic models used in the association tests, number of markers tested in the study, allele frequencies of the risk variants, effect sizes, genome control inflation rates, desired Type I error rates, type of study design (quantitative trait association, case-control studies with unrelated individuals or family-based trios, or sibling case-control designs) (71, 72). A number of tools are available to calculate the optimum sample size; some of the tools often used include Genetic Association Study (GAS) Power Calculator for one-stage genetic association studies http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/; (73), CaTS Power calculator http://csg.sph.umich.edu//abecasis/CaTS/index.html; (74) for two stage genome wide association studies; Quanto for various study designs (including the matched case-control, case-sibling, case-parent, and case-only designs) http://biostats.usc.edu/Quanto.

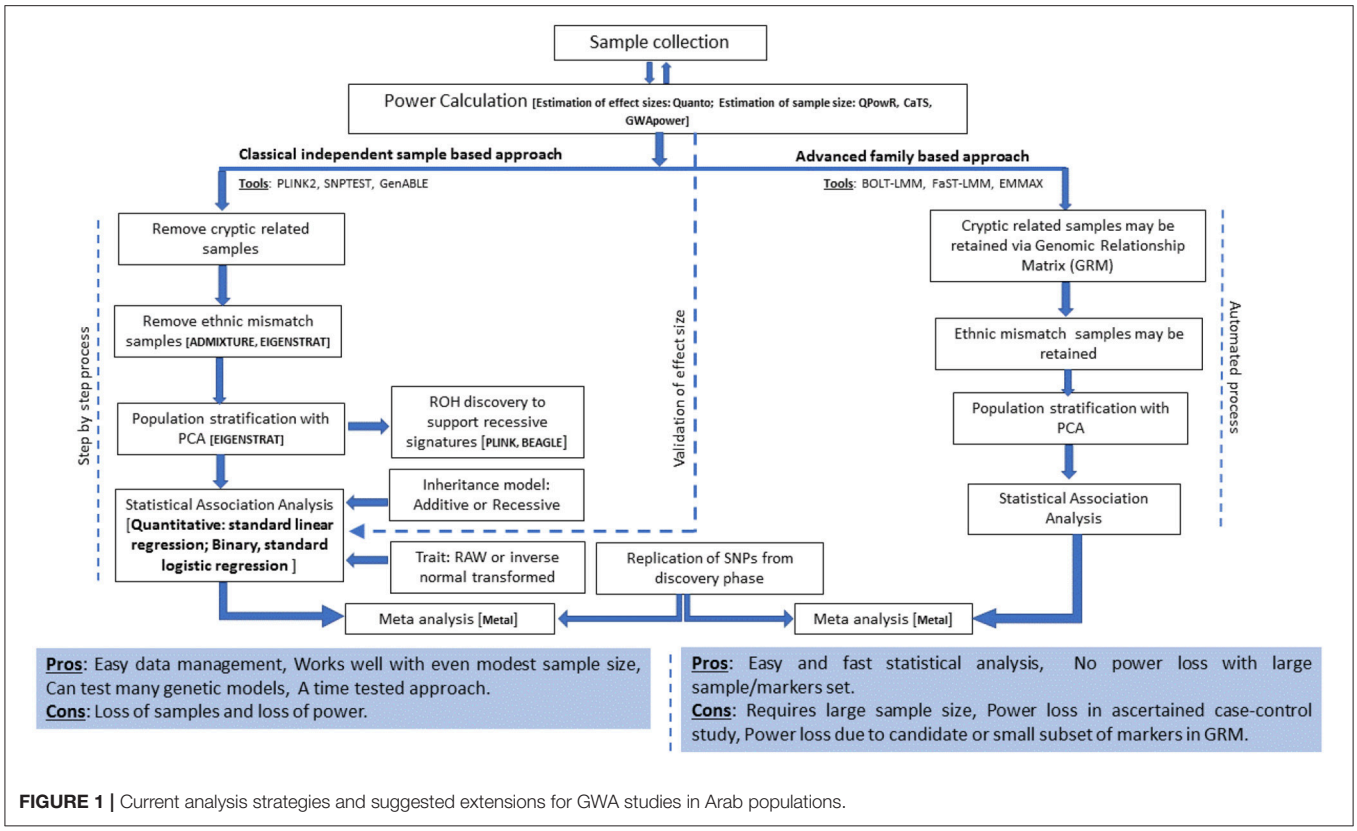

FIGURE 1 | Current analysis strategies and suggested extensions for GWA studies in Arab populations.

html; (75), and QpowR for two-stage study design with unrelated individuals https://msu.edu/~steibelj/JP_files/QpowR.html.

Differences in the Extent of Inbreeding Among Subgroups in the Arab Population

The rate of consanguinity and the extent of resultant inbreeding differ among Arab countries as well within a single country. For example, among the three genetic substructures of Kuwaiti Arab population (76–79), the subgroups of Saudi Arabian tribe ancestry and Persian ancestry exhibit high inbreeding coefficients (0.04226 and 0.025742, respectively) indicating endogamy, whereas the nomadic Bedouin subgroup exhibits lower inbreeding coefficient (0.00274), indicating heterogamy (76). Similar observations have been made with population substructures in Qatar (80). The genetic heterogeneity between the subgroups warrants subgroup-specific genetic association analysis using large sample sizes, particularly for those with higher rates of inbreeding.

Comorbid Conditions as Confounders

Diabetes is often comorbid with other complex chronic disorders (81); Most prevalent comorbid disorders, that occur in "concordant" form wherein both disorders represent parts of the overall identical pathophysiological risk profile, include hypertension, coronary artery disease and peripheral vascular disease (82). An example for comorbid disorders being influenced by unique environmental factors is an increased risk for diabetes in patients with learning disabilities, physical or sensory disabilities, and mental health problems. The co-occurrence of schizophrenia and diabetes may partly be driven by shared genetic factors (64). Comorbidities can also be consequences of hyperglycemia. Tests for genetic associations need to consider comorbid conditions. For advancements in metabolic disorder research in the Arabian Peninsula, the identification of overlaps between metabolic disorders and rare genetic as well as common disorders from existing literature and the formation of a catalog of causal factors/markers for overlapping traits are immediately required. A careful and extensive phenotyping in relation to rare and less frequent disorders must be prioritized during sampling. These variables can be further used as covariates in association tests.

Genetic Models Based on Recessive Mode of Inheritance for Association Tests

Consanguinity in successive generations cumulatively increases inbreeding levels, recessive alleles, and the proportion of homozygous gene regions in Arab populations (76, 83). An overwhelming proportion (63%) of the disorders documented in the Catalog for Transmission Genetics in Arabs (CTGA) (84) follows a recessive mode of inheritance. Therefore, it is not surprising to observe that the risk variants reported in our studies appeared when the genetic model based on a recessive mode of inheritance was used (**Table 2**), and majority of the risk variants were observed to be harbored in the runs of homozygosity (ROH) segments (24). However, when established markers appeared in

the Kuwaiti population data sets, it was invariably when the association tests used additive models. It is obvious that genetic pre-disposition of T2DM is not a recessive disorder, although in certain populations homozygosity of susceptibility alleles can further increase the population prevalence of the disease. It is recommended that population genetics in the Arab region use recessive models in addition to additive models, especially when large number of homozygous segments and/or of recessive risk genotypes are observed in sufficiently large number of individuals in the study cohort.

Estimation of Acceptable Effect Size

Large sample sizes enable the identification of causal variants with small effect size in GWA studies. Although the estimation of sample sizes for a 2-stage design (discovery phase followed by replication) is generally strictly followed in GWA studies, the estimation of a true effect size explainable by sample size is not strictly followed in several studies; the proper selection of associated variants in the discovery phase is not usually difficult, mainly because the use of additive models (wherein both heterozygote + rare homozygous genotypes are tested against reference homozygous genotypes with additive effect) does not unusually inflate the effect size values for common markers. However, in case of the recessive model, variants that show $\geq$5% frequency (i.e., associated with few rare homozygous genotypes) can show unusually large effect sizes. Therefore, an estimation of the acceptable effect size for a desired percentage of variance in a given trait (mean $\pm$ SD) and sample size at 80% power is pivotal for restricting SNP associations from undesirable high effect size and inappropriate *p*-values resulting from recessive effect.

Joint Analysis—Combining Results From Discovery and Replication Phases

GWA studies follow a 2-stage design irrespective of addressing quantitative or binary trait association. A strategy involving the joint analysis of data from both stages, which is currently increasingly used, has been advocated to result in an increased capability of detecting genetic associations (74). Such a meta-analysis increases the capacity for detecting weak genotypic effects. An example of the resultant increased power is observed in the case of three established markers from *CETP* (rs3764261, rs1864163, and rs1800775), which do not attain genome-wide significance in discovery cohort of our studies but in joint analysis (**Table 2**—Item V).

Heteroscedasticity and Trait Transformation

Quantitative traits (associated with complex disorders) used in association studies often violate the assumption of normality. Methods commonly used to handle non-normal traits include natural logarithm and rank-based inverse-normal transformations (85). Although the merits of such transformations are questionable (86), their use has been increasing. Elaborate assessments regarding the extent to which such transformations mask the true phenotype variability are lacking in literature. Owing to high inbreeding and prevalence of autosomal recessive disorders in the Arab population (87), the segregation of rare homozygous alleles may exert relatively larger effects on quantitative trait variations. Hence, earmarking any outlying data dispersion as heteroscedastic may conceptually be incorrect. Furthermore, performing trait transformations may adversely mask the actual effects of such loci on the variation of quantitative traits. Thus, to avoid any false positive or negative associations, it is advised to perform association tests, using appropriate genetic models, with both the raw and transformed traits, and to simultaneously adjust the models for disorders (rare and common) that overlap with the metabolic syndrome.

Relatedness and Loss of Samples

The imprecise modeling of genetic relatedness and population stratification among study subjects results in a substantial inflation of test statistics and spurious associations. Moreover, randomized sample sets in the Arab population exhibit rich relatedness due to the prevalent practices of polygamy and consanguinity. Therefore, detailed quality control procedures for relatedness and admixture must be performed in case of Arab studies. Relevantly, our studies are performed using the following steps: (i) assessment of relatedness among participants to the extent of third-degree relatives and removal of one sample per pair of related participants, (ii) performing ancestry estimations using ADMIXTURE (88) and removal of samples with abnormal deviations to the extent of component ancestry elements that have been previously established for the three Kuwaiti population subgroups (76), and (iii) delineation of principal components using EIGENSTRAT (89) and removal of outlying samples. These exhaustive steps, aimed at reducing false positive findings, lead to huge loss of samples. Thus, although the use of unrelated individuals in GWA studies is a norm, the use of recent sophisticated algorithms [such as BLOT-LMM (90) FaST-LMM (91), EMMAX (92)], which account for kinship structures and ancestries within a sample set, may offer larger power to the study by retaining more samples.

Quantitative Trait Association Studies Using "All" Diabetic Cohort

Quantitative trait association studies are usually conducted in population-based cohorts comprising both people with diabetes and diabetes-free individuals at the time of participant recruitment. Association tests are usually adjusted for obesity, diabetes, and medication regimen. However, it is important to extend the studies to cohorts comprising entirely of diabetic or prediabetic participants so that the prospects for use of identified genetic determinants in diabetes care and treatment become promising (93).

Case for Whole-Genome Homozygosity Association (WGHA) Methodologies

High inbreeding within the Arab population renders it a promising repository for providing a large scope for discovery of ROH and segments of identity by descent (IBD). ROH indicates an ancient shared common ancestry, whereas IBD indicates a recent ancestry. Both traits are reportedly effective in delineating population demography and recessive components of Mendelian and complex phenotypes (76). The risk loci identified

in our studies often overlay ROH regions, some of which are "novel." Currently, a promising new concept of "whole-genome homozygosity association (WGHA) methodology" in identifying genetic susceptibility loci harboring recessive variants (94) is being developed. Exemplary works include the identification of "risk ROH" for schizophrenia (95) and adult height (96). Tools such as LOHAS (97), which use either whole-genome sequence or genotype data in cohorts of either related or unrelated individuals, are now available for performing WGH association tests under the study designs for both case–control and quantitative trait association tests.

Case for Family-Based Genetic Association Studies in Arab Population

The large number of T2DM genetic loci identified to date using unrelated people explains only a relatively small proportion of observed heritability (familial clustering) of T2DM (8, 98). Possible explanations for "missing heritability" may originate from the role of rare variants, copy number variants, indels and more complex rearrangements, gene-environment interactions and epigenetics (98–102). Family-based designs allow the segregation of rare variants in a pedigree; multiple copies of such rare variants facilitate the detection of their effects. Family-based studies require a fewer number of samples than population-based studies and offer advantages in terms of quality control, robustness to population stratification, and uniformity in exposure to environmental factors or lineage-specific diseases. They offer the potential to combine linkage and association data. Arab population, which is largely consanguineous, offers a large potential for family-based designs as the population can show familial gene clustering for diabetes and metabolic traits. However, except for few studies, such as the "Oman Family Study" (103–106) and the study on an extended family from the UAE by Al Safar et al. (22), no notable familial study for diabetes risk loci has been reported on the Arab population. Both the abovementioned studies confirmed well-established gene loci, but failed to identify any novel "rare" variants. Considerable attention needs to be paid to appropriate study designs as family data continue to provide important information in the search for trait loci (107). It is ideal if the recruitment of large-pedigrees/extended families, particularly those containing several sub-families suitable for both parent-offspring design or for sibling design, with high inbreeding and roots traceable up to at least six generations with deduced consanguinity data is possible.

Epigenetic Mechanisms of T2DM Genetic Risk Factors and Environmental Factors in Arab Population

As mentioned earlier, the post-oil era witnessed in Arab population a rapid shift in the eating and physical activity habits. Environmental and lifestyle factors (including diet, obesity, physical activity, tobacco smoking and environmental pollutants) can influence epigenetic mechanisms, such as DNA methylation, histone acetylation, and microRNA expression; these modifications can result in altered gene expression with effects on regulation of specific genes. Epigenome-wide association studies (EWASs) that examine the role of epigenetic modifications in the etiology and progression of metabolic disorders (108–112) and diabetes (113–116) have recently emerged. Most of such EWASs with T2D and obesity are focused on Caucasian populations; however, a study emerged recently on Arab population from Qatar (117), which identified one novel CpG association at *DQX1* at genome-wide significance for T2D and replicated eight previously reported associations involving

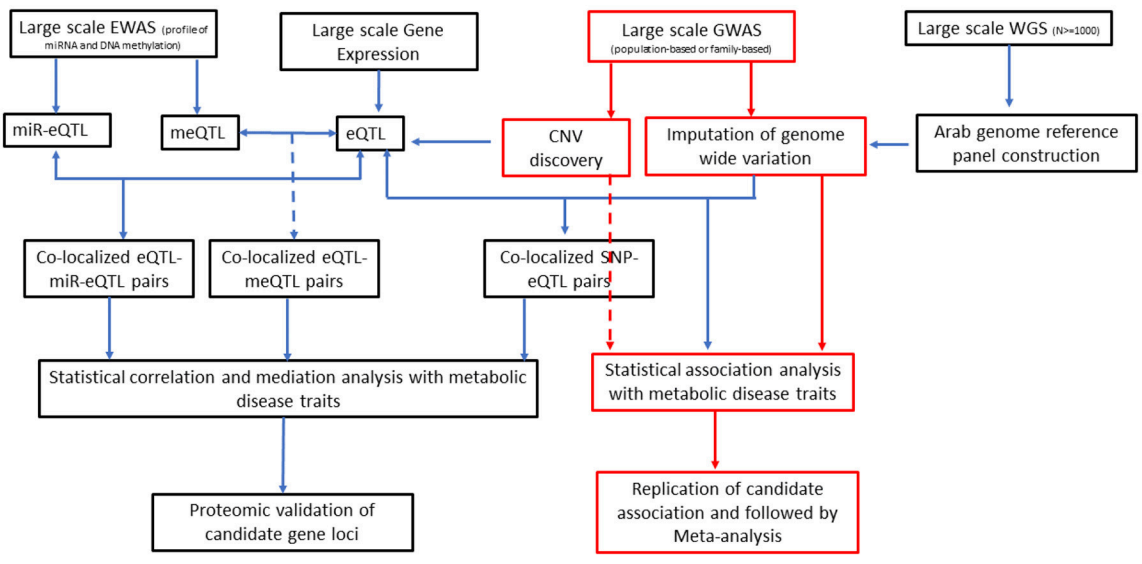

FIGURE 2 | An integrative approach as direction of future T2DM genetics research in Arab populations. EWAS, epigenome-wide association studies; GWAS, genome-wide association studies; WGS, whole-genome sequencing; miR-Eqtl, microRNA expression quantitative trait loci; meQTL, methylation quantitative trait loci; eQTL, expression quantitative trait loci; CNV, copy number variation; SNP, single nucleotide polymorphism.

TXNIP for T2D and *SOCS3, SREBF1, SBNO2, CPT1A, PRR5L, LY6G6E*, and an intergenic region on chromosome 17 for BMI *albeit* at suggestive *p*-values.

AN INTEGRATIVE APPROACH AS DIRECTION OF FUTURE T2DM GENETICS RESEARCH IN ARAB POPULATIONS

It has increasingly become evident that epigenetics, genetics, and environment are likely to interact with one another to define an individual's risk of diabetes and obesity (118). Integration of data on expression quantitative trait loci (eQTL), which represent regulatory loci, with genetic variants identified from GWA studies can give new insights into identification of causal genes for T2DM (119, 120). The ability of epigenetic modifications and expression of miRNA (and largely the non-coding RNAs) to manipulate gene expression has enabled incorporation of such data in research on pathogenesis of T2DM (121–123). Consideration of expression data and epigenome data along with large-scale GWA data on genotyped and imputed SNPs and copy number variations in association studies for T2DM is depicted as future directions for diabetes research (**Figure 2**).

CONCLUSION

The failure to convincingly replicate a large number of Euro-centric risk variants for T2DM in Arab populations may have resulted from several aspects, including study design and strength, low prevalence of causative Euro-centric risk variants in the Arab population, or from the gene–environment interactions that masked the effect of the Euro-centric risk variants. However, epidemiological studies have illustrated the deficit of global risk assessment tools fitted to the Arab population (124). The performance of global genetic risk assessment tools (based on Euro-centric markers) in other populations is also questionable (125). The discrepancy of marker relevance in the applicability of Euro-centric genetic risk variants to Arab population could be resolved by performing large-scale genome-wide surveys (a combination of GWAS, exome, and genome sequencing and imputation) of the Arab population with diabetes. Detailed functional assessments of loci identified in the Arab population to interact with Euro-centric risk loci as part of common gene networks or physiologic processes should also be performed.

AUTHOR CONTRIBUTIONS

TT and FA-M conceptualized the study design. TT and PH developed the manuscript. All the authors participated in discussions. JA, MA-F, JT, and FA-M critically reviewed 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/fendo.2019.00008/full#supplementary-material

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Insulin Treatment Is Associated With Improved Fetal Placental Vascular Circulation in Obese and Non-obese Women With Gestational Diabetes Mellitus

Giulia Barda[1,2], *Jacob Bar*[1,2], *Margarita Mashavi*[2,3], *Letizia Schreiber*[2,4] *and Marina Shargorodsky*[2,5]*

[1] Department of Obstetrics and Gynecology, Edith Wolfson Medical Center, Holon, Israel, [2] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [3] Department of Medicine, Edith Wolfson Medical Center, Holon, Israel, [4] Department of Pathology, Edith Wolfson Medical Center, Holon, Israel, [5] Department of Endocrinology, Edith Wolfson Medical Center, Holon, Israel

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Jehad Ahmed Abubaker,
Dasman Diabetes Institute, Kuwait

Reviewed by:
Julianne Toohey,
University of California, Irvine,
United States
Silvina Bocca,
Eastern Virginia Medical School,
United States

***Correspondence:**
Marina Shargorodsky
marinas@wolfson.health.gov.il

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Barda G, Bar J, Mashavi M, Schreiber L and Shargorodsky M (2019) Insulin Treatment Is Associated With Improved Fetal Placental Vascular Circulation in Obese and Non-obese Women With Gestational Diabetes Mellitus. Front. Endocrinol. 10:84. doi: 10.3389/fendo.2019.00084

Objective: The present study was designed to investigate the impact of carbohydrate restriction and insulin treatment on placental maternal and fetal vascular circulation in obese and non-obese women with gestational diabetes mellitus (GDM).

Design and methods: One Hundred Ninety-One women with GDM who gave birth and underwent a placental histopathological examination at Wolfson Medical Center, Israel, were included in the study: 122 women who were treated with carbohydrate/calorie restriction diet (Group 1) and 69 women who were treated with diet plus insulin (Group 2). Additionally, each group was divided into two subgroups according to pre-pregnancy BMI: non-obese and obese.

Results: Maternal vascular malperfusion lesions did not differ significantly between groups. Vascular lesions related to fetal malperfusion were significantly lower in GDM women treated by insulin and diet compared to women with diet alone ($p = 0.027$). Among fetal malperfusion lesions, villous changes consistent with fetal thrombo-occlusive disease (FTOD) were significantly lower in women treated with diet plus insulin and lowest in GDM women with pre-pregnancy BMI < 30 kg/m^2 ($p = 0.009$). In the logistic regression analysis, insulin treatment was significantly associated with a decreased rate of villous changes consistent with FTOD (OR 0.97, 95% CI 0.12–0.80, $p = 0.03$). Prevalence of gestational hypertension was higher in obese women of both treatment groups ($p = 0.024$).

Conclusion: Combination of obesity and GDM increased rate of FTOD and prevalence of gestational hypertension. Carbohydrate restriction diet plus insulin treatment was associated with improved fetal placental vascular circulation, especially in GDM women with pre-pregnancy BMI < 30 kg/m^2.

Keywords: insuli, diabetes gestational, obesity, gestational hypertension, placental circulation

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disease associated with maternal glucose intolerance, fetal hyperglycaemia/hyperinsulinaemia, abnormal placental vascular function, and adverse perinatal outcomes (1–3). Treatment of GDM with diet management to control for maternal glycaemia usually reduces glucose levels to the recommended levels (4). Although diet management in GDM pregnancies results in both the mother and the newborn being normoglycaemic at birth, several alterations in terms of fetoplacental vascular reactivity are evident (5, 6). Moreover, recent studies have found abnormal endothelial function due to altered expression of insulin receptors (IR) in the fetoplacental endothelium of pregnant women who underwent diet management, even with adequately controlled GDM (7). These adverse changes in placental structure and function have harmful consequences for neonatal and maternal pregnancy outcome.

A percentage of GDM women do not achieve the recommended values of glycaemia with diet management, and consequently receive insulin therapy until delivery. It has been shown that insulin treatment for GDM restores placental insulin receptors expression, leading to normalization of endothelial function. Moreover, placental insulin resistance, associated with a reduction in phosphorylated AKT (p-AKT), found in diet treated GDM, can be reversed by insulin treatment during pregnancy (8, 9).

Thus, therapeutic interventions in GDM pregnancies are crucial not only for the control of maternal and fetal glycaemia, but also for the prevention of fetoplacental vascular dysfunction as well as the subsequent risk reduction of neonatal complications and adulthood metabolic diseases (e.g., obesity, hyperlipidemia, and type 2 diabetes mellitus). Nevertheless, the impact of GDM treatment on placental histopathology has not been examined. The present study was designed to investigate the impact of insulin treatment on maternal and fetal placental vascular circulation, pregnancy complications, and neonatal outcome in obese, and non-obese women with GDM.

METHODS

One Hundred Ninety-One women with GDM who gave birth and underwent a placental histopathological examination between 2007 and 2013 at Wolfson Medical Center were included in the study. Exclusion criteria included pre-gestational type 1 and type 2 diabetes mellitus, multiple pregnancies, abnormal fetus karyotype, and labor before 37 weeks gestation.

Early pregnancy screening for gestational diabetes by the patient's medical history and fasting glucose levels was performed. If the result of initial testing was negative, a repeat screening at 24–28 weeks of gestation was performed.

Following the diagnosis of GDM, diet management was initiated. Diet management included a carbohydrate restricted diet (∼200 g carbohydrates per day maximum), which was focused on optimizing participants' consumption of vegetables, fruits, whole-grain products, low-fat dairy products, and a lower intake of sugar-rich foods. If treatment of GDM with diet management did not sufficiently reduce glucose levels to the recommended levels (fasting blood glucose <105 mg/dl) during the first trimester, insulin treatment (short- or long-lasting insulin) was initiated.The study consisted of two groups according to type of intervention: Group 1 included 122 pregnant women with GDM who were treated with diet management, while group 2 included 69 pregnant women with GDM who received diet management together with insulin treatment. Additionally, each group was divided into two subgroups according to pre-pregnancy BMI: Subgroup A included non-obese GDM women (BMI < 30 kg/m^2) and subgroup B contained obese pregnant women (BMI ≥30 kg/m^2).

The present study was approved by the ethics committee of the Edith Wolfson Institutional Review Board, Wolfson Medical Center, Israel. Since the study was observational, retrospective cohort study, informed consent from the participants of this study was not required.

Placental Examination

Placental histology was analyzed according to the criteria of the Society of Pediatric Pathology (10) with the 2016 Amsterdam Placental Workshop modifications (11). Placental findings were divided into maternal and fetal malperfusion lesions. Maternal mulperfusion lesions included marginal and retro-placental hemorrhages, vascular (i.e., acute atherosis and mural hypertrophy) and villous changes (i.e., villous infarcts, increased syncytial knots, and intervillous fibrin deposition). Fetal malperfusion lesions included vascular and villous lesions related to thrombo-occlusive disease. A representative image of fetal malperfusion abnormalities is shown in **Figure 1**. A single pathologist, who was blind to the type of gestational diabetes treatment and BMI, performed all the placental pathological examinations, using a previously described standard protocol (12, 13).

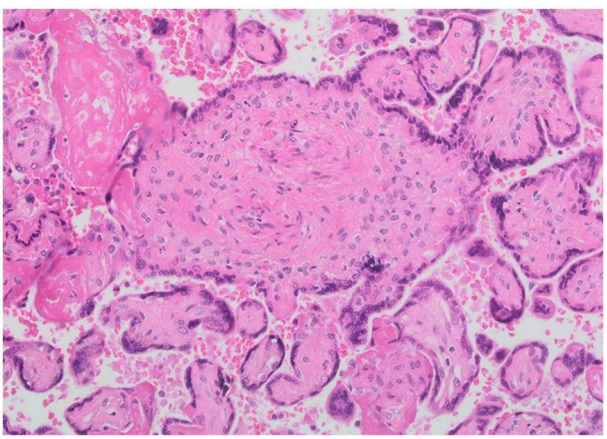

FIGURE 1 | Representative images of fetal placental vascular circulation abnormalities. Villous changes consistent with fetal thrombo-occlusive disease.

Statistical Analysis

Analysis of data was carried out using SPSS 9.0 statistical analysis software (SPSS Inc., Chicago, IL, USA, 1999). The Kolmogorov-Smirnov test was used for normalcy of distribution of continuous variables (cut off at $p = 0.01$). Categorical variables such as sex and co-morbidities were described using frequency distributions and are presented as frequency (%). Differences across the study groups were examined by one-way analysis of variance (ANOVA). Variables for which across-group differences were detected underwent *post hoc* pairwise testing using the Bonferroni test. The General linear model (GLM) was developed using a backward, stepwise approach and inclusion of variables is this model was based on univariate results.

RESULTS

Demographic and clinical characteristics of the four study groups according to type of treatment intervention and pre-pregnancy BMI are presented in **Table 1**. As shown, four groups were similar in terms of age ($p = 0.938$), gravidity ($p = 0.661$), and parity ($p = 0.199$). Pre-gestational BMI did not differ significantly between groups 1A and 2A ($p = 0.311$) as well as 1B and 2B ($p = 0.981$). Weight gain during pregnancy did not differ significantly between the study groups ($p = 0.062$). Mean fasting plasma glucose during the first trimester of pregnancy differed significantly between the study groups, and was significantly higher in insulin plus diet treated obese women ($p = 0.040$). Mean fasting plasma glucose did not differ significantly between groups during the second and third trimester of pregnancy.

As can be seen in **Table 1**, neonatal outcome parameters (i.e., birth weight, large for gestational age (LGA), macrosomia, first and fifth minute Apgar score, hypoglycemia, cord artery pH and ICU admission) did not differ significantly between groups. Among pregnancy complications, prevalence of gestational hypertension was higher in obese women in both treatment groups, compared to GDM women with pre-pregnancy BMI < 30 kg/m^2 ($p = 0.024$).

Placental Findings

Placental findings of the study groups according to type of intervention and pre-pregnancy BMI are presented in **Table 2**. As can be seen, maternal vascular malperfusion (MVM) lesions of the placental bed did not differ significantly between groups ($p = 0.230$), whereas vascular lesions related to fetal malperfusion were significantly lower in insulin plus diet treated patients, obese, and non-obese (Group 1A and 1B) than in diet managed women (Groups 2A and 2B) ($p = 0.027$). Among placental lesions related to fetal vascular malperfusion, villous changes consistent with fetal thrombo-occlusive disease (FTOD) were significantly

TABLE 1 | Maternal and neonatal characteristics according to the study groups.

Variables	Group 1 Insulin treatment		Group 2 Diet management		*p*-value
	Non-obese (A)	Obese (B)	Non-obese (A)	Obese (B)	
Age (y)	32.5 ± 4.7	32.5 ± 4.6	32.0 ± 5.3	32.0 ± 5.5	0.938
Gravidity	2.9 ± 1.9	2.9 ± 1.6	2.6 ± 1.7	3.0 ± 2.1	0.661
Parity	1.1 ± 1.4	1.3 ± 1.2	0.9 ± 1.0	1.2 ± 1.2	0.199
Pregestational BMI (kg/m²)	25.3 ± 3.1	36.7 ± 5.3	23.8 ± 3.0	35.4 ± 4.1	0.001
Weight at delivery (kg)	76.9 ± 17.3	90.5 ± 47.2	54.9 ± 33.6	73.0 ± 53.9	0.001
Weight gain during pregnancy (kg)	12.8 ± 6.4	10.3 ± 8.0	12.1 ± 6.7	8.7 ± 7.8	0.062
Gestational hypertension, *n* (%)	2 (6.1)	5 (13.9)	1 (1.3)	6 (13.9)	0.024
Preeclampsia, *n* (%)	2 (6.1)	1 (2.8)	6 (7.6)	8 (18.6)	0.067
Smoking, *n* (%)	5 (15.6)	6 (16.7)	10 (12.8)	8 (18.6)	0.853
Family history of diabetes, *n* (%)	15 (45.5)	17 (47.2)	33 (41.8)	16 (37.2)	0.812
Family history of hypertension, *n* (%)	5 (15.2)	9 (25.0)	15 (19.0)	5 (11.6)	0.452
1st trimester fasting glucose (mg/dl)	95.9 ± 16.2	103.6 ± 23.6	91.6 ± 18.8	97.0 ± 17.5	0.040
2nd trimester fasting glucose (mg/dl)	95.5 ± 22.5	115.5 ± 31.6	101.6 ± 27.3	104.2 ± 27.3	0.050
3rd trimester fasting glucose (mg/dl)	96.7 ± 16.4	101.6 ± 22.1	93.6 ± 28.1	100.0 ± 27.6	0.711
Birth weight (g)	3302.2	3568.4	3260.7	3570.5	0.823
Umbilical cord PH	7.0 ± 1.4	7.3 ± 0.1	7.3 ± 0.1	7.1 ± 1.2	0.435
Apgar at 1 min	8.8 ± 0.1	8.7 ± 0.2	8.6 ± 0.2	8.6 ± 0.2	0.796
Apgar at 5 min	9.9 ± 0.4	9.8 ± 0.2	9.9 ± 0.1	9.9 ± 0.2	0.456
Large for gestational age, *n* (%)	5 (15.1)	13 (36.1)	20 (25.3)	17 (39.5)	0.078
Macrosomia, *n* (%)	2 (6.1)	7 (19.4)	11 (13.9)	10 (23.3)	0.192
Hypoglycemia, *n* (%)	4 (12.1)	6 (17.1)	10 (12.7)	2 (4.7)	0.366
Neonatal Intensive Care Unit, *n* (%)	8 (24.2)	14 (40.0)	34 (43.0)	12 (27.9)	0.161

BMI, body mass index. By-group comparisons made using one way analysis of variance (ANOVA) simultaneously comparing all study groups.

TABLE 2 | Histopathological characteristic of placenta according to the study groups.

Variables	Group 1 Insulin treatment		Group 2 Diet management		
	Non-obese (A)	Obese (B)	Non-obese (A)	Obese (B)	
Placental weight (g)	503 ± 138.4	599 ± 136.0	521 ± 120.2	571 ± 125.9	0.003
Fetal Placental Ratio	8.3 ± 10.8	6.1 ± 0.9	6.5 ± 1.2	6.4 ± 0.8	0.158
Composite MVM rate, *n* (%)	19 (57.5)	13 (36.1)	42 (53.2)	19 (44.2)	0.230
Vascular lesions related to maternal malperfusion	2 (6.1)	2 (5.6)	7 (8.9)	2 (4.6)	0.811
Villous lesions related to maternal malperfusion	17 (51.5)	11 (30.6)	37 (46.8)	17 (39.5)	0.268
Composite FVM rate, *n* (%)	2 (6.1)	3 (8.3)	11 (13.9)	12 (27.9)	0.027
Vascular lesions with FTOD, *n* (%)	2 (6.1)	2 (5.6)	2 (2.5)	4 (9.3)	0.449
Villous lesions with FDOD, *n* (%)	0 (0.0)	1 (2.8)	9 (11.4)	9 (20.9)	0.009
MIR *n* (%)	4 (12.1)	1 (2.8)	9 (11.4)	5 (13.9)	0.467
FIR *n* (%)	3 (9.1)	0 (0.0)	5 (6.3)	2 (4.7)	0.590

By-group comparisons made using one way analysis of variance (ANOVA) simultaneously comparing all study groups.

lower in women treated with diet plus insulin and lowest in GDM women with pre-pregnancy BMI < 30 kg/m^2 ($p = 0.009$). Multiple regression analysis was arrived at using a backward, stepwise approach to identify variables independently associated with composite fetal malperfusion abnormalities. Variables were included in the model based on their associations in univariate analyses: mean fasting plasma glucose during the first trimester of pregnancy, gestational hypertension and insulin treatment. In this model, insulin plus diet treatment was significantly associated with a decreased rate of villous changes consistent with composite FVM (OR 0.35, 95% CI 0.12–0.99, $p = 0.049$). In the regression model of villous changes consistent with fetal thrombo-occlusive disease were included variables based on their associations in univariate analyses (mean fasting plasma glucose during the first trimester of pregnancy and insulin treatment). In this model, diet management with insulin treatment was significantly associated with a decreased rate of villous changes consistent with FTOD (OR 0.97, 95% CI 0.12–0.80, $p = 0.030$).

DISCUSSION

The major finding of the present study is that carbohydrate restricted diet management with insulin treatment was associated with improved placental vascular circulation of fetal origin in obese and non-obese women with GDM. Additionally, villous changes consistent with fetal thrombo-occlusive disease (FTOD) were lowest in GDM women with pre-pregnancy BMI < 30 kg/m^2 treated with diet plus insulin. Combination of obesity and GDM increased rate of villous changes consistent with FTOD and prevalence of gestational hypertension in both treatment groups. Thus, prevention of obesity throughout women's reproductive age may translate to improved placental circulation and potential positive effects on adulthood metabolic diseases.

Although insulin is not essential for the placental transfer of glucose, maternal insulin can bind to insulin receptors in the trophoblast membranes, and activate adenosine and insulin receptors (cAMP, PKA, MAPK, and PI3K/Akt) as well as increase placental expression of GLUT-4 and GLUT-9, subsequently affecting placental and fetal development (14, 15). Insulin treatment in GDM could result in restoration of the expression and activity of insulin and adenosine receptors and the l-arginine–NO signaling pathway as well as promote placental fatty acid transfer and overcome placental insulin resistance (7, 16, 17). Whereas, in trophoblast plasma membranes from gestational diabetic women treated with diet alone there is less expression of insulin receptors, in women treated with insulin there is greater expression of insulin receptors (18). The altered expression of IRs in the fetoplacental endothelium of GDM women leads to abnormal fetal microvascular and macrovascular circulation, even when their diet was adequately controlled. Insulin treatment restored IR expression in these patients, leading to normal endothelial function (7, 8). Our results further support previous findings demonstrating that insulin treatment improves insulin resistance, restores placental insulin and adenosine receptors expression, and positively impacts fetoplacental circulation and endothelial function.

It has been shown that women treated with insulin have a higher metabolic risk profile and lower insulin sensitivity, compared to diet-treated women with GDM. Reduced insulin sensitivity and beta-cell function in insulin-treated women, remained significant after adjustment for confounders such as age, BMI, ethnicity, and pregnancy weight gain (19). Impaired beta-cell compensation is probably chronic and leads to alterations in placental structure and function, neonatal complications, and adulthood metabolic diseases (20–22). In the present study, insulin treatment of gestational diabetes, characterized by more severe glucose intolerance, was not associated with an adverse impact on placental vascular circulation. Moreover, a beneficial effect of insulin treatment on fetal placental vascular circulation was observed. Although, mean fasting plasma glucose did not differ significantly between groups during the second and third trimester of pregnancy and in the regression model, mean fasting plasma glucose during the first trimester was not significantly associated with

a villous changes consistent with FTOD (OR 0.963, 95% CI 0.919–1.009, $p = 0.111$), the possibility that beneficial effect of insulin therapy in terms of placental vascular circulation is linked to a better diabetes control cannot be excluded. Since women with GDM needing insulin treatment, remain a high risk population, further research is necessary to determine whether beneficial effect on placental vascular circulation could lead to improved neonatal and pregnancy outcomes in this group. In the present study, insulin treatment of gestational diabetes was associated with improvement of placental vascular circulation but not pregnancy complications. However, the fact that both study groups were similar in terms of neonatal complications, could indicate improvement regarding clinical outcomes in insulin treated women because this group is characterized by more severe impairment of glucose homeostasis and we would have expected more pregnancy complications in these patients.

Obesity during pregnancy is associated with impaired endothelial function, increased pro-inflammatory cytokine expression and being associated with a higher risk of placental pathological lesions (23, 24). We previously reported that obesity, *per se*, emerged as a significant independent predictor of fetal vascular malperfusion and Willous maturation defect (25). In the present study, combination of GDM and obesity was associated with increased prevalence of villous changes consistent with fetal thrombo-occlusive disease and gestational hypertension in both treatment groups. Thus, addressing maternal pre-pregnancy BMI and recommending overweight women planning a pregnancy to return to a normal BMI, is reasonable and important.

There are advantages as well as limitations to our study. One advantage is that all placental pathological examinations were performed by a single pathologist, who was unaware of the GDM treatment approach, using validated placental pathological criteria. Another advantage is that the study groups were similar in terms of age, gravidity, parity and mode of delivery, ruling out these factors as confounders. The major limitation of the present study is retrospective cross-sectional design. Thus, prospective long term studies evaluating the impact of different treatment approach for GDM (i.e., diet, insulin, and oral hypoglycemic drugs) are needed to confirm these findings.

In conclusion, our findings favor insulin treatment in terms of placental vascular circulation, and support recently published guidelines indicating insulin as the preferred medication in gestational diabetes treatment. Addressing maternal weight control before and during pregnancy may translate to further improvement of placental vascular circulation and better pregnancy outcomes.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

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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The Complex Etiology of Childhood Obesity in Arabs Is Highlighted by a Combination of Biological and Socio-Economic Factors

Naser Elkum[1,2]*, *Monira Alarouj*[2], *Abdullah Bennakhi*[2] *and Azza Shaltout*[2]

[1] Research Department, Sidra Medicine, Doha, Qatar, [2] Department of Clinical Services, Dasman Diabetes Institute, Kuwait City, Kuwait

Objectives: To identify predictors of childhood and adolescent obesity in Kuwaitis with Arab ethnicity.

Methods: A cross-sectional sample of 6–18 year-old schoolchildren was randomly selected from 244 public schools across all six governorates in the State of Kuwait. Anthropometric data were measured from 6,574 Arab Kuwaiti schoolchildren, and a structured questionnaire was used to collect information on possible risk factors associated with obesity. Overweight and obesity were defined in accordance with the Center for Disease Control and Prevention criteria.

Results: The prevalence of overweight and obesity in children (aged 6–18 years) were 17.7% and 33.7%, respectively. The likelihood of childhood obesity increased with birth weights >4.0 Kg [odds ratio (OR) = 2.3; $p < 0.0001$], maternal employment (OR = 1.26, $p = 0.0006$), maternal age at pregnancy >30 years (OR = 1.24; $p = 0.0016$) and family size of <6 members (OR = 1.16, $p = 0.0106$).

Conclusions: Public health professionals should be aware that advanced maternal age, maternal employment, smaller family size, and high birthweight may predict the risk of obesity in Kuwaiti Arab children and adolescents.

Keywords: obesity, children, Arabs, prevalence, risk factors

Edited by:
Jehad Ahmed Abubaker,
Dasman Diabetes Institute, Kuwait

Reviewed by:
Aaron Hanukoglu,
Tel Aviv University, Israel
Nirav Dhanesha,
The University of Iowa, United States

***Correspondence:**
Naser Elkum
nelkum@sidra.org

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INTRODUCTION

Childhood obesity is one of the most serious public health challenges worldwide. It has been shown that progression of obesity from childhood to adulthood is associated with the development of type 2 diabetes (T2D) (1, 2) and cardiovascular diseases (CVDs) (3). Our previous report (4) has demonstrated a markedly high prevalence of childhood obesity in Kuwait that exceeded those reported in neighboring countries and North America. This public health crisis requires a serious investigation to identify the key factors contributing to the high prevalence of childhood obesity in Kuwait.

A number of childhood obesity-related etiological factors have been identified, such as genetic, environmental and socio-economic risk factors (5–7). In addition, it is becoming increasingly evident that birthweight, short sleep duration, physical inactivity, parental obesity, and parental income are important predictive factors of childhood overweight and obesity in various countries. However, the majority of these factors were identified and their effects were studied only in Western

populations. Therefore, in order to design meaningful prevention strategies, it is important to identify the contributing factors specific to each population and geographical location and to understand the reasons for the observed differences.

Few studies have investigated the factors associated with the prevalence of overweight and obesity in Kuwait, but differences in methodologies and target populations make comparison between these studies difficult. Improving our understanding of the factors associated with childhood overweight and obesity is crucial not only to improve child health but also to curb the increasing rates of adult obesity and its complications, such as T2D and CVDs. To address these challenges, we conducted a cross-sectional study among 6–18 year-old Arab Kuwaiti school children, to identify factors that may contribute to the development of childhood overweight and obesity.

SUBJECTS AND METHODS

Design

A cross-sectional study was conducted in a national sample of 6,574 Kuwaiti boys and girls aged 6–18 years between September 2012 and June 2013. The sampling methodology is described in detail in our previous publication (4). Briefly, the primary sampling unit was the school and the sampling frame included primary, intermediate and secondary schools for each gender in all six governorates in the State of Kuwait. The study sample was obtained by stratifying school children based on governorate, grade and gender. A two-stage stratified random sampling technique was used to select the sample. The study protocol was reviewed and approved by the Scientific Advisory Board and Ethical Review Committee of the Dasman Diabetes Institute in Kuwait City, Kuwait, and the study was conducted in collaboration with the Ministry of Education. Study procedures were explained to the school administration and teaching staff who contacted the students' parents to obtain an informed and signed written parental consent and the student's assent.

Anthropometric Measurements

Anthropometric measurements included body weight, height and waist-circumference (WC). Height and weight were measured with the participants being bare-footed and wearing light indoor clothing using calibrated portable electronic weighing scales (Seca, GmbH and Co., Hamburg, Germany) and portable inflexible height measuring bars, respectively. WC was measured using a constant tension tape at the highest point of the iliac crest on the mid-axillary line at the end of a normal expiration with arms relaxed at the sides. Body mass index (BMI) was calculated by dividing body weight (kg) by height squared (m). Weights were classified according to the BMI percentile charts for age and gender by the Centers for Disease Control and Prevention (CDC) (8), as underweight (BMI <5th percentile), normal weight (5th $\leq$ BMI <85th percentile), overweight (85th $\leq$ BMI < 95th percentile) and obese (BMI $\geq$ 95th percentile). Our research team was specifically trained in making these anthropometric measurements. Measurements were carried out in accordance with the standard operating procedures that were specifically developed for the study.

Questionnaires

The study questionnaire was divided into two-sections. The first section, completed in the school by the study team, included general demographic information, and anthropometric measurements and the second section, completed by the parents,

TABLE 1 | Demographic, lifestyle and anthropometric characteristics of the Kuwaiti students stratified by gender.

Parameter	Kuwaiti students (6–18) years		
	Boys $n = 2,601$	Girls $n = 3,973$	*P*-value trend
Age (years)	11.8 ± 3.3	12.1(3.5)	0.0007
Height (cm)	150.6 ± 17.9	145.9 ± 14.2	<0.0001
Weight (kg)	55.9 ± 25.4	50.0 ± 19.6	<0.0001
Waist (cm)	77.9 ± 17.9	74.3 ± 14.5	<0.0001
BMI (kg/m^2)	23.5 ± 7.1	22.8 ± 6.5	<0.0001
Age group, *n* (%)			
6–10	900 (34.6)	1,414 (35.6)	<0.0001
11–15	1,301 (50.0)	1,705 (42.9)	
16–18	400 (15.4)	854 (21.5)	
Birth weight (kg)			
Low (<2.5)	321 (12.3)	530 (13.3)	0.0625
Normal (2.5 - <4)	2,032 (78.1)	3125 (78.7)	
High (≥4)	248 (9.5)	318 (8.0)	
Breastfeeding (months)			
<2	1124 (43.2)	1,637 (41.2)	0.1953
2–6	566 (21.8)	926 (23.3)	
>6	911 (35.0)	1,410 (35.5)	
Mother age at pregnancy (years)			
<24	735 (28.3)	1,139 (28.7)	0.0035
24–30	875 (33.6)	1,470 (37.0)	
≥30	991 (38.1)	1,364 (34.3)	
Mother's working status, *n* (%)			
Working	1,354 (53.4)	2,063 (52.6)	0.5285
Not working	1,184 (46.7)	1,863 (47.5)	
Family size			
≤6	713 (27.4)	1,074 (27.0)	0.7348
>6	1,888 (72.6)	2,899 (73.0)	
Mother education, *n* (%)			
Illiterate (no schooling)	37 (1.3)	86 (1.9)	0.0734
1–12 years	1,713 (58.2)	2,575 (56.8)	
>12 years	1,196 (16.0)	1871 (41.3)	
Family income (dinar kuwaiti), *n* (%)			
<800	498 (23.6)	735 (23.6)	0.9994
801–2000	1,183 (56.1)	1,750 (56.2)	
Above 2000	427 (20.3)	631 (20.3)	
Fruit (times/week)	3.4 ± 2.2	3.3 ± 2.2	0.1071
Vegetable (times/week)	3.9 ± 2.5	4.1 ± 2.7	0.0196
Fast-food (times/week)	4.2 ± 3.1	3.7 ± 3.3	<0.0001
Drinks (times/week)	5.8 ± 3.2	5.3 ± 3.2	<0.0001
TV & Video (hours/week)	9.4 ± 3.6	9.0 ± 3.6	0.0026

BMI, body mass index; Waist, waist-circumference. Income: students average monthly household income for the last 12 months. Data are means ± SD unless noted otherwise.

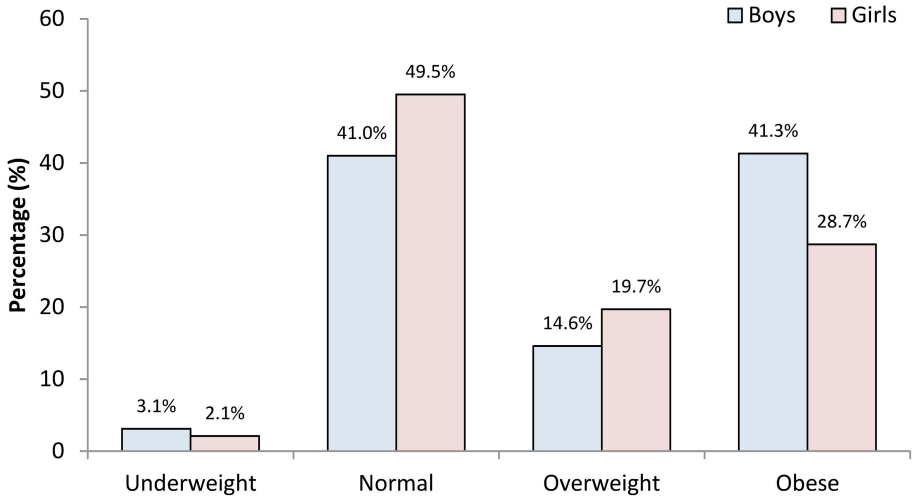

FIGURE 1 | The prevalence of childhood underweight, normal weight, overweight and obesity among Kuwaiti Arab children and adolescent boys and girls aged 6–18 years.

was intended for collecting information with regard to family demographics, socio-economic status, breastfeeding practices of the mother, birth history, eating habits, physical activity, and other health behaviors. In the eating habits section, the parents were asked how many times per week the child typically consumed fruit, vegetables, soft drinks and fast foods (burger, shawarma, etc.). In the sedentary behaviors section, the child was asked to state the average number of daily hours spent watching television and/or playing video and computer games.

Statistical Analyses

Data completeness and accuracy were verified. Descriptive statistics were performed and presented as means and standard deviation (±/SD) for continuous variables or as numbers and percentages for nominal/categorical variables. Student's *t*-test and chi-squared test were used to evaluate differences between continuous and categorical variables, respectively. Logistic regression analysis was performed to estimate odds ratios (ORs) and to examine the predictive effect of each factor on obesity risk. ORs and their 95% confidence intervals (95% CI) for associated factors were estimated. Research Electronic Data Capture was used for data collection and data management (9). All statistical assessments were two-sided and considered to be statistically significant at *p*-values <0.05. Data analysis was performed using SAS (version 9.4; SAS Institute, Cary, NC, USA).

RESULTS

Population Characteristics

Socio-demographic, lifestyle, and anthropometric characteristics of the study population are shown in **Table 1**. Among the 10,707 students approached, a total of 6,574 (61.4%) children and adolescents were recruited for the study; the majority were girls (3,973; 60.4%). The mean age of the study population was 12.0 ± 3.4 years (boys, 11.8 ± 3.3 years; girls, 12.1 ± 3.5 years). The study participants were stratified into three age groups: 6–10 years (35.2%), 11–15 years (45.7%) and 16–18 years (19.1%). Compared with girls, boys had a greater mean BMI (23.5 vs. 22.8 kg/m^2; $p < 0.0001$) and WC (77.9 vs. 74.3 cm; $p < 0.0001$). Compared with girls, boys also had significantly higher intakes of fast food and soft drinks and a significantly lower intake of vegetables (**Table 1**). Middle Eastern culture allows more freedom and eating out behavior for boys than girls

Body Weight

The prevalence of underweight, normal weight, overweight, and obesity among the entire sample was 2.5, 46.1, 17.7, and 33.7%, respectively. With regard to the overall gender differences, more girls than boys were proportionately overweight (19.7 vs. 14.6%). In contrast, 41.3% of boys were obese compared with 28.7% of the girls (**Figure 1**). The gender-specific distribution of BMI across different age groups (**Figure 2**) revealed a higher prevalence of underweight boys in all age groups compared with girls; at the same time, boys were comparatively more obese in all age groups (**Table 2**). In both genders, 11–15-year olds had the highest prevalence of overweight and obesity of all age groups examined.

Factors Associated With Obesity

Based on univariate logistic regression analysis, **Table 3** illustrates the estimated ORs of the association between obesity and potential risk factors. Male gender was significantly associated with obesity (OR = 1.35). Age was a strong predictor of obesity; participants aged 11–15 years had a 58% increased risk of obesity as compared with those aged <11 years (OR = 1.58). Birth weight was also strongly associated with obesity; children born with weights >4 kg (8.6%) were more likely to develop obesity as they grew older (OR = 2.1). There was also a significant difference between participants whose mothers were employed vs. those who were unemployed ($P < 0.0085$). Our findings also indicated

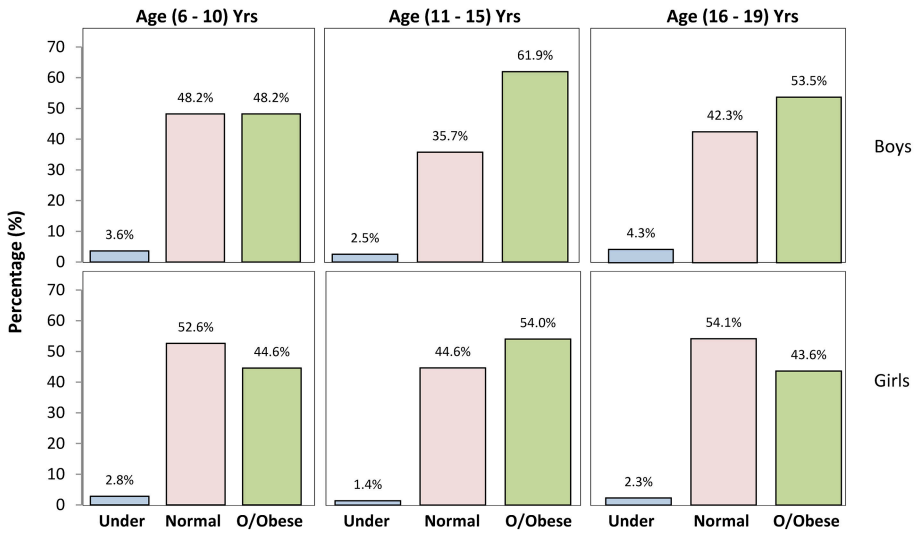

FIGURE 2 | The prevalence of underweight, normal weight and combined overweight and obese (o/obese) by age group and gender among Kuwaiti students.

TABLE 2 | Prevalence of overweight and obesity among children and adolescents stratified by gender and age.

Age, years	Weight status	Gender		*P*-value trend
		Male (%) *N* = 2601	Female (%) *N* = 3973	
6–10 ($n = 2314$)	Underweight	32 (3.6)	39 (2.8)	0.0006
	Normal weight	434 (48.2)	744 (52.6)	
	Overweight	114 (12.7)	231 (16.3)	
	Obese	320 (35.6)	400 (28.3)	
11–15 ($n = 3006$)	Underweight	32 (2.4)	24 (1.4)	<0.0001
	Normal weight	464 (35.7)	760 (44.6)	
	Overweight	207 (15.9)	374 (21.9)	
	Obese	598 (46.0)	547 (32.1)	
16–18 ($n = 1254$)	Underweight	17 (4.3)	20 (2.3)	<0.0001
	Normal weight	169 (42.3)	462 (54.1)	
	Overweight	58 (14.5)	179 (21.0)	
	Obese	156 (39.0)	193 (22.6)	

a strong correlation between childhood obesity and maternal age at pregnancy (OR = 1.24).

To analyze this relationship in more depth, we also investigated the correlation between duration of breastfeeding and maternal employment. As shown in **Supplementary Figure 1A**, a highly significant negative association between duration of breastfeeding and maternal employment was found. In addition, as shown in **Supplementary Figure 1B**, we found a significant difference in high birth weight (>4 kg) across maternity age categories at pregnancy.

In univariate analysis, there were no significant statistical associations between obesity and maternal education, duration of breastfeeding, family income, physical activity, fast food intake, or fruit and vegetable intake for both males and females (data not shown).

Multivariate logistic regression analysis (**Table 3**) showed that the following factors were strongly associated with obesity: male gender, older child age, smaller family size, high birth weight, maternal employment, and advanced maternal age at pregnancy. Children born to mothers who were aged >30 years at pregnancy and who were currently employed were 19 and 26% more likely to be obese, respectively. With regard to gender, boys had a 29% higher risk of obesity than girls. Similarly, children whose families had fewer than six members were at a 17% higher risk of being obese. Children with birth weight above 4.0 kg were 2.3 times more likely to be obese. Age-and gender-adjusted least squares means of maternity age and birth weight were significantly associated with both overweight and obesity in children (*P*-trend <0.0001).

DISCUSSION

Our findings suggest that childhood obesity is significantly related to age, male gender, birth weight, family size, maternal employment and maternal age at pregnancy. In both genders, age was a highly significant predictor of obesity, with the highest prevalence found in children aged 11–15 years. Compared with students aged 6–10 year obesity was 58% more common among those aged 11–15 years. We believe that the pubertal status of children in the 11–15 years age group may have influenced their body weight. This may also explain, at least in part, the higher prevalence of obesity in this group compared with that of the those in the 6–10 years age group (10). A large population-based longitudinal study has shown that obesity occurred early in life between 2 and 6 years in life and subsequently continued at a lower but still positive rate, which led to a greater degree of obesity to persist into adolescence, findings similar to ours

TABLE 3 | Estimated odds ratios (OR) for obesity by associated risk factors.

Risk factors	Obesity	Univariate logistic regression	Step-wise logistic regression	*P*-value
	Prevalence, %	OR (95% CI)		
Age (years)				
06–10	46.0	1	1	<0.0001
11–15	57.4	1.58 (1.42–1.76)	1.59 (1.42–1.78)	
16–18	46.7	1.03 (0.90–1.18)	1.06 (0.92–1.22)	
Gender				
Girls	48.4	1	1	<0.0001
Boys	55.9	1.35 (1.22–1.49)	1.29 (1.16–1.43)	
Mother's working status				
Not working	49.5	1	1	0.0006
Working	52.7	1.14 (1.04–1.26)	1.20 (1.08–1.33)	
Mother age at pregnancy				
<24	48.6	1	1	0.0016
24–30	51.0	1.10 (0.97–1.24)	1.11 (0.98–1.26)	
≥30	53.9	1.24 (1.10–1.40)	1.26 (1.11–1.43)	
Birth weight (kg)				
Low (<2.5)	46.9	1	1	<0.0001
Normal (2.5 – <4)	50.6	1.16 (1.00–1.34)	1.25 (1.07–1.46)	
High (≥4)	65.0	2.11 (1.69–2.62)	2.28 (1.81–2.86)	
Breastfeeding (months)				
<2	51.1	1	–	–
2–6	49.7	0.94 (0.83–1.07)		
>6	52.7	1.07 (0.95–1.19)		
Mother education, %				
>12 years	52.0	1.12 (0.97–1.28)	–	–
1–12 years	51.5	1.10 (0.96–1.25)		
Illiterate—no schooling	49.3	1		
Family size				
>6	50.3	1	1	0.0106
≤6	54.1	1.16 (1.04–1.30)	1.16 (1.04–1.30)	
Income, kuwaiti dinar				
<800	51.5	1	–	–
800–2000	52.2	1.03 (0.87–1.23)		
>2000	51.1	0.99 (0.84–1.16)		
Fruits/week				
≤1	51.6	1	–	–
>1	50.6	0.96 (0.85–1.09)		
Vegetable/week				
≤1	51.5	1	–	–
>1	50.8	0.97 (0.85–1.11)		
TV and video (hours/day)				
<1	51.0	1	–	–
1–2	50.0	0.92 (0.82–1.04)		
>2	53.7	1.11 (0.99–1.24)		
Sporting/(hours/day)				
≤1	51.5	1	–	–
>1	50.1	0.95 (0.80–1.11)		

For forward stepwise multivariate logistic analyses, values were adjusted for all significant parameters in univariate analysis. Sporting; physical activities e.g., walking, football, running, etc.

(11). This observation is consistent with a previous study conducted in Kuwait (12). Nevertheless, we observed that in both genders, compared with children aged 11–15 years, overweight and obesity was lower in those aged 16–18 years. This decline in overweight and obesity with age may be explained by the rapid increase in the height of participants in this stage of life (13). Moreover, as children gain mental maturity and their self-esteem becomes more tied to their body image, they maybe more motivated to change their lifestyle and eating habits.

Gender was also found to be associated with obesity at certain ages. Major differences were observed in the 11–15 years age group; boys were at a 30% greater risk of obesity than girls. This finding is consistent with that of previous reports from other countries in the surrounding region, such as Qatar and Lebanon (14, 15). This gender difference in obesity observed in the 11–15 years age group, persisted, although the prevalence was much lower, in the 16–18 years age group. Different eating patterns and food preferences in boys compared with girls may explain this observation. Other factors may explain the discrepancy in this finding as males tend to be masculine and consume food with high dense caloric content compared to female.

Maternal employment appears to be a significant predictor of childhood obesity in Kuwait. Our study indicated that children of mothers working full-time had higher BMIs and were more likely to be obese compared with children whose mothers were unemployed. Several factors may explain how maternal employment is associated with overweight and obesity in childhood. For instance, working mothers could be pressed for time, which might cause them to substitute healthy, home-cooked meals for fast food and/or restaurant meals. Given the fact that many families in Kuwait have hired domestic help, a lack of supervision at meal times might also be associated with childhood obesity. Children of mothers who worked more hours per week over the child's life (16) and who had higher work intensity in the period after the child's birth and before the child started school (17), were shown to have an increased likelihood of becoming overweight or obese later in childhood.

At present, the mechanism by which maternal employment influences childhood obesity is unknown. However, after examining the data in more depth, we found a highly significant association between duration of breastfeeding and maternal employment, wherein maternal employment was associated with shorter duration of breastfeeding. This may indicate that supplementary feeding at an early age is a possible pathway through which children may gain weight. Therefore, we recommend that communities should provide necessary support for working mothers in the upbringing of their children so that their children's health is not compromised. Policies that support extending maternal leave may contribute to a reduction in childhood obesity rates.

Previous studies have shown that advanced maternal age at pregnancy is associated with increased rates of complications such as cesarean delivery and adverse neonatal outcomes, as well as an increased risk of gestational diabetes mellitus (GDM), preeclampsia toxemia, preterm delivery and neonatal intensive care unit admission (18, 19). Our study indicates that in addition to these outcomes, pregnancy after 30 years of age is associated with a higher prevalence of obesity among schoolchildren and adolescents. This could be due to the fact that pregnant women over 30 years old tend to have a higher maternal BMI, which is in turn associated with childhood obesity. Maternal obesity in early pregnancy has been shown to be associated with childhood obesity and to double the risk of child obesity at 2–4 years of age (20).

Birth weight is a strong predictor of child BMI (21), and this relationship was confirmed in our study, which showed that children with a high birth weight (>4.0 kg) were 2.3 times more likely to develop obesity ($p < 0.0001$). Our results also revealed a significant difference in birth weight above 4.0 Kg across maternity age at pregnancy, as pregnant women at aged >30 years tend to give birth to babies with high birth weight. Several studies have shown that higher birth weight predicted an increased risk of overweight in adolescence (11, 22) and was positively associated with adult obesity (23). Birth weight may also be a strong indicator of GDM. It is well known that children born out of pregnancies complicated by GDM are at a risk of developing obesity in childhood (24) and that maternal obesity is associated with child birth weight above the 90th percentile (25).

In our study, children whose families had fewer than six members were significantly more likely to be obese. The average family size has been decreasing worldwide, and a number of studies have indicated that in smaller families, parents tend to pay more attention to their children and their dietary habits. Family size may also be an indicator of improved socio-economic status.

This study did not identify any association between breastfeeding and childhood/adolescent obesity. This finding is in agreement with a previous study from Kuwait, which reported that neither breastfeeding nor duration of breastfeeding was associated with obesity in children aged 3–6 years (26). Surprisingly, lifestyle factors found to be associated with childhood obesity in other studies, such as television watching and physical inactivity, were not shown to be risk factors for obesity in our sample of Kuwaiti children. Similarly, our data did not establish an association between maternal education and childhood obesity. This finding is consistent with other similar studies on Arab populations (27, 28).

As any other cross-sectional study, our survey had limitations with regard to the study design. The cross-sectional nature of our study made it impossible to determine any temporal relationship between the prevalence of obesity and possible associated etiological factors. A lifestyle intervention among children in the study sample who were found to be either overweight or obese is currently underway. We also plan to prospectively follow this cohort of children to determine causality and the directional effect of the various factors contributing to childhood overweight and obesity. A second study limitation was the lack of information collected about the students' mothers regarding the prevalence of GDM, maternal obesity or gestational weight gain, all of which have been associated with childhood obesity in previous research (29, 30).

Finally, we know that dietary and activity patterns established early in life persist later in life, highlighting the importance of early intervention to develop and maintain health-promoting behaviors and healthy weight throughout life

(31). Understanding risk factors and how they interact is important to inform interventions that aim to prevent obesity in early childhood (30).

CONCLUSION

In conclusion, this study provides knowledge of and insight into the risk factors related to the alarmingly high prevalence of childhood obesity in Kuwait. Interventions that can successfully alter the trajectory toward overweight status among high-risk children are critical if we have to effectively address this public health crisis (30, 32). Experience in several other countries has shown that successful behavior change during childhood can be achieved through a combination of population-based measures implemented both at the national level and as part of local school and community programs (33). Special attention should be placed on women with advanced maternal age, and support should be provided to working mothers to ensure the health and well-being of their children.

AUTHOR CONTRIBUTIONS

NE: the principal Investigator, participated in the conception, and overall supervision of the study, handled data management, data analysis and interpretation, and wrote the manuscript; AB: a co-investigator, and participated in the study conception; MA: a co-investigator, and participated in critical revision of the manuscript; AS: a co-investigator, participated in data interpretation and critical revision of the manuscript. All authors have read and approved the final 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/fpubh.2019.00072/full#supplementary-material

Supplementary Figure 1 | (A) age-gender adjusted least square means of duration of breastfeeding according to the maternal employment status. **(B)** frequency of high birth weight across maternity age at pregnancy.

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Genetic Associations With Diabetic Retinopathy and Coronary Artery Disease in Emirati Patients With Type-2 Diabetes Mellitus

*Sarah K. Azzam[1], Wael M. Osman[2], Sungmun Lee[1], Kinda Khalaf[1], Ahsan H. Khandoker[1], Wael Almahmeed[3,4], Herbert F. Jelinek[5] and Habiba S. Al Safar[1,2]**

[1] Biomedical Engineering Department, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, [2] Khalifa University Center of Biotechnology, Abu Dhabi, United Arab Emirates, [3] Institute of Cardiac Science, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates, [4] Heart and Vascular Institute, Cleveland Clinic, Abu Dhabi, United Arab Emirates, [5] Australian School of Advanced Medicine, Sydney and School of Community Health, Charles Sturt University, Macquarie University, Albury, NSW, Australia

Aim: Type 2 Diabetes Mellitus (T2DM) is associated with both microvascular complications such as diabetic retinopathy (DR), and macrovascular complications like coronary artery disease (CAD). Genetic risk factors have a role in the development of these complications. In the present case-control study, we investigated genetic variations associated with DR and CAD in T2DM patients from the United Arab Emirates.

Methods: A total of 407 Emirati patients with T2DM were recruited. Categorization of the study population was performed based on the presence or absence of DR and CAD. Seventeen Single Nucleotide Polymorphisms (SNPs), were selected for association analyses through search of publicly available databases, namely GWAS catalog, infinome genome interpretation platform and GWAS Central database. A multivariate logistic regression test was performed to evaluate the association between the 17 SNPs and DR, CAD, or both. To account for multiple testing, significance was set at $p < 0.00294$ using the Bonferroni correction.

Results: The SNPs rs9362054 near the *CEP162* gene and rs4462262 near the *UBE2D1* gene were associated with DR (OR = 1.66, p =0.001; OR = 1.37, $p = 0.031$; respectively), and rs12219125 near the *PLXDC2* gene was associated (suggestive) with CAD (OR = 2.26, $p = 0.034$). Furthermore, rs9362054 near the *CEP162* gene was significantly associated with both complications (OR = 2.27, $p = 0.0021$). The susceptibility genes for CAD (*PLXDC2*) and DR (*UBE2D1*) have a role in angiogenesis and neovascularization. Moreover, association between the ciliary gene *CEP162* and DR was established in terms of retinal neural processing, confirming previous reports.

Conclusions: The present study reports associations of different genetic loci with DR and CAD. We report new associations between CAD and *PLXDC2*, and DR with *UBE2D1* using data from T2DM Emirati patients.

Keywords: type 2 diabetes mellitus, diabetic retinopathy, coronary artery disease, single nucleotide polymorphism, United Arab Emirates, Arab population

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***Correspondence:**
Habiba S. Al Safar
habiba.alsafar@ku.ac.ae

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INTRODUCTION

Diabetes is among the largest global health emergencies in the twenty-first century, as it creates a major human and financial burden worldwide (1). The International Diabetes Federation (IDF) has estimated that 415 million people have been diagnosed with diabetes in 2015, and the number is expected to rise to 642 million by 2040. The financial burden associated with diabetes worldwide has been estimated to be 5–20% of total health expenditure (1). Within the United Arab Emirates (UAE), the prevalence of diabetes in adults between the age of 20–79 years old, was found to be 19.3% in 2015, with diabetes associated costs per individual reaching 2155.9 USD (1). Diabetes prevalence in the UAE is among the top prevalence rates in the Middle East and North Africa (MENA) region, with comparable levels to Saudi Arabia (20.0%) and Kuwait (20.0%); while showing higher prevalence levels as compared to other countries in the MENA region such as Egypt (16.7%), Jordan (11.7%), and Oman (14.8%) as reported by IDF(1).According to the IDF, type 2 diabetes mellitus (T2DM) is the most common type of diabetes (1), accounting for more than 90% of patients diagnosed with diabetes (2). Contributing to the human and financial costs of T2DM is that it is a complex multifactorial disease resulting from a combination of genetic, environmental, and behavioral risk factors that manifest in multiorgan dysfunction (3).

T2DM can lead to a number of long-term serious complications and health problems. Consistently high blood glucose levels (BGL) can seriously damage the heart, blood vessels, vision, the nervous system, and kidney. Therefore, T2DM is commonly associated with microvascular as well as macrovascular complications including diabetic retinopathy (DR), and coronary artery disease (CAD), respectively.

CAD is defined as a complex disease resulting from an interplay between lifestyle, environmental and genetic factors (4). Globally, diabetes is recognized as a major cause of CAD (5), which in turn is ranked as number one cause of mortality worldwide (4–6). Adults with diabetes are reported to have a higher death rate by 2 to 4-folds from CAD, as compared to adults without diabetes (5). Identified common risk factors associated with CAD include hypertension, smoking, increased age, diabetes mellitus, male-gender, diet high in fat, elevated low-density lipoprotein (LDL) cholesterol, reduced plasma high-density lipoprotein (HDL) cholesterol, increased triglycerides, and increased plasma total cholesterol (4, 6).

A study by Al-Maskari et al. reported CAD prevalence levels of 14.4% among UAE residents with diabetes in Al-Ain city, with age ($p = 0.04$), diabetes duration ($p = 0.002$), and hypertension ($p = 0.04$) reported as major risk factors (7). CAD prevalence of 17.8% among diabetic population from Yemen was reported while a prevalence of 23.7% for CAD among diabetic individuals was reported in Iran (8). Reduction in mortality and morbidity by 30–40% has been reported by studies targeting such risk factors and illustrating the importance of preventive measures against diabetes and CAD (6). Yet, genetic predisposition is estimated to account for 40–60% of CAD susceptibility (6) as concluded from familial and twin studies (5). Such studies highlight the importance of investigating not only common risk factors, but also genetic risk factors associated with CAD in patients with diabetes in order to provide early prevention schemes to reduce the mortality rates caused by CAD among patients with diabetes.

Diabetic Retinopathy (DR) is another serious complication of T2DM and is the most common cause of blindness for adults in developed countries (9). Deterioration of vision implicated in DR is a gradual process starting from mild non-proliferative diabetic retinopathy (mild-NPDR), to moderate and severe non-proliferative diabetic retinopathy (NPDR), finally to proliferative diabetic retinopathy (PDR) (10). Prevalence of DR is related to a number of common risk factors including diabetes duration, poor glycemic control, hypertension and dyslipidemia (5, 9–11). In the UAE, the prevalence of DR with diabetes was found to be 19% in Al-Ain (12). Prevalence of DR among different diabetic populations was reviewed by Zabetian et al. (8), and reported as follows; Saudi Arabia (30.0%), Qatar (23.5%), and Oman (16.2%). In addition to common risk factors, genetic risk factors have been reported to play an important role in the development of DR, where their impact accounts for 25–50% of DR risk (11).

Several Genome Wide Association Studies (GWAS) have identified possible genes associated with DR and CAD (13–16), with different degrees of genetic associations. For instance, the *GLUL* gene in human endothelial cells is associated with CAD in the T2DM European population (17), while the *GRB2* gene is associated with DR in T2DM Australian patients and upregulated in neovascularization and retinal stress (18). To date, GWAS has been conducted on different ethnic groups. However, no extensive studies from the Middle Eastern population have been conducted (19).

The aim of the present study is to investigate common genetic variants (17 single nucleotide polymorphisms, SNPs) that have been reported to increase risk of diabetic complications including diabetic retinopathy (DR), coronary artery disease (CAD), or a combination of these two (R+CAD) in a case-control study in an Arab population in the UAE. This can help in establishing a comprehensive prevention program in the future for the diabetic Emirati population by considering early detection for T2DM complications in patients with certain SNPs preventatively.

METHODS

Subjects and Sample Collection

Study subjects were enrolled in during routine visits to the endocrinology and cardiology clinics at Sheikh Khalifa Medical Centre (SKMC) and Mafraq Hospital in Abu Dhabi city, in the period between July 2014 and May 2015. The study cohort consisted of 407 (234 females and 173 males), unrelated patients diagnosed with T2DM from the UAE. The Institutional Ethics

Abbreviations: UAE, United Arab Emirates; T2DM, type 2 diabetes mellitus; BGL, blood glucose levels; DR, diabetic retinopathy; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; GWAS, Genome Wide Association Studies; SNPs, single nucleotide polymorphisms; WHO, World Health Organization; HWE, Hardy-Weinberg Equilibrium; UBE2D1, ubiquitin conjugating enzyme E2 D1; HIF1-α, hypoxia-inducible factor alpha subunit; VEGF, Vascular Endothelial Growth Factor; PLXDC2, Plexin domain-containing protein 2; PEDF, Pigment Epithelium Derived Factor.

Committees of SKMC and Mafraq Hospital both reviewed and approved the study (REC-04062014 and R292, respectively). All participants provided written consent in accordance with the Helsinki Declaration of ethical conduct in research.

A qualified physician confirmed the presence of diabetes associated complications, as outlined in the criteria by the World Health Organization (WHO) consultation group report (20). Subjects included in this study were UAE-born nationals, diagnosed with T2DM, can give consent and older than 18 years of age when enrolled in the study. Subjects who could not give consent, who were pregnant at the time of enrollment or who were diagnosed with other pathophysiology like cancer or psychosis, were excluded from this study.

Biochemistry tests were performed at the time of enrollment. Blood pressure was taken at two different time points, 48 h apart, and the average was taken. Diagnosis criteria of hypertension and dyslipidemia were previously defined in Jelinek et al. (21)

The major complications of T2DM considered in this study were coronary artery disease and retinopathy. Diagnosis of cardiovascular disease was obtained from the medical records using ICD-9 diagnostic codes 410–414, and verified by the consulting physician. Diagnosis of retinopathy was defined according to WHO criteria (22), by the presence of either white or red lesions (non-proliferative or proliferative retinopathy) or the presence of both in the retina.

Genotyping

The GWAS data were obtained from 490 individuals resident in the United Arab Emirates (UAE). Genotyping was performed on the Infinium Omni5ExomeHuman chip according to the manufacturer's protocols (Illumina Inc., San Diego, USA), and raw data was collated on the GenomeStudio v2010.3 (Illumina Inc., San Diego, USA). The microarray contained 4,641,218 SNPs. Quality control (QC) on the data was performed using the PLINK software (version 1.07) (23) to remove SNPs with a minor allele frequency (MAF) <0.05, with $>5\%$ missing genotype rate, failing the Hardy-Weinberg equilibrium (HWE) test at the 0.000001 significance level and Mendelian error. Approximately 39% of SNPs passed these QC criteria. Samples that failed quality control were also excluded from the analysis. The average call rate for the remaining 490 samples was 98.99%. Out of the 490 genotyped samples available in the Emirates Family Registry (EFR) database (24), 411 genotyped samples included available data on diabetes-associated complications. As a final exclusion step, four individuals were reported as patients diagnosed with type 1 diabetes mellitus (T1DM) and were excluded; hence, resulting in the inclusion of 407 individuals in the study.

SNPs Selection

A search was performed using different resources, with the aim of selecting SNPs that have been reported with CAD or DR in individuals with T2DM. Further SNPs reported in association with CAD or retinopathy (R) in individuals without T2DM, were also selected. The following resources were utilized: GWAS catalog: https://www.ebi.ac.uk/gwas/home, infinome genome interpretation platform: https://www.infino.me/, and GWAS Central database: http://www.gwascentral.org/. Forty-three SNPs were initially retrieved from searching the aforementioned resources, reported in association either with CAD or R. Next, a search was performed on the database established using Emirates Family Registry (EFR)(24), which resulted in selecting 17 SNPs with significant and suggestive associations with either CAD or R ($P < 5 \times 10^{-5}$). The 17 SNPs are reported in **Table 2**, as SNPs found in the Emirati cohort under study. The current study focused on DR and CAD as T2DM associated complications, given that DR was reported as the single most prevalent T2DM complication among Emiratis, followed by CAD prevalence, as reported by Jelinek et al. (21).

Statistical Analysis

Statistical analyses for demographic, clinical and laboratory data were performed using Microsoft Excel and R (28) software was utilized in performing graphical normality tests of distribution. Continuous variables results were expressed as mean ± standard deviation, or as median and interquartile range (IQR) for highly-skewed distributions; while categorical variables were presented as counts and percentages. For comparisons between cases and controls, the Pearson chi-square test was utilized for categorical variables or Fisher's exact test when expected frequencies were <5. For continuous variables statistical differences were assessed using two-tailed Student *t*-tests for normally-distributed data, or using the Wilcoxon rank-sum (Mann-Whitney) test for highly-skewed data. Results were considered of statistical significance when the *p*-value was <0.05. Data quality control was implemented using the Hardy-Weinberg Equilibrium (HWE) test via Plink Software (23), where a $P < 0.005$ indicates significant deviations from HWE, reflecting issues such as population stratification (23, 29) or genotyping errors (29). Such SNPs were excluded from the analysis to avoid biased conclusions. For the purpose of assessing genetic risk factors that are associated with development of diabetic retinopathy (DR), or coronary artery disease (CAD), or both of these complications (DR+CAD), a multivariate logistic regression test in Plink Software (23) was performed to evaluate the association between a T2DM complication as a result (response or dependent variable) and the presence of a SNP (predictor or independent variable). Furthermore, adjusting for covariates was considered in generating measures of association and significance within the statistical modeling, where covariate adjustment is based on factors which showed a significant difference between cases and controls. Covariate adjustment was conducted for each group in the study cohort as follows; for the R vs. no R group, age and diabetes duration were considered as covariates; for the CAD vs. no. CAD group, gender, age, hypertension, dyslipidemia and smoking behavior were adjusted for as covariates; finally for the CAD+DR vs. no CAD and no DR. group, age, diabetes duration, hypertension, and dyslipidemia were considered as covariates. The Bonferroni correction was performed to account for multiple testing that is conducted in this study, where the α-value is adjusted from 0.05 to a new α-value = (0.05/N) (30), where *N* refers to the number of statistical test performed. In the current study, 17 SNPs were investigated in association with certain T2DM complications. Therefore, a new α-value = (0.05/17) = 0.002941 is set. Results from our multiple testing

TABLE 1 | Demographic, clinical, and laboratory data of the study subjects.

Type of variables	Variable	DR‡ (*n* = 202)	No DR (*n* = 205)	*P**	CAD (*n* = 160)	No CAD (*n* = 245)	*P**	DR + CAD (*n* = 76)	No DR and No CAD (*n* = 121)	*P**
Demographic	Gender: Female†	124 (61%)	110 (54%)	0.11	74 (46%)	159 (65%)	**0.00021**	37 (49%)	73 (60%)	0.11
	Age (years)	63 ± 11	60 ± 11	**0.0034**	66 ± 9.3	59 ± 11	**< 0.0001**	67 ± 9.7	57 ± 12	**< 0.0001**
Clinical variables	Diabetes duration (years)**	14 (5.5, 20)	7 (4, 15)	**< 0.00001**	9 (5, 20)	12 (5, 20)	0.070	13 (5, 20)	9 (5, 16)	**0.022**
	Clinical hypertension†	169 (84%)	167 (82%)	0.71	152 (95%)	183 (75%)	**< 0.0001**	74 (97%)	89 (75%)	**0.0001**
	Dyslipidemia†	193 (96%)	184 (90%)	0.052	158 (99%)	218 (89%)	**0.0002**	75 (99%)	101 (84%)	**0.0005**
	Smoking†	50 (25%)	59 (29%)	0.34	53 (33%)	55 (23%)	**0.018**	25 (33%)	31 (26%)	0.29
	Diabetes family history†	136 (68%)	139 (68%)	0.88	100 (63%)	174 (71%)	0.21	48 (64%)	87 (72%)	0.51
	Diabetes complications family history†	53 (26%)	41 (20%)	0.12	32 (20%)	62 (25%)	0.38	19 (25%)	28 (23%)	0.81
	BMI (kg/m^2)	32 ± 6.1	32 ± 6.4	0.42	32 ± 6.0	32 ± 6.4	0.23	31 ± 5.7	32 ± 6.4	0.57
	Waist circumference (cm)	107 ± 12	106 ± 15	0.43	108 ± 14	105 ± 13	0.050	108 ± 12	104 ± 14	0.075
Laboratory variable Glycemic index	HbA1c (%)	7.8 ± 1.5	7.7 ± 1.7	0.52	7.9 ± 1.6	7.7 ± 1.6	0.34	7.8 ± 1.5	7.5 ± 1.7	0.32
Lipids profile (mg/dl)	Total-cholesterol	148 ± 37	153 ± 44	0.21	142 ± 38	156 ± 41	**0.0011**	139 ± 32	159 ± 43	**0.001**
	Triglycerides**	119 (90.3, 165)	117 (88.6, 154)	0.48	116 (90.8, 160)	118 (87.7, 164)	0.98	110 (90.3, 161)	113 (81.5, 154)	0.51
	HDL-cholesterol	47 ± 18	47 ± 21	0.83	45 ± 22	49 ± 18	0.052	45 ± 12	49 ± 15	0.073
	LDL-cholesterol	74 ± 30	81 ± 37	0.062	73 ± 33	80 ± 34	**0.045**	69.8 ± 28	85 ± 37	**0.0062**

P-value: for continuous variables, computed using two-tailed t-test for normally-distributed data, or using Mann-Whitney test for highly-skewed data. For categorical variables, computed using the Pearson chi-square test or Fisher's exact test for expected frequencies <5. †Categorical variables are presented as counts and percentages. All remaining continuous variables are expressed as mean ± standard deviation, or as median (IQR lower, upper) for highly-skewed distributions**. ‡BMI, Body Mass Index; CAD, Coronary Artery Disease; DR, Diabetic Retinopathy; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; n, number of individuals. Bold value indicates significance level and p < 0.05.*

TABLE 2 | The study selected SNPs according to their previous reports, together with reported associated traits and ethnicities.

SNP† ID	Gene(s)	Chr: BP	Associated population	Associated trait	References
rs7553035	*RD3*	1: 211691706	European	R‡	(25)
rs10004839	*LOC105377441*	4: 138154812	European	R‡	(25)
rs6472155	*LOC105375878; LOC105375879*	8: 65730207	European	R‡	(25)
rs17194885	*SRC*	20: 36068389	European	R‡	(25)
rs2811893	*MYSM1*	1: 59162148	Taiwanese	DR	(14)
rs17376456	*KIAA0825; LOC105379087*	5: 93557702	Taiwanese	DR	(14)
rs12219125	*PLXDC2*	10: 20593087	Taiwanese	DR	(14)
rs4838605	*ARHGAP22*	10: 49699957	Taiwanese	DR	(14)
rs4462262	*LOC105378313; LOC105378314*	10: 59189178	Taiwanese	DR	(14)
rs2038823	*HS6ST3*	13: 96951433	Taiwanese	DR	(14)
rs9362054	*LINC01611*	6: 85178268	Japanese	DR	(13)
rs9543976	*UCHL3*	13: 76136648	Chinese	DR	(26)
rs646776	*CELSR2*	1: 109818530	European	CAD‡	(15)
rs4977574	*CDKN2B-AS1*	9: 22098574	European	CAD‡	(15)
rs8055236	*CDH13*	16: 83212398	European(British)	CAD‡	(27)
rs10911021	*LOC105371642; ZNF648*	1: 182081960	European	CAD	(17)
rs7901695	*TCF7L2*	10: 114754088	African Americans	CAD	(16)

†BP, Base-pair position; CAD, Coronary artery disease; Chr., Chromosome number; DR, Diabetic retinopathy; R, retinopathy; SNP, Single Nucleotide Polymorphism. ‡Individuals diagnosed with R or CAD but without T2DM.

approach describe a SNP as "significantly" associated SNP when $p < 0.002941$. SNPs that show a direction of association (when a $p < 0.05$ yet did not reach significance of new α-value) are reported as SNPs that are "suggestively" associated with a complication. SNPs with suggestive association are worth investigating in the future with a larger cohort size.

RESULTS

Characteristics of Study Subjects

Demographic data, clinical and laboratory data of participants are summarized in **Table 1**. A total sample of 407 T2DM Emirati patients were recruited. Categorization of study population into 3 groups was performed based on the presence of diabetes and the presence or absence of diabetic retinopathy (DR) and coronary artery disease (CAD). For category 1, patients diagnosed with DR had significantly higher age and a higher reported diabetes duration as compared to controls without DR. In category 2, T2DM patients with CAD had also significantly higher age with higher total cholesterol, LDL-cholesterol, hypertension, and presence dyslipidemia. Significantly more male patients presented with CAD and indicated longer smoking history. For category 3, patients with both conditions had similar results to category 2, with higher reported diabetes duration (**Table 1**).

Association Between SNPs and T2DM Complications Under Study

The 17 SNPs within our database and associated with DR and CAD complications are listed in **Table 2**. The results of associations between SNPs and T2DM complications (DR and CAD) considering gender, age, diabetes duration, dyslipidemia, hypertension and smoking behavior as covariates are shown in **Table 3**, where covariate adjustment was performed for each group within the study cohort based on factors which showed a significant difference between cases and controls. Furthermore, ORs of confounding factors in **Table 3** give an indication of the relative contribution of each factor to the others. Rs9362054 near the *CEP162* gene, and rs4462262 near the *UBE2D1* gene were associated with increased risks of DR (DR vs. no DR) with rs9362054 having an odds ratio of nearly 2 and highly significant. For CAD (CAD vs. no CAD) rs12219125 near the *PLXDC2* gene showed suggestive association, with an increased risk of CAD with an odds ratio over 2. For the DR+CAD category (DR + CAD vs. no DR and no CAD), rs9362054 was significantly associated with the presence of both complications, while rs17376456 in the *KIAA0825* gene showed suggestive association with both complications, with rs17376456 having an odds ratio below 1 and indicative of a possible protective function with both complications less likely to occur.rs9362054 again doubling the odds ratio for presence of the two complications.

Comparison Between Emirati Population and Reference Populations

We next compared the results of the identified SNPs based on results that were previously reported in different ethnic groups, as shown in **Table 4**. The SNP rs9362054 near *CEP162* was previously reported by Awata et al. as a locus for DR in the Japanese population, with 1.40-fold increase in DR risk (13). Huang et al. reported significant association of the SNPs rs12219125 and rs17376456 with DR in the Taiwanese population with a 1.62- and 3.63-fold increases in DR risk, respectively (14). In the current Emirati patients study, rs12219125 is suggestively associated with CAD, with an increase of 2.26-fold.

TABLE 3 | Results of significant and suggestive associations between tested SNPs and T2DM complication: DR, CAD, or a combination of both.

SNP† ID	Gene(s)	Chr: BP	A1/A2	MAF Cases	MAF Controls	OR (CI 95%)‡	*P*-value
DR vs. no DR							
rs9362054	*LINC01611*	6: 85178268	A/G	39%	50%	1.66 (1.23–2.24)	**0.001**
						Age: 1.02 (1.00–1.04)	
						Diabetes duration: 1.05 (1.03–1.08)	
						Unadjusted: 1.62 (1.21–2.16)	***0.0011***
rs4462262	*LOC105378313; LOC105378314*	10: 59189178	A/G	39%	46%	1.37 (1.03–1.81)	0.031
						Age: 1.02 (1.00–1.70)	
						Diabetes duration: 1.05 (1.03–1.08)	
						Unadjusted: 1.31 (1.00–1.73)	*0.050*
CAD vs. no CAD							
rs12219125	*PLXDC2*	10: 20593087	A/C	3.8%	6.3%	2.26 (1.06–4.81)	0.034
						Gender: 0.43 (0.24–0.75)	
						Age: 1.06 (1.03–1.08)	
						Hypertension: 3.74 (1.65–8.48)	
						Dyslipidemia: 5.20 (1.12–24.2)	
						Smoking: 0.85 (0.47–1.54)	
						Unadjusted: 1.79 (0.89–3.59)	*0.10*
DR + CAD vs. no DR and no CAD							
rs17376456	*KIAA0825; LOC105379087*	5: 93557702	G/A	14%	9.9%	0.429 (0.193–0.952)	0.037
						Age: 1.08 (1.04–1.13)	
						Diabetes duration: 1.01 (0.97–1.05)	
						Hypertension: 5.60 (1.18–26.6)	
						Dyslipidemia: 4.56 (0.50–41.3)	
						Unadjusted: 0.607 (0.312–1.18)	*0.14*
rs9362054	*LINC01611*	6: 85178268	A/G	36%	50%	2.27 (1.35–3.83)	**0.0021**
						Age: 1.08 (1.04–1.13)	
						Diabetes duration: 1.02 (0.98–1.06)	
						Hypertension: 4.52 (0.95–21.5)	
						Dyslipidemia: 5.03 (0.55–45.7)	
						Unadjusted: 1.89 (1.21–2.93)	*0.0047*

†SNP, Single-nucleotide Polymorphism; Chr, chromosome number; A1, minor allele; A2, major allele; MAF, Minor Allele Frequency; OR, Odds Ratio; CI, Confidence Interval; DR, Diabetic Retinopathy; CAD, Coronary Artery Disease; LINC01611, long intergenic non-protein coding RNA 1611; LOC105378313, uncharacterized LOC105378313; LOC105378314, uncharacterized LOC105378314; PLXDC2, plexin domain containing 2; KIAA0825, protein-coding gene with uncharacterized protein KIAA0825; LOC105379087, uncharacterized LOC105379087. Adjusted OR values are illustrated, where adjustment was performed using factors that show a significant difference between cases and controls for each group within the study cohort. ‡A2, the major allele, was used as a reference allele in logistic regression tests; Bold and italic values indicates the significance level and $p < 0.05$.

DISCUSSION

Diabetic retinopathy and coronary artery disease are clinically significant complications of T2DM that lead to increased morbidity and mortality. Genetic risk factors were found to play an important role in the susceptibility of these complications. Previous studies have reported a 25–50% genetic risk for DR (11) and 40–60% risk for CAD (6). Genetic risk factors may act solely or in combination with primary risk factors such as hypertension and level of cholesterol, leading to these diabetic complications.

In the present study, we investigated genetic risk factors associated with DR, CAD or a combination of both complications in a case-control study of T2DM Emirati patients. In the following discussion, we will also present the possible functions of the genetic loci associated with each category in an attempt to identify possible pathways that may be involved in the etiology of DR or CAD. Demographic, clinical, and laboratory data in the present study indicated a different distribution of risk factors that may contribute to the development of the tested traits. Although some studies report the "male gender" as a risk factor for T2DM as reported by Nordstrom et al. (31) yet it is attributed it to the effect of differences in visceral fat mass between males and females; indicative of visceral fat mass effect rather than gender. Nevertheless, studies from the UAE either do not report gender as a risk factor as reported recently by Sulaiman et al. (32) or report female predominance in terms of T2DM incidence among UAE residents (33). As reported by Ali et al. (33) restricted outdoor physical activities due to sociocultural norms as well as the lack of culturally-sensitive exercise facilities contribute to lower physical

TABLE 4 | Comparison between SNPs reported with T2DM complication in this study: DR, CAD, or both, with previous reports.

SNP† ID	Chr. No.	Gene(s)	Ref. population *p*-value	Ref. population OR	Our reported *p*-value	Reported trait	Our reported trait	References
DR vs. no DR								
rs9362054	6	*LINC01611*	1×10^{-6}	1.40	0.001	DR, T2DM, Japanese	DR, T2DM, Emirati	(13)
rs4462262	10	*LOC105378313; LOC105378314*	9×10^{-8}	–	0.031	DR, T2DM,Taiwanese	DR, T2DM,Emirati	(14)
CAD vs. no CAD								
rs12219125	10	*PLXDC2*	9×10^{-9}	1.62	0.034	DR, T2DM,Taiwanese	CAD, T2DM, Emirati	(14)
DR + CAD vs. no DR and no CAD								
rs17376456	5	*KIAA0825; LOC105379087*	3×10^{-15}	3.63	0.037	DR, T2DM,Taiwanese	DR+CAD, T2DM, Emirati	(14)
rs9362054	6	*LINC01611*	1×10^{-6}	1.40	0.0021	DR, T2DM, Japanese	DR+CAD, T2DM, Emirati	(13)

†*CAD, Coronary artery disease; Chr.No, Chromosome number; DR, Diabetic retinopathy; KIAA0825, protein-coding gene with uncharacterized protein KIAA0825; LINC01611, long intergenic non-protein coding RNA 1611; LOC105378313,uncharacterized LOC105378313; LOC105378314,uncharacterized LOC105378314; OR, Odds ratio; PLXDC2,plexin domain containing 2; Ref, Reference; SNP, Single Nucleotide Polymorphism; T2DM, Type 2 diabetes mellitus.*

activity and higher predominance among female residents in the UAE.

The top SNP, rs9362054, significantly associated with DR in the T2DM Emirati cohort, is located on the *LINC01611* gene or alternatively called *RP1-90L14.1*, a long intergenic non-protein coding RNA 1611. Although the SNP, rs9362054 also shows a significant association with DR+CAD (in the DR+CAD vs. no DR and no CAD group), to our current knowledge its potential association with CAD has not yet been established in literature. Therefore, rs9362054 is discussed herein only in the context of DR. The SNP rs9362054 is an intronic variant, and was previously reported to be associated with DR in a Japanese population using the GWAS approach (13). The nearest protein-coding gene to *LINC01611* (using UCSC database), is *CEP162* (centrosomal protein 162), which is alternatively called QN1. A connection between the long non-coding RNA *LINC01611* gene and *CEP162* gene may be possible via *cis*-regulation exerted by *cis*-acting lncRNA, which controls expression of genes nearby the lncRNA transcription sites (34). CEP162 protein is utilized in promoting the assembly of the transition zone of the primary cilia found on the apical surface of the majority of mammalian cells in G0/G1 phase of the cell cycle (35). Primary cilia are important in cell signaling, thermo-, mechano- and chemosensation (30–32, 35, 36). Defects in cilia are found to be associated with several disorders, including retinal degeneration, liver, and kidney diseases, a group of disorders termed ciliopathies (36). Ciliary dysfunction affects retinal photoreceptors; where a transport process across the retinal connecting cilium is disrupted, leading to reduced survival of retinal photoreceptor cells (36, 37). CEP162 loss halts the ciliogenesis process, specifically the assembly of the connecting cilium stage (35), which in turn affects protein transport process and resulting in retinal degeneration (36) (**Figure 1**). However, the effect of dysfunctional neurons on retinal vasculature physiology and survival remains poorly understood (38). In transgenic rats (TGR) overexpressing a mutant cilia gene (38), encoding polycystin-2 protein, resulted in neuronal death and subsequent retinal degeneration. The ciliary protein polycystin-2 is located in the connecting cilium and its dysfunctional expression results in defective protein transport across the connecting cilium. The study further reported similar phenotypes to diabetic retinopathy including vasoregression, loss of endothelial capillary cells and pericytes (38). This association between a ciliary gene and DR was previously discussed in Awata et al. (13) and verifies our finding that a similar process is present in the Emirati patient group.

Another SNP suggestively associated with DR in our study is rs4462262. This is an intergenic SNP, which is located between the uncharacterized genes *LOC105378313* and *LOC105378314*. This SNP was previously reported to be associated with DR in a Taiwanese population by a GWAS approach (14). A protein-coding gene, *UBE2D1* (ubiquitin conjugating enzyme E2 D1), located near rs4462262 was previously investigated for function and possible involvement in the development of retinopathy. UBE2D1 is also known as UBCH5. UBE2D1 is one of the three classes of ubiquitination enzymes. It is a ubiquitin-conjugating enzyme (E2 class), which accepts ubiquitin from ubiquitin-activating enzymes (E1 class) and catalyzes the covalent attachment of ubiquitin to other proteins. Hence, contributing to the selective degradation of short-lived or abnormal proteins (39). UBE2D1 interacts with enzymes from E1 and E3 classes in the ubiquitination of hypoxia-inducible factor alpha subunit (HIF1-α). HIF1-α is an important regulator of oxygen homeostasis (40), and is associated with pathological conditions that are caused by hypoxia and retinal ischemia (41). The expression of HIF1-α is up-regulated by hypoxia during normal retinal development (41). In diabetes mellitus, elevated blood glucose levels negatively affect retinal capillaries, resulting in their incompetence on functional and anatomical levels that manifest as retinal hypoxia that can lead to proliferative retinopathy. Persistently-high glucose levels induce further damage to retinal capillaries and vessels that eventually result in hypoxia (41). Under hypoxic conditions, levels of

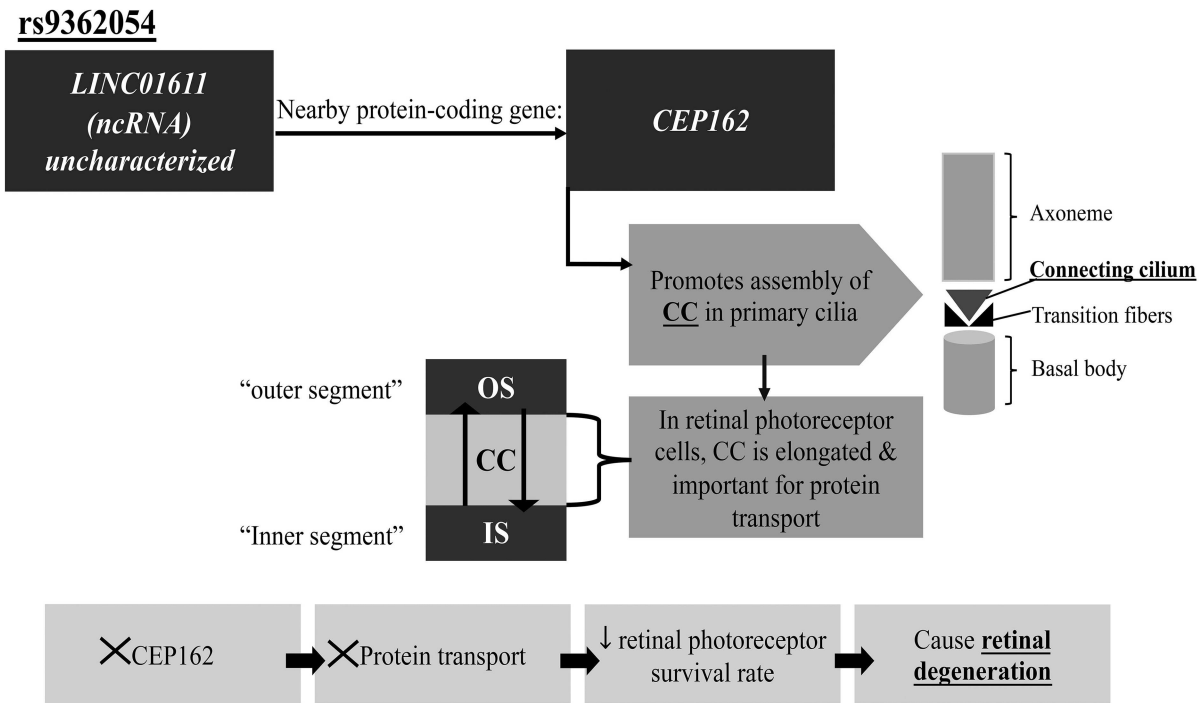

FIGURE 1 | Proposed pathway by which rs9362054 SNP can be associated with DR. CEP162, centrosomal protein 162; CC, Connecting cilium; IS, Inner segment; ncRNA, Non-coding RNA; OS, Outer segment. See text for further details.

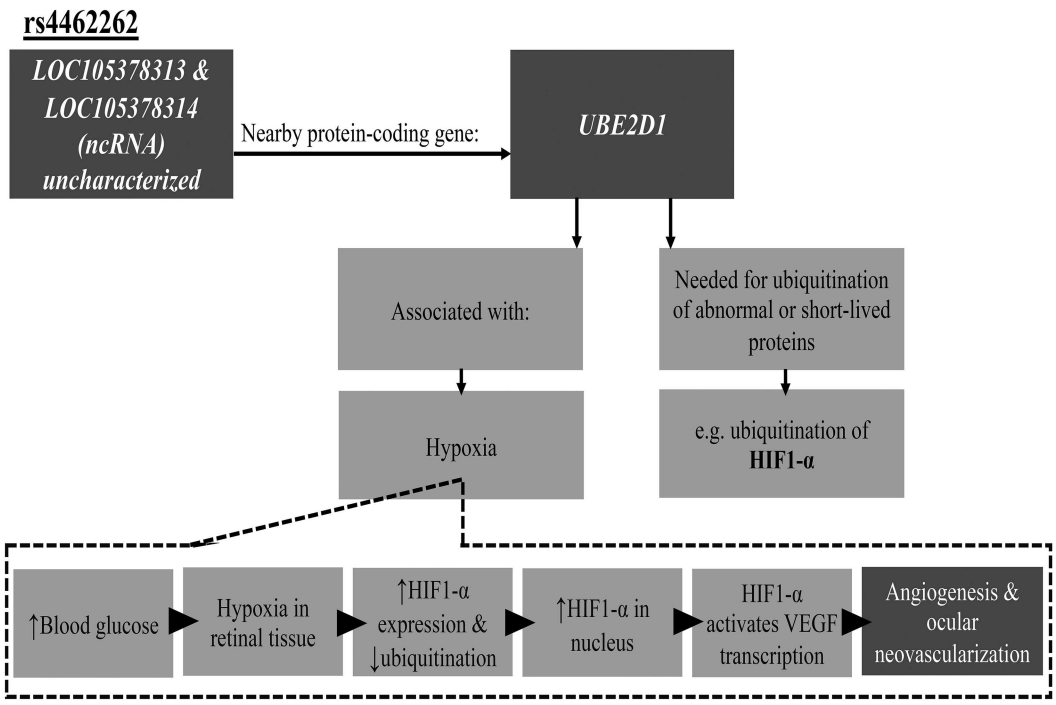

FIGURE 2 | Proposed pathway illustrating association of rs4462262 SNP with DR. HIF1-α, hypoxia-inducible factor 1-alpha; ncRNA, Non-coding RNA; UBE2D1, ubiquitin conjugating enzyme E2 D1; VEGF, Vascular Endothelial Growth Factor. See text for further details.

HIF1-α protein are elevated and its ubiquitinated portion decreases, which results in HIF1-α accumulation in the nucleus (**Figure 2**). Consecutively, HIF1-α activates the transcription of different target genes, including the Vascular Endothelial Growth Factor (*VEGF*), which is involved in angiogenesis and ocular neovascularization (41, 42), a characteristic of diabetic retinopathy. The detailed hypothetical model of the association between ubiquitin-conjugating enzyme UBE2D1 and HIF1-α in the development of DR is shown in **Figure 2**.

Finally, rs12219125 showed suggestive association with CAD in T2DM Emirati patients, and was previously reported to be significantly associated with DR in a Taiwanese population (14). This is the first study to investigate the association of the rs12219125 SNP with CAD in T2DM patients. This SNP is located in an intergenic region, and the nearest protein-coding gene is *PLXDC2*. The protein encoded by this gene, which is known as Plexin domain-containing protein 2, was suggested to play a role in tumor angiogenesis (43). Furthermore, a study by Cheng et al. identified the transmembrane protein PLXDC2 to be one of the cell-surface receptors for Pigment Epithelium Derived Factor (PEDF) (44). PEDF is expressed in different tissues including eye, brain, liver, heart, and lung, where it is an important inhibitor of angiogenesis, with an anti-tumorigenic, anti-thrombotic, and anti-metastatic function (44). It also has been utilized in the treatment of eye diseases including diabetic retinopathy, ischemic retinopathy and age-related macular degeneration, where reduced PEDF levels in the eye are associated with the susceptibility to these disorders (44). The hypothetical model for association between PLXDC2-PEDF and DR is shown in **Figure 3**. One previous study agreeing with our results, reported that elevated serum PEDF levels were observed in patients diagnosed with coronary artery disease (CAD) (45).These higher levels of PEDF may act as a protective mechanism in response to vascular damage and atherosclerosis (45). In addition to inhibition of angiogenesis, PEDF inhibits inflammation and cardiovascular remodeling and is an important target in the prevention of atherogenesis and necrotic core progression. Furthermore, PEDF serum level was found to be correlated with necrotic core progression during statin therapy, which suggests a protective response mechanism aimed at protecting against core progression (46).

Studies investigating the association of the SNPs rs9362054, rs4462262, rs12219125, and rs17376456 are summarized in **Table 4**, including this study. In the Emirati population under study, rs12219125 is suggestively associated with CAD with increased risk of 2.26-fold, whereas in the Taiwanese population it was associated with a 1.62-fold increased risk of DR (14). Discrepancies in reported risk levels between our population and

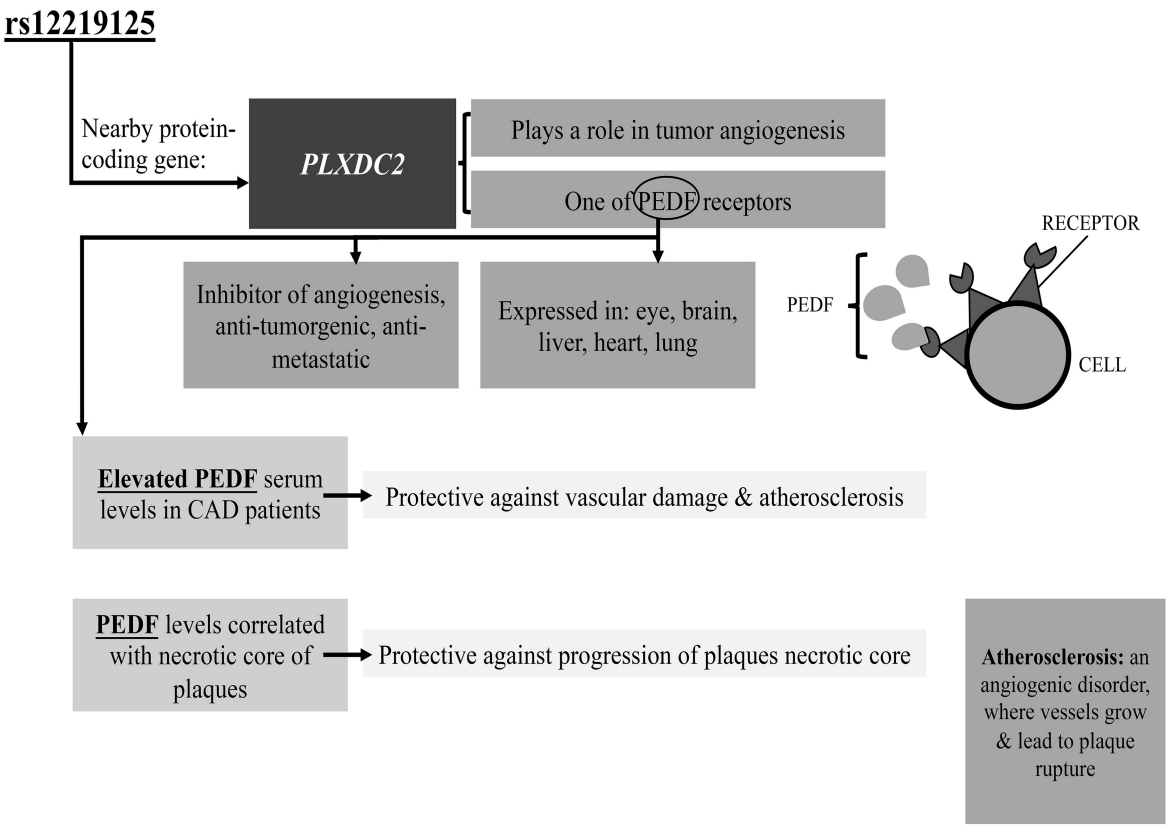

FIGURE 3 | Proposed pathway by which rs12219125 SNP may be associated with CAD as a T2DM complication. PEDF, Pigment Epithelium Derived Factor; PLXDC2, plexin domain containing 2. See text for further details.

reference populations can be explained by the larger number of subjects in the Japanese and Taiwanese GWA studies in comparison to our study. Moreover, differences in confounding factors for adjustment of logistic regression models between our study cohort, the Japanese and the Taiwanese cohorts can further contribute to discrepancies, which is dependent on each cohort under study and is based on factors that show significant difference between cases and controls. For example, adjusting for hypertension state as a confounding factor was performed in the current study, while it was not considered as a confounder in the Japanese and Taiwanese GWA studies despite significant difference between cases and controls. One of the strengths of the present study is that patients were recruited from the two largest hospitals in Abu Dhabi, UAE; making our sample fairly representative of Emirati patients. Nonetheless, a limitation of this study would be the small sample size ($n = 407$). Another limitation inherent to the current study is that the logistic analyses performed did not include treatment approaches as most patients are diagnosed with multiple conditions and are undertaking several treatments which would affect the analyses stability if multiple drug classes were to be adjusted for. A future study with a larger sample size is important in confirming these findings in association with T2DM in the Emirati population A future study with a larger sample size is important in confirming these findings in association with T2DM in the Emirati population which can help in the future for early detection of complication for T2D patients with certain SNPs for a preventative manner.

In conclusion, our study findings contribute to the understanding of the genetic susceptibility of CAD and DR in patients with T2DM. We report associations of CAD with the *PLXDC2* gene and DR with the *UBE2D1* gene, both of these genes may contribute to DR and CAD as part of diabetes progression by playing a role in angiogenesis and neovascularization. Moreover, association between the ciliary gene *CEP162* and DR was established in terms of neural processing in the retina, and confirms findings that were previously reported. Although our findings require further investigation using a larger sample size they present potential pathways that may contribute to the etiology of DR or CAD in patients with T2DM. Our study is the first in the Middle East region to report genetic risk factors associated with DR or CAD.

ETHICS STATEMENT

The Institutional Ethics Committees of Sheikh Khalifa Medical Center (SKMC) and Mafraq Hospital in Abu Dhabi city both reviewed and approved the study (REC-04062014 and R292, respectively). All participants provided written consent in accordance with the Helsinki Declaration of ethical conduct in research.

AUTHOR CONTRIBUTIONS

SA wrote the manuscript and performed genetic association tests with support from HA. HA contributed to manuscript writing, analyses and study design. WO contributed to genetic association analyses and study design. SL, KK, AK, WA, and HJ have all contributed to the study design. HA supervised the project. All authors discussed the results and contributed to the final manuscript.

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Prevalence of Undiagnosed Depression in Patients With Type 2 Diabetes

*Dina Siddiq Abdulhadi Alajmani[1], Amna Mohamad Alkaabi[1], Mariam Waleed Alhosani[1], Ayesha Abdulaziz Folad[1], Fawzia Ahmed Abdouli[1], Frederick Robert Carrick[2,3,4] and Mahera Abdulrahman[1]**

[1] Dubai Health Authority, Dubai, United Arab Emirates, [2] Department of Neurology, Carrick Institute, Cape Canaveral, FL, United States, [3] Department of Neurology, University of Central Florida College of Medicine, Orlando, FL, United States, [4] Centre for Mental Health Research in Association, University of Cambridge, Cambridge, United Kingdom

Introduction: Type 2 Diabetes Mellitus (T2DM) is the most prevalent type of diabetes among adults and constitutes around 90% of all cases. Substantial evidence demonstrates that depression in the context of diabetes is associated with a wide range of adverse consequences such as reduced adherence to the prescribed treatment regimen, lower quality of life, higher fasting glucose and HbA1c levels, and higher health expenditures.

Methods: This study was conducted to assess the depression among T2DM patients attending diabetic clinics, primary healthcare centers (PHC), Dubai Health Authority (DHA). Depressive symptoms were assessed by using both Arabic and English version of the Beck Depression Inventory.

Results: Out of 1,050 diabetic patients approached, 559 were within our inclusion criteria and agreed to participate in this study (Response rate of 53%). The mainstream of the participants had T2DM for <10 years (393, 70%), were under oral hypoglycemic treatment only (479, 86%), and had good medication adherence (526, 94%). The overall depression prevalence using a cutoff of 16 was 17%. When we assessed the level of depression amongst participants in association with their sociodemographic and clinical characteristics, there was a significant difference between age groups ($p < 0.00001$); gender ($p < 0.0001$); nationality ($p < 0.00001$); educational level ($p < 0.00001$); and employment status ($p < 0.0001$). The type of clinic in which the T2DM patients were attending (e.g., diabetes mini-clinic vs. General Family Clinic) was also significantly associated with depression ($p < 0.0001$).

Conclusion: Our results demonstrate that the intensive service being given in a diabetes mini-clinic compared to routine PHC clinics appears to benefit the psychological aspects of T2DM patients in the UAE population resulting in a lower incidence of depression than commonly seen in a diabetic population. We have identified a need for the establishment of these mini-clinics in each PHC clinics; and the development of campaigns and educational programs, both for health care providers and the public to decrease depression in T2DM patients in this region.

Keywords: diabetes, depression, mental health, glycemic control, United Arab Emirates

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****Correspondence:***
Mahera Abdulrahman
marad@dha.gov.ae

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Alajmani DSA, Alkaabi AM, Alhosani MW, Folad AA, Abdouli FA, Carrick FR and Abdulrahman M (2019) Prevalence of Undiagnosed Depression in Patients With Type 2 Diabetes. Front. Endocrinol. 10:259. doi: 10.3389/fendo.2019.00259

INTRODUCTION

There are differences and similarities between the diabetes-specialty clinics with respect to diabetes management and outcome underscoring the necessity for a protocol-driven treatment approach in ensuring improved diabetes care and outcome (1). We have developed diabetes-specialty clinics in UAE and desired to investigate and differences in outcomes of diabetes treatment in these clinics and in the general medical clinic.

Diabetes mellitus (DM) is a chronic, multifaceted, and progressive disease. Global estimates demonstrate an increase in the prevalence of DM from 422 million patients in 2016 to 642 million by the year 2040 (2). Type 2 DM (T2DM) is the most prevalent type of diabetes among adults and constitutes around 90% of all cases (2–4). Remarkably, 70% of these individuals live in developing countries (5, 6). According to the International Diabetes Federation (IDF), more than 35.4 million of people in the Middle East and North Africa region have diabetes and it is predicted that the number will rise to 72.1 million by 2040 (2).

The UAE is a newly developing country with massive changes in lifestyle and eating habits occurring over the last 47 years. After the discovery of oil in this region in the 1970s, urbanization and growth in prosperity brought major lifestyle changes to the native population. A survey from the United Arab Emirates (UAE) in 2009, found a diabetes prevalence of 23% (7), placing UAE among the countries in the world with the highest diabetes prevalence (2). According to IDF, the prevalence of DM in UAE was 15.6% in the year 2017, and it might reach to 23.4% by 2045; furthermore, there were over 1 million cases of diabetes in UAE in 2017 (2).

Depression is a common comorbidity in individuals with diabetes, compared to those without diabetes (8, 9), affecting approximately 20% of all patients (10, 11). Substantial evidence demonstrates that depression in the context of diabetes is associated with a wide range of adverse consequences such as reduced adherence to a prescribed treatment regimen (11), lower quality of life, higher fasting glucose, and HbA1c levels (12, 13), and higher health expenditures (14). Depressed and anxious individuals are less likely to comply with diabetes self-care recommendations and are more likely to follow a sedentary lifestyle with a probability of poor diabetes control and clinical outcomes. Comorbid depression in diabetic patients can be responsible for premature morbidity, mortality, developing complications, increased pain, and suffering and escalated costs (15).

This study determined the prevalence of depression and its association with socio-demographic and clinical factors in patients attending the Diabetic mini-Clinic located in primary health care centers (PHC) in the Dubai Health Authority. We expect that the findings of this study will be helpful in developing protocols and guidelines to be implemented in PHC to decrease depressive mental illness in the diabetic population in the UAE. Furthermore, the finding of the study can be used as a baseline for other researchers who desire to conduct larger scale studies.

TABLE 1 | Descriptive demographic characteristics of type 2 diabetic patients ($n = 559$).

Variable	*n* (%)
Gender	
Female	318 (57)
Male	241 (43)
Age (years)	
21–30	4 (1)
31–40	52 (9)
41–50	137 (25)
51–60	248 (44)
>60	118 (21)
Nationality	
UAE	412 (74)
Non-UAE	147 (26)
Marital status	
Single	31 (6)
Married	504 (90)
Divorced/widowed	24 (4)
Education level	
Less than high school	245 (44)
High school	208 (37)
Diploma or Bachelor's degree	93 (17)
Post Grad or Higher	13 (2)
Employment status	
Employed	266 (48)
Unemployed	293 (52)

METHODS

Study Design

This Cross-sectional survey was conducted in Dubai, from December 2017 to November 2018.

TABLE 2 | Patient distribution per primary health care clinic.

PHC	Frequency	Percent	Cumulative
1	384	68.69	68.69
2	25	4.47	73.17
3	6	1.07	74.24
4	3	0.54	74.78
5	2	0.36	75.13
6	46	8.23	83.36
7	30	5.37	88.73
8	2	0.36	89.09
9	49	8.77	97.85
10	8	1.43	99.28
11	4	0.72	100.00
Total	559	100.00	

TABLE 3 | Mixed-effects ML regression.

Diabetes total score	Coef.	Std. err.	*Z*	*P*	95% conf. interval
PHC	−0.2072795	0.3093964	−0.67	0.503	−0.8136852, 0.3991262
cons	8.271454	2.011414	4.11	0.000	
Number of observations = 559, Number of groups = 11					
Random-effects parameters	**Estimate**	**Std. err.**		**95% conf. interval**	
Clinic:Independent					
Sd (Clinic)	0.0005027	0.0036905		2.84e-10, 890.8998	
Sd (cons)	2.554172	0.8453436		1.335155, 4.886172	
Sd (Residual)	6.896664	0.2079046		6.500982, 7.316428	

Study Procedure

Participants for the study were recruited from diabetic clinics and primary healthcare centers in the Dubai Health Authority (DHA). Patients with T2DM, between the age of 20 and 65 years and composed of both UAE citizens (nationals) and expatriates, were included in the study. Patients with type I DM, those who had the previous history of psychiatric illness or presently receiving any form of psychiatric treatment, or those who had cognitive impairment or a family history of depression, were excluded. Exclusions were made to avoid confounding due to any effects of ongoing psychiatric treatment. The sample size of our study, cross-sectional design, was calculated using epidemiological information for a population of 8,568 (e.g., total cases of T2DM in PHC for the year 2017 in DHA), with an alpha of 5% maintaining a 95% confidence level with 80% power. We calculated that we needed a minimum required sample size of 368 (16). The participants were assured of the confidentiality of the information provided and the protection of their rights to privacy, mandated by the research ethics guidelines of the human research ethics committees.

Survey Design (Evaluation Tools)

A structured questionnaire was designed and developed by a multidisciplinary team after thorough review of the literature from relevant studies (11, 17–19). The evaluation tool was then pre-tested among 20 adults to assess ease of understanding and time required for completion. The survey consisted of three functional domains: socio-demographic characteristics, DM data, and depression analysis. The demographic data included age, gender, nationality, marital status, educational level, occupation, and employment status. Diabetic data included the duration of DM, type of treatment, practice of exercise, smoking, glycemic control, presence of any comorbidities, and DM complications. Depressive symptoms were assessed using both the Arabic and English version of the Beck Depression Inventory (BDI) (20, 21). The Beck Depression Inventory (BDI) contains 21 multiple-choice questions, and each option has a range of 0–3 scores; higher scores indicate the severity of depression. This questionnaire has a maximum score of 63 points in which 0–10 represents a normal, 11–16 indicates mild mood disturbance, 17–20 borderline clinical depression, 21–30 indicates moderate depression, and 31–63 indicates severe depression. The BDI demonstrates high internal consistency with a mean coefficient alpha of 0.86 reported for psychiatric groups and 0.81 for non-psychiatric groups (22). Questionnaires were administered during face-to-face interviews conducted in Arabic or English by physician researchers.

Data Analysis and Statistics

Statistical analysis of the data was performed using STATA 14 (StataCorp College Station, TX), maintaining an alpha (α) 0.05 with 80% power to decrease the possibility of making Type 1 and 2 errors. Descriptive statistics were computed for the socio-demographic variables. The overall responses to each item of the survey were recorded as a percentage of the total. The percentage differences in the total responses were determined using the Chi-square test and statistical significance recorded for non-parametric data. For all tests, linear and logistic regression models were fitted to search for any statistically significant predictors of diabetes and depression in the sample. A linear mixed model exploring the total diabetic score with each individual clinic was fit and estimated with a restricted maximum likelihood (REML) approach. Correlations between variables were calculated, and the discrimination of fitted logistic models was analyzed.

Ethics Statement

The study was approved by the institutional review boards of Dubai Health Authority, Dubai (Ethics approval # DSREC/RRP/2017/22). Participants were not compensated, and all participants gave informed consent before participation. Aggregate reporting of data was assured to enhance confidentiality and accurate reporting by the respondents. The return of the completed survey also guaranteed the anonymity of participation constructs to an administrator; independent and blinded to the study hypothesis. A code linking respondents to their surveys was kept isolated from the investigators.

RESULTS

Out of 1,050 diabetic patients approached, 559 were within our inclusion criteria and agreed to participate in this study (Response rate of 53%). The interview was conducted both in the Diabetes Mini-Clinic and the General Family Clinic,

TABLE 4 | Clinical characteristics of type 2 diabetic (T2DM) patients participated in this study, $n = 559$.

Variable	*n* (%)
Where the interview conducted	
Mini Diabetes Clinic	249 (45)
General Family Clinic	310 (55)
How long you are diagnosed with T2DM	
1–10 years	393 (70)
11–20 years	126 (23)
>20 years	40 (7)
Type of treatment	
None or diet only	6 (1)
Oral hypoglycemic only	479 (86)
Insulin only	8 (1)
Insulin and oral hypoglycemic	66 (12)
Medication adherence (e.g., taking your medications) regularly	
Yes	526 (94)
No	33 (6)
Do you exercise	
Yes	275 (49)
No	284 (51)
Do you smoke	
Yes	65 (12)
No	494 (88)
How is you glycemic control	
Poorly controlled (HbA1c ≥7)	270 (48)
Well controlled (HbA1c <7)	247 (44)
I don't know	42 (8)
Latest HbA1c	
Poorly controlled (HbA1c ≥7)	265 (47)
Well controlled (HbA1c <7)	294 (53)
How often do you attend your diabetes clinic	
Every 3 months	416 (75)
Every 6 months	69 (12)
Once a year	30 (5)
First time	44 (8)
Do you have any other chronic medical illness	
No	
Yes*	482 (86)
Hypertension	38 (7)
Dyslipidemia	151 (27)
Ischemic heart disease	5 (1)
Dyslipidemia and hypertension	208 (38)
Dyslipidemia and ischemic heart disease	11 (2)
Complication of diabetes	
None	482 (86)
Peripheral neuropathy	30 (5)
Ischemic heart disease	11 (2)
Eye involvement	24 (4)
Kidney involvement	14 (2)
Delayed wound healing	7 (1)
Amputation	1 (0.1)
Others (obesity, gastroparesis, erectile dysfunction)	4 (0.5)
Levels of depression	
Normal	330 (59)
Mild mood disturbance	135 (24)
Borderline clinical depression	46 (8)
Moderate depression	45 (8)
Severe depression	3 (0.5)
Extreme depression	0

Respondents were given the choice to mention more than one complication.

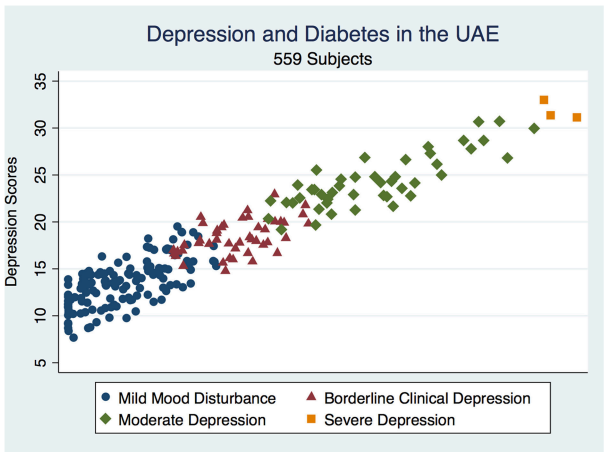

FIGURE 1 | Depicts a scatterplot of the distribution of depressive illness in a diabetic population in the UAE.

and participants were recruited equally in both (45% vs. 55%, respectively). The majority of our T2DM respondents were female (318, 57%), aging >50 years (366, 65%), UAE national (412, 74%), married (504, 90%), having high school or less certificate (453, 81%), and unemployed (293, 52%), **Table 1**.

The largest number of patients (384 subjects) representing 68.69% of subjects were seen in one of the eleven (11) primary healthcare centers (PHC) involved in this study, **Table 2**. We did not find any difference in the type of patients seen between each of the 11 PHCs in our study. A linear mixed model exploring the total diabetic score with each individual clinic was fit and estimated with a restricted maximum likelihood (REML) approach. We estimated the fixed effects as $b\_0 = 8.27$ and $b\_1 = -0.21$. The estimated variance components revealed a Sigma∧u was estimated as 0.00 with a standard error of 0.00. We obtained a Wald test (0.45) comparing the coefficient's estimated value with the estimated standard error for the coefficient where the coefficient's estimate was expected to be normally distributed (z-test $= -0.67$, $P = 0.50$). A likelihood-ratio test comparing the model to ordinary linear regression is highly significant

for these data (LR test vs. linear model: chi2(2) = 21.21; $P >$ chi2 = 0.00) **Table 3**.

The mainstream of the participants had T2DM for <10 years (393, 70%), were under oral hypoglycemic treatment only (479, 86%), had good medication adherence (526, 94%), were not smokers (494, 88%), but were not practicing exercise (284, 51%). However, although the majority of the participants assumed that their glycemic control was poor (270, 48%), their HbA1c was well controlled (294, 53%), and they were attending the diabetic clinic at least once every 3 months (416, 75%). The vast majority of participants did not have any DM complications (482, 86%); **Table 4** shows types of comorbidities and diabetes complications among contributors.

The overall depression prevalence using a cutoff of 16 (23) was 17% (**Figure 1**). When we assessed the level of depression amongst participants in association with their sociodemographic and clinical characteristics, there was a significant difference between different aged groups ($p < 0.00001$); gender ($p < 0.0001$); nationality ($p < 0.00001$); educational level ($p < 0.00001$); and employment status ($p < 0.0001$) (**Table 5**). The discrimination of fitted logistic models, via receiver operating characteristic (ROC) curves of the total depression score and the gender, nationality and education of all subjects revealed good discrimination values. (**Figures 2–4**). Although none of the clinical factors were significantly associated with a higher risk of depression, the type of clinic in which the T2DM patients were attending (e.g., diabetes mini-clinic vs. General Family Clinic) was significantly associated with depression ($p < 0.0001$) (**Table 5**).

DISCUSSION

Depressive symptoms that are not severe enough to warrant a diagnosis of clinical depression are highly prevalent in the diabetic population and are associated with both poor well-being and poor diabetes self-management (24). A cutoff of 16 or higher on the Beck Depression Inventory was suggested to be accurate in diabetes (23), representing an overall depression prevalence of 32.8% in diabetes (25). The overall prevalence of depression (borderline, moderate, and severe depression) in our studied population was much less at 17% (94/559). We did not find any difference in the type of patients seen between each of the 11 PHCs in our study. We compared the prevalence of depression in our sample to that of patients with T2DM throughout the world: 43.5% in Pakistan (5), 40% in Palestine (26), 7.8% in Nigeria (27), 13–61% in Ethiopia (28), and 37% in Turkey (29). A meta-analysis of 42 studies found that approximately 20–40% of individuals with T2DM have comorbid depression, a prevalence at least double that found in the general population (30). Hence, our results of 17% seem to show a lower rate of depression compared to worldwide reports.

Our study shows a higher prevalence of depression in T2DM females than males (14.5 vs. 18.5%, $p < 0.0001$); this is in

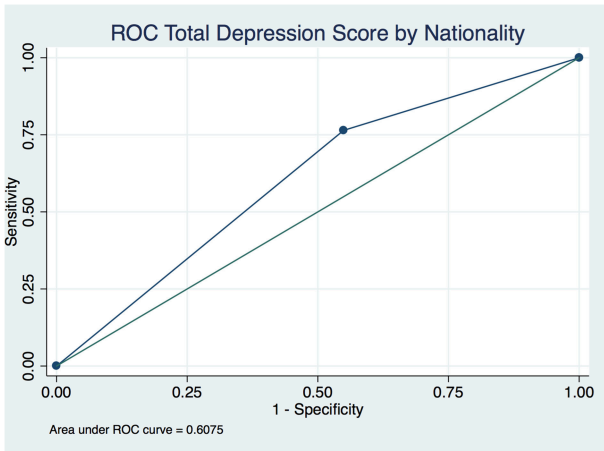

FIGURE 3 | Receiver operating characteristic (ROC) curve of total depression in type 2 diabetic patients by nationality.

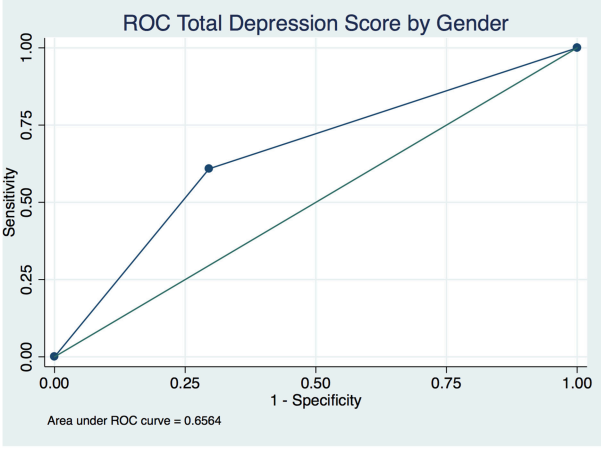

FIGURE 2 | Receiver operating characteristic (ROC) curve of total depression in type 2 diabetic patients by gender.

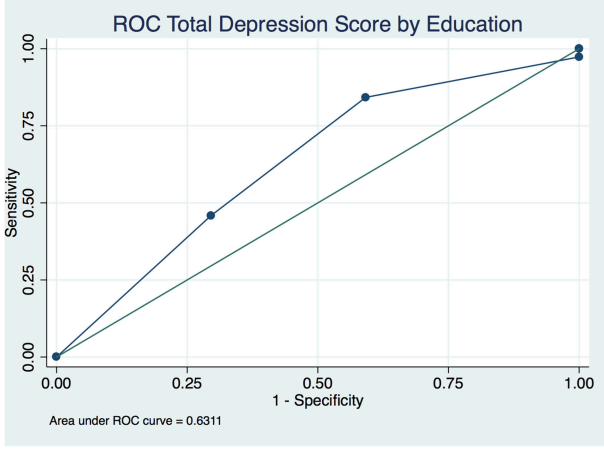

FIGURE 4 | Receiver operating characteristic (ROC) curve of total depression in type 2 diabetic patients by level of education.

TABLE 5 | The relationship between sociodemographic characteristics and depression status among T2DM Patients in UAE ($n = 559$).

Variable	Normal (score ≤10)		Mild mood disturbance (score 11–16)		Borderline clinical depression (score 17–20)		Moderate depression (score 21–30)		Severe depression (score 31–40)		*P-Value*
	n %	Total	*n* %	Total	*n* %	Total	*n* %	Total	*n* %	Total	
AGE											
21–30	1	330	1	135	1	46	1	45	0	3	0.00001
31–40	31		12		5		3		1		
41–50	84		32		10		11		0		
51–60	147		61		21		19		0		
>60	67		29		9		11		2		
GENDER											
Male	163 (49)	330	43 (32)	135	20 (43)	46	15 (33)	45	0		0.0001
Female	167 (51)		92 (68)		26 (57)		30 (67)		3 (100)	3	
NATIONALITY											
UAE	226 (68)	330	106	135	37 (%)	46	40 (%)	45	3 (100)	3	0.00001
Non UAE	104 (32)		29		9 (%)		5 (%)		0		
EDUCATION LEVEL											
Less than high school	136 (41)	330	59 (44)	135	25 (54)	46	23 (51)	45	2 (67)	3	0.00001
High school	115 (35)		56 (42)		16 (35)		20 (45)		1 (33)		
Diploma/bachelor degree	74 (23)		14 (10)		4 (9)		1 (2)		0		
Postgraduate/higher degrees	5 (1)		6 (4)		1 (2)		1 (2)		0		
EMPLOYMENT STATUS											
Employed	172(52)	330	54 (40)	135	23 (50)	46	16 (35)	45	1 (33)	3	0.0001
Unemployed	158 (48)		81 (60)		23 (50)		29 (65)		2 (67)		
CLINIC											
Diabetes mini-clinic	165	330	60	135	13	46	9	45	2	3	0.0001
PHC* clinic	165		75		33		36		1		

*$p < 0.05$, significance determined using Montecarlo 2 tailed significance at 95% CI. Only significant results are presented. *Primary health care clinic.*

agreement with other studies reported worldwide (31–33). We found a significant association between depression prevalence in T2DM patients and their education level and employment status (those not employed and with less education had higher rates of depression compared to their counterparts ($p < 0.0001$ and $p < 0.00001$, respectively). Other reports also revealed that a higher level of educational standard attained has a protective effect against both anxiety and depression (34, 35). We realized that patients with a low level of education may leave some questions unanswered during written administration of this questionnaire. We intentionally avoided this problem by administering it during direct face-to-face interviews. We found that the clinic in which the patient was getting treatment was a modifiable risk factor that contributed to depression. The mini-diabetes clinic is a specialist clinic located in some of the primary health care centers, initiated in Dubai health authority since 2004 and staffed by family physicians providing comprehensive medical care to diabetic patients through a multidisciplinary team approach specific to diabetes. In addition to the family physician, the team at each mini-diabetes clinic consists at least of a registered nurse, a health educator, a nutritionist, and a pharmacist. The criteria to refer patients with type 2 diabetes mellitus from family medicine clinics to mini-diabetes clinics are as follows: HbA1c ≥7% on 2 consecutive visits apart for 3 months despite proper management; elderly patients (65 years and above) with HbA1c ≥7.5% on 2 consecutive visits apart for 3 months despite proper management. All patients with T2DM are required to attend the clinic once per year for comprehensive diabetes care. The uniqueness of the diabetes mini-clinic includes the time allocated to the patient: 40 min vs. 15 min in ordinary PHC clinics. This allows more time for comprehensive examination and encourages the patients to participate in the decision-making process while enhancing the patient's concordance with medical care regimens. Another difference is that, at every visit, the patient undergoes an extensive complete review of his/her diabetic wellbeing. The services provided include weight and body mass index assessment, colored fundus photography, foot care, cardiovascular risk assessment with Electrocardiography if appropriate, review of smoking habits and renal functions, immunization status, as well as medication review and diet education. Retinal camera screening, which started in PHC in April 2015 is obtained at least annually for all diabetic patients registered in PHC facilities. Our results suggest that the intensive service being given to the T2DM patients in diabetes

mini-clinics in PHC is not only comprehensive in the clinical service and diagnosis but it also appears to affect the long-term improvement of the psychological outcome of the patients. Further investigation is warranted to clarify possible mechanisms of improvement.

CONCLUSIONS

This study is the first to examine depression in patients with T2DM in the United Arab Emirates. Our results demonstrate that the intensive service being given in diabetes mini-clinics compared to routine PHC clinics benefits the psychological aspects of T2DM patients in the UAE population and is associated with a lower incidence of depressive mental illness. There is a need for the establishment of these mini-clinics in each PHC clinic and the development of more campaigns and educational programs, both for health care providers and public on depression in T2DM patients. We recommend that healthcare administration and public health policymakers in the UAE should utilize more educational tools, in order to increase the awareness of the community on the risk of depression in diabetes patients. Finally, we suggest that new policies need to be established that focus more on increasing community awareness of diabetes and depression preventive measures in UAE.

LIMITATIONS

This study has several limitations. It focused on patients in governmental sectors in Dubai, UAE so it may not be generalized to all populations. Therefore, the results of this study should be interpreted with caution. This study used a cross-sectional design that speculates on the causal relationship between the variables. It used convenience sampling so that the results might be unrepresentative of the general population. However, despite these limitations, the results of this study provide a basis for further planning and future in-depth research needs to assist in the development of educational materials and planning training-based interventions for further boosting psychological care and clinical management of patients with T2DM in UAE.

ETHICS STATEMENT

The study was approved by the institutional review boards of Dubai Health Authority, Dubai (Ethics approval # DSREC/RRP/2017/22). Participants were not compensated, and all participants gave informed consent before participation. Aggregate reporting of data was assured to enhance confidentiality and accurate reporting by the respondents. The return of the completed survey also guaranteed the anonymity of participation constructs to an administrator; independent and blinded to the study hypothesis. A code linking respondents to their surveys was kept isolated from the investigators.

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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Salivary N1-Methyl-2-Pyridone-5-Carboxamide, a Biomarker for Uranium Uptake, in Kuwaiti Children Exhibiting Exceptional Weight Gain

Jo Max Goodson[1], Markus Hardt[1], Mor-Li Hartman[1], Hend Alqaderi[1,2], Daniel Green[1], Mary Tavares[1], Al-Sabiha Mutawa[3], Jitendra Ariga[2], Pramod Soparkar[1], Jawad Behbehani[4] and Kazem Behbehani[5]*

[1] Department of Applied Oral Sciences, The Forsyth Research Institute, Cambridge, MA, United States, [2] Kuwait School Health Program, Kuwait City, Kuwait, [3] Ministry of Health, Kuwait City, Kuwait, [4] Faculty of Dentistry, Kuwait University, Kuwait City, Kuwait, [5] The Dasman Diabetes Institute, Kuwait City, Kuwait

In a longitudinal study of 6,158 Kuwaiti children, we selected 94 for salivary metabolomic analysis who were neither obese (by waist circumference) nor metabolic syndrome (MetS) positive (<3 diagnostic features). Half (43) remained healthy for 2 years. The other half (51) were selected because they became obese and MetS positive 2 years later. In the half becoming obese, metabolomic analysis revealed that the level of salivary N1-Methyl-2-pyridone-5-carboxamide (2PY) had the highest positive association with obesity ($p = 0.0003$, AUC = 0.72) of 441 salivary biochemicals detected. 2PY is a recognized uremic toxin. Also, 2PY has been identified as a biomarker for uranium uptake. Considering that a relatively recent military conflict with documented uranium contamination of the area suggests that this weight gain could be a toxicological effect of long-time, low-level uranium ingestion. Comparison of salivary 2PY in samples from the USA and Kuwait found that only Kuwait samples were significantly related to obesity. Also, the geographic distribution of both reported soil radioactivity from ^{238}U and measured salivary 2PY was highest in the area where military activity was highest. The prevalence pattern of adult diabetes in Kuwait suggests that a transient diabetogenic factor has been introduced into the Kuwaiti population. Although we did not measure uranium in our study, the presence of a salivary biomarker for uranium consumption suggests potential toxicity related to obesity in children.

Keywords: adolescent obesity, kuwaiti children, N1-Methyl-2-pyridone-5-carboxamide, 2PY, nicotinate metabolism, uranium toxicity, metabolic syndrome

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****Correspondence:***
Jo Max Goodson
mgoodson@forsyth.org

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INTRODUCTION

Kuwait is a small country (4.1 million) that lies at the northern end of the Persian Gulf between Iraq and Saudi Arabia. As with many countries of the Middle East, discovery of oil has precipitated a rise in lifestyle disorders such as obesity, hypertension and type 2 diabetes. At the time of this study (2012–2014) the prevalence of adult obesity in Kuwait was 43.4% (Male) to 58.6% (Female) of the population (1), the prevalence of adult hypertension was ∼26.3% (2) and the prevalence of type 2 diabetes in adults was about 23.9%, the sixth highest of any country in the world (3).

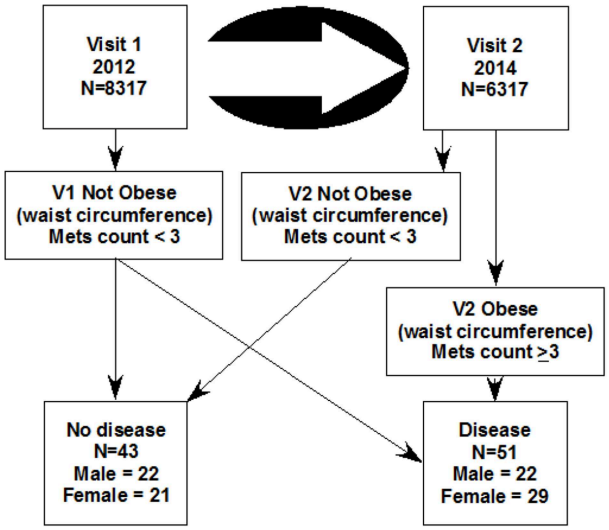

FIGURE 1 | The design of this study which selected 94 children from a longitudinal study of two visits. The metabolic disease category was selected such that 51 were not obese at the first visit and became obese 2 years later at the second visit (the disease group). The 43 control subjects (the healthy group), were selected for not having obesity at either visit. Mets count is the sum of 4 binary metabolomic syndrome characteristics (obesity by waist circumference + high blood pressure + high salivary glucose + low salivary cholesterol).

In the current study, we bring together data suggesting that uranium consumption may have contributed to the development of obesity in Kuwait children. Although uranium does not naturally occur at high levels in Kuwait, an estimated 286 tons of depleted uranium was used in Kuwait (4) as munitions during the Gulf War (1990–1991). Compounds associated with obesity-related diabetes can be identified through the measurement of urine samples in a US population (NHANES 1991–2010). Using inductively coupled plasma mass spectrometry this work has demonstrated that uranium uptake is significantly associated with diabetes (5).

Uranium is a potent renal toxin, with the element accumulating in calcified tissues, livers, and kidneys as a result of both natural and anthropogenic exposure (6, 7). While uranium toxicity can be radiological, uranium chemical toxicity is more acute, and particularly affects the liver, kidneys, and lungs. Chemical toxicity likely involves altered glomerular tubule function or damage, the disruption of cellular ion transport mechanisms, and the inhibition of aerobic oxidative phosphorylation. Renal impairment and uranium poisoning can be caused at dosages as low as 50 ppb to 20 ppm (7–9). Uranium in dust is the largest source of uranium-based radiation exposure in uranium processing facilities (9, 10).

N1-Methyl-2-pyridone-5-carboxamide (2PY) is found at higher concentrations in the serum of patients suffering from renal failure (11, 12). Levels of 2PY in renal tissues of healthy people are typically 1.37 mg/L (± 0.68), whereas concentrations in patients with uremia average 4.02 mg/L (± 3.28) with measurements as high as 7.80 mg/L (± 3.59) (13, 14). In rats, 2PY is one of a number of metabolites whose concentration in urine is associated with prolonged low-dose exposure to uranium (15, 16).

Kuwait has one of the highest obesity and type II diabetes levels in the world for reasons that are not fully understood (1). The objective of this study is to evaluate the development of metabolic disease in Kuwaiti children. We measured blood pressure, height, weight and collected saliva samples from 8,317 children in 2012 and 6,317 again in 2014. From these samples, we selected 94 children with data at both visits, all who were neither obese nor MetS positive at the first visit and approximately half of whom became both obese and MetS positive at the second visit. Saliva samples were tested by metabolomic analysis to determine biomarkers that discriminate these two groups.

METHODS

Anthropomorphic Measurements

Height measured by stadiometer, weight measured by a calibrated bathroom scale, systolic, and diastolic blood pressure measured by a pediatric automated arm cuff were combined with salivary glucose (17) and salivary high-density lipoprotein cholesterol (HDLC) (18).

Subject Selection

The study of 8,317 Kuwaiti children (V1, 4th, or 5th grades in 2011–2012) was approved by the Dasman Diabetes Institute Ethical Review Committee in Kuwait. Two years later (2013–2014) 6,317 of the same group were examined using the same methods a second time (V2). Arabic language informed consent was signed by parents/guardians in advance. Subject assent was obtained the day of the visit. Ninety-four children were selected for metabolomic analysis. In the healthy category, 43 children were selected to be metabolic syndrome (MetS) negative at both visits. In the disease category, 51 children were considered healthy on the first visit and developed MetS 2 years later (**Figure 1**).

For each subject, metabolic disease was evaluated by determining four binary characteristics that define MetS. These included obesity, high blood pressure, and salivary estimates of high blood glucose and low HDLC (19). The diagnosis of MetS positive was defined as having at least three of these four characteristics. Obesity was defined as a waist circumference greater than or equal to the 90th percentile for European children. High blood pressure was defined as either systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg. Salivary glucose higher than 1.13 mg/dL, a value determined to be equivalent to 100 mg/dL in plasma (17) was considered hyperglycemic. Salivary HDLC <0.6 mg/dL, a value determined to be equivalent to 40 mg/dL in plasma (18) was designated as low HDLC.

Saliva Collection

Fasting whole saliva was collected using standard methods (20) in a 15 ml screw-top test tube between 8:30 and 9:00 in the morning before breakfast. Children were asked to rinse and swallow with 15 ml water before collection of 3 ml saliva by drooling. Samples

TABLE 1 | Anthropometric values of children at V1 and V2.

					V2–V1 Difference		
	Metabolic disease ($n = 51$)	**Healthy ($n = 43$)**	**Disease-healthy difference**	***p*-value (disease vs. healthy)**	**Metabolic disease**	**Healthy**	***p*-value (V2–V1)**
Age V1(y)	9.8 ± 0.6	9.8 ± 0.6	−0.03	0.8	2.08 ± 0.13	2.1 ± 0.12	0.4
Age V2(y)	11.9 ± 0.6	11.9 ± 0.6	−0.05	0.7			
Waist circumference V1 (cm)	68.4 ± 5.4	60.1 ± 5.8	8.30	<0.0001	20.65 ± 6.19	8.26 ± 7.87	<0.0001
Waist circumference V2 (cm)	89.1 ± 4.6	68.4 ± 9.2	20.68	<0.0001			
BMI V1(Kg/m^2)	21.0 ± 2.2	17.1 ± 1.8	3.93	<0.0001	5.02 ± 1.78	1.43 ± 1.69	<0.0001
BMI V2 (Kg/m^2)	26.0 ± 1.5	18.5 ± 2.4	7.51	<0.0001			
Systolic BP V1(mmHg)	112.9 ± 14.9	103.7 ± 15.4	9.23	0.004	16.69 ± 18.01	5.35 ± 22.72	0.008
Systolic BP V2(mmHg)	129.6 ± 11.7	109.0 ± 16.0	20.57	<0.0001			
Diastolic BP V1(mmHg)	77.4 ± 13.0	67.7 ± 10.3	12.39	0.0001	12.39 ±17.17	8.58 ±16.5	0.3
Diastolic BP V2(mmHg)	89.8 ± 11.7	76.3 ± 13.5	8.58	<0.0001			
Height V1 (cm)	138.3 ± 6.7	134.3 ± 7.5	4.08	0.006	13.12 ± 3.70	13.16 ± 3.70	0.95
Height V2 (cm)	151.5 ± 6.7	147.4 ± 9.0	4.03	0.02			
Weight V1(Kg)	40.3 ± 5.6	31.1 ± 6.2	9.19	<0.0001	19.57 ± 4.29	9.5 ± 4.58	<0.0001
Weight V2(Kg)	59.9 ± 6.4	40.6 ± 8.5	19.26	<0.0001			
Male	22	22					
Female	29	21					

P-values were computed by t-test and indicate the probability that differences between healthy and metabolic disease children were due to chance alone. Mean values ± standard deviations are listed for metabolic disease and healthy subjects. BMI, Body mass index; BP, Blood pressure.

TABLE 2 | Analysis of salivary metabolite associations with obesity (second visit) for $p \leq 0.01$ in predicting obesity (Mann-Whitney *U*-test).

Biochemical	**Super_Pathway**	**Sub_Pathway**	***p***	**AUC**
INCREASE				
N1-Methyl-2-pyridone-5-carboxamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	0.0003	0.72
Urate	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.0005	0.71
Sphingomyelin (d18:1/24:1, d18:2/24:0)	Lipid	Sphingolipid metabolism	0.002	0.64
Gamma-glutamylphenylalanine	Peptide	Gamma-glutamyl amino acid	0.002	0.56
Acisoga	Amino acid	Polyamine metabolism	0.005	0.67
Phosphate	Energy	Oxidative phosphorylation	0.01	0.65
Threonylphenylalanine	Peptide	Dipeptide	0.01	0.65
Acetylcarnitine	Lipid	Fatty acid metabolism(Acyl Carnitine)	0.01	0.65
Arginine	Amino acid	Urea cycle; Arginine and Proline metabolism	0.01	0.65
DECREASE				
1-stearoyl-GPC (18:0)	Lipid	Lysolipid	0.0002	0.73
docosahexaenoate (DHA; 22:6n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	0.001	0.69
N-acetylglycine	Amino Acid	Glycine, Serine and Threonine Metabolism	0.001	0.69
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)	Lipid	Phospholipid Metabolism	0.002	0.69
1-stearoyl-GPE (18:0)	Lipid	Lysolipid	0.002	0.68
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	Lipid	Phospholipid Metabolism	0.003	0.68
oleoylcarnitine	Lipid	Fatty Acid Metabolism(Acyl Carnitine)	0.003	0.64
2'-deoxyinosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.004	0.65
cholesterol	Lipid	Sterol	0.005	0.67
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	Lipid	Phospholipid Metabolism	0.008	0.66
isobutyrylcarnitine	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.009	0.56
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	Lipid	Phospholipid Metabolism	0.009	0.66
1-palmitoyl-GPC (16:0)	Lipid	Lysolipid	0.010	0.74
arachidonate (20:4n6)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	0.01	0.65
sphingomyelin (d18:1/14:0, d16:1/16:0)	Lipid	Sphingolipid Metabolism	0.01	0.60
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)	Lipid	Plasmalogen	0.01	0.58

The top ten biochemicals (upper panel) all increased in obese children. Sixteen biochemicals that significantly decreased in the saliva of obese children are listed in the lower panel.

TABLE 3 | Analysis of salivary metabolite associations with obesity (first visit) for $p \leq 0.01$ in predicting obesity (Mann-Whitney *U*-test).

Biochemical	Super_Pathway	Sub_Pathway	*p*	AUC
INCREASE				
Nicotinate ribonucleoside	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	0.00007	0.73
Xanthosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.004	0.67
Phenol sulfate	Amino Acid	Phenylalanine and Tyrosine Metabolism	0.004	0.67
Phosphoenolpyruvate (PEP)	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.006	0.66
DECREASE				
Sulfate	Xenobiotics	Chemical	0.001	0.70
Caffeine	Xenobiotics	Xanthine metabolism	0.005	0.55
Ribitol	Carbohydrate	Pentose metabolism	0.01	0.55
Phenylacetate	Amino Acid	Phenylalanine and tyrosine metabolism	0.01	0.65
4-hydroxyphenylacetate	Amino Acid	Phenylalanine and tyrosine metabolism	0.01	0.65

The top four biochemicals (upper panel) all increased in obese children. Five biochemicals that significantly decreased in the saliva of obese children are listed in the lower panel.

were maintained on ice until centrifuged at 2,800 RMP at 20 min at 4°C.

Supernatant aliquots were transferred to screw-cap 2D barcoded storage tubes (Thermo Scientific) read by a barcode reader (Thermo Scientific VisionMate ST). The barcode was captured with the subject number to a spreadsheet. The sample vials were sealed by a torque-controlled tube capper (Thermo Scientific 8-Channel Screw Cap Tube Capper), placed in a 96-vial rack (Thermo Scientific Latch Rack) and frozen at −80°C. Racks were air-transferred from Kuwait under temperature monitored dry ice (Biocair, Boston MA) to the Forsyth Institute and maintained at −80°C until assay.

Metabolomic Analysis

Aliquots of saliva supernatants (both the USA and Kuwait) and of plasma samples (only from USA) from each participant (120 μL) were assayed. Relative levels of metabolites were obtained by integrating peaks detected on an untargeted metabolic profiling platform (Metabolon®, Durham, North Carolina) which used high-performance liquid chromatography, tandem mass spectrometry, and gas chromatography-mass spectrometry for volatile species (21). Compounds were identified by matching chromatographic retention times and mass spectral fragmentation signatures with reference library data created from authentic standards.

Statistical Analysis

Analysis was directed toward biochemicals that were most closely related to the development of MetS in the second visit. Anthropomorphic data was evaluated by a two-sample *t*-test. Comparison of salivary biomarkers between diseased and healthy children was computed by the Mann-Whitney *U*-test using the values from the second visit. Analysis by receiver operating curve (ROC) was performed by using the ROC Curve explorer and tester software (22). This software provides the univariate area under the curve (AUC) analysis to predict the transition from health to disease. We computed differences between the USA and Kuwait mass spectrometric analysis of 2PY for each subject from total ion count values divided by the median scaled data for each biochemical. *p*-values were computed for each metabolite.

TABLE 4 | Analysis of covariance in the prediction of BMI and systolic blood pressure by salivary biomarkers ($N = 94$, correlation coefficients = 0.48 for BMI and 0.42 for systolic bp).

	p-Value	
Source	**BMI**	**Systolic BP**
Age	0.60	0.24
Sex	0.27	0.57
N1-methyl-2-pyridone-5-carboxamide (2PY)	0.01	0.12
Urate	0.45	0.04
Acisoga	0.65	0.81
Phosphate	0.53	0.81
Isobutyrylcarnitine	0.19	0.03

Only those with $p \leq 0.01$ were included for analysis. In this case, calculated *p*-values were used only to identify those biochemicals most closely associated with obesity not to estimate the true significance level which by Bonferroni adjustment for the 421 biochemicals identified would be $p \leq 0.0001$. For analysis of covariance, we included the effect of age, sex, five metabolites, and systolic blood pressure.

RESULTS

We evaluated children who were 9.8 years old at V1 and at 11.9 years old at V2, an elapsed time of ∼2 years (**Table 1**). A significant increase in waist circumference, BMI, systolic blood pressure, and body weight occurred over these 2 years. Height and diastolic blood pressure did not significantly change.

We identified 421 biochemicals in the saliva samples. Each was tested for their probability of identifying obese children by non-parametric analysis (p) and the area under the receiver operating curve predicting obesity (AUC). By this analysis, N1-methyl-2-pyridone-5-carboxamide (2PY) was identified as the biochemical increasing with obesity most strongly associated with obese children (**Table 2**). Other metabolites including urate, a sphingomyelin, gamma-glutamylphenylalanine, acisoga,

TABLE 5 | The difference in the normalized spectral abundance of N1-Methyl-2-pyridone-5- carboxamide in saliva samples from obese and not obese children from the USA compared to Kuwait.

				Obese		Not obese			
Study	**Country**	**Average age (y)**	***N***	**Mean**	***N***	**Mean**	**Ntotal**	***p***	**Date of analysis**
1	USA	10.7	22	1.187	46	1.037	68	0.46	2/23/2011
2	USA	12.2	15	1.181	26	0.960	41	0.16	8/16/2012
3	Kuwait	10.1	50	1.008	100	0.675	150	0.0006	3/26/2013
4	Kuwait V2	11.9	51	1.367	43	0.948	94	0.0003	11/3/2015

Saliva samples from Kuwait exhibited a highly significant difference between obese and those not obese whereas this difference in samples from the USA was not significant by t-test. Study 4 is the study analyzed in this manuscript and described in ***Tables 1–4****.*

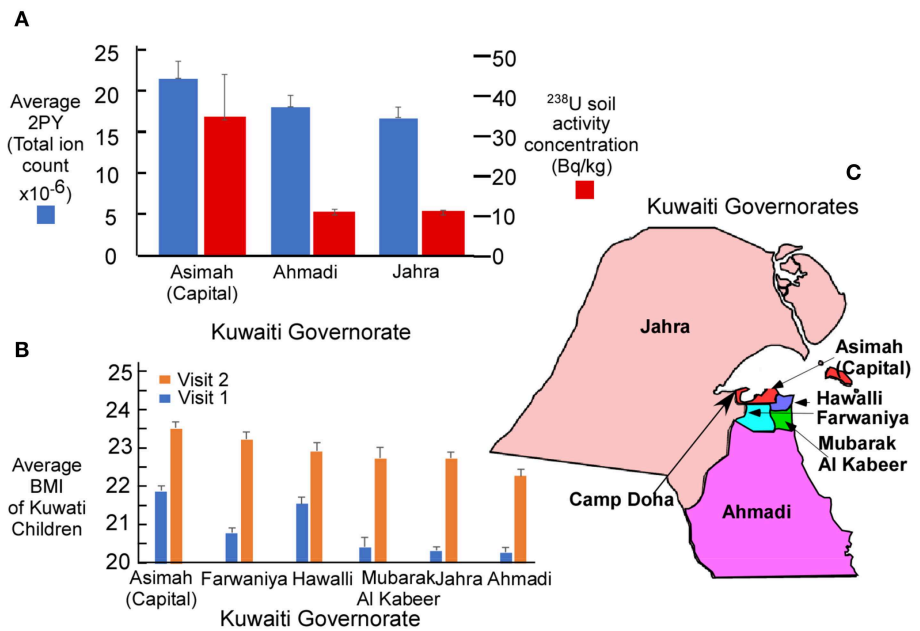

FIGURE 2 | (A) Average salivary N1-Methyl-2-pyridone-5-carboxamide (2PY) in Kuwaiti children by mass spectrometry and ^{238}U activity concentration (4) in soil following remediation completed after the Gulf War. The principal military facility was located at Camp Doha in the Al Asimah governorate (capital) where both 2PY and ^{238}U soil radioactivity were greatest. **(B)** Average BMI of children in each Kuwaiti governorate for V1 (n = 8,317) and V2 (n = 6,317). **(C)** Relative position of each governorate to Camp Doha. Graphic whiskers in **(A,B)** represent the standard error of the mean for each governorate.

phosphate, threonylphenylalanine, acetylcarnitine, and arginine also increased but to a lesser degree. Elevated uric acid has been associated with MetS, renal, and cardiovascular diseases (23). Elevated sphingomyelin suggests plasma membrane destruction. The dipeptide gamma-glutamylphenylalanine suggests that proteolytic activity is also increased. Acisoga is a spermidine metabolite which has been associated with oxidative stress (24) and the induction of beige adipocytes (25). Phosphate has been previously reported as an obesity-related biochemical (26). An increase of acylcarnitines with obesity, a measure of incomplete fatty acid oxidation, has also been recognized (27). Arginine has not been reported to be associated with obesity. Metabolomic biomarkers decreasing in obese children were mainly lipids suggesting that they arise from intraoral sources rather than from plasma. Previous metabolomic studies indicate that many the biochemicals that decreased in the saliva of obese children were likely the products of oral metabolism (bacterial or mucosal) since none have been found to have a significant correlation between saliva and blood.

In this manuscript, we focus principally on 2PY which has been recognized as a uremic toxin associated with metabolic disturbances (28), cancer, and thrombocytopenia (29). 2PY is of interest in this population because of its characterization as a biomarker for uranium uptake (16) and the history of Kuwait which includes a likely source for uranium resulting from a recent (August 1990–February 1991) military conflict (the Gulf War) as a potential source for uranium.

We found 2PY in the saliva of all children that we have analyzed by metabolomics both in the USA and Kuwait, so that differences between individuals appeared as a relative magnitude, not presence or absence. Analysis of the first visit data in the same manner (**Table 3**) exhibited increased levels of

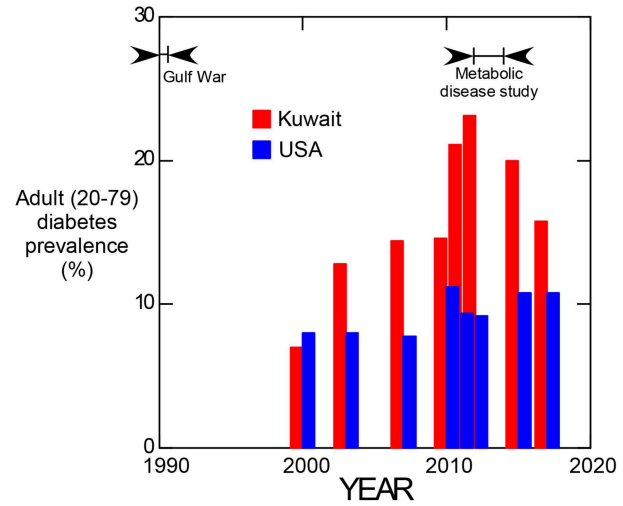

FIGURE 3 | Prevalence of adult (20–79 years old) diabetes in Kuwait and the United States of America from 2000 to 2017. (Data from the International Diabetes Federation, https://www.idf.org/).

nicotinate ribonucleoside (like 2PY, also a nicotinate metabolite), xanthosine, phenol sulfate, and phosphoenolpyruvate. We also observed reductions related to obesity of sulfate, caffeine, ribitol, phenylacetate, and 4-hydroxyphenylacetate.

An analysis of covariance (**Table 4**) indicates that of those salivary biomarkers that prominently increase with BMI, only 2PY is able to predict elevated BMI with statistical confidence. Systolic blood pressure was also elevated in children with elevated BMI (**Table 1**). Systolic blood pressure, however, was significantly associated with urate and isobutyl carnitine but not with 2PY. Neither sex nor age were significantly associated covariates with metabolic disease in either case.

We compared the non-targeted metabolomic analysis of 2PY in the saliva of USA children with that of Kuwaiti children in **Table 5**. In samples from the USA, association with obesity was not significant whereas those of Kuwait were highly significant. Blood samples were taken only from USA subjects. Correlation in 2PY between saliva and blood samples was significant in both study 1 ($r = 0.50$, $p = 0.0001$) and 2 ($r = 0.73$, $p = 0.00000005$). Study 4 with values at both the first and second visits is the study described in this paper. In this group of 94 subjects, 2PY was not associated with obesity in the first visit ($p = 0.75$) but was significantly associated at the second visit ($p = 0.0003$).

The reported level of soil ^{238}U measured following reclamation is compared with salivary levels of 2PY for three Kuwaiti governorates (**Figure 2A**). Highest levels of both ^{238}U and 2PY were in governates close to the U.S. military base (camp Doha). The average BMI of children (**Figure 2B**) was also highest in those governorates closest to Camp Doha (Asimah, Farwaniya, Hawalli, and Mubarak Al Kabeer) and least in those most distant (Jahra and Ahmadi). The relative location of each of the governates is illustrated in **Figure 2C**.

Since 2000, adult obesity has been estimated for all countries of the world by the International Diabetes Federation. Comparative prevalence estimates from 2000 to 2017 for Kuwait and the United States are shown in **Figure 3**. These data suggest that a dramatic increase in the prevalence of diabetes occurred in Kuwait starting at about 2000, reached a maximum in 2012 and has since tended to return to values more comparable to that of the United States.

DISCUSSION

N1-methyl-2-pyridone-5-carboxamide (2PY) is one of the major metabolites of nicotinamide, a commonly consumed vitamin found in meat, fish, nuts, and mushrooms. 2PY levels are elevated in chronic kidney disease patients where it may act as a uremic toxin by inhibiting poly (ADP-ribose) polymerase-1 (PARP-1) (29). PARP-1 is involved in repair of DNA damage. To our knowledge, 2PY has not been reported to be associated with obesity. Laboratory studies have demonstrated that 2PY inhibits poly(ADP-ribose) polymerase-1 (PARP-1) with an IC50 value of 8 μM (1.2 mg/L).

The appearance of pyridine metabolites (2PY, **Table 2**, and nicotinate ribonucleoside, **Table 3**) in saliva with a strong obesity association, suggests that nicotinate and nicotinamide metabolism may be at least in part responsible for these children becoming obese within the 2 years of our study.

We did not find that niacin was consumed to a higher degree in obese children by nutritional analysis (data not shown) and increased niacin consumption is generally associated with weight loss rather than obesity (30). Among other conditions suggested that could trigger this response, consumption of low doses of uranium over 9 months has been found to increase 2PY levels by seven times control in rat urine (16). Elevated levels of 2PY, however, is not uniquely associated with uranium consumption since it was also elevated following high-fat diet protocol without uranium (31).

In support of the uranium hypothesis, between 1990 and 1991, Kuwait was contaminated by an estimated use of 286 tons of depleted uranium during the Gulf War. Measured levels of ^{238}U activity concentration in the capital governorate (Asimah) of 13,200 becquerel/kg (Bq/kg)before remediation (4) leaves little doubt of the magnitude of uranium contamination in Kuwait. Geographic distribution (**Figure 2C**) of salivary 2PY was consistent with the expectation that uranium, salivary 2PY (**Figure 2A**) and BMI (**Figure 2B**) were highest in the Asimah governorate where the U.S. military camp was located (Camp Doha) and lower in outlying governorates (Ahmadi and Jahra).

Uranium measured by plasma mass spectrometry, even at the low levels seen in the U.S. population, is a recognized risk factor for diabetes (5). It appears that chemical toxicity of uranium may be of greater concern than radioactivity (32). Urinary uranium was not found to be related to insulin resistance, and it has been suggested that heavy metal toxicity and not radioactivity may be the result of direct β-cell damage (33). Data on occupational exposure indicates that the risk for both kidney disease and cancer are also increased with uranium

uptake (34). It should be noted however, that association of uranium exposure with salivary 2PY in humans has not been demonstrated.

It should be noted that association of uranium exposure with salivary 2PY in humans has not been demonstrated, and direct measurement of uranium in salivary or blood tissues would be needed to confirm its role in elevated 2PY levels and obesity. As a further caution, Kuwaiti and American populations may differ in a variety of respects influencing the etiology of obesity. It is therefore possible that factors other than relative uranium exposure are responsible for elevated 2PY levels in obese Kuwaiti children, when these levels are not seen in obese American children.

Considering the temporal values of adult diabetes prevalence in Kuwait (**Figure 3**), evidence for the occurrence of a transient response to an etiologic factor is suggested. Individuals representing the maximum difference between Kuwait and the United States seen at 2012 would have been 22 years old if born during the Gulf War, indicating that the diabetogenic effect may have been manifest in childhood since diabetes prevalence was not elevated relative to the USA in 2000. Although uranium is implicated by the salivary biomarker data, associated effects such as an influx of westernized diet following the Gulf War also contributed to this effect.

An analysis of covariance for BMI (**Table 4**) indicated that of the top metabolites only 2PY was significantly associated with obesity. In contrast, elevated systolic blood pressure was significantly associated to both urate and isobutyrylcarnitine.

As part of our study, two validation studies were conducted in Massachusetts and Maine children that collected both saliva and blood of comparable age (17). We found that only in the saliva of Kuwaiti children salivary 2PY was significantly associated with obesity. A critical characteristic of this biomarker is that saliva levels of 2PY were found significantly correlated with plasma levels. This suggests that salivary 2Py levels may serve as a surrogate for plasma 2PY levels.

Elevation of salivary levels of 2PY was only found significant in saliva samples from Kuwait (**Table 5**). Only the saliva values of studies 3 and 4 demonstrated a significant difference between obese children and non-obese children. In study 4 (the primary study of this manuscript), salivary 2PY levels at V1 when all subjects were neither obese nor MetS positive did not show a significant difference between groups. At V2 the group that became obese and MetS positive had significantly higher levels of 2PY ($p = 0.0003$).

CONCLUSIONS

A juxtaposition of the data indicating that salivary 2PY is higher in Kuwaiti children with the recognition that 2PY is a biomarker for low-level uranium exposure in animals suggests the possibility that obesity in Kuwaiti children could be in part due to uranium toxicity. Uranium toxicity is especially relevant considering that urinary levels have been convincingly associated with diabetes (5).

ETHICS STATEMENT

Although the underlying cause may not be related to uranium consumption, this work demonstrates that elevation of 2PY, a suspected uremic toxin, occurred in these children in association with their exceptional weight gain.

This study was carried out in accordance with the recommendations of

- Nuremberg Code of Ethics
- Belmont Report- Ethical Principles and Guidelines for the Protection of Human Subjects
- World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects

The Committee also takes guidance from applicable international ethical guidelines for biomedical research, such as the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (GCP) and the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Biomedical Research Involving Human Subject and Epidemiological Studies. The study was approved by the Dasman Diabetes Institute Ethical Review Committee. It was also reviewed and approved by the Forsyth Institutional Review Board. Written informed consent was obtained from parents or guardians of each child before initiation of the study. In addition, a signed assent form was obtained from each child at the time of examination.

AUTHOR CONTRIBUTIONS

The study was designed by JG, JB, and KB. M-LH, JG, HA, MT, JA, and PS directed the work. HA, JA, AM, and PS directed clinical research activity in Kuwait. MH and DG reviewed and critiqued the metabolomic analysis portion of the study.

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The Status of Metabolic Control in Patients With Type 2 Diabetes Attending Dasman Diabetes Institute, Kuwait

Mohammad Qaddoumi[1,2]*, *Yousuf Al-Khamis*[1], *Arshad Channanath*[1], *Jaakko Tuomilehto*[1,3,4] *and Dalia Badawi*[1]*

[1] Dasman Diabetes Institute, Kuwait City, Kuwait, [2] Faculty of Pharmacy, Kuwait University, Kuwait City, Kuwait, [3] Center for Vascular Prevention, Danube-University Krems, Krems an der Donau, Austria, [4] Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland

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Jan Polák,
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Jan Brož,
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Javier Ena,
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***Correspondence:**
Mohammad Qaddoumi
mohammad.qaddoumi@dasmaninstitute.org;
m.qaddoumi@hsc.edu.kw
Dalia Badawi
daliaab@yahoo.com;
dalia.badawi@dasmaninstitute.org

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Purpose: To evaluate metabolic control in patients with type 2 diabetes at Dasman Diabetes Institute (DDI, Kuwait), a specialist diabetes clinic and research center, and to investigate its association with patient demographics and clinical characteristics.

Methods: Data from 963 patients with type 2 diabetes were retrospectively collected from the Knowledge Based Health Records maintained at DDI for patients who attended DDI during 2011–2014. The collected data included patient demographics, clinical characteristics, and anti-diabetic medications. Student's *t*-test was used to evaluate the differences in mean values between poor and good glycemic control groups. Categorical variables were assessed using chi-square analysis with Fisher's exact test for small data sets.

Results: The patients' mean age was 53.0 ± 9.5 years with equal number of males and females. Females (34.4 ± 7.2 kg/m^2) had a higher mean body mass index than males (32.1 ± 6.4 kg/m^2). The mean fasting blood glucose and HbA1c levels were 9.6 ± 3.8 mmol/L and 8.5 ± 1.8%, respectively. Dyslipidemia (46%) and hypertension (40%) were the most common comorbidities, whereas nephropathy (36%) and neuropathy (35%) were the most common diabetic complications. The most commonly used anti-diabetic medication was metformin (55%). Factors significantly associated with poor glycemic control (HbA1c level ≥ 7%) included insulin use; neuropathy or foot ulcers as diabetic complications; and elevated systolic blood pressure and total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and fasting blood glucose levels. Factors significantly associated with good glycemic control included metformin use and elevated high-density lipoprotein cholesterol level. The proportion of patients with good glycemic control (HbA1c level < 7%) was 29.5%. A large proportion of the patients with poor glycemic control were only administered monotherapy drugs, and two-thirds of the patients were obese. Further, the American Diabetes Association (ADA) recommendations for blood pressure and LDL cholesterol level were met (62 and 63%, respectively) by follow-up year 4.

Conclusion: The therapeutic management of type 2 diabetes in Kuwait is suboptimal. Therapeutic strategies should ensure better adherence to ADA guidelines, evaluate the high obesity rates, and adherence to lifestyle recommendations by patients, and continually promote diabetes education and self-empowerment.

Keywords: type 2 diabetes, glycemic control, HbA1c, anti-diabetic medication, diabetic complications

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that is known to have affected 415 million people worldwide in 2015. It has been projected that >600 million people will acquire the disease by 2040 (1). Kuwait is one of the countries with the highest prevalence of diabetes mellitus globally, with an estimated prevalence of 25.4% reported among adults in 2013 (2). The recent increase in the prevalence from 14.8% in 1998 in Kuwait is alarming (3). Generally, 90–95% of patients with diabetes are classified as having type 2 diabetes characterized by the lack of response to the effects of insulin by the human body or its inability to produce enough insulin (4).

Patients with diabetes are prone to the development of microvascular complications, such as nephropathy, neuropathy, and retinopathy, and macrovascular complications, such as coronary artery disease, stroke, and heart failure. These diabetic complications result in marked disability, mortality, and an enormous national economic burden if not managed well (5). One way of reducing diabetic complications associated with type 2 diabetes and improving its long-term outcome is ensuring tight control of blood glucose level and blood pressure. For instance, the UK Prospective Diabetes Study (UKPDS) demonstrated that intensive glycemic control with anti-diabetic medications is vital for preventing the chronic complications associated with type 2 diabetes. Furthermore, tight blood pressure control in patients with hypertension and type 2 diabetes reduced the risk of death related to diabetes and its complications and reduced the progression of diabetic retinopathy and blindness (6, 7).

According to the American Diabetes Association (ADA), a glycated hemoglobin (HbA1c) level of <7% indicates good glycemic control in patients with diabetes. It has been shown that an improvement in HbA1c level by 1% in patients with type 2 diabetes reduced the risk of microvascular complications by 37% and those of heart failure and myocardial infarction by 16 and 14%, respectively (8).

However, attainment of glycemic control by patients with diabetes has not been adequate worldwide. The cross-sectional PANORAMA study that analyzed the data for adults with type 2 diabetes from nine European countries showed that only 37.4% of these patients achieved the target HbA1c level of <7% (9). Furthermore, a large prospective study that observed a combination of diabetic patients from 141 study centers located in the Czech Republic and Slovakia determined that only 29.9% of patients with type 1 diabetes and 33.4% of those with type 2 diabetes attained the desired target HbA1c level of <7% (10). Similarly, a local study that collected data from patients with type 2 diabetes from 28 health centers in Saudi Arabia showed that only 27% of these patients reached the target HbA1c level of <7% (11). Hence, the purpose of this retrospective study was to evaluate the level of metabolic control in patients with type 2 diabetes at a specialist diabetes clinic and research center located in Kuwait and to investigate associations between glycemic control and patient demographics, clinical characteristics, and anti-diabetic medications.

METHODS

Study Population

In this study, data from a total sample of 1,191 patients with type 2 diabetes were obtained from the Knowledge Based Health Records (KBHR), an electronic health record system maintained at Dasman Diabetes Institute (DDI), a specialist diabetes clinic and research center in Kuwait. The inclusion criteria were type 2 diabetes patients (excluding pregnant patients) aged 18–70 years who attended the clinics at DDI from 2011 to 2014 and were enrolled at DDI for ≥1 year prior to this period, had at least three endocrinologist appointments per year, and had their HbA1c levels measured at least twice annually. Based on the inclusion criteria, the total sample was 963 patients with type 2 diabetes. The collected data included patient demographics, clinical characteristics, anti-diabetic medications dispensed by the pharmacy, and laboratory results such as measured serum creatinine, lipid profile, glycated hemoglobin level, and fasting blood glucose level.

The included patients were stratified into categories according to their last recorded HbA1c level and/or prescription patterns taken in their last appointment for each year. Good glycemic control was defined as HbA1c <7% and poor glycemic control as HbA1c ≥7%. The outcomes of interest were evaluated by examining the patients' latest laboratory results. Annual adherence of patients to performance indicators was evaluated every 12 months, and values were placed in 12 month block intervals using their measurements obtained at the initial visit as the reference starting point. Prescription patterns were classified into three main categories: monotherapy, combination therapy, and total therapy (sum of monotherapy and combination therapy). Prescription pattern was defined as the number of anti-diabetic medications prescribed in the latest prescription. For example, if a patient was started with metformin but later switched to a sulfonylurea, his/her treatment was categorized into a sulfonylurea monotherapy. If a patient started with metformin and later a sulfonylurea was introduced, then the patient was categorized into a combination therapy. Six indicators were adopted to measure

the performance in relation to diabetes management, three of which were process indicators and three were outcome indicators.

Process Indicators

The following process indicators were used: glycosylated hemoglobin (1) HbA1c management, percentage of patients who underwent ≥1 HbA1c tests annually; (2) cholesterol/lipid measurement, percentage of patients who underwent ≥1 low-density lipoprotein (LDL) cholesterol test annually; and (3) annual screening of nephropathy, percentage of patients who underwent ≥1 test for urinary microalbumin level measurement during the measurement year. The urinary microalbumin test is a urine test that measures the amount of albumin in the urine. When kidney damage occurs, albumin leaks into the bloodstream and it is present in urine.

Outcome Indicators

The following outcome indicators were used: (1) HbA1c control, percentage of patients with the most recent HbA1c level of <7%; (2) LDL cholesterol control, percentage of patients with the most recent LDL level of <2.6 mmol/L; and (3) blood pressure control, percentage of patients with the most recent blood pressure level of <140/90 mmHg.

Statistical Analysis

Results are expressed as mean ± SD or frequencies (and proportions). Student's *t*-test was performed to evaluate the differences in mean values between the poor and good glycemic control groups. Categorical variables were assessed by performing chi-square analysis with Fisher's exact test when the number of data points was small. For differences among variables, a $p < 0.05$ was considered statistically significant. All analyses were performed using R version 3.5.1: A language and environment for statistical computing.

Ethical Approval

The study obtained ethical approval from the Ethical Review Committee at DDI in 2014 to conduct it and to permit access to the patient data from the KBHR electronic health record database. All patients attending DDI signed a consent form, which allowed their information to be used for any research purpose. To maintain privacy and anonymity, all patient data were extracted without identifying name, address, or national ID number and a unique identification was assigned to each participant. The patient data will be kept confidential by the study investigators, and all paper and electronic records of the patients will be stored securely and limited only to authorized study investigators.

RESULTS

Population Characteristics

Out of a total of 1,191 patients with type 2 diabetes, 963 (81%) patients met the inclusion criteria and their detailed demographic and clinical data were collected. The demographics and clinical characteristics of the patients with type 2 diabetes at baseline are presented in **Table 1**. Among these 963 patients, the number of females and males was similar. The overall mean age of the cohort was 53.0 ± 9.5 years. The mean body mass index (BMI) was higher in female (34.3 ± 7.0 kg/m^2) than in male patients (32.1 ± 6.1 kg/m^2). The mean levels of total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were 4.7 ± 1.1, 2.8 ± 0.9, 1.1 ± 0.3, and 1.8 ± 1.4 mmol/L, respectively. Further, the mean fasting blood glucose and HbA1c levels were 9.6 ± 3.8 mmol/L and 8.5 ± 1.8%, respectively. Among all comorbidities, dyslipidemia (46.5%) and hypertension (40.4%) were the most common in the study population, whereas the most common diabetic complications were nephropathy (36.7%) and neuropathy (35.4%) followed by retinopathy (21.7%).

Table 2 summarizes the characteristics of the medicines administered to the study patients. As shown, the majority of the patients received monotherapy with an oral drug without insulin. The most common anti-diabetic medication administered as

TABLE 1 | Demographic and clinical characteristics of T2D patients (n = 963).

Variable	Number (%)
Age	
mean ± SD (years)	53.0 ± 9.5
<50	274 (28.5)
50–65	656 (68.1)
>65	33 (3.4)
Sex	
Male	483 (50.0)
Female	480 (49.8)
BMI, Mean ± SD (KG/M^2)	
Male	32.1 ± 6.1
Female	34.3 ± 7.0
SBP, mean ± SD (mm/Hg)	134.4 ± 17.7
DBP, mean ± SD (mm/Hg)	75.8 ± 11.3
Total cholesterol, mean ± SD (mmol/L)	4.7 ± 1.1
LDL cholesterol, mean ± SD (mmol/L)	2.8 ± 0.9
HDL cholesterol, mean ± SD (mmol/L)	1.1 ± 0.3
Triglyceride, mean ± SD (mmol/L)	1.8 ± 1.4
Fasting blood sugar, mean ± SD (mmol/L)	9.6 ± 3.8
HbA1c level, mean ± SD (%)	8.5 ± 1.8
Creatinine, mean ± SD (μmol/L)	85.3 ± 32.8
Comorbidities and diabetic complications	
Dyslipidemia	449 (46.52)
Hypertension	391 (40.4)
Nephropathy	353 (36.7)
Neuropathy	341 (35.4)
Retinopathy	209 (21.7)
Coronary heart disease	95 (9.9)
Foot ulcer	54 (5.6)
Stroke	15 (1.6)
Kuwaiti	720 (74.8%)
Average diabetes appointment per year	2.3

Mean values of LDL, HDL, HbA1c, and BP levels are calculated on the basis of the values measured at baseline of the study patients.

TABLE 2 | Medicine characteristics of T2D patients.

Anti-diabetic medication	Number (%) of patients receiving the therapy
MONOTHERAPY	
Metformin	202 (21.0)
Insulin	196 (20.3)
DPP-IV inhibitors	80 (8.3)
Sulfonylureas	63 (6.5)
GLP-1 agonists	16 (1.6)
Meglitinides	10 (1.0)
Glitazones	2 (0.2)
1 oral drug without insulin	373 (38.7)
COMBINATION THERAPY	
Metformin + insulin	94 (9.8)
Metformin + DPP-4 inhibitors	67 (7.0)
Metformin + sulfonylureas	25 (2.6)
Metformin + GLP-1 agonists	5 (0.5)
Sulfonylureas + DPP-4 inhibitors	5 (0.5)
Sulfonylureas + insulin	4 (0.4)
1 oral drug with insulin	117 (12.1)
Metformin + insulin + DPP-4 inhibitors	61 (6.3)
Metformin + sulfonylureas + DPP-4 inhibitors	29 (3.0)
Metformin + sulfonylureas + insulin	5 (0.5)
2 oral drugs with insulin	80 (8.3)
2 oral drugs without insulin	109 (11.3)
≥3 oral drugs with insulin	20 (2.1)
≥3 oral drugs without insulin	39 (4.0)
TOTAL THERAPY (MONOTHERAPY AND COMBINATION)	
Metformin	532 (55.2)
Insulin	413 (42.9)
DPP-IV inhibitors	294 (30.5)
Sulfonylureas	145 (15.0)
GLP-1 agonists*	35 (3.6)
Meglitinides	32 (3.3)
Glitazones	8 (0.8)
Patients aged >40 years + taking statin drug	672 (76.6)

**GLP-1 agonists given only to obese patients with diabetes (BMI > 30 kg/m^2).*

monotherapy was metformin (21.0%) followed by insulin (20.3%) and DPP-4 inhibitors (8.3%), with glitazones (mainly pioglitazone) being the monotherapy medication administered to the least number of patients (0.2%). Regarding combination treatment, most patients received one oral drug with insulin (12.1%) followed by two oral drugs without insulin (11.3%). The most common combination treatment was metformin with either insulin (9.8%) or a DPP-4 inhibitor (7%) or both (6.3%). The least used drug combination was a sulfonylurea with insulin and a DPP-4 inhibitor (0.1%). Further, only 3.1% of the patients received no anti-diabetic therapy and were managed on diet and/or with lifestyle changes. The most common anti-diabetic medication prescribed in total (as monotherapy or combination treatment) was metformin (55.2%) followed by insulin (42.9%).

TABLE 3 | Demographic and clinical characteristics distributed according to HbA1c level.

Variable	HbA1c < 7%	HbA1c ≥ 7%	*P*-value
Number of patients (%)	284 (29.5%)	679 (70.5%)	
Age, mean ± SD (years)	52.8 ± 9.3	53.1 ± 9.6	0.59
Sex, n (%)			
Male	151 (53.2%)	332 (48.9%)	0.25
Female	133 (46.8%)	347 (51.1%)	
BMI, mean ± SD (kg/m^2)	32.8 ± 7.1	33.3 ± 6.4	0.28
SBP, mean ± SD (mm/Hg)	132.2 ± 15.6	134.9 ± 16.3	0.035*
DBP, mean ± SD (mm/Hg)	71.7 ± 10.9	70.9 ± 11.1	0.32
TC, mean ± SD (mmol/L)	4.0 ± 0.9	4.2 ± 1.0	<0.001*
LDL-C, mean ± SD (mmol/L)	2.2 ± 0.8	2.3 ± 0.9	<0.01*
HDL, mean ± SD (mmol/L)	1.2 ± 0.4	1.1 ± 0.4	<0.01*
TG, mean ± SD (mmol/L))	1.3 ± 0.6	1.7 ± 1.1	<0.001*
FBS, mean ± SD (mmol/L)	7.0 ± 1.7	9.8 ± 3.5	<0.001*
Creatinine, mean ± SD	93.0 ± 70.9	88.9 ± 40.4	0.36
Dyslipidemia, n (%)	125 (27.8%)	324 (72.2%)	0.33
Hypertension, n (%)	115 (29.4%)	276 (70.6%)	1.0
Nephropathy, n (%)	103 (29.2%)	250 (70.8%)	0.93
Neuropathy, n (%)	72 (21.1%)	269 (78.9%)	<0.001*
Retinopathy, n (%)	54 (25.8%)	155 (74.2%)	0.22
CHD, n (%)	31 (32.6%)	64 (67.4%)	0.55
Foot ulcer, n (%)	8 (14.8%)	46 (85.2%)	<0.03*
Stroke, n (%)	5 (33.3%)	10 (66.7%)	0.78

**Statistically significant (P < 0.05).*

Factors Associated With Glycemic Control in Patients With Type 2 Diabetes

Table 3 presents the demographics and clinical characteristics of the study patients with diabetes divided into two groups (good glycemic control, HbA1c < 7%; poor glycemic control, HbA1c ≥ 7%). Among the demographics and clinical characteristics, only the levels of systolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, and fasting blood glucose had a significant association with glycemic control. Most clinical characteristics, except HDL cholesterol level, had a positive association with glycemic control; the patients with poor glycemic control (HbA1c level ≥ 7%) were likely to have higher systolic blood pressure and total cholesterol, LDL cholesterol, triglyceride, and fasting blood glucose levels. On the other hand, patients who had high HDL cholesterol levels were associated with good glycemic control (HbA1c level < 7%). Regarding comorbidities, neuropathy and foot ulcers were significantly associated with HbA1c levels; 79% of the patients with neuropathy and 85% of those with foot ulcers had poor glycemic control (HbA1c level ≥ 7%).

Table 4 presents the association between anti-diabetic medication and glycemic control in patients with type 2 diabetes. Two-thirds of the patients with diabetes with good glycemic control were significantly more likely to receive metformin as monotherapy or in combination. On the other hand, insulin use as monotherapy and total therapy was found to be significantly

TABLE 4 | Association between anti-diabetic medication and glycemic control in T2D patients.

Anti-diabetic medication	HbA1c < 7% (*n* = 284)	HbA1c ≥ 7% (*n* = 679)	*P*-value
MONOTHERAPY			
Metformin	102 (35.9%)	100 (14.7%)	<0.001*
Insulin	45 (15.8%)	151 (22.2%)	0.031*
DPP-IV inhibitors	17 (6.0%)	63 (9.3%)	0.118
Sulfonylureas	18 (6.3%)	45 (6.6%)	0.982
GLP-1 agonists	2 (0.7%)	14 (2.1%)	0.171
Meglitinides	1 (0.4%)	9 (1.3%)	0.296
Glitazones	0 (0%)	2 (0.3%)	1
1 oral drug without insulin	140 (49.3%)	233 (34.3%)	<0.001*
COMBINATION THERAPY			
Metformin + insulin	20 (7.0%)	74 (10.9%)	0.085
Metformin + DPP-4 inhibitors	24 (8.5%)	43 (6.3%)	0.299
Metformin + sulfonylureas	6 (2.1%)	19 (2.8%)	1
Metformin + GLP-1 agonists	4 (1.4%)	1 (0.1%)	0.028*
Sulfonylureas + DPP-4 Inhibitors	1 (0.4%)	4 (0.6%)	1
Sulfonylureas (SU) + insulin	1 (0.4%)	3 (0.4%)	1
1 oral drug with insulin	23 (8.1%)	94 (13.8%)	0.017*
Metformin + insulin + DPP-4 inhibitors	11 (3.9%)	50 (7.4%)	0.060
Metformin + SU + DPP-4 inhibitors	8 (2.8%)	21 (3.1%)	0.983
Metformin + sulfonylureas + insulin	1 (0.4%)	4 (0.6%)	1
2 oral drugs with insulin	14 (4.9%)	66 (9.7%)	0.019*
2 oral drugs without insulin	36 (12.7%)	73 (10.8%)	0.453
≥3 oral drugs with insulin	4 (1.4%)	16 (2.4%)	0.490
≥3 oral drugs without insulin	10 (3.5%)	29 (4.8%)	0.720
TOTAL THERAPY			
Metformin	184 (64.8%)	348 (51.3)	<0.001*
Insulin	86 (30.3%)	327 (48.2%)	<0.001*
DPP-IV inhibitors	69 (24.3%)	225 (33.1%)	0.008*
Sulfonylureas	38 (13.3%)	107 (15.8%)	0.400
GLP-1 agonists*	8 (2.8%)	27 (4.0%)	0.491
Meglitinides	6 (2.1%)	26 (3.8%)	0.245
Glitazones	0 (0%)	8 (1.2%)	0.113

**Statistically significant (P < 0.05).*

associated with poor glycemic control. Similarly, the use of DPP-4 inhibitors in total was associated with poor glycemic control. We further divided our patient sample based on insulin and non-insulin treatment to observe any association with glycemic control (**Table 5**). Patients treated with insulin had significantly higher fasting blood glucose, BMI, and HbA1c level as well as diabetes-related microvascular and macrovascular complications and had poorer glycemic control than those treated with oral anti-diabetic drugs.

Adherence to Performance Indicators

Table 6 presents the level of adherence of patients with type 2 diabetes to performance indicators set by DDI and ADA. The proportion of patients with good glycemic control (Hb1AC level < 7%) significantly improved in the second year but became steady at 32.5% thereafter. Similarly, the proportion of patients with diabetes attaining optimal levels of LDL cholesterol (<2.6 mmol/L) and blood pressure (<140/90 mm/Hg) significantly increased over the first 3 years. In contrast, the proportion of patients with diabetes whose urine microalbumin or LDL cholesterol levels were measured at least once yearly decreased significantly after the first year and subsequently leveled off.

DISCUSSION

This retrospective study was conducted to determine the level of metabolic control in patients with type 2 diabetes attending a specialist diabetes clinic in Kuwait and to investigate the factors that affect metabolic control. Our findings showed that most of the patients with diabetes (70.5%) did not attain the recommended target HbA1c level according to the ADA definition (<7%), with a mean HbA1c level of 8.5 ± 1.8%. This finding is in agreement with those of other studies conducted on patients with type 2 diabetes in several Gulf countries, whereby the prevalence of poor glycemic control ranged from 65 to 75% (12–14). In developed countries, several studies have reported that 35–67% of patients with type 2 diabetes have poor glycemic control (9, 10, 15–17).

It is recognized that tight glycemic control (HbA1c level < 7%) is necessary to reduce the risk of diabetes-related microvascular and macrovascular complications, as demonstrated by the UKPDS Group (7). Although the percentage of patients with HbA1c level of <7% improved dramatically after 1 year of attending our clinic (from 22.4 to 32.5%), it did not improve in the subsequent years. Despite the high obesity rates in our patients (65%), we observed no association between BMI and poor glycemic control. Further, several studies have showed the effect of weight on glycemic control (18, 19), but many studies have not observe this association (9, 20, 21). Another possible factor influencing poor glycemic control, which was not obtained in this study, was the duration of type 2 diabetes. Reportedly, patients with a type 2 diabetes duration of >10 years are likely to have a 15% higher HbA1c level than those with type 2 diabetes for a shorter duration (22).

Of the anti-diabetic drugs used by our patients with diabetes, metformin was most commonly prescribed and was used by >50% of the patients as monotherapy or in combination. Although our finding is in agreement with that of a previous study (23), a high proportion of patients have not been treated with metformin. In our study, the use of metformin as monotherapy or in combination was significantly associated with good glycemic control. This finding concurs with those of a systematic review of 35 double-blinded randomized controlled trials showing that metformin use as monotherapy, compared with placebo, was associated with an HbA1c reduction of 1.1% (24). The UKPDS Group has shown that metformin therapy for patients with type 2 diabetes reduced diabetic complications and death (7). Our data were not segmented based on diabetic complications, but our findings showed

TABLE 5 | Association between insulin treatment and glycemic control in T2D patients.

Variable	Insulin treatment ($n = 413$)	Non-insulin treatment ($n = 550$)	*P*-value
Age, mean ± SD (years)	53.2 ± 9.8	52.8 ± 9.2	0.49
BMI, mean ± SD (kg/m^2)	34.0 ± 7.1	32.5 ± 6.1	<0.01*
SBP, mean ± SD (mm/Hg)	135.5 ± 16.4	133.2 ± 15.8	0.05
DBP, mean ± SD (mm/Hg)	69.3 ± 11.9	72.4 ± 10.3	<0.001*
Total cholesterol, mean ± SD (mmol/L)	4.10 ± 1.1	4.2 ± 1.0	0.3
LDL, mean ± SD (mmol/L)	2.3 ± 0.9	2.3 ± 0.8	0.4
HDL, mean ± SD (mmol/L)	1.1 ± 0.4	1.2 ± 0.4	0.1
Triglycerides, mean ± SD (mmol/L)	1.7 ± 1.1	1.6 ± 1.0	0.3
FBG, mean ± SD (mmol/L)	9.4 ± 3.9	8.4 ± 2.8	<0.001*
HbA1c level, mean ± SD (%)	8.4 ± 1.7	7.7 ± 1.5	<0.001*
Patients with HbA1c <7%, n (%)	86 (20.8)	198 (36.0)	<0.001*
Macrovascular complications, n (%)	49 (11.9)	33 (6.0)	<0.03*
Microvascular complications, n (%)	284 (68.8)	317(57.6)	<0.001*

**Statistically significant ($p < 0.05$). FBS, Fasting blood glucose. Diabetic macrovascular complications include coronary heart disease and stroke. Diabetic microvascular complications include nephropathy, retinopathy, neuropathy, and foot ulcers.*

TABLE 6 | Adherence of patients with type 2 diabetes to performance indicators by year.

Variable	Year 1 $N = 881$ (%)	Year 2 $N = 840$ (%)	Year 3 $N = 771$ (%)	Year 4 $N = 661$ (%)	*P*-value
HbA1C measurement (≥1)	98.64	95.95	97.79	97.28	<0.10
LDL measurement (≥1)	94.89	88.21	89.49	88.35	<0.001
Urine microalbumin (≥1)	89.21	70.12	74.97	71.56	<0.001
HbA1C control (<9%)	65.36	79.28	77.32	77.60	<0.001
HbA1c control (<7%)	22.44	32.51	30.50	32.66	<0.01
Obese (BMI ≥ 30 kg/m^2)	65.21	65.05	62.87	64.91	0.80
LDL-C level (<2.6 mmol/L)	43.03	55.83	62.99	63.14	<0.001
Blood pressure (<140/90 mmHg)	56.95	65.88	70.21	62.58	<0.001

that patients treated with oral anti-diabetic drugs had fewer microvascular and macrovascular complications than those treated with insulin.

There is a high proportion of patients treated with insulin monotherapy, i.e., 20%, which is higher than that reported in previous studies (23, 25) and is not consistent with the ADA and European Association for the study of Diabetes (EASD) guidelines (26). Unlike metformin, insulin use as monotherapy or in combination with 1–2 oral anti-diabetic agents by our patients with diabetes was a predictor of poor glycemic control. Further stratification showed that insulin-treated patients had reduced probability of attaining glycemic targets of HbA1c <7% (21%) compared with those treated with oral anti-diabetic drugs (36%). Our findings are in agreement with those of some previous studies (9, 27–29), with one particular study demonstrating that insulin use is associated with an increase of 22.4% in HbA1c level relative to the use of diet or an oral anti-diabetic drug (22). Our findings indicate that a high proportion of patients with HbA1c >7% (∼45%) are treated with monotherapy, highlighting the need to closely follow the ADA and EASD guidelines in the future. Although the deterioration in glycemic control is probably attributed to the progressive nature of diabetes, the choice of medications and their doses may also have important roles.

The clinical characteristics of patients with diabetes may also influence glycemic control, as suggested previously (12, 22). In our study, approximately 50% of the patients with diabetes had dyslipidemia as the most common comorbidity. Elevated lipid profile marker (LDL-C, total cholesterol, and triglycerides) levels were significantly and positively associated with poor glycemic control. According to Yurgin et al. (22), for every increase of 0.65 mmol/L in the total cholesterol level, the HbA1c value was higher by 2.6%. On the other hand, HDL cholesterol levels had a significant and positive influence on the improvement in HbA1c levels in our patients with diabetes. Hypertension was the second most common comorbidity in our patients (40%). This result is similar to those reported in studies conducted on patients with type 2 diabetes in a similar age group (17, 30, 31). According to these studies, the prevalence of hypertension increases to 60% by the age of 75 years. Similar to the effect of lipid marker levels, we observed a significant and positive association between systolic blood pressure and glycemic control. Our finding is in agreement with that of a large cross-sectional study on patients with type 2 diabetes conducted in Malaysia in which elevated

blood pressure (≥130/80 mmHg) was found to be associated with poor glycemic control. In Singapore, a study on a large sample of patients with type 2 diabetes indicated that prehypertension levels are associated with poor glycemic control (32). It is recognized that intensive management of cholesterol and blood pressure is effective in preventing macrovascular disease in type 2 diabetes (6, 33).

As in other studies, the percentage of patients with microvascular complications in our study was higher than that of patients with macrovascular complications. Although nephropathy was the most common microvascular complication, only neuropathy and foot ulcers (manifestations of neuropathy) showed significant association with glycemic control. Compared with patients with diabetes with good glycemic control, those with poor glycemic control were 3–4-times more likely to have neuropathy and foot ulcers as microvascular complications, which is consistent with the findings of other studies (17, 18). In contrast, other investigators have shown that the presence of neuropathy did not significantly decrease the odds of achieving optimal glycemic control (34).

The ADA recommendations for blood pressure and LDL cholesterol levels were met by 62 and 63% of the patients, respectively, by follow-up year 4. Certainly, adherence to LDL cholesterol standards significantly improved in these patients in the past 4 years, which may indicate an aggressive lipid-lowering therapy approach. Nonetheless, only 5% of our patients met the triple targets for glycemia, blood pressure, and LDL cholesterol levels.

Our study has several limitations that are worth mentioning. First, the retrospective study design prevented us from determining a causal relationship between the clinical characteristics of the patients and HbA1c glycemic control. Second, we were not able to report the duration of diabetes for our patients because many patients had a late diagnosis and most of the patients were referred to our specialist diabetic center from primary care clinics with insufficient health data. Third, our study lacked data on physical activity and adherence to diet and lifestyle changes by our patients with diabetes, thus making it difficult to conduct a thorough assessment of diabetes management and the factors affecting glycemic control. Fourth, our study did not collect data on self-monitoring of blood glucose levels or detailed data on medicine dosage and adherence to treatment. Finally, glycemic control also depends on factors other than those assessed in this study, which were not assessed because they were beyond the scope of our study.

In conclusion, the results of this retrospective study indicate that the therapeutic management of type 2 diabetes in Kuwait is suboptimal. Therapeutic strategies should ensure better adherence to ADA guidelines and evaluate high obesity rates and any lifestyle changes followed by patients. Emphasis on diabetes education and self-empowerment is the key to successful management of this disease. Further longitudinal studies are warranted to observe the trends of diabetes and its glycemic control and the associated short- and long-term complications.

ETHICS STATEMENT

This study was carried out after ethical approval from the Ethical Review Committee at DDI in 2014 to allow access to the patient data from the KBHR electronic health record database. All patients attending the clinic gave a written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

YA-K contributed to conception and design of the study. DB and YA-K facilitated regulatory approval for this study and data retrieval from the KBHR as well as overall organization of the study. AC conducted statistical analysis with the assistance of MQ. MQ drafted the first version of the manuscript. MQ and JT contributed to the revision of the manuscript. All authors contributed and approved the final version of the manuscript.

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The Prevalence of Overweight and Obesity in an Adult Kuwaiti Population in 2014

Elisabete Weiderpass[1], *Edoardo Botteri*[2,3], *Joseph C. Longenecker*[4,5]*, *Abdullah Alkandari*[6]*, *Rihab Al-Wotayan*[4], *Qais Al Duwairi*[6] and *Jaakko Tuomilehto*[6,7]

[1] International Agency for Research on Cancer, The World Health Organization, Lyon, France, [2] Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway, [3] Norwegian National Advisory Unit for Women's Health, Women's Clinic, Oslo University Hospital, Oslo, Norway, [4] Primary Health Care Department, Ministry of Health, Kuwait City, Kuwait, [5] Faculty of Public Health, Kuwait University, Kuwait City, Kuwait, [6] Research Sector, Dasman Diabetes Institute, Kuwait City, Kuwait, [7] Department of Public Health, University of Helsinki, Helsinki, Finland

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Undurti Narasimha Das,
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Hospital Marina Baixa, Spain

***Correspondence:**
Joseph C. Longenecker
jlongene@hsc.edu.kw
Abdullah Alkandari
abdullah.alkandari@dasmaninstitute.org

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Background: According to World Health Organization (WHO) estimates, Kuwait is ranked amongst the top countries in the world in obesity prevalence. This study aims to describe the prevalence of overweight, obesity, and various types of adiposity in Kuwaiti adults.

Methods: This cross-sectional study of 3,915 Kuwaiti adults aged 18–69 years used the STEP-wise approach to surveillance of non-communicable diseases, a WHO Instrument for Chronic Disease Risk Factor Surveillance. We assessed demographic information, lifestyle, personal and family history of diseases and physical measurements (height, weight, waist, and hip circumferences). All participants with valid height and weight measurements ($n = 3,589$) were included in the present analysis. Overweight was defined as BMI 25–29.9 kg/m^2 and obesity as BMI $\geq$30 kg/m^2.

Results: Obesity prevalence was 40.3% [95% confidence interval, 38.6–42.0%] (men, 36.5%; women, 44.0%); and overweight prevalence was 37% [35.4–38.7%] (men, 42%; women, 32.1%). The median BMI was 28.4 kg/m^2 among men and 29.1 kg/m^2 among women. Obesity prevalence was directly associated with female sex, age, history of diabetes, and being married in both men and women; and was inversely associated with education level in women. The prevalence of elevated waist-to-hip ratio was 46.9% among men and 37.9% among women. Waist circumference, waist-hip and waist-height ratios were directly associated with diabetes in both men and women, and inversely associated with education level in women.

Conclusion: Almost eight in ten Kuwaiti adults were overweight or obese. Urgent public health action is warranted to tackle the obesity epidemic in Kuwait.

Keywords: overweight, obesity, body mass index, prevalence, Kuwait, Middle East, WHO STEPS survey

INTRODUCTION

The epidemic of obesity does not show signs of halting in most countries of the world (1). Obesity and overweight are responsible for an estimated 4 million deaths globally and estimated to be increasing (2). The Eastern Mediterranean Region (EMR) in particular is heavily affected by the obesity epidemic and its consequences. The Global Burden of Diseases (GBD) 2015 collaborators estimate that prevalence of obesity in adults in the EMR increased from 15% in 1980 to 21% in 2015, which is far higher than the global average of 12% in the 2015. The prevalence of obesity in adults in Kuwait according to the GBD estimate is 41% in men and 49% in women in 2015; being second only to Qatar in leading the highest obesity prevalence rates within the EMR (3). Previously, the prevalence of obesity in Kuwaiti adults was reported to be 23% among men and 39% among women in the 1980s (3) and 36% amongst men and 48% amongst women aged 20–64 years in 2006 (4).

A nationally representative survey of adults was conducted in Kuwait in 2014 to assess the prevalence of non-communicable diseases and their risk factors (5). This study reports on the prevalence of overweight and obesity in Kuwaiti adults and its associated risk factors. In addition to body mass-index (BMI), we also describe for the first time data on other measures of adiposity in the Kuwaiti population.

METHODS

Study Design and Sampling

This study analyzed data from the 2014 STEPS survey on non-communicable diseases (NCD) risk factors in Kuwait, which was supported by the Kuwait Ministry of Health (MOH) and the World Health Organization (WHO) (5). The STEPS survey methods have been previously described in detail (6). In brief, the target population of this cross-sectional survey included adult Kuwaiti nationals from all Kuwait governorates. The Kuwait Ministry of Information randomly sampled Kuwaiti citizens aged 18–69 years from the national civil registration rolls, with separate random sampling according to eight sub-groups stratified by sex and four age groups (18–29, 30–44, 45–59, and 60–69 years). The study estimated that 3,842 participants were needed to achieve a 5% acceptable margin of error within each stratum, assuming $Z = 1.96$, a 50% prevalence of a specific characteristic and equal representations in the eight strata. To account for incomplete data and non-responders, this sample selection estimate was increased by 12.5%, yielding a sample selection size of 4,391. The response rate for the questionnaire and physical examination steps of the study was 89% ($n = 3{,}915$; women, 96%; men, 80%). The analysis dataset included the 3,589 participants who had valid measurements of both height and weight. Of these, 307 were missing waist and hip circumference, so analyses of waist-hip ratio (WHiR) and waist-height ratio (WHtR) included 3,282 participants.

Data Collection

The WHO STEP-Wise Approach to Surveillance survey methods were used, including a structured questionnaire (Step 1), physical examination (Step 2), and a blood draw (Step 3). Data from Steps 1 and 2 were used for the present analysis. The questionnaire, administered by face-to-face interview, included information regarding sociodemographic factors, tobacco smoking, exercise, and medical history, among other characteristics. The physical examination included measurement of weight (0.1 kg precision) and height (0.5 cm precision) using the Growth Management Scale, a device suitable for survey purposes (employing a weight scale and a height gauge with laser). BMI was calculated as weight (kilograms) divided by the height (meters) squared and classified according to the WHO cut-points for underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), Class I obesity (30.0–34.9 kg/m^2), Class II Obesity (35.0–39.9 kg/m^2), and Class III obesity (≥ 40 kg/m^2) (7). Weight and height for all those with an outlying BMI were checked for biological plausibility. Of 33 BMI values >50 kg/m^2, 13 had biologically impossible combinations of weight and height; their BMI, weight and height were therefore recoded as missing. All those with BMI <17 kg/m^2 had biologically plausible weight and height combinations. Waist circumference was measured using a MioTape device (a non-extensible tape measure, with ± 1 mm precision), with the tape placed on the bare midriff midway between the lowest rib and the superior iliac crest while ensuring the tape was horizontal across the back. Bare hip circumference was measured using the same device placed at the maximum circumference of the buttocks. WHiR and WHtR were computed for all respondents consenting to the measurement except for pregnant women. Fourteen individuals had a WHiR ≤ 0.55 and 10 individuals had a WHiR ≥ 1.8, all of whom had biologically inconsistent waist and hip circumferences in relation to their weight and height. Therefore, waist and hip circumference and WHiR were coded as missing for these outliers. Cut-off values for elevated WHiR were ≥ 0.90 for males and ≥ 0.85 for females, according to WHO recommendations.

Teams composed of physicians, nurses, dieticians, social workers, and phlebotomists collected data at MOH primary healthcare centers within each governorate. The selected survey candidates were contacted and informed regarding the scope and purpose of the study, after which they were invited to come to the center for full informed consent and data collection procedures. Participants were enrolled from March 2014 to September 2014.

Data Management and Statistical Analyses

Statistical analyses were conducted using SAS Statistical Package (version 9.4), including the package survey commands to account for the sampling weights and provide population-based estimate, and the STATA Statistical Package (version 14). Sample weights for each of the eight study strata defined by age (18–29 years; 30–44 years; 45–59 years, and 60–69 years) and sex were calculated as the product of the sample selection weight (population number/sample number), the non-response weight (1/response rate) and the population weight (population proportion/sample proportion). The Chi-square test, Chi-square test for trend and Mann-Whitney-U test were used to assess the statistical significance of sex (men vs. women) with categorical, ordinal and continuous variables, respectively (**Table 1**). Statistical significance for the trend of

TABLE 1 | Characteristics of the study population, overall and by sex (unweighted analyses).

Characteristic	Total (n = 3,589) 100%		Men (n = 1,381) 38.5%		Women (n = 2,208) 61.5%		p-value[a]
	n	(%)	*n*	(%)	*n*	(%)	
Age (years)							
≤25	686	(19.1)	304	(22.0)	382	(17.3)	0.061
26–35	1,131	(31.5)	425	(30.8)	706	(32.0)	
36–45	859	(23.9)	289	(20.9)	570	(25.8)	
>45	913	(25.4)	363	(26.3)	550	(24.9)	
Median (IQR)	35	[27–46]	34	[26–46]	36	[27–45]	0.015
Highest level of education							
Primary (or less)	190	(5.3)	30	(2.2)	160	(7.2)	<0.001
Intermediate	423	(11.8)	204	(14.8)	219	(9.9)	
High	672	(18.7)	327	(23.7)	345	(15.6)	
University	1,217	(33.9)	336	(24.3)	881	(39.9)	
Post-graduate	931	(25.9)	401	(29.0)	530	(24.0)	
Marital status							
Single	828	(23.1)	336	(24.3)	492	(22.3)	<0.001
Married	2,466	(68.7)	997	(72.2)	1,469	(66.5)	
Separated/divorced	178	(5.0)	35	(2.5)	143	(6.5)	
Widowed	116	(3.2)	13	(0.9)	103	(4.7)	
Work status							
Employed	2,658	(74.1)	1,063	(77)	1,595	(72.2)	<0.001
Student	255	(7.1)	125	(9.1)	130	(5.9)	
Homemaker	336	(9.4)	0	(0.0)	336	(15.2)	
Retired/unemployed	338	(9.4)	191	(13.8)	147	(6.7)	
Smoking status							
Never	2,784	(77.9)	678	(49.4)	2,106	(95.6)	<0.001
Former	190	(5.3)	163	(11.9)	27	(1.2)	
Current <20 cig/day	164	(4.6)	138	(10.1)	26	(1.2)	
Current 20–39 cig/day	249	(7.0)	243	(17.7)	6	(0.3)	
Current ≥40 cig/day	81	(2.3)	78	(5.7)	3	(0.1)	
Current shisha/pipe/cigar	107	(3.0)	72	(5.2)	35	(1.6)	
Fruits/vegetables (portions/day)							
<1	660	(18.6)	231	(17.0)	429	(19.6)	<0.001
1–1.9	930	(26.2)	316	(23.3)	614	(28.1)	
2–2.9	687	(19.4)	278	(20.5)	409	(18.7)	
3–4.9	703	(19.8)	272	(20.0)	431	(19.7)	
≥5	564	(15.9)	260	(19.2)	304	(13.9)	
Physical activity (hours/week)							
None	2,077	(57.9)	660	(47.8)	1,417	(64.2)	<0.001
0.1–3.0	584	(16.3)	226	(16.4)	358	(16.2)	
3.1–7.0	423	(11.8)	204	(14.8)	219	(9.9)	
>7.0	466	(13.0)	275	(19.9)	191	(8.7)	
Body mass index (kg/m^2)							
<18.5	41	(1.1)	13	(0.9)	28	(1.3)	<0.001
18.5–24.9	750	(20.9)	271	(19.6)	479	(21.7)	
25–29.9	1,299	(36.2)	580	(42.0)	719	(32.6)	
30.0–34.9	913	(25.4)	344	(24.9)	569	(25.8)	
≥35	586	(16.3)	173	(12.5)	413	(18.7)	
History of cardiovascular disease (% yes)[b]	222	(6.2)	95	(6.9)	127	(5.8)	0.17
History of diabetes (% yes)[c]	400	(11.2)	165	(12.0)	235	(10.6)	0.22

[a] *Men vs. women.*

[b] *Self-reported myocardial infarction, angina, or stroke.*

[c] *Self-reported history of diabetes.*

P-values for categorical variables were obtained by the Chi-square test, for continuous variables by the Mann-Whitney-U test. Manufactured or hand-rolled cigarettes (cig) were combined.

continuous anthropometric measures across the four age groups (**Table 2**) was evaluated using univariable linear regression models, with the anthropometric measure as the dependent continuous variable and age group entered as a continuous independent variable.

Two multivariable logistic regression models were used to estimate the association of the population characteristics with overweight and obesity (BMI $\geq$ 25 kg/m^2) and obesity (BMI $\geq$ 30 kg/m^2), included in the models as the binary dependent variables (**Table 3**). The enter method was used for covariate selection, with each model including the following independent variables: age, health region (estimates not reported), education, marital status, work status, smoking status, fruit, and vegetable consumption, physical activity, self-reported history of myocardial infarction, angina, or stroke and self-reported history of diabetes. Adjusted odds ratios (AORs) and corresponding 95% confidence intervals (CIs) are reported. Linear trends for education, smoking status (excluding those in the category "Current shisha/pipe/cigar"), fruit and vegetable consumption and physical activity were assessed entering those variables as continuous variables in the multivariable logistic regression models. All logistic regression models adhered to the goodness-of-fit test (p-value $>$ 0.05). Analysis of variance was used to obtain the weighted age-adjusted means of the anthropometric measures according to participant characteristics (**Table 4**).

Ethical Considerations

The study followed the principles of the Declaration of Helsinki and was approved by the MOH Standing Committee for the Coordination of Medical and Health Research. Written informed consent was obtained from each participant prior to enrolment in the study after explanation of the study procedures.

RESULTS

The median age of participants was 35 years and the proportion of women was 61.5% **(Table 1)**. The weighted mean age was 36.3 (±12.3) years. There was a similar distribution of age among men and women. Compared to men, a larger proportion of women were university graduates (39.9 vs. 24.3%), homemakers (15.2 vs. 0%), never smokers (95.6 vs. 49.4%), sedentary (64.2 vs. 47.8%), and with BMI $\geq$ 35 kg/m^2 (18.7 vs. 12.5%). More men than women were married (72.2 vs. 66.5%) and ate five or more portions of fruits and vegetables per day (19.2 vs. 13.9%). There was a similar proportion of men and women with self-reported previous history of heart diseases (total 6.2%) and diabetes (total 11.2%).

Detailed anthropometric measures including medians and inter quartile ranges by sex and age are presented in **Table 2**. All measures were statistically significantly associated with age in men and women (p for trend <0.001, with p-values for trend obtained from univariate linear regression models between each measure and age category, both analyzed as continuous variables). Among men, the median WHtR was 0.54, median WHiR was 0.89, and median BMI was 28.4 kg/m^2. Among

TABLE 2 | Anthropometric measures by sex and age (weighted[a] analysis).

			Age group							
	All men (*n* = 1,381)		**≤25 years (*n* = 304)**		**26–35 years (*n* = 425)**		**36–45 years (*n* = 289)**		**>45 years (*n* = 363)**	
Men	**Median**	**[IQR]**	**Median**	**[IQR]**	**Median**	**[IQR]**	**Median**	**[IQR]**	**Median**	**[IQR]**
Height, cm	172	[167–177]	172	[168–177]	174	[169–178]	172	[167–176]	170	[165–175]
Weight, kg	85	[74–95]	80	[70–90]	84	[74–93]	87	[77–98]	86	[78–97]
Body mass index (BMI), kg/m^2	28.4	[25.5–31.7]	26.7	[23.9–30.1]	27.8	[25.3–30.9]	29.4	[27.1–33.3]	29.8	[27.0–33.4]
Waist circumference, cm	92	[85–102]	86	[77–96]	90	[83–98]	98	[90–105]	100	[90–109]
Hip circumference, cm	105	[98–112]	101	[94–110]	104	[98–111]	107	[100–114]	107	[100–114]
Waist to height ratio	0.54	[0.49–0.60]	0.50	[0.45–0.56]	0.52	[0.48–0.57]	0.57	[0.53–0.62]	0.59	[0.54–0.64]
Waist to hip ratio	0.89	[0.84–0.94]	0.86	[0.80–0.91]	0.87	[0.82–0.92]	0.91	[0.87–0.95]	0.93	[0.89–0.97]
Elevated waist-to-hip ratio, ≥0.90 (n, %)	609	(46.9%)	89	(32.4%)	136	(35.8%)	153	(56.7%)	231	(70.0%)
Women	**All women [*n* = 2,208]**		**≤25 years [*n* = 382]**		**26–35 years [*n* = 706]**		**36–45 years [*n* = 570]**		**>45 years [*n* = 550]**	
Height, cm	158	[154–162]	159	[156–163]	159	[155–163]	159	[155–163]	157	[153–160]
Weight, kg	73	[63–85]	66	[56–76]	70	[62–80]	78	[69–88]	78	[70–88]
Body mass index (BMI), kg/m^2	29.1	[25.2–33.5]	25.6	[22.7–30.0]	27.4	[24.5–31.2]	30.8	[27.0–35.1]	32.0	[28.5–35.8]
Waist circumference, cm	88	[79–98]	80	[70–90]	83	[76–91]	90	[82–99]	96	[88–103]
Hip circumference, cm	106	[98–115]	102	[93–110]	104	[96–110]	109	[101–117]	110	[102–119]
Waist to height ratio	0.55	[0.49–0.62]	0.50	[0.45–0.56]	0.52	[0.48–0.57]	0.57	[0.52–0.62]	0.61	[0.56–0.67]
Waist to hip ratio	0.79	[0.77–0.89]	0.79	[0.74–0.84]	0.79	[0.75–0.85]	0.83	[0.77–0.88]	0.87	[0.81–0.92]
Elevated waist-to-hip ratio, ≥0.85 (n, %)	783	(37.9%)	79	(22.3)	179	(27.0%)	212	(40.2%)	313	(59.7%)

[a]*Median values and interquartile range (IQR) are reported (except for elevated waist-to-hip ratios, which are frequencies), weighted by sampling weights to allow population-based estimates. All measures were statistically significantly associated across age group, in men and in women ($P_{trend} < 0.001$), the p-values for which were obtained from univariate linear regression models between each continuous measure and age group, both analyzed as continuous variables.*

women, the median WHtR was 0.55, the WHiR was 0.79 and median BMI was 29.1 kg/m^2. Overall, the mean BMI was 29.4 ± 6.0 kg/m^2 (29.8 ± 7.0 among women and 29.1 ± 5.0 among men; $p < 0.001$).

The weighted prevalence of obesity was 40.3% [95% confidence interval, 38.6–42.0%] **(Figure 1)**. The weighted prevalence of Class I obesity was 24.9% [23.5–26.4%], Class II obesity was 9.9% [8.9–10.9%], and Class III was 5.5% [4.8–6.3%] in Kuwaiti adults. The prevalence of overweight was 37.0% [35.4–38.7%]. Amongst Kuwaiti men, the prevalence of obesity was 36.5% (24.3, 8.3, and 3.9% in Class I, II, and III, respectively) and the prevalence of obesity amongst Kuwaiti women was 44.0% (25.6, 11.4, and 7.0% in Class I, II, and III, respectively). The weighted prevalence of overweight was 42.0% in men and 32.1% in women. Only 22.7% of Kuwaiti adults had a BMI of <25 kg/m^2 (23.9% of women and 21.5% of men). The combined prevalence of overweight and obesity was 77.3% [75.8, 78.7] (Men: 78.5%; Women: 76.1%).

Age was strongly associated with BMI (**Figure 2**). The prevalence of obesity was 25.8% in the 18–25-year group, compared with 58.3% in people aged 45 years or over and the prevalence of overweight and obesity combined from 60.5 to 89.6%, in these age groups, respectively. This strong statistically significant ($p < 0.001$) trend across age was seen in both men and women.

Multivariable analysis of population characteristics associated with overweight and obesity by sex are presented in **Table 3** and **Supplementary Table 1**. Age was significantly associated with an increased prevalence of overweight and obesity in both sexes. Men with primary education only were less obese than men with post-graduate education, while among women there was a significant linear inverse trend of obesity with educational levels. Married men and women had significant increased odds of being overweight or obese when compared with single individuals. Current smoking of ≥40 cigarettes per day was associated with obesity in men compared with those never smoking. A significant inverse linear trend was found between physical activity (hours/week) and obesity in men but not in women. The self-reported history of diabetes was significantly associated with overweight and obesity in both sexes.

Table 4 and **Supplementary Table 2** show age-adjusted means, weighted by sampling weights to allow population-based estimates, for selected anthropometric measures, according to other participants characteristics. Among women, but not among men, there was a statistically significant inverse linear association of all these anthropometric measures with education. Analysis by occupational status did not reveal any patterns among men, while it confirmed that women who were homemakers had higher mean BMI, waist circumference, WHiR, and WHtR than other occupational categories. There were no clear associations between adiposity indicators and smoking

TABLE 3 | Multivariable analysis: population characteristics in association with overweight and obesity by sex.

Characteristic[a]	Overweight/obesity (BMI ≥ 25)		Obesity (BMI ≥ 30)	
	Men AOR (95% CI)	**Women AOR (95% CI)**	**Men AOR (95% CI)**	**Women AOR (95% CI)**
Age (years); +10 year increase	**1.3 (1.1–1.6)**	**1.8 (1.6–2.1)**	**1.3 (1.1–1.5)**	**1.6 (1.4–1.8)**
Education				
Primary	0.4 (0.1–1.1)	0.9 (0.4–2.0)	**0.4 (0.2–0.9)**	1.6 (0.9–2.8)[c]
Intermediate	0.8 (0.5–1.2)	1.5 (0.9–2.5)	0.9 (0.6–1.2)	**1.6 (1.1–2.3)**[c]
High	1.0 (0.7–1.5)	1.2 (0.9–1.8)	1.0 (0.8–1.4)	**1.5 (1.1–2.1)**[c]
University	0.8 (0.5–1.1)	1.2 (0.96–1.6)	0.8 (0.6–1.1)	1.2 (0.9–1.5)[c]
Post-graduate	[Reference]	[Reference]	[Reference]	[Reference]
Marital status				
Single	[Reference]	[Reference]	[Reference]	[Reference]
Married	**1.6 (1.1–2.3)**	**1.7 (1.3–2.2)**	1.0 (0.7–1.5)	1.3 (0.96–1.6)
Separated/divorced	0.8 (0.3–1.8)	1.3 (0.8–2.1)	0.5 (0.2–1.3)	1.2 (0.8–1.9)
Widowed	0.7 (0.2–3.5)	0.8 (0.4–1.6)	0.4 (0.1–1.7)	0.7 (0.4–1.2)
Work status				
Employed	[Reference]	[Reference]	[Reference]	[Reference]
Student	0.7 (0.4–1.2)	0.9 (0.6–1.4)	**0.5 (0.3–0.9)**	0.8 (0.5–1.3)
Homemaker	–	1.3 (0.7–2.3)	–	1.2 (0.8–1.8)
Retired/unemployed	0.8 (0.5–1.3)	1.0 (0.6–1.7)	1.1 (0.8–1.7)	1.3 (0.9–1.9)
Smoking status				
Never	[Reference]	[Reference]	[Reference]	[Reference]
Former	1.1 (0.7–1.7)	0.7 (0.3–1.8)	0.9 (0.6–1.4)[c]	0.9 (0.4–2.1)
Current < 20 cig/day	0.9 (0.6–1.4)	0.6 (0.3–1.5)	1.1 (0.7–1.6)[c]	0.5 (0.2–1.2)
Current 20–39 cig/day	0.9 (0.6–1.4)	1.6 (0.2–15.3)	1.3 (0.9–1.8)[c]	1.2 (0.20–6.6)
Current ≥ 40 cig/day	1.8 (0.8–3.6)	–	**2.0 (1.2–3.3)**[c]	1.7 (0.1–21.0)
Current shisha/pipe/cigar	0.9 (0.5–1.6)	0.9 (0.4–2.1)	1.1 (0.6–1.8)	0.7 (0.3–1.6)
Physical activity (hours/week)				
None	[Reference]	[Reference]	[Reference]	[Reference]
0.1–3.0	0.7 (0.5–1.04)	0.8 (0.6–1.1)	0.8 (0.6–1.1)[c]	0.8 (0.6–1.03)
3.1–7.0	0.9 (0.6–1.4)	1.0 (0.7–1.4)	**0.7 (0.5–0.97)**[c]	1.0 (0.7–1.4)
>7.0	0.7 (0.5–1.1)	0.9 (0.6–1.4)	**0.7 (0.5–0.96)**[c]	1.2 (0.9–1.7)
History of diabetes[b] (Y vs. N)	**1.8 (1.02–3.3)**	**1.9 (1.1–3.4)**	**1.8 (1.3–2.7)**	**2.1 (1.5–3.0)**

[a] *Neither obesity nor overweight/obesity were associated with history of heart disease or consumption of fruits and vegetables among men and women. The full tabulation of estimates can be found in* ***Supplemental Table 1****.*

[b] *Self-reported history of diabetes.*

[c] *Significant linear trend (for smoking the category "Current shisha/pipe/cigar was excluded").*

Values in bold are statistically significant.

among men, but women currently smoking ≥ 40 cigarettes per day had a higher WHiR (1.00) than other women. Physically active men had a significantly lower WHiR and WHtR while such association was not seen in women. Self-reported history of diabetes was significantly associated with higher anthropometric measurements (all variables) for men and women, while self-reported history of heart diseases was associated with higher waist circumference and WHtR in women only.

DISCUSSION

This cross-sectional survey found that among Kuwaiti adults the prevalence of overweight and obesity are extremely high and rank among the highest reported throughout the world. Only 22% of men and 24% of women had a BMI <25 kg/m^2, while 37% of men and 44% of women were obese. In both sexes, the prevalence of overweight and obesity were associated with increasing age, being married and self-reported diabetes. Amongst men, having a low educational level, being employed, currently smoking ≥40 cigarettes per day and being physically inactive were associated with either overweight and obesity. Among women there was a lower prevalence of obesity with increasing education level, while there were no clear associations with other additional characteristics studied.

The importance of such a high prevalence of overweight and obesity on public health in Kuwait, both now and in the future, cannot be overstated. The high obesity prevalence has undoubtedly contributed substantially to the 19% prevalence of diabetes reported among Kuwait adults, and the 63% prevalence in those aged 60–69 years (6). However, diabetes is only one of the subsequent waves of longer-term comorbidities to result from increased obesity prevalence. Over time, concomitant increases will likely follow in the incidence of hypertension (8), cardiovascular disease (9), cognitive decline (10), and many cancers (11). The prevalence of risk factors is directly proportional to the population attributable risk (PAR) of outcomes. With a prevalence of overweight and obesity above 75% in the adult Kuwaiti population, it will contribute to an increasing percentage of PAR over time, even for outcomes for which obesity has weak to modest associations.

Also of great public health importance is our finding of ∼60% prevalence of overweight and obesity in participants aged 18–25, suggesting that the majority of overweight and obesity in Kuwait has already become established by the time adulthood is reached. One of the main policy implications of this finding is that the focus of public health efforts must be directed to children and adolescents. This is consistent with the recommendations of the World Health Organization that "developing coherent public policies from production to consumption and across relevant sectors, through forming a cross-governmental task force, to oversee the development and/or strengthening of policies to ensure healthy diets throughout the life-course" (12). Behavioral interventions, together with public policies, regulation of industry and health education, will be necessary to stem the tide of increasing obesity (13). The Kuwait MOH and the Kuwait Public Authority for Food and Nutrition are both actively focusing on the issue of childhood obesity within their

TABLE 4 | BMI, waist circumference, waist to height and waist to hip ratio age-adjusted means according to sex and other participant characteristics[a].

Variable	Men				Women			
	BMI	**Waist circum.**	**Waist to height**	**Waist to hip**	**BMI**	**Waist circum.**	**Waist to height**	**Waist to hip**
Education								
Primary	26.5	90.9	0.54	0.93	31.4	94.5	0.60	0.86
Intermediate	29.1	94.5	0.55	0.89	31.3	92.0	0.58	0.85
High	29.4	94.0	0.55	0.89	30.0	88.5	0.56	0.84
University	29.1	93.4	0.54	0.89	29.6	87.5	0.55	0.83
Post-graduate	29.3	95.7	0.56	0.90	28.8	87.1	0.55	0.82
p-value	0.12	0.15	0.32	0.06	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Marital status								
Single	28.9	93.5	0.55	0.89	29.3	87.0	0.55	0.82
Married	29.3	95.1	0.55	0.90	30.1	89.2	0.56	0.83
Separated/Divorced	28.1	89.1	0.53	0.89	29.8	89.4	0.57	0.84
Widowed	25.9	79.0	0.46	0.82	28.6	89.5	0.57	0.84
p-value	0.07	**<0.01**	**<0.01**	**0.03**	**<0.01**	**0.04**	0.10	0.20
Work status								
Employed	29.3	94.4	0.55	0.89	29.6	87.4	0.55	0.82
Student	27.8	93.0	0.55	0.90	28.6	86.3	0.55	0.82
Homemaker	–	–	-	–	31.5	94.2	0.60	0.85
Retired/unemployed	29.6	95.1	0.56	0.90	29.7	90.3	0.57	0.86
p-value	**0.03**	0.57	0.35	0.52	**<0.01**	**<0.01**	**<0.01**	**<0.01**
History of diabetes[b]								
No	29.0	93.9	0.56	0.89	29.5	87.7	0.55	0.83
Yes	30.5	98.1	0.58	0.91	32.1	96.2	0.61	0.87
p-value	**0.01**	**<0.01**	**<0.01**	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Age-adjusted means are reported, weighted by sampling weights to allow population-based estimates. [a]The anthropometric measures were not consistently associated with smoking status, consumption of fruits/vegetables, physical activity, or history of heart disease among men and women. The full tabulation of estimates can be found in ***Supplemental Table 2****.*
[b]Self-reported history of diabetes.
Values in bold are statistically significant.

own domains. Such programs need to be supported, expanded to other stakeholders and linked together, as it is evident that a substantial reduction in adult obesity can only occur by addressing the obesity epidemic at earlier ages.

A few other population-based surveys reporting obesity estimates in the Kuwaiti population have been conducted in Kuwait since 2001. The estimates of overweight, obesity and combined overweight and obesity, respectively, were reported as follows: in 2001, estimates of 35, 24, and 58% (from a household survey of 7,609 Kuwaiti nationals aged 15–84 years) (14); in 2006, estimates of 34, 42, and 75% (from the first STEPS survey conducted among 2,280 Kuwaitis aged 20–64 years) (4); in 2008–9, estimates of 33, 43, and 76% (a national nutrition household survey of 1,704 Kuwaitis aged 19–86 years) (15); and in 2013, estimates of 38, 37, and 75% (a World Health Survey household survey of 2,995 Kuwaiti households) (16). The prevalence estimates of overweight, obesity, and overweight and obesity combined in the present study were 37, 40, and 77%.

The 2001 household survey estimates should not be directly compared to the other studies referenced above largely because one-third of the 2001 sample was between age 15 and 24 years, thus lowering the prevalence estimates compared with our study by an unknown amount, and the study did not use population sampling weights. Two other studies which analyzed the 2006 STEPS study data (17, 18) reported higher prevalence estimates than the official 2006 Kuwait STEPS monograph itself. However, both studies may have overestimated the true population prevalence since the mean age in the sample was substantially higher than that of the target population, and the study analyses did not utilize the population sampling weights. When unweighted estimates are used, they reflect the prevalence in the sample itself and do not necessarily provide accurate inferences for the target population. A comparison of the estimates from the four surveys between 2006 and 2014 indicates a slight increase in prevalence of overweight and obesity combined during this 8-year period from 75 to 77%. However, caution is warranted when making direct comparisons and inferences regarding temporal trends.

The estimates of the prevalence of overweight and obesity are high across the Arabian Gulf countries (3). Several risk factors likely explain the high prevalence of overweight and obesity in the region. These include genetic susceptibility, diet with excessive energy, low physical activity and cultural barriers for physical activity (19). Recent decades were marked by rapid urbanization and economic development as well as changes in traditional cultural factors in Kuwait, resulting in the adoption of more sedentary lifestyles and high energy intake diets (15), contributing to the increasing prevalence of overweight and obesity.

As expected, consistent age-adjusted direct associations of the anthropometric measures with diabetes were seen in both men and women. Consistent associations of the measures with being

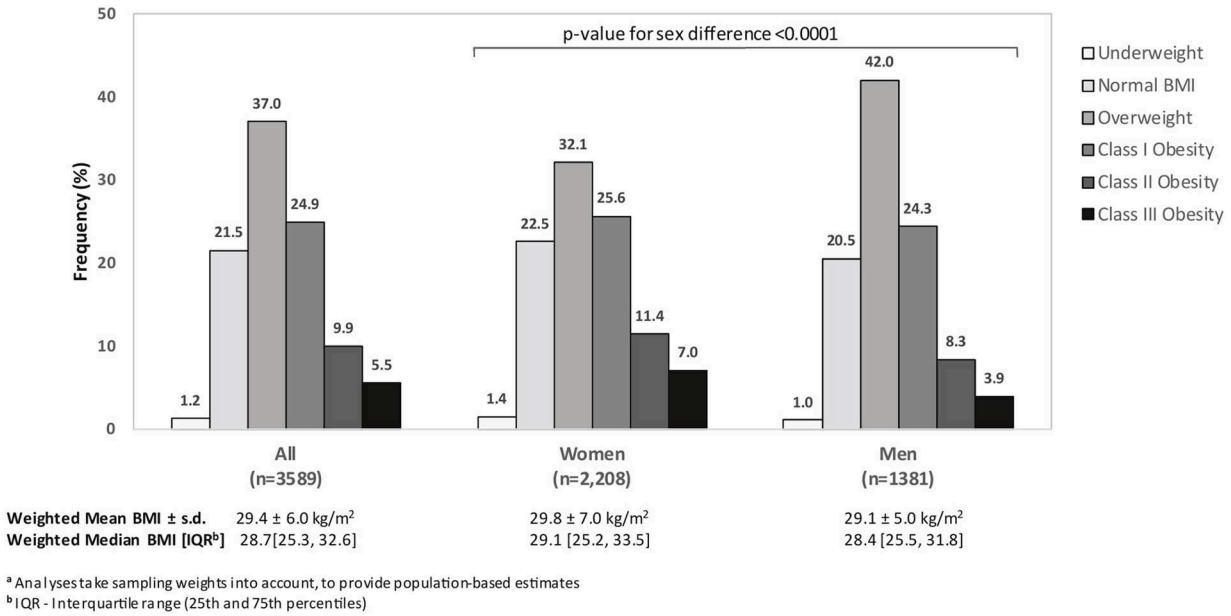

FIGURE 1 | Weighted[a] distribution of WHO body mass index (BMI) category among Kuwaiti citizens, according to sex, STEPS Study, 2014.

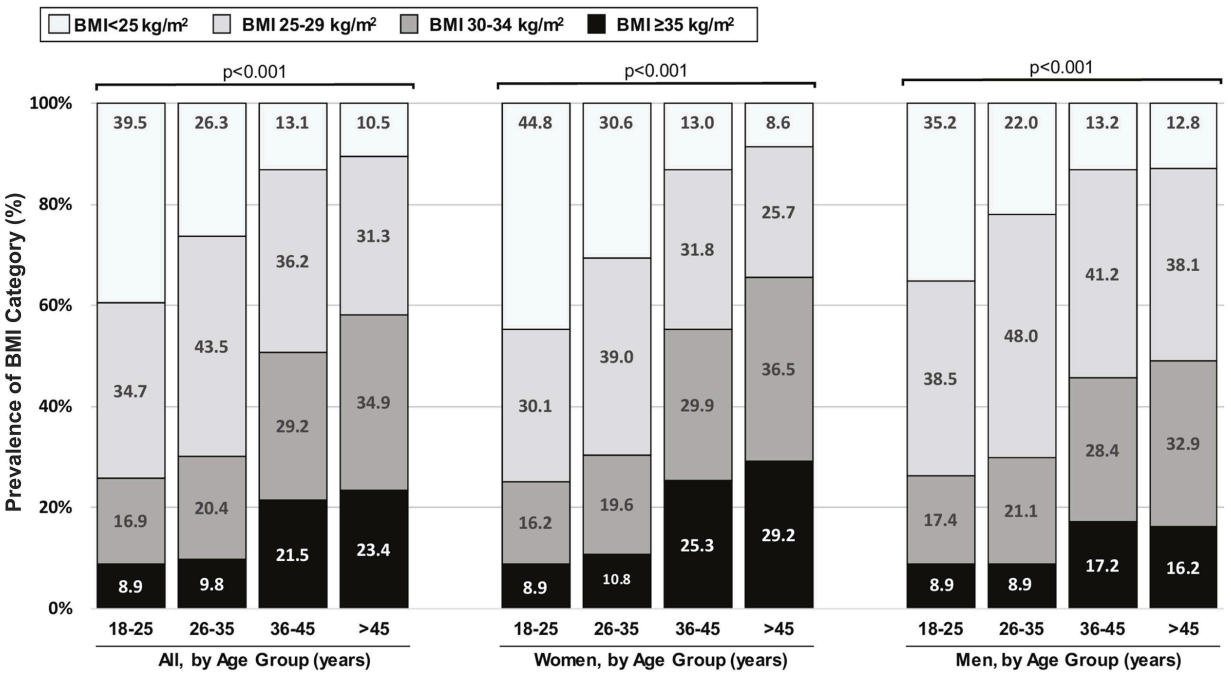

FIGURE 2 | Weighted[a] distribution of WHO body mass index (BMI) category among Kuwaiti citizens, according to age group and sex, STEPS Study, 2014.
[a] Analyses take sampling weights into account, to provide population-based estimates.

married were present primarily in men, and less consistently among women suggesting that marriage, especially for men, may be associated with dietary changes. Future qualitative studies could explore this finding further and provide evidence for health education programs that target families. We also found consistent inverse associations with education and direct associations with being a homemaker among women (no men identified as homemakers). These findings also can be used to inform health education programs that target women's dietary knowledge and practices. No consistent associations of the anthropometric measures were seen with smoking, fruit and vegetable consumption, physical activity, or cardiovascular

disease (**Supplementary Table 1**). There were a few scattered statistically significant associations of specific fatness measures with these characteristics, but no firm inferences should be made due to the possibility of chance findings due to the multiple comparisons.

There is no consensus about which anthropometric parameter best predicts diabetes or other NCDs (20–22). The association of diabetes with increasing BMI has been well-established (23, 24), as is the validity of BMI in predicting body fat mass and morbidity (25). However, BMI is an imperfect measurement of adiposity as it does not distinguish between lean and fat tissue (25), and has a J-shaped association with cardiovascular mortality and all-cause mortality, a phenomena known as the "obesity paradox" (26). In contrast, waist circumference, WHiR, and WHtR all predict diabetes incidence, cardiovascular mortality and all-cause mortality more directly (9, 27, 28). Nevertheless, the significant and consistent associations of the various anthropometric characteristics and self-reported diabetes suggest that measuring body fatness—regardless of the technique used—may be useful in identifying people at risk of diabetes.

An inverse association between smoking and obesity among men has been reported in other populations (29). In a previous study in Kuwait, smoking was associated with decreased risk of overweight but not obesity (30). In contrast, we found smoking $\geq$40 cigarettes a day increased obesity prevalence in men. Among women, an association between smoking and anthropometric measures was captured but only when using WHiR, which was highest amongst smokers of 40 or more cigarettes per day. This is consistent with findings from other studies that have reported higher waist circumference and WHiR in smokers than in non-smokers (31, 32). In contrast, a study on smoking and obesity among women in the USA reported smokers had decreased odds of being overweight or obese (33).

The strengths of this study include the population-based sampling design, use of the internationally-accepted methods of the WHO STEPS studies and a sufficiently large sample size for the purpose of a cross-sectional NCD risk factor survey. The study also had some limitations. This survey included only Kuwaiti nationals, who make up <30% of the country's total population of nearly 4.5 million (34). Future population health surveys in Kuwait should sample both national and non-national residents in Kuwait to provide data for the entire population living in the country. A previous study reported that prevalence of overweight and obesity amongst non-Kuwaitis living in Kuwait was lower than that amongst Kuwaiti nationals (35). Another limitation is that the study did not reach the target sample size, which was calculated to achieve a 5% margin of error in obesity prevalence within each of the eight sex-age categories. Therefore, the margin of error around estimates may be wider than 5%, and comparisons of binary data among these groups may fail to detect some weaker associations that are present. However, the power for detecting associations with continuous variables (e.g., WHiR, etc.) is substantially higher, since the power for parametric statistics is greater for continuous variables than it is for binary variables.

In conclusion, our findings highlight the major challenge facing Kuwait, similarly to other countries in the Gulf region, in controlling the obesity epidemic in this region. Being overweight or obese is the norm rather than an exception amongst Kuwaitis. Public health action to decrease overweight and obesity in Kuwait are urgently needed. The long-term health consequences and economic burden of obesity in the Kuwaiti society and health care system will likely be overwhelming in the decades to come. Recommendations on physical activity and healthy diet were discussed at the conference on Healthy Lifestyles and Non-Communicable Diseases in the Arab World and the Middle East in 2012 and included in the Riyadh declaration (36), but these recommendations have not yet been fully implemented.

As Kuwaiti adults rank amongst the highest for diabetes burden in the world, life-course weight waist circumference monitoring may be considered as a population-level intervention. Primary health care should aim at helping individuals avoid becoming obese, or losing weight, as needed. Monitoring of body weight and waist circumference should necessarily be followed by nutritional and lifestyle education and support.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Ministry of Health Standing Committee for Health Research, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ministry of Health Standing Committee for Health Research.

AUTHOR CONTRIBUTIONS

RA-W and QA organized and coordinated the data collection. EW, EB, and JL performed the data analysis. EW, EB, JL, AA, and JT contributed to the interpretation of the data and drafting the article. All authors were involved in the critical evaluation and final approval of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00449/full#supplementary-material

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ORIGINAL RESEARCH
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Higher Levels of ANGPTL5 in the Circulation of Subjects With Obesity and Type 2 Diabetes Are Associated With Insulin Resistance

Ghazi Alghanim[1†], Mohamed G. Qaddoumi[2,3†], Nouf Alhasawi[1], Preethi Cherian[2], Irina Al-Khairi[2], Rasheeba Nizam[1], Fadi Alkayal[1], Muath Alanbaei[4], Jaakko Tuomilehto[5], Jehad Abubaker[2], Mohamed Abu-Farha[2*] and Fahd Al-Mulla[1*]*

[1] Genetics and Bioinformatics Department, Dasman Diabetes Institute, Kuwait City, Kuwait, [2] Biochemistry and Molecular Biology Department, Dasman Diabetes Institute, Kuwait City, Kuwait, [3] Pharmacology and Therapeutics Department, Faculty of Pharmacy, Kuwait University, Kuwait City, Kuwait, [4] Department of Medicine, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait, [5] Research Division, Dasman Diabetes Institute, Kuwait City, Kuwait

Objective: The family of angiopoietin-like proteins (ANGPTLs) is composed of eight ANGPTLs members that are involved in regulating various metabolic processes and have been implicated in type 2 diabetes (T2D) and obesity. ANGPTL5 is an understudied member of this family that has been suggested to regulate triglyceride metabolism with a potential role in obesity. This study was designed to investigate the expression levels of ANGPTL5 protein in the circulation of subjects with obesity and T2D.

Methods: A total of 204 subjects were enrolled in this cross-sectional study, of which 95 had diagnosed T2D and 109 did not (non-T2D). Within the non-T2D group, 39 subjects were obese (BMI $\geq$ 30 Kg/m^2) and 70 were not (BMI $<$ 30 Kg/m^2). Among subjects with T2D, 61 were obese and 34 were non-obese. Circulating ANGPTL5 plasma levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: In this study, we showed that ANGPTL5 levels were higher in the plasma of subjects with T2D [mean $\pm$ standard error of the mean (SEM): 5.78 $\pm$ 2.70 ng/mL] compared with individuals without T2D (mean $\pm$ SEM: 4.42 $\pm$ 2.22 ng/mL; $P < 0.001$). Obese and non-T2D subjects had significantly higher levels of ANGPTL5 (mean $\pm$ SEM: 5.115 $\pm$ 0.366 ng/mL) compared with non-obese, non-T2D subjects (mean $\pm$ SEM: 4.02 $\pm$ 0.271 ng/mL; $P = 0.003$). Similarly, among subjects with diagnosed T2D, those who were obese had higher ANGPTL5 plasma levels than non-obese subjects, although this difference did not reach statistical significance ($P = 0.088$). Correlation analyses revealed that ANGPTL5 levels positively associated with fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), triglycerides (TGL), and insulin resistance as measured by HOMA-IR.

Conclusion: our data shows for the first time that circulating ANGPTL5 levels were higher in obese individuals and those with T2D. Further analysis will be required to better understand the interaction between ANGPTL5 and other metabolic related biomarkers to shed more light on its role in diabetes and obesity.

Keywords: obesity, insulin resistance, angiopoietin-like proteins, lipid metabolism, type 2 diabetes mellitus

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***Correspondence:**
Jehad Abubaker
jehad.abubakr@dasmaninstitute.org
Mohamed Abu-Farha
mohamed.abufarha@dasmaninstitute.org;
mafarha@gmail.com
Fahd Al-Mulla
fahd.almulla@dasmaninstitute.org

[†]These authors have contributed equally to this work

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INTRODUCTION

Type 2 Diabetes Mellitus (T2D) is a metabolic disorder characterized by an increase in circulating glucose levels, arising due to impaired insulin secretion and/or the resistance of peripheral tissue to insulin action (1). Factors such as a sedentary lifestyle and weight gain contribute to an increased demand for insulin secretion. Under normal conditions, the pancreatic beta cells increase the secretory output of insulin to meet this increased demand, through a mechanism termed "beta cell compensation." However, when beta cell compensation fails–coupled with a decrease in peripheral insulin sensitivity—T2D is manifested (2). Obesity is a major risk factor for the development of T2D, with ∼90% of T2D cases attributed to obesity (3). Another major risk factor associated with T2D is dyslipidemia. In ∼60–70% of cases of obesity, dyslipidemia positively correlates with obesity (4). In fact, risk factors for the development of obesity-induced diabetic vascular complications are changes in triglycerides, and low-density and high-density lipoproteins (HDL). These lipid abnormalities are typically found in cases of metabolic syndrome (5).

Obesity-induced T2D leads to macrovascular complications—such as coronary artery disease, peripheral arterial disease, increased stroke risk, and impaired wound healing—and microvascular complications—such as diabetic retinopathy, neuropathy, and nephropathy–, which are major causes of morbidity and mortality in patients with T2D (6). A key mediator of these diabetic vascular complications is angiogenesis, a process by which new blood vessels are formed via the proliferation of existing endothelial cells. Excessive angiogenesis is a key characteristic of microvascular problems (7), while inadequate angiogenesis gives rise to macrovascular complications (8). This fine balance is mediated by many pro- and anti-angiogenic growth factors (9). The angiopoietin-like protein (ANGPTL) family is composed of eight proteins, named ANGPTL1 through to ANGPTL8 (10). These proteins show structural homology to angiopoietins and display an N-terminal coiled-coil domain and a fibrinogen-related domain toward the C-terminus, except for ANGPTL8, which lacks the latter domain (11). All ANGPTL are secreted glycoproteins with pro-angiogenic effects, despite not binding to Tie receptors (12). Some ANGPTL family members have been implicated in obesity, insulin resistance, and diabetes. ANGPTL3 and 4 are amongst the most well-studied members of this family, based on their role in regulating lipoprotein lipase activity (13, 14). Another member that has been shown to regulate lipoprotein lipase activity is ANGPTL8, through its interaction with ANGPTL3 (15). Recent findings suggest that ANGPTL5, along with ANGPTL7, may be involved in the growth of hematopoietic stem cells (16, 17). However, the role of ANGPTL5 in angiogenesis and lipid metabolism remains ill-defined and recent findings suggest that, in contrast to most ANGPTLs, ANGPTL5 may not regulate angiogenesis (18).

This study investigated the expression level of ANGPTL5 in the circulation of obese and non-obese subjects, with and without T2D, and its association with glycemic and lipid metabolic clinical markers.

RESEARCH DESIGN AND METHODS

Study Population and Ethical Consent Statement

The study cohort comprised 204 subjects, including 95 subjects diagnosed with T2D and 109 subjects without T2D. Participants were stratified according to their Body Mass Index (BMI), and classified as non-obese ($19.5 \leq$ BMI < 30 kg/m^2) or obese ($30 \leq$ BMI < 40 kg/m^2). All subjects signed a written informed consent before participating in the study which abides by the Declaration of Helsinki and was approved by the Ethical Review Board of Dasman Diabetes Institute. Subjects with prior major illness or taking any medication and/or supplement known to influence the body composition or bone mass were excluded from the study. Morbidly obese subjects (BMI ≥ 40 kg/m^2) or subjects with Type 1 diabetes were also excluded from the study as previously reported (19–21).

Blood Collection and Biochemical Measurements

Blood samples from all 204 study subjects were collected and plasma was prepared using vacutainer EDTA tubes. Plasma samples were aliquoted and stored at −80°C until assayed as described previously (22–24). Fasting plasma Glucose (FPG), triglycerides (TGL), total cholesterol (TC), low density lipoprotein (LDL) and HDL were measured with Siemens Dimension RXL chemistry analyzer (Diamond Diagnostics, Holliston, MA, USA). Glycated hemoglobin (HbA1c) levels were measured using the Variant^{TM} device (Bio-Rad, Hercules, CA, USA). Insulin resistance was calculated using the HOMA-IR formula: FPG (mmol/L) × fasting insulin (mU/L)/22.5.

Plasma Levels of ANGPTL5

ANGPTL5 plasma levels were measured using ELISA kit from Wuhan EIAAB Science Co. Ltd (China). Plasma samples were thawed on ice and centrifuged at 10,000 g for 5 min at 4°C to remove any debris (22–24). The ELISA kit was validated using recombinant ANGPTL5 at a known concentration in the plasma. A plasma dilution of 1:25 showed linearity and was used in the assay. Intra-assay coefficients of variation were 7.5 to 9.2%, while the inter-assay coefficients of variation were 7.9 to 9.6%.

Plasma Level of Obesity Biomarkers

Plasma levels of leptin, adiponectin, and plasminogen activator inhibitor (PAI) were measured by multiplexing immunobead array as outlined by the manufacturer's instructions (R&D Systems, MN, USA). The data were processed using the Bio-Plex Manager Software version 6 (Bio-Rad, CA, USA), with five-parametric curve fitting. High Sensitivity C-Reactive Protein (HsCRP) was measured by ELISA as previously reported (25, 26).

Statistical Analysis

Comparisons between non-obese and obese subjects were made by Student's *t*-test. Comparisons between non-obese non-T2D, non-obese T2D, and obese T2D subjects were made by one-way ANOVA. Spearman's correlation coefficients were estimated to determine the associations between ANGPTL5 levels and

glycaemic and metabolic biochemical variables. All data are reported as mean ± standard error of the mean (SEM). Statistical assessments were two-sided and considered to be significant when $P < 0.05$. All analyses were performed using SAS (version 9.r; SAS Institute, Cary, NC).

RESULTS

Study Population Characteristics

The clinical and biochemical characteristics of the study subjects are outlined in **Table 1**. Subjects with T2D had significantly higher BMI, age, waist/hip ratio, TGL, FPG, HbA1c, HsCRP, and insulin levels, and significantly lower adiponectin and HDL levels ($P < 0.05$). On the other hand, total cholesterol, LDL, and leptin were not significantly different among subjects with and without T2D. **Tables 2**, **3** present the characteristics of obese and non-obese subjects with and without T2D. Leptin was significantly increased in obese subjects compared to non-obese subjects, regardless of T2D status. **Table 4** presents the characteristics of non-obese non-T2D, obese non-T2D, and obese T2D subjects.

Higher ANGPTL5 Plasma Levels of Obese Subjects and Subjects With T2D

Obese subjects had significantly higher levels of ANGPTL5 compared with non-obese subjects (mean ± SEM: 5.74 ± 0.25 ng/mL vs. 4.38 ± 0.24 ng/mL; $P < 0.001$; **Figure 1A**). Similarly, circulating levels of ANGPTL5 were higher in subjects with T2D compared with non-T2D (mean ± SEM: 5.78 ± 0.27 ng/mL vs. 4.42 ± 0.22 ng/mL; $P < 0.001$), as demonstrated in **Figure 1B**. In subjects without T2D, we observed that obese subjects had a significantly higher levels of ANGPTL5 compared with non-obese (mean ± SEM: 5.12 ± 0.37 vs. 4.03 ± 0.27 ng/mL; $P = 0.003$; **Figure 2A**). On the other hand, among subjects with T2D, those who were obese tended to have higher levels of ANGPTL5 compared with non-obese subjects (mean ± SEM: 6.15 ± 0.32 vs. 5.12 ± 0.46 ng/mL; $P = 0.088$; **Figure 2B**). Further analysis of the study population revealed that ANGPTL5 levels were significantly higher in obese subjects with T2D, when compared with non-obese non-T2D subjects (mean ± SEM: 4.027 ± 0.271 vs. 6.145 ± 0.317 ng/mL; $P < 0.0001$ **Figure 2C**). Furthermore, obese subjects with T2D had higher

TABLE 1 | Demographics and characteristics of the study based on their diabetes status.

	Non-diabetic N = 109	T2D N = 95	*P*-value
Age (years)	42.3 ± 1.8	52.5 ± 1.0	<0.001
BMI (kg/m^2)	28.26 ± 0.50	31.57 ± 0.43	<0.001
Waist/Hip Ratio	0.855 ± 0.01	0.96 ± 0.02	<0.001
TC (mmol/L)	5.11 ± 0.09	4.88 ± 0.14	0.184
HDL (mmol/L)	1.36 ± 0.04	1.16 ± 0.05	0.002
LDL (mmol/L)	3.21 ± 0.09	2.99 ± 0.12	0.142
TGL (mmol/L)	1.22 ± 0.09	1.67 ± 0.12	0.004
FPG (mmol/L)	5.33 ± 0.12	7.94 ± 0.29	<0.001
HbA1c (DCCT%)	5.60 ± 0.07	7.60 ± 0.18	<0.001
Insulin (U/L)	9.44 ± 066	15.14 ± 1.2	<0.001
Leptin (μg/mL)	6.67 ± 0.46	6.82 ± 0.48	0.816
Adiponectin (μg/mL)	4.99 ± 0.28	3.78 ± 0.33	0.005
HsCRP (μg/mL)	2.05 ± 0.18	3.82 ± 0.31	<0.001

Data are presented as mean ± SEM. P-values were calculated using Student's t-test. BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HsCRP, high sensitivity c-reactive protein; LDL, low-density lipoprotein; N, number of subjects; TC, Total cholesterol; TGL, triglycerides.

TABLE 2 | Demographics and characteristics of subjects without T2D.

	Non-obese N = 70	Obese N = 39	*P*-value
Age (years)	40.0 ± 1.4	46.4 ± 2.1	0.01
BMI (kg/m^2)	25.1 ± 0.4	33.9 ± 0.5	<0.001
Waist/Hip Ratio	0.83 ± 0.02	0.89 ± 0.02	0.01
TC (mmol/L)	5.07 ± 0.11	5.18 ± 0.17	0.579
HDL (mmol/L)	1.39 ± 0.05	1.31 ± 0.05	0.299
LDL (mmol/L)	3.14 ± 0.10	3.33 ± 0.15	0.31
TGL (mmol/L)	1.20 ± 0.13	1.25 ± 0.10	0.791
FPG (mmol/L)	5.22 ± 0.16	5.53 ± 0.17	0.183
HbA1c (DCCT %)	5.54 ± 0.09	5.70 ± 0.10	0.24
Insulin (U/L)	9.18 ± 0.78	10.23 ± 1.23	0.474
Leptin (μg/mL)	5.42 ± 0.47	9.12 ± 0.86	<0.001
Adiponectin (μg/mL)	5.29 ± 0.36	4.46 ± 0.43	0.15
HsCRP (μg/mL)	1.69 ± 0.20	2.76 ± 0.32	0.006

Data are presented as mean ± SEM. P-values were calculated using Student's t-test. BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HsCRP, high sensitivity c-reactive protein; LDL, low-density lipoprotein; N, number of subjects; TC, total cholesterol; TGL, triglycerides.

TABLE 3 | Demographics and characteristics of the subjects with T2D.

	Non-obese N = 34	Obese N = 61	*P*-value
Age (years)	51.4 ± 1.7	53.1 ± 1.2	0.405
BMI (kg/m^2)	26.9 ± 0.4	34.2 ± 0.3	<0.001
Waist/Hip Ratio	0.92 ± 0.02	0.98 ± 0.03	0.069
TC (mmol/L)	4.89 ± 0.29	4.88 ± 0.15	0.996
HDL (mmol/L)	1.24 ± 0.11	1.11 ± 0.04	0.292
LDL (mmol/L)	3.04 ± 0.24	2.97 ± 0.14	0.781
TGL (mmol/L)	1.56 ± 0.20	1.730 ± 0.16	0.494
FPG (mmol/L)	7.01 ± 0.33	8.44 ± 0.39	0.006
HbA1c (DCCT %)	6.59 ± 0.22	8.13 ± 0.22	<0.001
Insulin (U/L)	16.84 ± 2.26	14.11 ± 1.41	0.31
Leptin (μg/mL)	5.79 ± 0.71	7.70 ± 0.63	0.048
Adiponectin (μg/mL)	3.98 ± 0.82	3.67 ± 0.29	0.725
HsCRP (μg/mL)	3.08 ± 0.51	4.25 ± 0.39	0.073

Data are presented as mean ± SEM. P-values were calculated using Student's t-test. BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HsCRP, high sensitivity c-reactive protein; LDL, low-density lipoprotein; N, number of subjects; TC, total cholesterol; TGL, triglycerides.

TABLE 4 | Demographics and characteristics of non-obese non-T2D, obese non-T2D, and obese T2D subjects.

	Non-T2D		T2D	*P*-value
	Non-obese (*n*)	Obese (*n*)	Obese T2D (*n*)	
Age (years)	39.96 ± 1.36 (70)	46.44 ± 2.05 (39)	53.10 ± 1.18 (61)	<0.001
BMI (Kg/m^2)	25.11 ± 0.37 (70)	33.92 ± 0.47 (39)	34.18 ± 0.31 (61)	<0.0001
Waist/Hip Ratio	0.83 ± 0.02 (40)	0.89 ± 0.02 (27)	0.98 ± 0.03 (47)	0.0001
TC (mmol/L)	5.07 ± 0.11 (68)	5.18 ± 0.17 (39)	4.88 ± 0.15 (60)	0.343
HDL (mmol/L)	1.39 ± 0.05 (68)	1.31 ± 0.05 (39)	1.11 ± 0.04 (59)	0.0002
LDL (mmol/L)	3.14 ± 0.10 (68)	3.33 ± 0.15 (39)	2.97 ± 0.14 (58)	0.1836
TGL (mmol/L)	1.21 ± 0.13 (67)	1.25 ± 0.10 (39)	1.73 ± 0.16 (60)	0.0127
FPG (mmol/L)	5.22 ± 0.16 (67)	5.53 ± 0.17 (39)	8.44 ± 0.39 (61)	<0.0001
HBA1C (DCCT %)	5.54 ± 0.09 (63)	5.70 ± 0.10 (39)	8.12 ± 0.22 (61)	<0.0001
Insulin (U/L)	9.18 ± 0.78 (54)	10.23 ± 1.23 (18)	14.11 ± 1.41 (46)	<0.0001
Leptin (μg/ml)	5417.90 ± 465.83 (55)	9124.90 ± 856.74 (28)	7695.24 ± 625.56 (31)	0.0001
Adiponectin (μg/ml)	5285.66 ± 361.29 (62)	4464.58 ± 434.45 (34)	3669.38 ± 285.85 (56)	0.0031
HsCRP (μg/mL)	1687.09 ± 200.18 (61)	2764.78 ± 321.95 (31)	4248.08 ± 387.63 (56)	<0.0001

Data are presented as mean ± SEM. P-values were calculated using one-way ANOVA. BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HsCRP, high sensitivity c-reactive protein; LDL, low-density lipoprotein; n, number of subjects; TC, Total cholesterol; TGL, triglycerides.

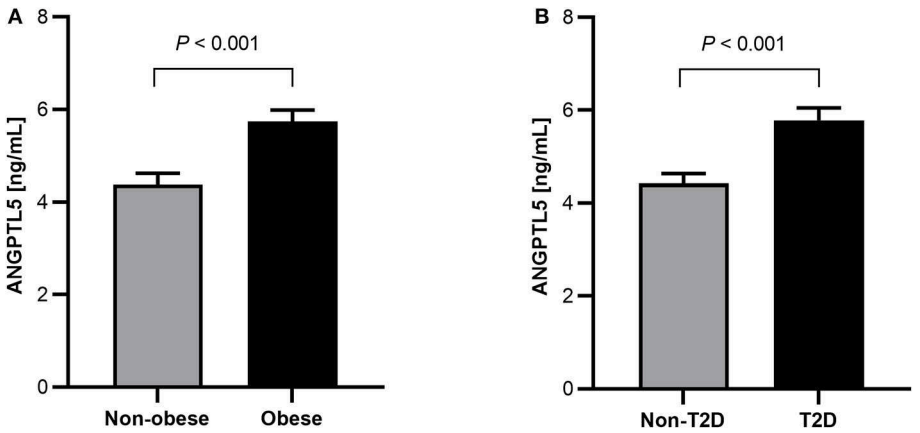

FIGURE 1 | (A) Circulation level of ANGPTL5 in non-obese ($n = 104$) vs. obese subjects ($n = 100$) as measured by ELISA. **(B)** Circulation level of ANGPTL5 in non-diabetics ($n = 109$) vs. T2D subjects ($n = 95$) as measured by ELISA. $P < 0.05$ was considered significant as determined using student's *t*-test.

levels of ANGPTL5 than obese subjects without T2D, although without achieving statistical significance (mean ± SEM: 5.119 ± 0.366 vs. 6.145 ± 0.317 ng/mL, $P = 0.075$; **Figure 2C**). Further stratification on T2D status of patients showed that ANGPTL5 levels were higher in subjects diagnosed with pre-diabetes compared with non-T2D subjects, but lower than in subjects with T2D (**Figures 3A,B**).

Correlation of ANGPTL5 Levels With Anthropometric and Clinical Markers

ANGPTL5 plasma levels positively correlated with BMI ($r = 0.304$; $P < 0.001$) and waist/hip ratio ($r = 0.227$, $P = 0.008$; **Figure 4**). Plasma ANGPTL5 levels positively correlated with FPG ($r = 0.329$, $P < 0.001$), HbA1c ($r = 0.275$, $P < 0.001$) and insulin resistance, measured by HOMA-IR ($r = 0.192$, $P = 0.014$; **Figure 5**). Correlation analysis of lipid profile markers with ANGPTL5 levels showed a significant positive correlation between ANGPTL5 and plasma TGL ($r = 0.218$, $P = 0.002$), but not with total cholesterol, LDL, or HDL (**Figure 6**). The obesity markers leptin and adiponectin did not show any correlation with ANGPTL5 in our study population. However, higher ANGPTL5 levels correlated with higher HsCRP ($r = 0.188$; $P = 0.012$; **Figure 7**).

DISCUSSION

While ANGPTL5 has been suggested to be involved in TGL metabolism, its role in diabetes and obesity remains to be elucidated. Here, we report the results of a cross-sectional study showing the association of ANGPTL5 plasma levels with obesity and T2D. In this study, we observed higher plasma levels of ANGPTL5 in obese subjects, which positively correlated with

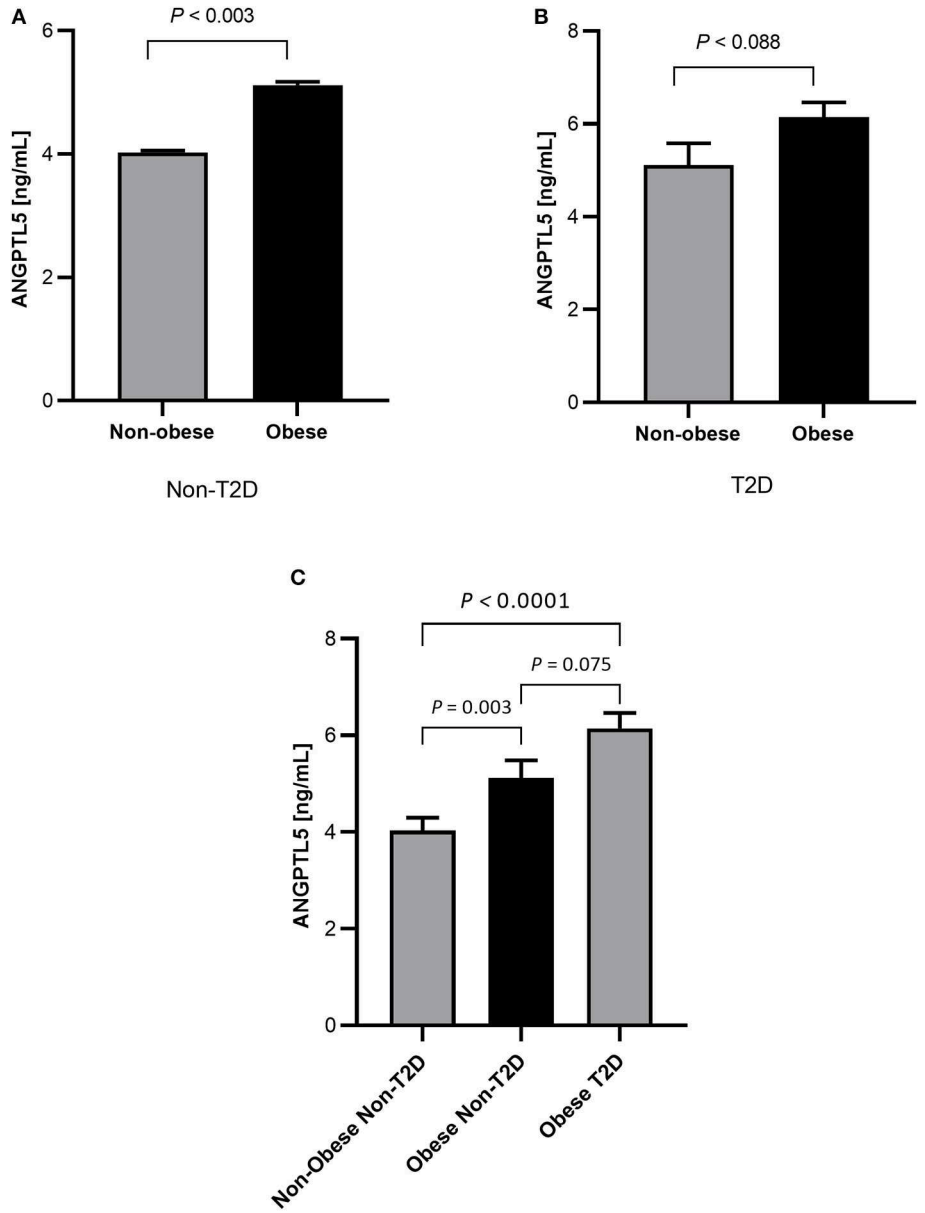

FIGURE 2 | (A) Circulation levels of ANGPTL5 in non-obese ($n = 70$) vs. obese subjects ($n = 39$) as measured by ELISA in non-diabetic people. **(B)** Circulation levels of ANGPTL5 in non-obese ($n = 34$) vs. obese subjects ($n = 61$) as measured by ELISA in people with T2D. $P < 0.05$ was considered significant as determined using student's *t*-test. **(C)** Circulation levels of ANGPTL5 in non-obese non-T2D ($n = 70$), obese non-T2D ($n = 39$), and obese T2D ($n = 61$) as measured by ELISA. $P < 0.05$ was considered significant as determined using one-way ANOVA.

increased BMI and waist/hip ratios. Additionally, subjects with pre-diabetes and T2D had elevated ANGPTL5 levels. ANGPTL5 plasma levels also showed positive correlation with insulin resistance as measured by HOMA-IR. There was a significant increase in TGL levels in subjects with T2D, which showed a positive correlation with ANGPTL5 levels. ANGPTL5 levels did not correlate with total cholesterol and LDL levels, or with the significant change in HDL level observed in subjects with T2D. Similarly, the significant changes observed in HsCRP in subjects with T2D and obese subjects without T2D, positively correlated with ANGPTL5 levels. The significant change in leptin in obese subjects, seen in subjects with and without T2D, did not correlate with ANGPTL5 levels. Similarly, the significant change in adiponectin levels in subjects with T2D did not correlate with ANGPTL5.

The roles of the other angiopoietin-like proteins in obesity and T2D have been studied previously, with some ANGPTLs having a marked role. Elevated levels of ANGPTL4, for example, has been shown to positively correlate with BMI, TGL, HOMA-IR, and HbA1c (27); interestingly, polymorphisms found in ANGPTL4

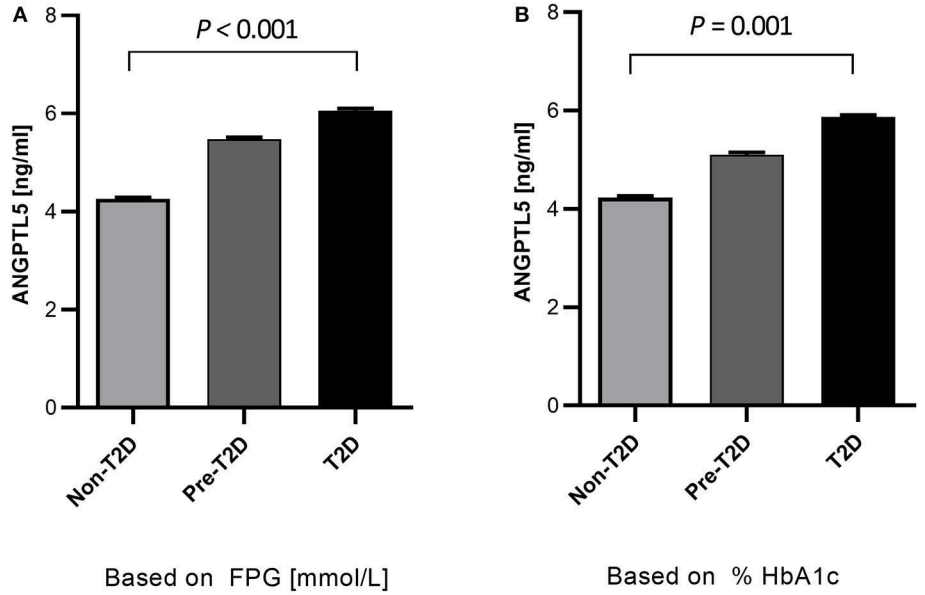

FIGURE 3 | Quantitative analysis of ANGPTL5 plasma levels according to prediabetes and T2D diagnosis based on either FPG **(A)** and HbA1c **(B)**. $P < 0.05$ was considered significant as determined using student's *t*-test. Pre-diabetes level was based on ADA criteria.

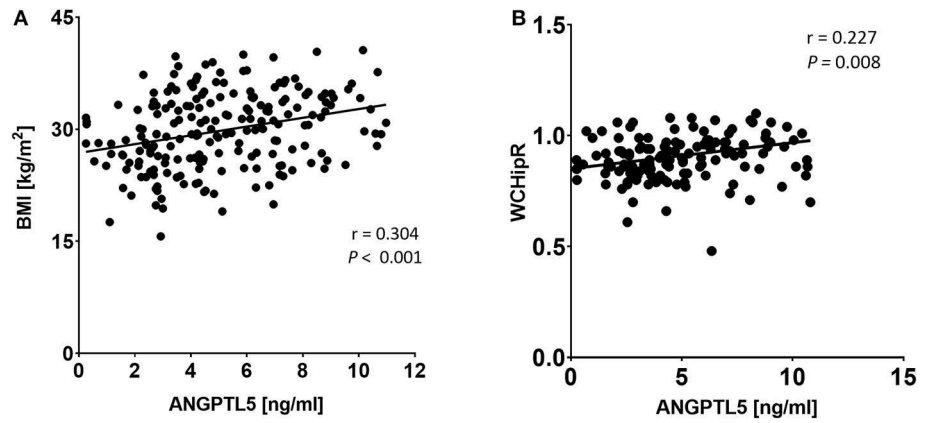

FIGURE 4 | Spearman's correlation analysis of ANGPTL5 levels and BMI **(A)** and waist/hip ratio measurements **(B)**. $P < 0.05$ was considered significant as determined by Spearman's correlation.

had been shown to positively correlate with body fat (28). In fact, genetic inactivation of ANGPTL4 was shown to reduce risk of T2D, improve insulin sensitivity and glucose homeostasis (29). Similarly, ANGPTL8 was shown to be positively associated with T2D, and correlated with many risk factors such as FPG, HbA1c, and HOMA-IR in non-T2D subjects, and thus may be a good predictor of T2D (30). In our study, subjects with T2D had significantly higher ANGPTL5 plasma levels, which positively correlated with FPG, HbA1c, and insulin resistance. While a difference was markedly evident in obese subjects without T2D when compared with non-obese subjects, in subjects with T2D we observed a non-significant elevation of ANGPTL5 in both obese and non-obese subjects. In fact, levels of ANGPTL5 found in non-obese subjects with T2D were comparable to those found in obese subjects without diagnosed T2D. The lack of significant changes in ANGPTL5 levels between obese and non-obese subjects with T2D may be due to effect of antidiabetic medication and a conscious effort to control obesity. For example, ANGPTL7 levels, previously shown to be increased in obese subjects, were shown to be significantly reduced upon exercise (19). We also observed that subjects who were at risk of developing T2D, i.e., pre-diabetes, had higher levels of ANGPTL5 than those without T2D, but lower than those with T2D. As ANGPTL5 levels also correlated positively with HbA1c, these observations, indicate that ANGPTL5 levels could be associated with poor glycemic control and increase the risk for T2D.

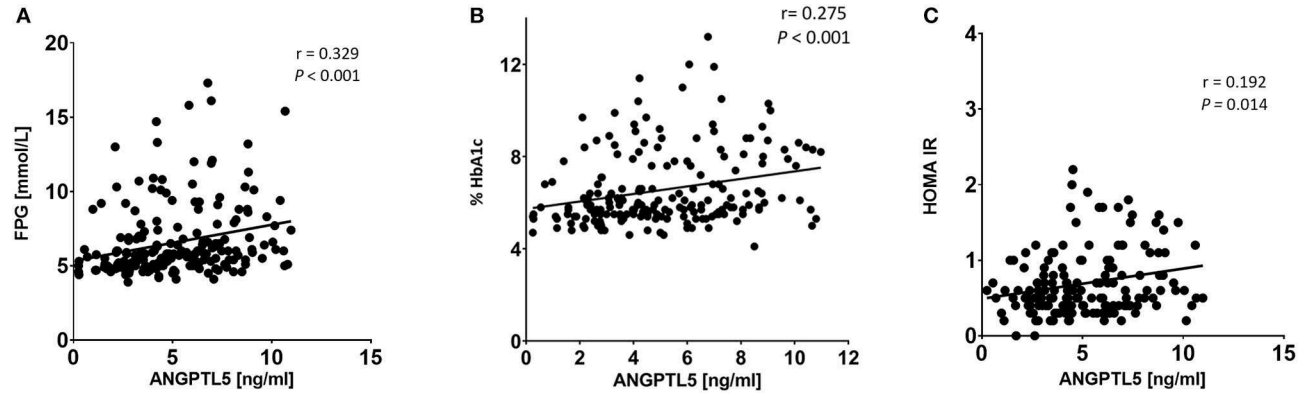

FIGURE 5 | Spearman's correlation analysis of ANGPTL5 levels and blood glucose metabolites including FPG **(A)**, HbA1c **(B)**, and HOMA-IR **(C)**. $P < 0.05$ was considered significant as determined by Spearman's correlation.

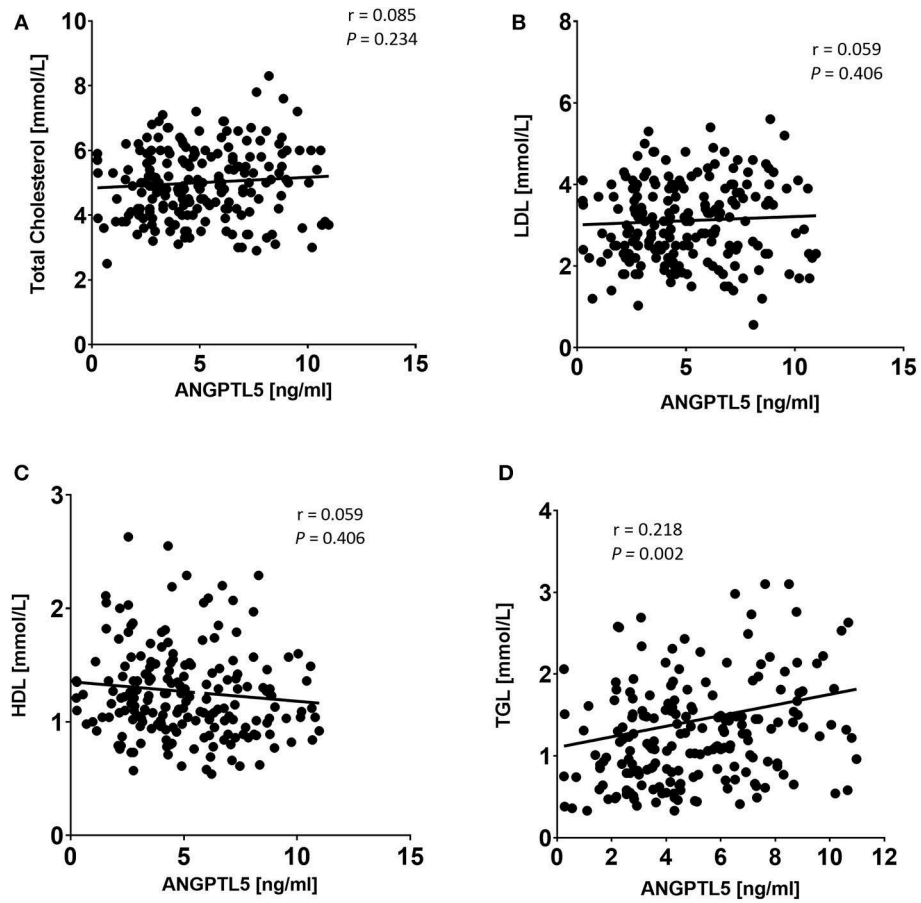

FIGURE 6 | Spearman's correlation analysis of ANGPTL5 levels and TC **(A)**, LDL **(B)**, HDL **(C)**, and TGL **(D)**. $P < 0.05$ was considered significant as determined by Spearman's correlation.

Previously, we have shown that obese subjects had significantly higher ANGPTL7 levels in both plasma and adipose tissue, which also correlated with increased TGL levels (19). ANGPTL2 was also found to be associated with obesity and insulin resistance, and to positively correlate with TGL levels (31). Conversely, ANGPTL6 had been shown to negatively correlate with obesity and insulin resistance, alongside a marked increase in energy expenditure (32), in a manner independent

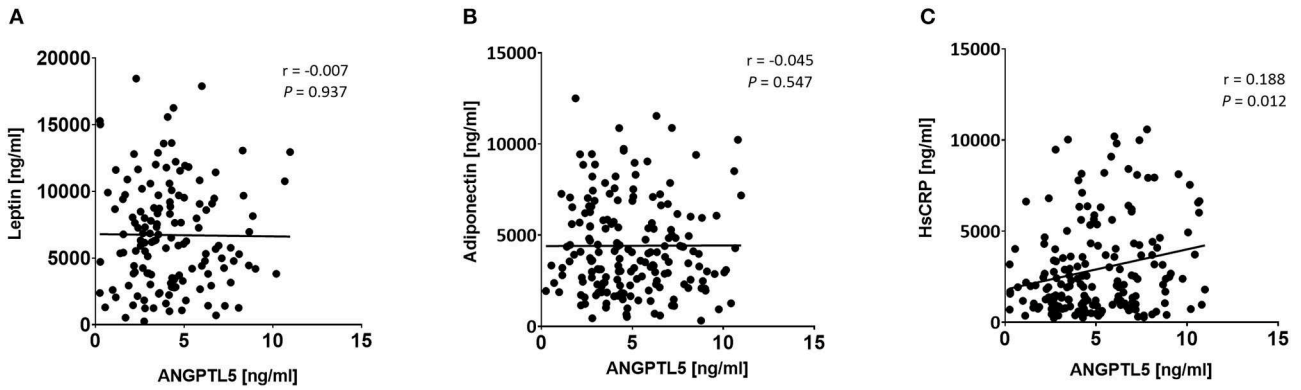

FIGURE 7 | Spearman's correlation analysis of ANGPTL5 levels and obesity markers including leptin **(A)**, adiponectin **(B)**, and HsCRP **(C)**. $P < 0.05$ was considered significant as determined by Spearman's correlation.

of lipoprotein lipase and lipid metabolism regulation (33). Although observed changes in LDL, HDL, and total cholesterol did not correlate with ANGPTL5, higher TGL levels significantly correlated with ANGPTL5 levels in subjects diagnosed with T2D. This positive correlation with TGL levels was previously observed with ANGPTL3,−4, and−7. Genetic work have illustrated that loss-of-function mutations in ANGPTL3,−4, and−5, but not ANGPTL6, correlates with lower TGL levels (34). As both ANGPTL3 and ANGPTL4 are important in lipid metabolism through their interaction with lipoprotein lipase (35), our data suggest a role for ANGPTL5 in TGL metabolism in obesity.

Interestingly, there were no significant correlations between ANGPTL5 and leptin. As an anti-obesity hormone (36), leptin was observed to be increased in obesity irrespective of T2D status. Whilst we observed a positive correlation between obesity and ANGPTL5, leptin did not correlate with increasing ANGPTL5. Other ANGPTL's were shown to correlate with leptin levels; ANGPTL3 is increased in leptin-resistant or leptin-deficient mice, highlighting a negative correlation with leptin (37). ANGPTL4, on the other hand, was previously shown to differentially correlate with leptin in a tissue-specific manner. In adipose tissue, leptin suppressed ANGPTL4 expression levels, whilst in the hypothalamus, leptin increased ANGPTL4 expression levels (38). Likewise, with adiponectin's role in regulating glucose levels and triglyceride clearance (39, 40), decreased adiponectin levels have been associated with the development of T2D and obesity (41). We observed a decrease in adiponectin levels in subjects with T2D in our study population, however there was no significant correlation between adiponectin and ANGPTL5. Given this lack of correlation between ANGPTL5 levels and both leptin and adiponectin, we suggest that the role of ANGPTL5 in obesity and T2D occurs independently of leptin and adiponectin.

Finally, we investigated the correlation between ANGPTL5 and HsCRP, a marker for low-grade chronic inflammation that may affect TGL metabolism and may correlate with increased TGL levels (42). HsCRP has been shown to be increased in obesity (43) and is a marker for cardiovascular disease and metabolic syndrome (44). Here, we show that HsCRP positively correlated with ANGPTL5, an observation also seen with ANGPTL3, ANGPTL4 (45), and ANGPTL7 (45). The higher HsCRP levels observed in obese subjects in our study population were statistically significant in those without T2D, but not in subjects with T2D. Serum levels of HsCRP were previously shown to be increased in obese subjects in a pre-diabetic state, indicating a role for HsCRP as a predictor for the development of T2D (46).

Whilst the functions and roles of other members of the angiopoietin-like family had been previously elucidated, ANGPTL5 function is yet to be established. As we have shown here, our data suggest that ANGPTL5 has an important role in obesity, TGL metabolism and T2D, and may be a possible indicator of a pre-diabetes state or metabolic syndrome. To the best of our knowledge, this study is the first to investigate the relationship between ANGPTL5 with obesity and T2D. However, due to the nature of observational studies, these findings have their limitations. As a non-randomized, cross-sectional study, it is limited in its predictions and thus, is only hypothesis generating. Without longitudinal data, the causal correlation between ANGPTL5 and T2D remains undefined, and therefore, mechanistic studies are required to further our understanding of the relationship of ANGPTL5 with T2D. Further functional studies to elucidate the mechanism of action of ANGPTL5, such as establishing its role in lipoprotein lipase activity, are needed to confirm its involvement in obesity and T2D and may support its apparent importance in TGL metabolism and as an indicator of a pre-T2D and predictor of T2D.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All subjects signed a written informed consent before participating in the study which abides by the Declaration of Helsinki and was approved by the Ethical Review Board of Dasman Diabetes Institute.

AUTHOR CONTRIBUTIONS

GA, MQ, and NA: data interpretation and manuscript writing. IA-K, PC, RN, and FA: ELISA assay. JT and MA: data analysis and management. JA: study design, data interpretation, and critical revision of the manuscript. MA-F: study design, data interpretation, and directed the laboratory investigation. FA-M: clinical data collection, and critical revision of the manuscript.

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Use of Non-invasive Parameters and Machine-Learning Algorithms for Predicting Future Risk of Type 2 Diabetes: A Retrospective Cohort Study of Health Data From Kuwait

Bassam Farran[1†], *Rihab AlWotayan*[1,2], *Hessa Alkandari*[1,3], *Dalia Al-Abdulrazzaq*[1,4], *Arshad Channanath*[1] *and Thangavel Alphonse Thanaraj*[1*]

[1] *Research Division, Dasman Diabetes Institute, Kuwait City, Kuwait,* [2] *Department of Primary Health Care, Ministry of Health, Kuwait City, Kuwait,* [3] *Department of Pediatrics, Farwaniya Hospital, Al Farwaniyah, Kuwait,* [4] *Department of Pediatrics, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait*

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***Correspondence:**
Thangavel Alphonse Thanaraj
alphonse.thangavel@dasmaninstitute.org

†Present address:
Bassam Farran, McLaren Applied Technologies, London, United Kingdom

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Objective: In recent decades, the Arab population has experienced an increase in the prevalence of type 2 diabetes (T2DM), particularly within the Gulf Cooperation Council. In this context, early intervention programmes rely on an ability to identify individuals at risk of T2DM. We aimed to build prognostic models for the risk of T2DM in the Arab population using machine-learning algorithms vs. conventional logistic regression (LR) and simple non-invasive clinical markers over three different time scales (3, 5, and 7 years from the baseline).

Design: This retrospective cohort study used three models based on LR, *k*-nearest neighbours (k-NN), and support vector machines (SVM) with five-fold cross-validation. The models included the following baseline non-invasive parameters: age, sex, body mass index (BMI), pre-existing hypertension, family history of hypertension, and T2DM.

Setting: This study was based on data from the Kuwait Health Network (KHN), which integrated primary health and hospital laboratory data into a single system.

Participants: The study included 1,837 native Kuwaiti Arab individuals (equal proportion of men and women) with mean age as 59.5 ± 11.4 years. Among them, 647 developed T2DM within 7 years of the baseline non-invasive measurements.

Analytical methods: The discriminatory power of each model for classifying people at risk of T2DM within 3, 5, or 7 years and the area under the receiver operating characteristic curve (AUC) were determined.

Outcome measures: Onset of T2DM at 3, 5, and 7 years.

Results: The k-NN machine-learning technique, which yielded AUC values of 0.83, 0.82, and 0.79 for 3-, 5-, and 7-year prediction horizons, respectively, outperformed the most commonly used LR method and other previously reported methods. Comparable results were achieved using the SVM and LR models with corresponding AUC values of (SVM: 0.73, LR: 0.74), (SVM: 0.68, LR: 0.72), and (SVM: 0.71, LR: 0.70) for 3-, 5-, and 7-year

prediction horizons, respectively. For all models, the discriminatory power decreased as the prediction horizon increased from 3 to 7 years.

Conclusions: Machine-learning techniques represent a useful addition to the commonly reported LR technique. Our prognostic models for the future risk of T2DM could be used to plan and implement early prevention programmes for at risk groups in the Arab population.

Keywords: body mass index, prognosis, type 2 diabetes, hypertension, logistic regression, support vector machine, k-nearest neighbours

INTRODUCTION

During the last two decades, the Arab world, and particularly countries in the Gulf Cooperation Council, has experienced an unprecedented increase in the prevalence of type 2 diabetes mellitus (T2DM). Wealth accumulated during the oil era in these countries contributed to rapid urbanisation resulting in dramatic changes in dietary habits and lifestyle. The resultant population-wide increase in sedentary lifestyle habits was a major contributor to the high prevalence of T2DM (1, 2).

Early intervention and prevention strategies are needed to curb this urgent health crisis, and such strategies rely on the ability to identify individuals at future risk of diabetes. Previous reported trials, including studies of impaired glucose tolerance (IGT) testing and fasting biomarker levels (3), have demonstrated that lifestyle modifications or the use of medication can substantially reduce the risk of T2DM in people with IGT or elevated fasting and post-load plasma glucose concentrations (4, 5). However, these tests are relatively invasive, time-consuming, costly and inconvenient. Therefore, diabetes risk models based on known non-invasive risk factors and statistical analyses have been generated to identify individuals at future risk of developing T2DM (6–8). Such prognosis models can help to correctly identify individuals who should be targeted by intervention programmes and to avoid burdening low-risk individuals with invasive assessments, prevention, and treatment regimens. In other words, such models could improve the efficacy and cost-effectiveness of T2DM prevention programmes.

Existing diabetes prognosis models differ in terms of the extent of prediction horizons, techniques and types of assessed variables. Some models are based on basic non-invasive parameters, while others include invasive biomarkers. Although, the former may be more successful (7), the latter are more easily implementable and convenient on a large scale. Furthermore, previous studies have demonstrated good discrimination when using non-invasive models to predict the future risk of incident T2DM over a 10-years period; for example, Abbasi et al. (7) reported that the most basic prediction models (i.e., those that use non-invasive parameters) could distinguish people at high risk of developing diabetes within a time span of 5–10 years but not in shorter timeframes. However, most basic models overestimate the actual risk of diabetes. Abbasi et al. concluded that although existing prediction models could successfully distinguish individuals at high risk, they could not adequately quantify the actual future risk of diabetes.

Existing models are also limited by the fact that most were generated using populations of white American or European populations, and only a few have been externally validated in different populations (6). A risk score tends to exhibit a weaker discriminatory performance in an external population while overestimating the risk in the initial target population (9). Therefore, model performance must be evaluated broadly within the intended target population. In the Arab world, the prevalence of obesity is very high, and diabetes is more frequently associated with obesity (10) than with β-cell dysfunction (11). Obesity contributes significantly to T2DM pathogenesis through various mechanisms. In addition to the risk of diabetes, obesity increases the risks of developing hypertension, cardiovascular disease and some types of cancers, which account for ∼50% of all deaths in the Arab region (12). Obesity-linked diabetes is a preventable disease, and a reduction in body weight decreases the risk of T2DM and its complications (13–15). These findings have encouraged collaboration among decision-makers from different Arab nations to limit the rise in obesity-related diabetes (10).

A number of mathematical techniques are used by researchers to build prognostic and predictive models in the field of biomedical applications. Apart from the techniques of logistic and Cox regression models that are often used in the field (7), machine-learning techniques have been demonstrated to have great potential (16, 17). Such machine-learning techniques include random forest (18), boosted regression tree (19), k-nearest neighbour (20), and support vector machines (SVMs) (21). Of these, the k-nearest neighbour and support vector machine are simplest classification systems having good discriminatory power (9).

In this study, we used data from a native Kuwaiti Arab population to develop prognostic models that could predict the risk of developing diabetes within three different time frames (3, 5, and 7 years) according to the body mass index (BMI) measurement at a given age. We implemented SVMs (22), k-nearest neighbours (k-NN) (23), and logistic regression (LR) (24) techniques to develop three models based on non-invasive parameters, which are age, BMI, family history of diabetes and hypertension, sex, and pre-existing hypertension.

Abbreviations: AUC, receiver operating characteristic curve; ROC, receiver operating characteristic; T2DM, type 2 diabetes mellitus; BMI, body mass index; k-NN, k-nearest neighbours; LR, logistic regression; SVM, support vector machine; KHN, Kuwait Health Network.

MATERIALS AND METHODS

Data From the Kuwait Health Network

The data used in this study were extracted from the Kuwait Health Network (KHN), which was collaboratively developed by the Dasman Diabetes Institute, Ministry of Health and the Public Authority of Civil Information of Kuwait. This network integrated health data from primary health centres and hospitals across Kuwait (9). The state clinics provide free primary health care and are located in all residential areas throughout Kuwait. Services include medical and dental care. These clinics are equipped to handle emergencies, as well as routine medical problems. The clinic has its own doctor or general practitioner who either provides treatment at the clinic or refer to one of the general hospitals. The primary contact for the patients and diagnosis are carried out at the primary health care centres; KHN integrates the patient data from these centres with data from hospital information system and laboratory information system when available for the patients. Thus, all the participants are from primary health centres.

The data records forming the research extract of the KHN were retrospective over a 9-year period, and all patients' names and civil identification numbers were anonymised before the data were provided to the researchers. Access to data from the KHN was approved by the Ethical Review Committee at the Dasman Diabetes Institute.

Data Content

The research extract from the KHN contains data on 107,821 native Kuwaiti participants without T2DM and 40,773 native Kuwaiti patients with T2DM. The participants without diabetes visit primary health centres and hospitals for various other ailments. The diagnosis for diabetes is generally carried out at the primary health centres (and then were referred to hospitals, if required) unless the participants were already visiting the hospitals for other ailments. The diagnoses of diabetes and hypertension were ascertained through clinical procedures. The outcome, T2DM, was defined from clinical records. The diagnosis was validated by way of using recorded blood glucose levels during visits at and around the diagnosis of diabetes. The extracted data included demographic information, anthropometric values, vital signs, and clinical laboratory measurements (the latter values were sparse). Not all data items were available for all the participants. This limited the size of the data pool and restricted the number of study subjects. It is possible that patients with missing data have different risk profiles as compared with patients included; however, the missing data were most often due to the reason that the integration of data by KHN was partial and ongoing.

The present study applied an inclusion criterion of a visit by the participant to a primary care centre and/or hospital at least 3 years prior to the diagnosis of T2DM with a recorded BMI measurement; as regards the participants marked as controls, it was required that the participant was continuously monitored for diagnosis of diabetes over at least 7 years since the first visit with recorded BMI measurement. This has markedly reduced the number of study subjects eligible for the study. The resultant data set comprised 1,837 native Kuwaiti patients with complete records of the following measurements: sex, ethnicity, family history of hypertension, family history of diabetes, pre-existing hypertension, BMI measurement (kg/m^2) and date of measurement (i.e., study entry point), age at BMI measurement and interval between the date of BMI measurement and diagnosis of diabetes (in months). Family histories of diabetes and hypertension were limited to first-degree relatives. Pre-existing hypertension was ascertained from clinical diagnostic data. The time point of obtaining the data for predictor variables such as sex, ethnicity, family history, and pre-existing condition of hypertension were at the study entry point (date of measurement of the first BMI measurement).

Classification of Data Sets for the Study

For categorizing participants according to BMI, the classification system approved by the WHO was used: normal weight (BMI = 18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), mildly obese (30.0–34.9 kg/m^2), moderately obese (35.0–39.9 kg/m^2), and severely obese ($\geq$40 kg/m^2). For categorizing participants according to age, the following classification system commonly used in the community [example as in: "Middle Age: definition of middle age in Oxford dictionary (American English) (US)". *Oxforddictionaries.com*. Retrieved 2018-11-09] was adopted: adolescence (13–19 years), early adulthood (20–45 years), middle adulthood (45–65 years), and old age (>65 years).

Statistical Analysis

Data mining and machine-learning calculations were performed using software from the R Project for Statistical Computing (https://www.r-project.org/). The models used in this study were explained in our previous publication (9) and are summarised below:

Logistic Regression

LR describes the relationship between an event and one or more independent variables by estimating probabilities and is used to formulate a generalised linear model. The number of regression coefficients corresponds to the number of measurements related to each hospital visitor. This statistical technique is widely used in the field of health research (24) to explain the associations among a set of explanatory variables with a binary response variable. The association of predictors with the diabetes status is measured using Odds Ratio (OR).

k-Nearest Neighbours

The k-NN is a simple classification algorithm that searches an entire training set for the *k*-closest neighbours and classifies new cases based on a majority vote (23). To determine closeness, Euclidean distance is used in the case of continuous variables and Hamming distance for binary data. We used the caret package in

R and five-fold cross-validation to test multiple values of k and determine the optimal value for the data.

Support Vector Machine

SVM is a supervised machine-learning technique based on supervised learning algorithms and used for classification and regression analyses. The classification algorithm learns from the data input (e.g., health records of patients with and without diabetes) and divides the data into two categories (e.g., diabetic and non-diabetic groups) by maximising the margin between the support points. Subsequently, the algorithm predicts which of the two possible classes should include each new data point. The success of SVM can be attributed to its ability to maximise the *margin*, which denotes the distance between an example and the decision boundary (22). As the unseen examples (test cases) will be similar to the training examples, this large margin ensures better generalisation to the test cases. The programme is set to select arbitrary values for a cost variable, C, which controls the trade-off between training errors and margin maximisation.

Denoting Discriminatory Power of the Models

The performance of a model was assessed by way of calculating the area under the receiver operating characteristic (ROC) curve (AUC). ROC curves compare sensitivity vs. specificity across a range of values for the ability to predict a dichotomous outcome. It is one the most common statistical technique used to quantify how well the model can distinguish between two states, i.e., in the context of the presented study, people who will or will not develop diabetes in the prediction horizon.

RESULTS

Descriptive Statistics of the Data Sets Used for the Analysis

The descriptive statistics of the participants are presented in **Table 1**. The cohort comprised 1,837 native Kuwaiti Arab participants (of whom 49.5% were men) with a complete record of the following measurements: age at baseline, BMI, family history of diabetes, family history of hypertension, diagnosis of hypertension, sex, and the time interval (in months) from the time of study entry to diabetes diagnosis (the last variable was not included in the analysis).

Of these 1,837 participants, 647 had developed diabetes within 7 years since the date of the baseline BMI measurement (including 290 and 468 who had developed diabetes within 3 and 5 years, respectively). The remaining 1,190 participants did not develop diabetes even after 7 years since the date of the baseline measurement.

At baseline, the mean age of all participants was 59.5 ± 11.4 years, The participants exhibited the following distribution into age categories at baseline: adolescence: 2 (0.1%); early adulthood: 144 (7.8%); middle adulthood: 1,116 (60.8%); and old age: 575 (31.3%). Patients who developed T2DM were more often from middle adulthood age group: adolescence: 2 (0.3%); early adulthood: 90 (13.9%); middle adulthood: 446 (68.9%); and old age: 109 (16.8%).

At baseline, the participants had a mean BMI of 31.6 ± 7.1 kg/m^2, and were distributed into the following BMI categories: underweight: 0.3%; normal weight: 12.1%; overweight: 34.1%; mildly obese: 28.3%; and moderately and severely obese: 25.2%. In the overall data set, 587 and 371 of the participants had a family history of diabetes or hypertension, respectively, and 71.6% (1316/1837) were hypertensive at baseline. For comparison, 41% of the 40,773 T2DM patients present in the initial research extract from KHN presented with comorbid hypertension. Higher proportion of the study individuals being hypertensive is because the study participants are predominantly from late adulthood and old age–hypertension is typically prevalent in such an age group.

Table 2 presents comparative descriptive statistics between the group of participants with T2DM onset within 7 years since the study entry point and the group of participants without onset of diabetes within the same time duration. The mean age and BMI at study entry point, pre-existing condition of hypertension and family history of hypertension were significantly different between the two groups. The mean age at the baseline BMI measurement was 54.9 ± 11.1 years among those who developed T2DM and 61.9 ± 10.8 years among those who did not develop T2DM. Notably, 67% of participants that developed T2DM within 7 years and 74% of those that did not develop T2DM were hypertensive at baseline. The mean BMI at study entry point was significantly higher for individuals who developed T2DM within 7 years compared to those who did not develop T2DM (32.95 ± 8.45 vs. 30.82 ± 6.19; $p < 0.001$).

Prognostic Models for T2DM

We constructed three different models using the LR, k-NN, and SVM techniques for predictions over three different time horizons: 3, 5, and 7 years.

3-Year Prediction Horizon

Two hundred and ninety cases and 1,547 controls were available for the 3-year prediction horizon. The performance results of the three models are presented in **Table 3** and **Figure 1A**. Using the LR function and five-fold cross-validation, we achieved an AUC value of 0.737. Using the SVM technique (with default parameters), we achieved a similar AUC value (0.729). The use of five-fold cross-validation to select the optimal hyper-parameter for k-NN yielded a k value of 8 and an AUC of 0.831, which was significantly better than the LR and SVM models. In other words, the k-NN model was significantly discriminatory.

The 1,547 individuals forming the controls are those that did not develop T2DM in the 3-years period from study entry point; the results did not differ when we experimented with having only the 1,190 individuals, who did not develop T2DM during the entire study period, as controls.

5-Year Prediction Horizon

Four hundred and sixty eight cases and 1,369 controls were available for the 5-year prediction horizon. The performance results of the three models are presented in **Table 3** and **Figure 1B**. The discrimination of the LR model did not change when applied to a 5-year prediction horizon, which yielded a

TABLE 1 | Descriptive statistics of the participants.

Total number of participants	1,837
Sex (Male:Female)	909:928 (49.5%:50.5%)
Number of participants with a family history of T2DM	587 (32.0%)
Number of participants with a family history of hypertension	371 (20.2%)
Number of participants who were baseline hypertensive	1,316 (71.6%)
Number of participants with T2DM considering the 3-year horizon	290 (15.8%)
Number of participants with T2DM considering the 5-year horizon	468 (25.5%)
Number of participants with T2DM considering the 7-year horizon	647 (35.2%)
Mean age of participants at T2DM onset considering the 3-year horizon (years)	55.1 ± 11.0
Mean age of participants at T2DM onset considering the 5-year horizon (years)	56.7 ± 11.5
Mean age of participants at T2DM onset considering the 7-year horizon (years)	58.4 ± 11.5
Mean BMI of participants considering the 3-year horizon (kg/m^2)	33.6 ± 10.2
Mean BMI of participants considering the 5-year (kg/m^2)	33.2 ± 8.9
Mean BMI of participants considering the 7-year horizon (kg/m^2)	33.0 ± 8.5
Mean interval from study entry point to diabetes diagnosis considering the 3-year horizon (months)	17.9 ± 11.5
Mean interval from study entry point to diabetes diagnosis considering the 5-year horizon (months)	29.6 ± 17.9
Mean interval from study entry point to diabetes diagnosis considering the 7-year horizon (months)	41.5 ± 24.8

T2DM, type 2 diabetes mellitus; BMI, body mass index.

TABLE 2 | Descriptive statistics of participants who became diabetic within 7 years since study entry point vs. those who did not become diabetic.

	Diabetic group (n = 647)	Non-diabetic group (n = 1,190)	p-value
Male	311 (48.1%)	598 (50.3%)	0.4
Mean age at entry point (years)	54.92 ± 11.05	61.9 ± 10.8	<0.001
Mean BMI at entry point (kg/m^2)	32.95 ± 8.45	30.82 ± 6.19	<0.001
Positive diagnosis for hypertension at entry point	431 (66.6%)	885 (74.3%)	<0.001
Family history of diabetes	191 (29.5%)	396 (33.2%)	0.110
Family history of hypertension	165 (25.5%)	206 (17.3%)	<0.001

TABLE 3 | AUC values obtained using logistic regression, k-nearest neighbours, and Support vector machine models designed for predicting the risk of T2DM over three different prediction horizons.

Prediction horizons	Logistic regression	k-nearest neighbours	Support vector machine
3-year	0.737 95% CI: 0.7049–0.7692	0.8308 95% CI: 0.8079–0.8537	7286 95% CI: 0.696–0.7611
5-year	0.7161 95% CI: 0.6886–0.7435	0.818 95% CI: 0.7973–0.8389	0.6823 95% CI: 0.6514–0.7132
7-year	0.7039 95% CI: 0.6791–0.7286	0.7903 95% CI: 0.7694–0.8112	0.7059 95% CI: 0.6812–0.7306

AUC, area under the curve; T2DM, type 2 diabetes mellitus; CI, confidence interval.

slightly lower AUC (0.716) than what was obtained for the 3-year prediction horizon. Similarly, although the discriminatory power of the SVM model decreased slightly from 3 to 5 years (0.729–0.682, respectively), this decrease was not statistically significant (p = 0.08). By contrast, a five-fold cross-validation yielded a k value of 8 and an AUC of 0.818, which was slightly lower than that obtained with the 3-year prediction horizon. Again, the k-NN method performed better than the LR and SVM models.

The 1,369 individuals forming the controls are those that did not develop T2DM in the 5-years period from study entry point; the results did not differ when we experimented with having only the 1,190 individuals, who did not develop T2DM during the entire study period, as controls.

7-Year Prediction Horizon

Six hundred and forty-seven cases and 1,190 controls were available for the 7-year prediction horizon. The performance results of the three models are presented in **Table 3** and **Figure 1C**. Here, the LR model yielded a lower AUC (0.70) for the 7-year prediction horizon than for the 3- (0.74) and 5-year horizons (0.72). The SVM model yielded an AUC of 0.71 at 7 years, which was slightly higher and lower than the values obtained for the 5- and 3-year horizons, respectively (0.68 and 0.73, respectively). Although, a five-fold cross-validation for k-NN hyper-parameter selection again yielded a k value of 8, this method yielded a lower discriminatory power (AUC = 0.79) for the 7-year horizon relative to the 5- (0.82) and 3-year (0.83) horizons. Still, the k-NN model performed better than the LR and SVM models.

Coefficients Identified as Significant When Applying Logistic Regression Model to the Three Prediction Horizons

Among the multiple LR coefficients deduced with the models for 3- and 5-year prediction horizons, sex, and family history of diabetes were insignificant factors (**Table 4**); however the family

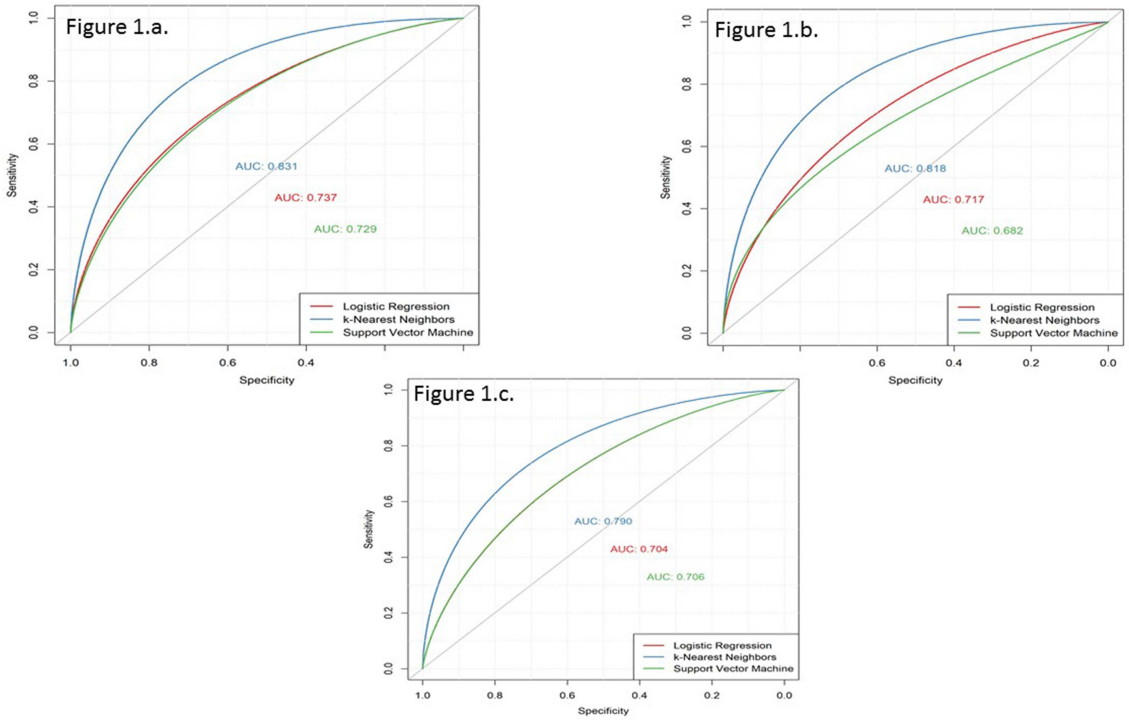

FIGURE 1 | Receiver operating characteristic (ROC) curves derived for prediction horizons of 3, 5, and 7 years using the three models based on logistic regression (LR), k-nearest neighbours (k-NN), and support vector machine (SVM). **(a)** 3-year prediction horizon. **(b)** 5-year prediction horizon. **(c)** 7-year prediction horizon.

history of diabetes became a significant variable in the 7-year model, and sex remained the only insignificant variable for this time horizon.

Other Performance Metrices (Such as Sensitivity and Specificity) for the Models

In order to derive other performance metrices such as sensitivity and specificity of the model, we matched the sizes of the case and control. Best measure for sensitivity was obtained with SVM model at 44% for 7-year prediction horizon and at 35% for 5-year prediction horizon (**Table S1**). AUC was always higher with k-NN model in all the three prediction horizons, but the accuracy was comparatively lower for k-NN. This is because the measures of sensitivity, specificity and accuracy characterize the true positive rate and true negative rate at the threshold value of 0.5, while AUC is computed by adding all the "accuracies" computed for all the possible threshold values. Hence, AUC is an average (expected value) of those accuracies when computed for all threshold values.

DISCUSSION

As noted, Arab countries have seen increase in the incidence rates of obesity, diabetes, and metabolic syndrome (25–28) in recent decades. Accordingly, tools that can be used to accurately identify high-risk individuals as targets for early intervention and prevention programmes are urgently needed. In this retrospective cohort study, we analysed a data set from native Arab individuals who were predominantly middle-aged and older and among whom more than half were either obese or very obese. We demonstrated that prognostic models developed using six non-intrusive parameters (baseline age, BMI, family histories of diabetes and hypertension, pre-existing hypertension, and sex) could identify patients at a high risk of developing T2DM within 3–7 years. In our study, the k-NN machine-learning technique outperformed the most commonly used LR, as well as another tested model based on SVM. For all models, however, the discriminatory power decreased as the prediction horizon increased.

Table 5 lists the previously published T2DM risk assessment tools and classification models developed using non-invasive parameters. These studies, which were mostly based on LR models, yielded AUC discrimination values of 0.76–0.78, which were lower than the values obtained with our k-NN models (0.79–0.83) but higher than the values obtained with our LR (0.70–0.74) and SVM models (0.70–0.73). We note that all previously reported studies included lifestyle data (e.g., smoking, physical activity, diet, and medication) in addition to the standard non-invasive parameters used in our models. Accordingly, our newly developed k-NN model outperformed these reported studies.

The uses of tools such as the presented models are two-fold. First, at the individiuals level, these models identify subjects at high risk for T2DM; such subjects can be targeted for prevention programmes that address issues such as awareness, fitness and nutrition. At the population level, the application of these

TABLE 4 | Variables identified as significant (shown in bold) when applying the logistic regression model to the three prediction horizons.

Terms from logistic regression model	Coefficient	Odds ratio (95% CI)	*p*-value
3-YEAR PREDICTION HORIZON			
Constant	**1.636**		**0.007**
Baseline age	**−0.068**	**0.93 (0.92, 0.94)**	**<0.001**
Baseline BMI	**0.0357**	**1.06 (1.04, 1.08)**	**<0.001**
Family history of diabetes	−0.094	0.89 (0.67, 1.17)	0.547
Family history of hypertension	**0.984**	**1.73 (1.28, 2.33)**	**<0.001**
Diagnosis for baseline hypertension	**−0.557**	**0.57 (0.44, 0.75)**	**<0.001**
Sex	−0.11	0.99 (0.77, 1.28)	0.447
5-YEAR PREDICTION HORIZON			
Constant	**1.937**		**<0.001**
Baseline age	**−0.0623**	**0.94 (0.93, 0.95)**	**<0.001**
Baseline BMI	**0.031**	**1.05 (1.03, 1.07)**	**<0.001**
Family history for diabetes	−0.1523	0.89 (0.71, 1.12)	0.239
Family history of hypertension	**0.853**	**1.61 (1.25, 2.08)**	**<0.001**
Diagnosis for baseline hypertension	**−0.408**	**0.63 (0.5, 0.8)**	**<0.01**
Sex	−0.171	0.92 (0.75, 1.15)	0.156
7-YEAR PREDICTION HORIZON			
Constant	**2.0934**		**0.0000**
Baseline age	**−0.0589**	**0.94 (0.93, 0.95)**	**0.0000**
Baseline BMI	**0.0295**	**1.05 (1.03, 1.06)**	**0.0004**
Family history of diabetes	**−0.2389**	**0.84 (0.68, 1.03)**	**0.0400**
Family history of hypertension	**0.8325**	**1.64 (1.3, 2.06)**	**0.0000**
Diagnosis for baseline hypertension	**−0.3106**	**0.69 (0.56, 0.85)**	**0.0137**
Sex	−0.1650	0.92 (0.76, 1.11)	0.1261

BMI, body mass index.

programmes would greatly reduce the national economic burden associated with diabetes care, as these programmes are far less expensive than the treatment of diabetes and its complications. Second, patients identified as high-risk for developing T2DM comprise an interesting cohort from a research perspective. These patients can be monitored in the context of prevention programmes, using more detailed data (such as biochemical markers). Furthermore, these risk assessment tools can also be introduced to the public via online platforms, which would allow individuals to check their risk levels from the comfort of their homes. Such platforms could decrease the number of low-risk patients visiting healthcare facilities and increase the number of high-risk patients that might otherwise have remained ignorant of their risk status. These latter patients could then be invited to participate in a more detailed assessment based on invasive biomarkers.

Interestingly, pre-existing condition of hypertension was associated with a reduction in the likelihood of developing diabetes in all the three prediction horizons (see **Table 4**) though it is known that hypertension and diabetes are "concordant" disorders and represent parts of the overall identical pathophysiological risk profile (41); Positive family history of hypertension was associated with higher odds of developing diabetes in all the three prediction horizons (**Table 4**). Positive family history of diabetes was associated with lower odds of developing diabetes in all the three prediction horizons, though the odds ratio was statistically significant only in the case of 7-year prediction horizon. In a similar manner, increased age at baseline was associated with a lesser risk of developing diabetes (**Table 4**); the study subjects were mostly from the higher risk group of late adulthood and old age (mean age of all participants at baseline was 59.5 ± 11.4 years) and the mean age at baseline was significantly higher for individuals who did not develop diabetes compared to that for individuals who developed diabetes within 7 years from study entry point (61.9 ± 10.8 vs. 54.92 ± 11.05; $p < 0.001$) (**Table 2**). The observed inverse association with age can be partly explained by the general observations (from literature) that in Kuwait the age of onset of T2DM is relatively low—for example, we have earlier reported the mean onset age for T2DM from a larger data set of Kuwaiti natives as 48.63 ± 12.12 years (25).

As mentioned earlier, ROC is the standard technique used to measure test performance and to compare the performance among different models. However, to understand the clinical applicability of the developed models, reporting other performance metrics such as sensitivity and specificity is becoming necessary (42). Our work identified that the best value for sensitivity was obtained with SVM compared to the other two models (LR and k-NN) is only 44% (**Table S1**) and that if the prediction model is applied in clinical practice, it would mean than >50% of the cases will be missed. Though, the models are not readily usable for the clinical predictions, it

TABLE 5 | Comparison of the presented prognostic models with models reported in the literature.

	Study title, citation (prediction horizons)	Model; outcome measure; population; sample size	Predictors used	AUC
1	Current study (3, 5 and 7 years)	k-nearest neighbours; Reports AUC; Population: native Arabs from Kuwait; Sample size: 1,837	Age, BMI, family history of diabetes, hypertensive status, family history of hypertension, and sex	0.83 (3-year), 0.82 (5-year), 0.79 (7-year)
2	Current study (3, 5 and 7 years)	Logistic regression; Support vector machine; Reports AUC; Population: native Arabs from Kuwait; Sample size: 1,837	Age, BMI, family history of diabetes, hypertensive status, family history of hypertension, and sex	LR: 0.74 (3-year), 0.72 (5-year), 0.70 (7-year) SVM: 0.73 (3-year), 0.68 (5-year), 0.71 (7-year)
3	Alssema et al. (29): "The evaluation of screening and early detection strategies for type 2 diabetes and impaired glucose tolerance (DETECT-2) update of the Finnish diabetes risk score for prediction of incident type 2 diabetes" (5 years)	Logistic regression; Reports AUC; Population: Finnish; Sample size: 18,301	Age, BMI, Waist circumference, physical activity, diet, use of antihypertensive medication, history of high blood glucose level, sex, smoking, and family history of diabetes (parent, sibling, or both)	0.77
4	Wannamethee et al. (30): "The potential for a two-stage diabetes risk algorithm combining non-laboratory-based scores with subsequent routine non-fasting blood tests: results from prospective studies in older men and women" (7 years)	Logistic regression; Reports AUC; also reports sensitivity and specificity in the top quintile of the score; Population: British; Sample size: 3,523 men and 3,404 women	Age, sex, family history of diabetes, smoking status, BMI, waist circumference, and hypertension	AUC: 0.77. Sensitivity: 50.3%; specificity: 81.4%
5	Rathmann et al. (31): "Prediction models for incident type 2 diabetes mellitus in the older population: KORA S4/F4 cohort study" (6 years)	Logistic regression; Reports AUC; Population: Germans; Sample size: 1,353	Age, sex, BMI, parental diabetes, smoking, and hypertension	0.76
6	Chen et al. (32): "AUSDRISK: an Australian Type 2 Diabetes Risk Assessment Tool based on demographic, lifestyle and simple anthropometric measures" (5 years)	Logistic regression; Reports AUC; also sensitivity, specificity, and positive predictive values; Population: more than 85% of participants were born in Australia, New Zealand, or the United Kingdom; Sample size: 6,060	Age, sex, ethnicity, parental history of diabetes, history of high blood glucose level, use of antihypertensive medications, smoking, physical inactivity, and waist circumference	AUC = 0.78. Sensitivity = 74% Specificity = 68% Positive predictive value = 13%
7	Rosella et al. (33): "A population-based risk algorithm for the development of diabetes: development and validation of the Diabetes Population Risk Tool (DPoRT)" (9 years)	Logistic regression; Reports C-statistics which is AUC; Population: residents of Ontario in Canada; Sample size: 19,861	BMI, age, ethnicity, hypertension, immigrant status, smoking, education status, and heart disease	0.77
8	Joseph et al. (34): "Incidence of and risk factors for type-2 diabetes in a general population: The Tromsø study" (6 years)	Cox proportional hazard models; Reports hazards ratio; Population: Caucasian subjects from Norway; Sample size: 12,431 men and 13,737 women	Age, BMI, triglycerides, high-density lipoprotein cholesterol, hypertension, family history of diabetes, low education, and smoking	–
9	Kahn et al. (35): "Two risk-scoring systems for predicting incident diabetes mellitus in U.S. adults age 45 to 64 years" (10 years)	Proportional hazard models; Reports risk score derived from proportional hazard coefficients; Population: USA adults with European or African ancestry; Sample size: 12,729	Waist circumference, maternal diabetes, hypertension, paternal diabetes, short stature, black race, age 55 years or older, increased weight, rapid pulse, and smoking history	–
10	Hippisley-Cox et al. (36): "Predicting risk of type 2 diabetes in England	Cox proportional hazards models; Reports hazard ratios;	Ethnicity, age, sex, body mass index, smoking status, family history of	–

(Continued)

TABLE 5 | Continued

	Study title, citation (prediction horizons)	Model; outcome measure; population; sample size	Predictors used	AUC
	and Wales: prospective derivation and validation of QDScore" (10 year)	Population: multi-ethnic from UK; Sample size: 2,540,753 (Model development); 1,232,832 (Model validation)	diabetes, townsend deprivation score, treated hypertension, cardiovascular disease, and current use of corticosteroids	
11	Balkau et al. (37): "Predicting diabetes: clinical, biological, and genetic approaches: data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR)" (9 years)	Logistic regression; Reports AUC; Population: French; Sample size: 1,863 men and 1,954 women	Waist circumference and hypertension in both sexes, smoking in men and diabetes in the family in women	0.71 for men, 0.83 for women
12	Simmons et al. (38): "Do simple questions about diet and physical activity help to identify those at risk of Type 2 diabetes?" (5 years)	Logistic regression; Reports AUC; Population: British; Sample size: 25,633	Physical activity, diet, age, BMI, and family history	0.76
13	Wilson et al. (39): "Prediction of incident diabetes mellitus in middle-aged adults. The Framingham offspring study" (7 years)	Logistic regression; Reports AUC; Population: white and non-Hispanic; Sample size: 3,140	Age, sex, parental history of diabetes, and BMI	0.72
14	Lindström et al. (40): "The diabetes risk score: a practical tool to predict type 2 diabetes risk" (10 years)	Logistic regression; Reports AUC and sensitivity and specificity; Population: Finnish; Sample size: 4,746 (Model development); 4,615 (Model validation)	Age, BMI, waist circumference, history of antihypertensive drug treatment, high blood glucose, physical activity, and diet	0.85 (model development); (0.87) model validation. Sensitivity, 0.78; specificity, 0.77

is to be noted that the work illustrated that machine learning algorithms classify the subjects better than the logistic regression model. It is to be further noted that the models used only a small number of predictor variables (age, sex, BMI, family history of diabetes, pre-existing condition of hypertension, and family history of hypertension); making use of data (when available) on further predictor variables, such as on physical activity and lifestyle, in building the models is expected to improve the performance metrics.

Our study featured a notable strength. While most reported diabetes risk assessment tools were based on LR [see **Table 5** and Abbasi et al. (7)], our study reported models based on machine-learning approaches, specifically the k-NN, with a consistently high discriminatory power. However, our study also had some limitations. First, the selection criteria of subjects for the study may cause bias in the dataset: (i) at baseline, the mean age of all participants was 59.5 $\pm$ 11.4 years, indicating that the study subjects are mostly from the higher risk group of late adulthood and old age who tend to seek medical care for pre-diabetes ailments. (ii) The subjects included in the study were required to have their BMI measurements recorded; physicians usually tend to check the BMI when they suspect obesity or other related ailments in the subjects; and hence the study subjects were already obese or over-weight leading to under-representation of normal-weight and lean subjects. Second, as up to 72% of the participants in this study had pre-existing hypertension at baseline, the study was limited by the absence of information regarding antihypertensive medication use; the observed higher prevalence of hypertension in the study subjects is not surprising as a large number of them were of late adulthood or old age and hypertension is prevalent in such an age group. Third, we did not have access to data regarding lifestyle factors, medication use, and other obesity indicators. Fourth, the models in this study did not account for variations in BMI that might occur during the interval from the date of the baseline measurement to the date of T2DM diagnosis, other changes (e.g., transition from non-hypertension to hypertension) or the administration of a new medication regimen or implementation of lifestyle modifications during the interim period. Fifth, extensive data quality assurance was missing. Finally, the predictive power of the models was not tested in the younger age group and was not validated in an external independent cohort.

In conclusion, our study findings demonstrate that the future risk of diabetes in Arab population can be predicted using non-invasive clinical parameters. Notably, our model based on the machine-learning technique k-NN outperformed those based on LR and SVM, as well as previously reported models, thus demonstrating the need to extend existing models using these machine-learning techniques. However, future studies should concentrate on developing similar models predicting future risk of T2DM in younger age groups to plan prevention programmes as early as possible.

AUTHOR CONTRIBUTIONS

TT undertook the study design, directed the reported work, and directed the development of the manuscript. BF performed all machine-learning algorithms and calculations. AC handled data extraction, created the different data sets, and performed the calculations. TT, BF, and AC developed the manuscript. RA represents Ministry of Health which participated in the generation of Kuwait Health Network data. RA, HA, and DA-A critically reviewed the manuscript and participated in the discussions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00624/full#supplementary-material

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A Systematic Review of Childhood Diabetes Research in the Middle East Region

Saras Saraswathi[1†], *Sara Al-Khawaga*[1,2†], *Naser Elkum*[3] *and Khalid Hussain*[1*]

[1] Division of Endocrinology, Department of Pediatrics, Sidra Medicine, Doha, Qatar, [2] College of Health & Life Sciences, Hamad Bin Khalifa University, Qatar Foundation, Education City, Doha, Qatar, [3] Biostatistics Section, Clinical Research Center, Research Services, Sidra Medicine, Doha, Qatar

Background: Diabetes mellitus (DM) is a common chronic disorder in children and is caused by absolute or relative insulin deficiency, with or without insulin resistance. There are several different forms of childhood DM. Children can suffer from neonatal diabetes mellitus (NDM), type 1 diabetes (T1DM), type 2 diabetes (T2DM), Maturity Onset Diabetes of the Young (MODY), autoimmune monogenic, mitochondrial, syndromic and as yet unclassified forms of DM. The Middle East has one of the highest incidences of several types of DM in children; however, it is unclear whether pediatric diabetes is an active area of research in the Middle East and if ongoing, which research areas are of priority for DM in children.

Objectives: To review the literature on childhood DM related to research in the Middle East, summarize results, identify opportunities for research and make observations and recommendations for collaborative studies in pediatric DM.

Methods: We conducted a thorough and systematic literature review by adhering to a list recommended by PRISMA. We retrieved original papers written in English that focus on childhood DM research, using electronic bibliographic databases containing publications from the year 2000 until October 2018. For our final assessment, we retrieved 429 full-text articles and selected 95 articles, based on our inclusion and exclusion criteria.

Results: Our literature review suggests that childhood DM research undertaken in the Middle East has focused mainly on reporting retrospective review of case notes, a few prospective case studies, systemic reviews, questionnaire-based studies, and case reports. These reported studies have focused mostly on the incidence/prevalence of different types of DM in childhood. No studies report on the establishment of National Childhood Diabetes Registries. There is a lack of consolidated studies focusing on national epidemiology data of different types of childhood DM (such as NDM, T1DM, T2DM, MODY, and syndromic forms) and no studies reporting on clinical trials in children with DM.

Conclusions: Investing in and funding basic and translational childhood diabetes research and encouraging collaborative studies, will bring enormous benefits financially, economically, and socially for the whole of the Middle East region.

Keywords: T1DM, Middle-East, childhood, MODY, insulin-resistance, prevention, epidemiology, registry

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***Correspondence:**
Khalid Hussain
khussain@sidra.org

[†]*These authors share first authorship*

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INTRODUCTION: OVERVIEW OF CHILDHOOD DIABETES MELLITUS

Burden of Diabetes

Recent trends have indicated that the incidence of diabetes is increasing rapidly worldwide (1), with a dramatic increase in prevalence in the Middle Eastern countries, among adults (2) and in children alike (3, 4). This trend is evidenced and emphasized by a 3% increase in the occurrence of this disease in children, in whom it manifests itself in many debilitating ways (1, 4).

According to the IDF Diabetes Atlas—Seventh Edition (3), the number of children (0–14 years) with T1DM in the Middle East and North Africa (MENA) Region is 60,700 and the number of newly diagnosed children each year is 10,200. According to the IDF Diabetes Atlas—eighth Edition (4), the number of adolescents in Qatar with T1DM is 592, and the number of newly diagnosed children and adolescents, per 100,000 children per year is 12.2. Other data, such as for undiagnosed cases of DM, mortality rates, and health care expenditure due to DM are given only for adults. The total health expenditure for the adult population is expected to go from 17.1 billion in 2015 to 30 billion in 2040 (3). There are many consequences due to DM and complications such as stroke, blindness, heart attacks, kidney failure, and amputations can occur. There is a major social cost due to this disease and the risks increase with age, genetic factors, and family history. Those with diabetes are likely to have double the amount of health expenditure than others (3).

It is therefore important to identify the causes of this trend and develop newer therapies through improved research that could result in the development of better treatment and care. Hence in this study, we thoroughly reviewed the published literature to try and understand the types of research reported in childhood DM in the Middle East.

Nature of the Disease—Diabetes

Diabetes mellitus (DM) is a complex, chronic metabolic condition that results in hyperglycemia and is caused by an absolute or relative insulin deficiency with or without insulin resistance. Neonatal diabetes mellitus (NDM) occurs before 6 months of age and is relatively rare. Although Type 1 DM (T1DM) is the commonest form of DM observed in children, Type 2 DM (T2DM) is becoming more prevalent for this age group where the rising numbers are mostly driven by the obesity epidemic (5). Maturity Onset Diabetes of the Young (MODY) can also present during childhood. Autoimmune monogenic forms of DM are a relatively new group of diseases described in children associated with multiple autoimmunities. Other rare forms of DM observed during childhood include mitochondrial DM, syndromic forms of DM and as yet unclassified forms. Cystic Fibrosis (CF) related to diabetes, also known as (CFRD) develops in many patients over time (6). Considering that current epidemiology data about CF in the Middle East is between one in 2000 and 5,800 live births, this is an important area of research (7).

Type 1 Diabetes Mellitus

In a six-center study conducted in the USA, 80% of DM cases were for T1DM for those <9 years of age and 6–76% for those between 10 and 19 years of age (5). T1DM accounts for nearly two-thirds of newly diagnosed patients in the United States, who are <19 years of age (5). In 2006, the number of children with T1DM was estimated by the International Federation of Diabetes (IDF) to be 440,000, an annual increase of 3%, with 70,000 newly diagnosed cases per year (4). Furthermore, the prevalence of cases in individuals younger than 15 years of age is estimated to rise by 70% (8, 9). These epidemiological data suggest an "accelerating" epidemic and serves as a useful indicator of the future burden of T1DM.

T1DM is the most common form of childhood DM and is due to a combinations of factors, such as defective autoimmunity, genetics, and environmental factors. T1DM occurs during early through mid-childhood when pancreatic beta-cells are destroyed, as a result of an autoimmune process, resulting in a lack of insulin production. The autoantibodies facilitate the destruction of the beta-cells over the years, which results in metabolic abnormalities ranging from asymptomatic hyperglycemia to frank DM. The underlying genetic or other mechanisms that trigger the onset of T1DM are not known, but ∼50% of the familial clustering of genes, which increase the susceptibility risk of inheriting T1DM, are located within or in the human leukocyte antigen (HLA) complex on chromosome 6 (10). The highest risk haplotypes (such as HLA-DR4-DQ8 and DR3-DQ2) are known to confer the greatest risk for developing T1DM, particularly when occurring together. However, ∼10% of patients with DM do not carry any of these high-risk HLA class II haplotypes (10).

Autoimmunity in T1DM relies on the detection of insulitis, islet cell antibodies (ICA) and activated beta-cell-specific T lymphocytes (11). These beta-cell-specific autoantibodies are thought to be the molecular markers of the diabetogenic process. Although the type of antibody a patient has is an important indicator of the disease, a patient's progression to develop DM can be predicted more accurately if they have an increasing number of antibodies (12). Insulin Autoantibodies (IAA) tend to appear early on in a child's life with other antibodies [such as Glutamic Acid Decarboxylase (GAD65), insulinoma-2 antigen antibodies (IA-2A), and Zinc transporter-8 (ZnT8)] appearing later (13). The presence of one or more of these autoantibodies increases the risk of developing T1DM (12).

A recent study (14) estimated the prevalence ZnT8A in juvenile-onset T1DM, to establish its utility as a standalone marker for T1DM in subjects who tested negative for other antibodies. This study (14) also investigated ZnT8A's co-existence with other antibodies such as GADA and insulinoma-2 antigen antibodies (IA-2A). When compared to other antibodies, prevalence of ZnT8a (31.8%) was lower than that of GADA (64.7%), but higher than IA2A (19.3%) (14). While 45% of newly diagnosed patients tested positive for ZnT8A, it was uniquely present in 26% of these patients (where patients tested negative for GADA and IA-2A) (14), which was a much higher value when compared to the unique presense of IA-2A in just two patients. Hence, this study (14) found that the combined presence of GADA and ZnT8A were better predictors of T1DM (at 97%) when compared to IA-2A. In one study (15) 32% of cases (in 12 out of 38 children in the study) with T2DM were antibody positive, where the patients were primarily obese and females of pubertal age.

Type 2 Diabetes Mellitus

T2DM is a chronic disease, which is complex and heterogeneous in its manifestations (16). Its risk factors vary with environmental, social and behavioral patterns and are also susceptible to genetic variations. Childhood obesity is the primary cause of T2DM at a young age. The increased prevalence of obesity over the last two decades has increased the number of patients who have T2DM. In the Arab world, it is estimated that the number of diabetic patients (adults and children) will increase by 96.2% by 2035, mostly driven by the increase in T2DM (17). Although genetic factors may be contributing for the increased number of T2DM cases being diagnosed in children in the Middle East, changing the lifestyle that has resulted in urbanization, unhealthy and sedentary life and obesity due to rich food intake, have also contributed to the increased prevalence of T2DM (18).

Maturity Onset Diabetes of the Young (MODY)

Maturity Onset Diabetes of the Young (MODY) occurs due to defects in a single gene. It can affect about 4% of diabetes patients. MODY generally occurs before the age of 25 and typically several family members might be affected (autosomal dominant inheritance pattern). Mutations in 12 different genes have been identified as causative of MODY (19). Encoding the commonest causes of MODY are mutations in the genes Hepatic Nuclear Factor 1 Alpha (HNF1A) and HNF4A and the enzyme Glucokinase (GCK) (15, 19, 20). MODY is commonly misdiagnosed as T1DM or T2DM. A diagnosis of MODY based on genetic testing can benefit patients as some of these patients can be managed by oral sulphonylreas (21).

Neonatal Diabetes

Neonatal diabetes mellitus (NDM) is classified as an early-onset (below 6 months of life) and rare form of diabetes that affects newborns with an increased rate of incidence of 1:90,000 which is nearly four times the number previously reported (19, 22, 23). Transient NDM (TNDM) and permanent NDM (PNDM) are the two main forms of NDM, which are classified according to the duration of the insulin dependency. About 50–60% of the cases are TNDM and the disease is generally expected to resolve in <18 months (24).

NDM in western countries is caused by defects in the *KCNJ11/ABCC8* genes, which encode for the pancreatic beta-cell K_{ATP} channel (25). However, NDM in the Middle East, among Arabic populations has a different genetic basis when compared to westerners (26). Mutations in the Glucokinase (*GCK*) gene is a frequent form of NDM in countries with high consanguinity rate since a homozygous or a compound heterozygous mutation in this gene leads to complete glucokinase deficiency that results in PNDM (27). Higher rate of consanguinity among Arabs makes PNDM mostly likely to occur as part of a recessively inherited syndrome such as Wolcott-Rallison syndrome, Fanconi-Bickel syndrome, and thiamine-responsive megaloblastic anemia and hearing loss (also known as Rogers's syndrome) (26).

Maternally Inherited Diabetes

Organelles such as the Mitochondria, contain circular DNA, called mtDNA. They are inherited through the maternal allele since they are present only in the oocytes. Defects in mtDNA are suspected to cause many diseases that include diabetes (28). The defective mtDNA can gradually cause damage to the beta-cells. The m.3243A>G mutation in the mtDNA (that codes for tRNA leucine) is the cause of this disease in over 85% of the patients. Since this disease is inherited only from the mother, it is called maternally inherited diabetes (29).

Syndromic Forms of Diabetes Mellitus

DM may also be associated with some rare syndromes involving other pancreatic features. Some of these rare syndromes include Wolfram (or DIDMOAD for diabetes insipidus, diabetes mellitus, optic atrophy, and deafness), Wolcott-Rallison, Alstrom, Bardet–Biedl, and Rogers's syndrome. Wolfram syndrome is the association between DM, diabetes insipidus, optic atrophy and sensorineural deafness (30), caused by defects in the WFS1 gene that is the negative regulator of endoplasmic reticulum signaling. Wolcott-Rallison occurs due to an autosomal recessive condition (that is rare), which results in an early presentation of DM accompanied by skeletal dysplasia, growth retardation, and multisystem clinical manifestations due to defects in the EIF2AK3 gene (31). Alstrom syndrome results in loss of vision and hearing, dilated cardiomyopathy and DM (32), caused by defective ALMS1 gene. Rogers's syndrome is due to defects in the SLC19A2 gene. Rogers's syndrome comprises of megaloblastic anemia, DM and sensorineural deafness (33). The clinical features of Bardet-Biedl include rod-cone dystrophy, with childhood-onset visual loss preceded by night blindness, postaxial polydactyly, truncal obesity, and DM (34).

Autoimmune Monogenic DM

Autoimmune monogenic DM is a relatively new group of diseases, where DM is associated with multiple autoimmune defects in these four genes: autoimmune regulator (AIRE) part of autoimmune polyendocrine syndromes (APS) (35, 36), forkhead box P3 (FOXP3) (37), sirtuin 1 (SIRT1) (38), and signal transducer and activator of transcription 3 (STAT3) (39). Defects that occur in any one of these genes can cause autoimmune diabetes that can affect many other organs, suggesting that in some patients, diabetes may be part of a complex autoimmune process involving multiple organs.

Rationale and Scope of This Study

There are several reviews published in the literature which were specially tailored to look at studies published under DM in the Middle East (ME). **Appendix A** lists nine of these reviews. The first review investigates the burden imposed by DM on the Saudi population and recommends ways to mitigate this disease (17). Other reviews discuss the increasing prevalence of DM and advocate a better understanding of the epidemiology and early detection and control of DM among subgroups in the population (40–42). A third review (43) recognizes the paucity of DM related research and publications in the Middle East when compared to other advanced countries. The recommendations

of this review are also along the lines of control of DM through diet and changes in lifestyle (43). A fourth review (44) recognizes that consanguineous marriages, a customary practice peculiar to the Arabic regions, can predispose the population to novel and unique genetic mutations that can cause DM. They emphasize the need for establishing a diabetes registry (44), based on Arab populations that encompasses 22 Arabic speaking countries. They reiterate that the information related to genetic variants in non-Arabic populations discovered elsewhere, will probably be irrelevant for understanding the epidemiology and underlying genetics in the ME populations. They point out that very few registries are currently available among the ME countries and is one of the studies that advocate a collaborative approach to research in DM. The remaining two reviews report on the alarming trends in DM which seem to affect an increasing number of urban, female and younger populations (45, 46).

Although many review papers have been published, they are limited to certain types of diabetes such as T1DM, T2DM (40, 47), and diabetic ketoacidosis (DKA) (48). There is no mention of other types of diabetes such as MODY, autoimmune monogenic forms and other rare forms such as mitochondrial DM and syndromic forms that can affect children. Some of these reviews are limited to only certain ME countries, where the studies took place (43, 49). The nature of these articles are quite diverse but are mostly limited to observational studies that advocate disease control. Some articles provide information on DM in areas outside of the Middle East (50, 51) and some of them do not cover pediatric populations exclusively (41–45). Hence there is a need for a comprehensive review that encompasses all the topics of interest discussed above that pertain only to children and adolescents.

Our study aims to cover all manifestations of childhood diabetes research that has been reported in the Middle East countries. It will investigate the state of research for all the subtypes of DM disease in children, to provide a consolidated and comprehensive view of the current state of affairs in DM. We are not aware of any previously published systematic reviews that have addressed these fundamental research questions on childhood DM in the Middle East.

Aims of This Study

Although childhood DM is common in the Middle East, T1DM, NDM, and syndromic forms of diabetes have a high incidence rate in this region (17, 45). The prevalence of diabetes has steeply increased over the years in the Middle East and the region has been increasingly burdened with childhood DM. The existing reviews do not include studies on all types of DM and there is very little information on studies that investigate the molecular basis of the disease. Hence, we undertook a systematic review of publications that relate to research on childhood diabetes in the Middle East. The key questions we wanted to address were:

a) What types (basic, clinical, and translational) of research has been reported in childhood DM?
b) What impact does this research have on the local population of children in the Middle East?
c) What research strategies are in place to tackle the burden of childhood DM in the Middle East?
d) What funding opportunities are available for childhood DM research in the Middle East?
e) What collaborations exist between different Middle Eastern countries in childhood DM research?

We hope to make recommendations and suggestions for collaborative research related to childhood DM in the Middle East, based on the knowledge gained from this study.

OBJECTIVES

- To systematically review the literature on childhood (aged between 0 and 18 years) DM research in the Middle East region, published between the years 2000 and 2018.
- To summarize the results of studies reporting on childhood DM research in the Middle East.
- To identify key areas and opportunities for research in childhood DM in the Middle East.
- To make recommendations for collaborative research opportunities in childhood DM based on our identification of key areas that need attention to improve diabetic care.

METHODS

We aim to review the state of research in pediatric diabetes in the Middle East region. We broadly follow the guidelines provided by *Agency for Healthcare Research and Quality (AHRQ) Methods Guide* for this comparative effectiveness review (52, 53) and *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)* (54).

Literature Search Strategy and Study Selection

Initially, we identified our objectives (section Objectives) and predefined our search criteria for articles based on these objectives. Four months were earmarked for the literature search and collation of articles by two analysts (SS and SAK). Three months were earmarked for the analysis and review of the articles by senior authors (KH and NE).

We undertook an extensive literature search as suggested by PRISMA (54), to recover articles of primary interest that were published in English. We used the internet to search the electronic bibliographic databases for publications reporting research studies in the Middle East that addressed problems related to diabetes in children and adolescents. The dates included in the search for these studies were over a period of 18 years, between January 1st, 2000 and October 31st, 2018. Search terms and these search strategies are detailed in **Table 1**. Articles that had any of these search terms in their titles, abstracts or keywords list, were collected. EndNote©, a reference management software, was used to share and keep track of the titles and abstracts of articles of interest. A systematic list of articles detailing the eligibility/selection criteria for each of the articles was also maintained in Microsoft© Excel and categorized

TABLE 1 | Search terms and search strategy.

Search terms and search strategy		
Publications	Medical databases such as PubMed and Medline of the National Institute of Health (NIH), Pubtator (a web-based tool that uses advanced text mining methods on PubMed), journals such as NEJM, BMJ, Web of Science, Embase **(**a biomedical literature database**)**, Science Direct, journals related to endocrinology, diabetes and metabolism, Google scholar, global and local pediatric publications in GCC (Gulf Cooperation Council)	
Population	"Children" OR "Adolescents" OR "Childhood" OR "Infants" OR "Teens" OR "Teenagers" OR "Youths"	
and		
Arab countries	"Qatar" OR "Saudi Arabia" OR "KSA" OR "Bahrain" OR "Emirate" OR "UAE" OR "Kuwait" OR "Oman" OR "Egypt" OR "Yemen" OR "Iran" OR "Iraq" OR "Arabian Gulf" OR "GCC" OR "Middle East" OR "Arab"	
and		
Outcome	Adolescent diabetes mellitus	Insulin-dependent diabetes
	Autoantibody and/or antibody	Ketoacidosis
	Autoimmune	MODY
	Childhood	Monogenic
	Childhood diabetes in developing countries	NDM
	Continuous glucose monitoring	Neonatal diabetes
	Diabetes mellitus	Non-insulin-dependent
	Diabetic complications	Prevalence
	Diabetic risk factors	Risk factors
	Diabetic syndrome	Risk of diabetes
	Endocrine and/or polyendocrine	Risk of diabetes in Arabian populations
	Epidemiology	Syndromic
	Gene mutations and/or mutations	T1D
	HLA and/or HLA haplotype	T1DM
	Hypoglycemia	T2DM
	IDDM and/or insulin-dependent diabetes mellitus	Type 1
	Incidence	Type 2
	Insulin pumps	Type 1 diabetes
	Insulin-dependent	Type 2 diabetes

according to year of publication, age, study type, study design, study size, and prevalence of each subcategory of the disease.

Four hundred and eighty-six (486) articles were initially identified through a database search in PubMed, Medline, NEJM, BMJ, Pubtator, Science Direct, and Google scholar (**Figure 1**). We obtained an additional thirty-seven (37) articles through other means such as Google search. Two analysts performed independent analysis of the titles and abstracts to eliminate articles that were unrelated or duplicated, to finally obtain four hundred and fifty-seven (457) abstracts. After removing another twenty-eight (28) irrelevant abstracts, we assessed four hundred and twenty-nine (429) full-text articles for inclusion criteria in this study.

Full-content articles were downloaded for the 429 abstracts and were examined and critically analyzed by two senior reviewers (KH and NE), to evaluate their suitability and to determine any bias in the selection of articles. Articles were removed if their content related to non-Middle-East regions, non-diabetic studies, written in a language other than in English, patient age was above 18, case reports or not peer-reviewed (conference presentations). Once agreement on the included articles was reached, data was extracted from the full-text articles. We used the PRISMA methodology (54) to select the final set of articles for qualitative synthesis in our review. Ninety-five (95) articles that included previously published reviews and reference materials were collected for qualitative synthesis, where the reviews were used only for content comparison. Finally, fifty-three (53) articles, that were published in the Middle East Region that pertained only to diabetic-related clinical or molecular studies were selected for quantitative synthesis (listed in **Appendix B**). Of these articles, nine (9) studies were previously published reviews that were used to determine their contribution to existing knowledge and to identify gaps that are to be filled. The remaining forty-four (44) articles were then separated into sub-groups according to the nature of the disease, as seen in subtitles in section Results of this paper.

Inclusion Criteria

We included all articles that were published in the Arab countries that were related to diabetic studies on children below 18 years of age. We included only full-text publications and original articles that were published in the peer-reviewed journals (**Table 1**) between the years of 2000 and 2018. Articles that were printed locally in Arab countries (**Table 1**), in journals that related to diabetic studies on children below 18 years of age, were also included in our studies. The initial research included all types of research study designs such as meta-analysis, randomized controlled trials (RCT), observational studies, case-control, and cross-sectional studies, although those that were finally selected consisted of mostly prospective or retrospective observational studies.

Exclusion Criteria

We excluded studies that were based only on adult populations, articles that were not written in English, review articles (used only for discussing their content), conference abstracts, and case reports (with one exception that illustrates the use of diabetic monitoring devices).

Data Collection and Quality Determination for Individual Studies

For study identification and data collection, two analysts (SS and SAK) extracted and stored details of the underlying data in an excel database: These details were then used for analysis and scoring of the quality of the studies and quantitative synthesis later on. **Appendix C** lists sample data collection forms that were the basis for the collection of data in each study. **Appendix D** contains the evidence table for the 44 studies, which holds the collection of actual data from all 53 studies selected for quantitative synthesis (nine of these studies are previous reviews used for content discussion). **Appendix E** shows the meta-analysis for collaborative studies that involve multiple countries.

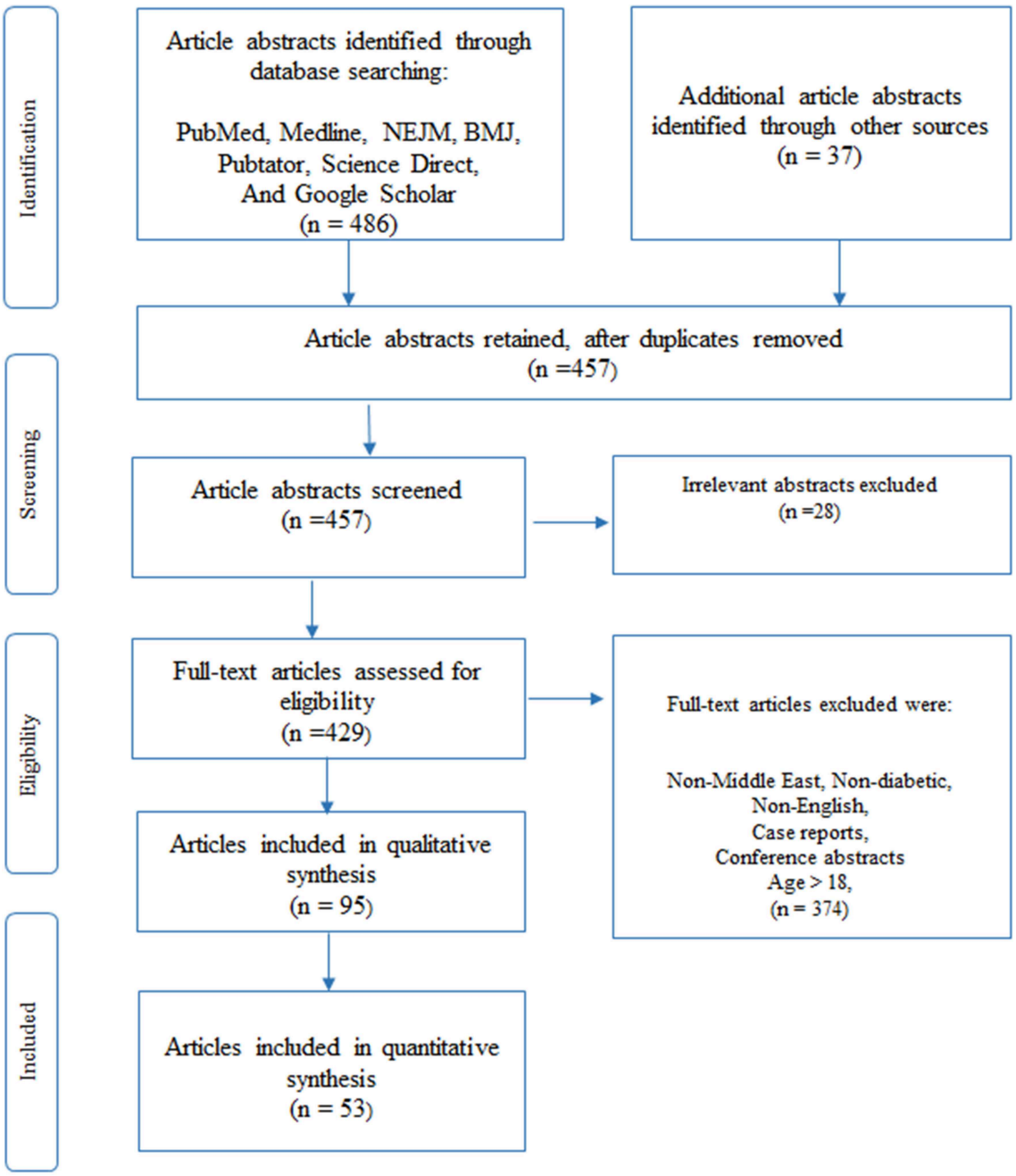

FIGURE 1 | Article selection using PRISMA (54) methodology, as described under the Methods section, was used to select 95 articles for our review of which 53 articles relate to previous reviews (10), clinical and molecular research studies (43) in DM. The given figure is a modified version of the PRISMA methodology (54).

Several criteria were used to determine the quality of each study and the final score was used to classify the selected articles in this study as belonging to levels 1–4, where level-1 studies are of highest quality and level-4 is the lowest, as per the guidelines that are given by *AHRQ methods guide* (52, 53). Quality scores for individual studies are given in **Appendix D** in the evidence table. The following data and quality metrics were collected from each study. A combination of these scores was used to assign the final quality score (levels 1–4) to each study.

- **Study type**: Was it retrospective/prospective observational study or did it involve molecular work? A higher value was given for prospective studies compared to retrospective studies, while molecular studies were given the highest preference.

- **Study design:** Was the type of study regional/national/worldwide? was it a single/multi-center study? A higher value was given for national and/or multi-center studies.
- **Length of study:** Studies that lasted over a year had a higher value.
- **Patient characteristics:** studies with clearly stated details of participant numbers, age and gender were allocated a higher value. Studies with >1,000 participants, even distribution of age groups and equal representation of each gender were valued more.
- **Study quality:** Were outcomes predefined? Any presence of confounding elements/bias? Did the studies include many types of DM? Higher values were given for studies that had defined outcomes, had minimum bias and covered many types of DM included in the study.
- **Study standard:** Did the study have ethical approval, declaration of no conflict of interest (COI) by all authors and was the study funded? Higher values were assigned if the studies had all or at least any two of these standards satisfied.
- **Study outcome:** Did the study have a clear outcome/conclusion that matched its declared aim? Studies that satisfied these criteria were valued more.
- **DM occurrence:** Incidence and prevalence of DM (per 100,000 per year), if these figures were given. Not many studies gave these values clearly in their conclusions. Hence this information was not used to rank the studies, to maintain uniformity.

The studies reviewed were widely varied in many of the criteria listed above such as the number of participants, length of study, type of study, outcomes, and disease covered. This variability did not make it meaningful to combine and compare them under one single criterion. Each study was scored under the common sets of criteria outlined above, as recommended by the *AHRQ methods guide* (52, 53), that *pertain to our study*. Level-1 is ranked as the highest rating and level-4 as the lowest rating. **Figure 2** shows that there were 13 studies categorized as "level-1," which essentially had two of three criteria such as ethics approval, COI declared and/or had funding, in addition to being long term studies with large cohort. There were seven studies that were classified as "level-2," 17 studies which were labeled as "level-3" and six studies that did not meet most of the above criteria, were classified as "level-4." Technology oriented studies were not ranked. Four Molecular studies were automatically assigned high values. If there were no known COI declared then the studies did not come up to a "level-1" grade (most of these studies were from previous years, from older studies). All studies were included in this review since missing information was not a criterion used to eliminate studies. These rankings were used to determine the degree of the contribution that each study made to our article as a whole.

Data Synthesis and Analysis

For the methodological assessment, the following aspects were evaluated: Differentiation and classification between T1DM and T2DM, NDM, MODY, syndromic, autoimmune monogenic, insulin-dependent, estimation of prevalence and incidence.

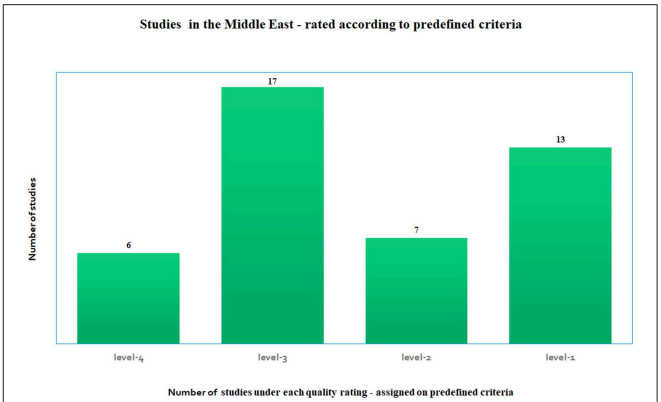

FIGURE 2 | Number of studies under each quality rating—based on predefined quality criteria as satisfied by each study, where level-1 is the highest rating and level-4 is the lowest rating, where the rating is determined according to the *AHRQ methods guide* recommended standards for meta-analysis (52, 53).

RESULTS

Type 1 Diabetes Mellitus in the Middle East

Studies Reporting on the Incidence and Prevalence of T1DM in the Middle East

The rates for childhood T1DM (prevalence and incidence) across the top ranked 10 countries is shown in **Tables 2**, **3** for the year 2015 for age <15 years and for the year 2017 for age <20 years (3, 4).

The International Diabetes Federation has reported (4) that Saudi Arabia has one of the highest numbers (35,000) of children and adolescents (aged 0–19 years) with T1DM. **Table 2** shows the numbers for the prevalence of T1DM in the top 10 countries, where Saudi Arabia's prevalence value increases from 16,200 for ages <15 years (ranked 7th in 2015) to 35,000 in 2017 for ages <20 years (4), although it falls by one rank to 8th place. Although the numbers for 2017 are for a larger group of age <20 (instead of age <15 years), the increase in numbers is much larger even after accounting for the additional number of adolescents this would bring for the years 15–20.

All values discussed below are incidence per 100,000/year. The rates for childhood T1DM across European countries vary between 40 and 67 for Sardinia (40), Sweden (47) and Finland (>60). This study found a higher incidence of T1DM occurring in males (1.3–2.0 times) compared to females, for children aged >15 years (55). The global trend has generally shown a steady increase in childhood-onset of T1DM while the age of onset is much earlier than seen before (55).

In a study conducted in Eastern Saudi Arabia, over a period of 18 years between 1990 and 2007, the average incidence rate for T1DM (438 patients, <15 years) rose from 18.05 in the first 9 years to 36.99 in the second half of the study, for an average

TABLE 2 | Top 10 countries for children diagnosed with T1DM for age <15 years of age in 2015 and for age <20 in 2017 (3, 4, 55).

(Rank) country/territory for <15 years	Number of pediatric patients diagnosed with T1DM in 2015 for age <15 years (3)	(Rank) country/territory for <20 years	Number of pediatric patients diagnosed with T1DM in 2017 for age <20 years (4)
1. USA	84,100	1. USA	169,900
2. India	70,200	2. India	128,500
3. Brazil	30,900	3. Brazil	88,300
4. China	30,500	4. China	47,000
5. United Kingdom	19,800	5. Russian Federation	43,100
6. Russian Federation	18,500	6. Algeria	42,500
7. Saudi Arabia	16,200	7. United Kingdom	40,300
8. Germany	15,800	8. Saudi Arabia	35,000
9. Nigeria	14,400	9. *Morocco	31,800
10. Mexico	13,500	10. Germany	28,600
GLOBAL: NUMBER OF CHILDREN DIAGNOSED WITH T1DM			
Global: number of children (<15 years) with T1DM	542,000	Global: number of children (<15 years) with T1DM	586,000
		Global: number of children (<20 years) with T1DM	1,106,200

*The numbers for 2017 are for a larger group of age <20, but the increase in numbers is much larger than the difference in the additional number of adolescents would bring, for years 15–20. *The data for Morocco, extrapolated from Algeria.*

TABLE 3 | Top 10 countries for children with incidence rates (per 100,000 per year) for T1DM, for age <15 years of age in 2015 and for age <20 in 2017 (3, 4, 55).

(Rank) country/territory	Incidence of T1DM (per 100,000 per year) in 2015: for age <15 years (3)	(Rank) country/territory	Incidence of T1DM (per 100,000 per year) in 2017: for age <20 years (4)
1. Finland	62.3	1. Finland	57.2
2. Sweden	43.2	2. Kuwait	44.5
3. Kuwait	37.1	3. Sweden	39.5
4. Norway	32.5	4. Saudi Arabia	33.5
5. Saudi Arabia	31.4	5. Norway	29.8
6. United Kingdom	28.2	6. Algeria	26.0
7. Ireland	26.8	7. Morocco*	26.0
8. Canada	25.9	8. United Kingdom	25.9
9. Denmark	25.1	9. Ireland	24.3
10. USA	23.7	10. Denmark	23.0
GLOBAL: NEW CASES OF T1DM			
Global: number of new cases of children (<15 years) with T1DM	86,000 (annual increase is 3%)	Global: number of children (<15 years) with T1DM	96,100
		Global: number of new cases of children (<20 years) with T1DM	132,600

*The numbers for 2017 are for a larger group of age <20, but the increase in numbers is much larger than the difference in the additional number of adolescents would bring, for years 15–20. This data is illustrated in **Figure 3**. *The data for Morocco, extrapolated from Algeria.*

increase of 27.52 per year (56). No significant increase in the incidence of T1DM was found in patients below 5 years of age (21% of the cohort) when compared to patients in the age group over 5 years of age. In a 5 years study (2004–2009) in North-West Saudi Arabia in Al-Madinah (57) on children below 12 years of age (419 patients) the mean age of onset was 6.9 ± 3.5 years, with an incidence rate of 29. This study found a higher incidence rate for children between 10 and 12 years of age, with the rate higher in girls (33) than in boys (22), but they did not find any significant annual increase in incident rates (57).

In a study conducted in the Al-Baha region in Saudi Arabia (58) over 10 years (2007–2016), on 471 children below 19 years of age, the prevalence rate of T1DM was dramatically high at 355, which could be cause for great concern. The female to male ratio in the cohort was 1:1.36 where T1DM was more common among girls at 57.5% compared to 42.5% among boys. This number is much higher than those given for other countries outside the Middle East (55).

The incidence of childhood T1DM varies from one country to another globally, as given for the top 10 countries in **Figure 3**

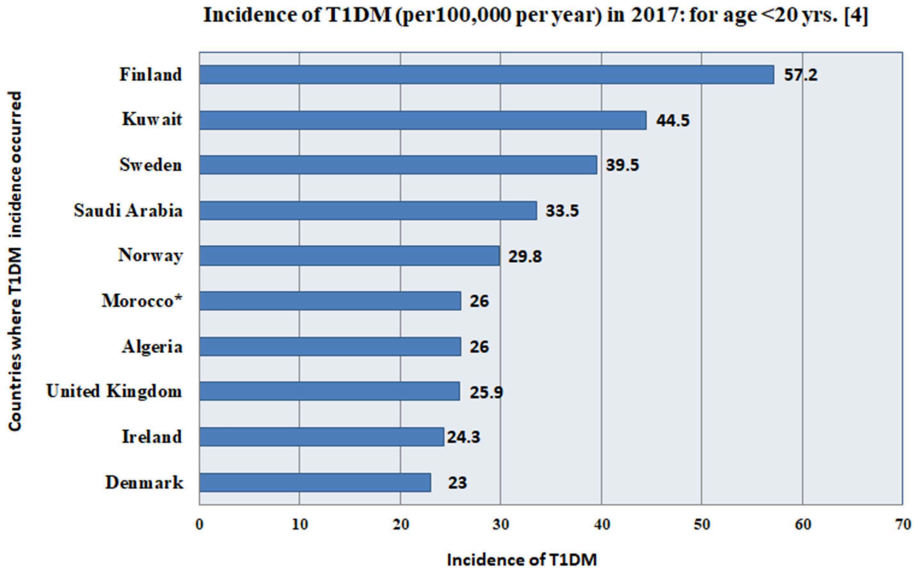

FIGURE 3 | This figure gives the incidence (per 100,000 per year) for the top 10 countries in the world, according to the International Diabetes Federation (4), where, Kuwait and Saudi Arabia rank 2nd and 4th in the world with incidence rates of 44.5 and 33.5 per 100,000, for ages of children and adolescents < 20 years. *The data for Morocco, was extrapolated from Algeria. The details for all 10 countries are shown in **Table 3**.

and **Table 3**. Kuwait and Saudi Arabia ranked 2nd and 4th in the world with incidence rates of 44.5 and 33.5 per 100,000 per year, for ages of children and adolescents <20 years. Kuwait jumped from 3rd rank in 2015 to 2nd rank in 2017, while Saudi Arabia rose from rank 5 to rank 4 (although previous ranks in 2015 were for ages <15 years). The details for all countries are shown in **Table 3**.

Another study from the Eastern Province of Saudi Arabia found no etiological influences in children with new-onset T1DM for any of the following factors such as, maternal age at birth, birth order, birth weight, early introduction of cow's milk and cereals, infections and vaccines as well as nitrate levels in drinking water (59). These factors did not explain the rising incidence of T1DM in this population (59). Data on the national prevalence of T1DM, T2DM, and pre-diabetes in childhood is limited in Saudi Arabia. In one study, the incidence rate was calculated as 109.5 per 100,000 for Saudi Arabia and fifty adolescents and children were newly identified as having T1DM (60). The prevalence rate was highest at 243 per 100,000 for the age group 13–16 years of age, in this study. This study reports that the highest rate of prevalence for T1DM was 162 in the central region, and the lowest was 48 (per 100,000) in the eastern region (60).

The Saudi Abnormal Glucose Metabolism and Diabetes Impact Study (SAUDI-DM) was used to assess the prevalence of T1DM and T2DM, as well as impaired fasting glucose (IFG) among children and adolescents (61). Socioeconomic and demographic information, clinical details and measurements on common tests [weight, Body Mass Index (BMI), and height] were collected from randomly selected adolescents and children who were <18 years of age. The prevalence of diabetes in this cohort was 10.84 and 0.45% were known to have both T1DM and T2DM. Nearly 90% of the people in this study were not aware that they had diabetes. 10.39% of those in this study were newly diagnosed with either having diabetes (4.27%) or impaired fasting glucose (IFG) (6.12%). The statistics obtained from this study indicated that T1DM and T2DM were prevalent at a much higher rate than what was reported by international organizations and this included data on newly identified cases. There were many significant at-risk factors for developing DM and IFG which included data on age, gender, obesity, urbanization, higher income and the presence of lipids which are known to occur as a result of unhealthy diet and lifestyle (61).

Table 4 summarizes the statistics from the reviewed studies that report the incidence and prevalence of DM in Middle East countries such as Saudi Arabia, Kuwait, and Qatar. The incidence for Kuwait is 41.70 in 2017 for children <14 years of age (62). Qatar has lower prevalence at 28.39 for children between 6 months and 14 years of age when compared to Kuwait and the Kingdom of Saudi Arabia (KSA) which have higher prevalence at 39.5 and 29.8, respectively (**Table 3**). But, it still shows a 5.75% increase between the years 2012 and 2016 (**Table 4**) compared to 3–4% increase in Childhood T1DM worldwide (55) in **Table 2**. The rates for East Asian and Native Americans are very low at 0.1 and 8, which are nowhere near the higher rates for all other countries. Perhaps a comparative study that includes differences in the genome, food habits and environmental factors between these countries and those in the Middle East can help to identify causative factors that can help with DM management.

A study from Kuwait reported the incidence of childhood-onset T1DM during the years 2011–2013 for children who were below age 14 (47, 62) and compared it with a previous study done in 1992–1997. This study detected an increasing trend in the incidence of T1DM from 17.7 in the previous study to 40.9 per 100,000 per year in 2011–2013 (2.3 times higher), as

TABLE 4 | Statistics from the reviewed studies on the occurrence of T1DM in the Middle East.

Disease-country	Study period	Age (year)	Study type and design	Study size	Incidence (per 100,000)	References
Kingdom of Saudi Arabia (KSA)	(1990–1998) to (1999–2010)	<19	Observational, case	119	18.05–36.99✖	(3, 4, 56, 57, 59)
KSA Nationwide	2001–2007	<19	Survey, case	45,682	48–162#	(60)
KSA	2010	<15	Observational, case	438	27.5–36.99	(56, 57)
KSA	2018	<19	Observational, case	471	355	(58)
Kuwait	2017	<14	Observational, case	515 (247 boys, 268 girls)	39.3 (boys) 41.70 (girls)	bib62
Qatar (T1DM)	2006–2011	<14	Prospective, case	440	23.15	(47)
Qatar (T1DM)	2012–2016	<14	Prospective, case	440	28.39✖	(47)
Qatar T2DM	2012	5 < age < 14	Prospective. Case	45	1.82⅄	(47)
Qatar T2DM	2012–2011	5 < age < 14	Prospective, case	45	2.7–2.9✖	(47)
Iran	2000–2015	<15	Observational, case	988	13.35☒	(49)

✖*Unadjusted rate. This is an increase of 5.24 since 2011, with 90% CI of 31.82–40.03.*
#On an average the incidence rate was 109.5.
⅄ *With no incidence prior to 2008 recorded.*
✖*A doubling in incidence of 18.94 between the years 1998 and 2010 and* ✖*an increase of 0.88 since 2012.*
☒*Rapid rise from 89 to 134 to 691 new cases.*

detailed in **Table 4** (62). They found a higher incidence rate for girls (44.1) when compared to boys (39.3) (47, 62). Another study from Kuwait aimed to understand the social and metabolic characteristics [lipids, lipoproteins, apolipoproteins, lipoprotein (a), and total sialic acid] and predisposing factors in 6–18-year old Kuwaiti children with T1DM (63). Children's metabolic and social characteristics were affected negatively when compared to those who were normal controls (63).

A prospective cohort study was performed in Qatar to estimate the occurrence of T1DM and T2DM among patients who were below 14 years (47). The aim was to ascertain all new cases of T1DM and T2DM in Qatar, in the only tertiary care center treating children with DM in Qatar. The results, given in **Table 4**, indicate there is an increase in the incidence rates of T1DM and T2DM between the years 2006 and 2016 (47).

Another study from Qatar compared the difference between familial T1DM and non-familial T1DM in terms of the clinical aspects and other biochemical measures such as lab results. This retrospective study, conducted between 2012 and 2016, across a cohort of children and youth with T1DM ($n = 424$), aged between 6 months and 16 years, concluded that familial T1DM was more prevalent in boys than girls (1.4:1, respectively). The prevalence of non-familial T1DM (1:1.1, respectively) did not differ between genders (64). Familial T1DM occurred relatively early in childhood (40.7% before the age of 4 years and 72% before 9 years of age) vs. non-familial T1DM which occurred relatively later in life (80% when they are over 4 years old and 40% after they were 9 years or older). Familial T1DM was more prevalent in boys vs. girls and occurred earlier in childhood compared to non-familial T1DM (64).

TABLE 5 | Country-wise statistics on the occurrence of T1DM in the Middle East from IDF Diabetes Atlas: Country reports 8th Edition (4).

Country	Year	Age	# of children and adolescents with T1DM; (# of newly diagnosed children and adolescents in 2017, per 100,000)
KSA	2017	<19	34,981; (33.5)
Kuwait	2017	<19	5,496; (44.5)
Qatar	2017	<19	592; (12.2)
Iran	2017	<19	9,009; (4.0)
Oman	2017	<19	355; (2.7)
Bahrain	2017	<19	96; (2.7)

A recent study from Iran also found that the annual incidence of T1DM for children under 15 years of age, between the years 2000 and 2015, was 13.35/100,000 (49). Adding the 2nd study period (15 years in total), the study disclosed a rapid rise of incidence as 89, 134, and 691 new diabetes cases for the 1st, 2nd and 3rd years respectively, over a period of 5 years (where new cases were at the rate of 5 per year) (49).

Table 5 reports on country-wise statistics on the occurrence of T1DM in the Middle East from IDF Diabetes Atlas: Country reports 8th Edition (4).

Studies Reporting on the Autoantibody Status in the Middle East

There is limited data on autoantibody status in T1DM in the Middle East. A study was conducted on patients diagnosed

with T1DM and T2DM to determine the prevalence of autoantibodies GAD65 (GADA) and IA-2 antibodies (IA-2A) of Saudi diabetic patients living in Jeddah (65). Eight out of 99 patients who had T2DM tested positive for GADA and three of these patients (who had the disease for a shorter time) were also positive for IA-2A. Here, the association of these autoantibodies was in patients who had an early-onset of T2DM, where GADA was positive in 54% of T1DM and IA-2A was positive in 27%. All patients who had T2DM and who tested autoantibody-positive were treated with insulin therapy (65). In the Middle East, autoantibodies are likely to be found if the disease-onset is at a younger age (65). Female patients with T1DM were more likely to have GADA present (65).

The study from Qatar (as given in **Table 6**) (66) reviewed all clinical and biochemical data, including beta-cell autoimmunity (GADA, ICA, and IAA) over 5 years. These values were analyzed and the results were compared with other studies to measure the prevalence of autoantibodies and their relationship to related diseases. This study reports a higher rate of T1DM occurrence for Qatar compared to the other countries and they reported that these incidences increased over the study period. They also report a higher prevalence of diseases related to the autoimmune abnormalities as shown in **Table 6** and recommend regular screening of patients for these disorders (66). It can be seen from the values in this table that a larger percentage of the T1DM patients have severe autoimmune response compared to the T2DM group, for each of the categories, except for Anti-insulin Ab, where T2DM percentage for prevalence is higher.

Studies Reporting on the HLA Haplotypes Among the Middle East Populations

Table 7 shows the heterogeneity in HLC class II haplotype distribution found among Lebanese and Bahrainis. This table lists the alleles, haplotypes, differing associations, and frequency of homozygous alleles (67). The results of this study, indicate that, when determining a patient's susceptibility to T1DM, with respect to a specific HLA haplotype, the patient's ethnic and racial background needs to be taken into consideration.

T1DM patients in Bahrain have similar associations between DRB1 and DQB1 alleles and diabetes as was found in European populations (who may or may not have Arab descent), such as individuals in Turkey (68), Spain (69), and the United Kingdom (70). These data suggest that diabetes that occurs in children below 5 years of age indicates a high familial risk (70). On the contrary, there was only a weak association between DRB1*040101-DQB1*0302 with T1DM in the Bahraini population and there was no negative association with DRB1*1501-DQB1*06 with T1DM (71).

TABLE 6 | Results of a cross-sectional study conducted in Qatar, on the antibody status in 490 T1DM and T2DM patients during 2012–2016 (66).

Antibody status in T1DM and T2DM (Age 0.5–16 years) over 5 years—2012–2016		
β-cell autoimmunity	**TIDM (431 patients)**	**T2DM (59 patients)**
Anti-GAD (Anti-glutamic acid decarboxylase)	75.5	29.3
Anti β-islet (Ab) (antibody)	53.4	29.4
Anti-insulin Ab (antibody)	40.4	58.3
All 3 antibodies listed above, together in a patient	18.4	No one
Thyroid function	**TIDM**	**T2DM**
Hypothyroidism (FT4 <11.5 pmol/L)	10.6	10.0
Subclinical hypothyroidism	3.5	8
High TPO with normal thyroid function	22.7	23.1
High anti TPO	27.2	34.6
ATT IgA	5	8.7
ATT IgG	4.4	Not detected
Celiac Disease in ATT IgA and IgG positive patients	75% of patients (9/12)	

Studies Reporting on Diabetes Complications

It is estimated that around 96,000 children who are <15 years old will develop T1DM every year and between 13 and 80% of these children are expected to have DKA when they are diagnosed with T1DM. The highest number of cases were found in the UAE, KSA, and Romania and the lowest occurrences were in Canada, Sweden, and the Slovak Republic (48). The frequency of DKA is significantly greater in T1DM adolescents with a higher HbA1c level, lipodystrophy and those who had discontinued insulin treatment (72). Most of the studies relating to DKA in the Middle East are from Saudi Arabia. The UAE and Saudi Arabia have the highest frequencies (80 and 44.9% respectively) of DKA in children at the time of presentation (73).

Infections were the most common precipitating factor for DKA (82.1%) in Al-Baha, Saudi Arabia (74). An episode of DKA was the first clinical presentation of diabetes among 52 (65%) patients (74). In a retrospective study from a single center in Saudi Arabia, the predominant precipitating cause of DKA were viral infections and non-compliance to the insulin regimen of the diagnosed diabetic cases (75). In Al-Madina, Saudi Arabia, DKA affected 55.3% of the patients on disease-onset, where the average age of the patients was 6.7 years and the female: male ratio was 1.4: 1 (76).

In a study from Kuwait of all children diagnosed with T1DM, 36.7% had DKA with young children (0–4 years) at the highest risk (77). Data which was obtained from the Eastern province of Saudi Arabia shows that three-quarters of patients with T1DM had ketoacidosis on presentation (78). In a study from the Pediatric Endocrinology Clinic of the Maternity and Children Hospital, Jeddah, from 2000 to 2014, the most significant independent predictors of DKA were poor compliance with a healthy lifestyle and an excess intake of sweets (79).

Studies Reporting on the Incidence and Prevalence of T2DM

A retrospective cross-sectional study addressed the prevalence of hyperinsulinism and T2DM in overweight and obese Saudi children (80). The overall prevalence of T2DM was 9.04% (80). Among children and adolescents with T2DM, the majority (62.86%) had a body mass index (BMI) $\geq$ 85th percentile, 37.14% had a BMI $\geq$ 95th percentile (80).

A retrospective study from the Al-Ain hospital from UAE characterized the features of T2DM among children and

TABLE 7 | HLA class II haplotypes distribution among Bahraini and Lebanese T1DM patients (67).

Heterogeneity in HLA class II haplotypes in T1DM patients		
Types	**Bahraini-alleles and haplotypes (252 subjects)**	**Lebanese-alleles and haplotypes (189 subjects)**
SUSCEPTIBLE ALLELES AND HAPLOTYPES		
Susceptible Alleles-shared	DRB1*030101, DQB1*0201	DRB1*030101, DQB1*0201
Susceptible Alleles	DRB1*040101	DRB1*130701
Susceptible Haplotype-shared	DRB1*030101-DQB1*0201	DRB1*030101-DQB1*0201
PROTECTIVE ALLELES		
Protective Alleles-shared	DRB1*100101, DQB1*030101	DRB1*100101, DQB1*030101
Protective Alleles		DQB1*050101
Protective Haplotypes-shared	DRB1*070101-DQB1*0201 and DRB1*110101-DQB1*030101	DRB1*070101-DQB1*0201 and DRB1*110101-DQB1*030101
DIFFERENTLY ASSOCIATED		
Susceptible or neutral	DRB1*040101-DQB1*0302 and DRB1*040101-DQB1*050101	
Protective		DRB1*040101-DQB1*0302 and DRB1*040101-DQB1*050101
THE FREQUENCY OF HOMOZYGOUS ALLELES		
Higher	DRB1*03011-DQB1*0201	
Higher		DRB1*110101-DQB1*030101
GENOTYPES		
Major genotype	DRB1*030101-DQB1*0201/DRB1*040101-DQB1*0201	
Less frequent genotype		DRB1*030101-DQB1*0201/DRB1*040101-DQB1*0201

adolescents. Of 96 young people newly diagnosed with DM, 11% were identified as having T2DM (81). The clinical characteristics were: pubertal onset, female preponderance, obesity, strong family history of T2DM, high plasma glucose at presentation, adequate beta-cell reserve and antibody negativity.

A study from Kuwait determined the prevalence of T2DM among patients between the ages of 6–18 years. Children with T2DM were identified at 182 schools (50 primaries, 63 intermediate, and 69 secondaries), randomly selected, using the 2000/2001 educational districts' registers as a sampling frame. T2DM was identified in 45 of the 128,918 children surveyed, thereby giving an overall prevalence of 34.9 per 100,000, with significantly different prevalence for males at (47.3, 95%) compared to females (26.3, 95%), with a trend for increased prevalence with age ($p = 0.026$). The final age-adjusted prevalence values for the Kuwaiti population for T2DM, in the year 2002, was 33.2, 41.6, and 24.6 for overall, male and female groups, respectively (82).

GWAS studies have successfully identified over 80 variants found in T2DM patients with small effect size where the risk for T2DM diabetes increased between 5 and 40%. A majority of these genes regulate insulin secretion while a few regulate insulin sensitivity (83). But, a recent study has indicated that T2DM variants (rs7903146 and rs4506565) in Asian and European populations are not predictors of T2DM in the Qatari population (84). Hence this study suggests that Qatari population might have different variants that might be risk factors for T2DM in this region.

Studies Reporting on the Incidence and Causes of Neonatal Diabetes Mellitus

Several studies conducted in the Gulf region have reported higher incidences of NDM compared to worldwide estimates reaching 1:260,000 live births (85). Incidence of PNDM is 1:31,900 in the United Arab Emirates (UAE) (86), and 1 in 21,196 live births in KSA (85), mainly as part of rare autosomal recessive syndromes. A study from Iran found that Wolcott-Rallison syndrome was a common cause of PNDM (87). In a study published in Oman, genetic abnormalities were found in 15/24 (62.5%) of their patients with PNDM (88).

Maturity Onset Diabetes of the Young

The incidence of monogenic forms of diabetes in childhood has not been identified in Saudi Arabia or any of the Middle East countries (89).

Studies Reporting on Syndromic Forms of Diabetes Mellitus

Autosomal recessive syndromic disorders that are generally considered rare, are highly prevalent in the Arabian Gulf region. The highest incidences worldwide were reported from Saudi Arabia in association with PNDM (85). Fourteen out

of 17 (82.4%) patients had been affected due to inheriting defective genes that cause Wolcott-Rallison syndrome (41%), NDM and hypothyroidism (29.4%), Fanconi-Bickel syndrome (5.8%), and thiamine-responsive megaloblastic anemia (5.8%). Another study from Emirates reported nine out of 25 patients with PNDM (36%) with Wolcott-Rallison Syndrome (86).

Mitochondrial Diabetes Mellitus

No studies have reported the incidences of childhood mitochondrial DM in the Middle East region.

Miscellaneous Forms of Diabetes Mellitus

Very little data is available on the prevalence of mutations in FOXP3, AIRE, SIRT1, and STAT3 in the Middle East, except for a few case-reports from children in the Arabian Gulf region (90).

Studies Reporting on the Use of Technology to Improve Diabetes Management

Added Value in Using Insulin Pumps

A study was conducted in UAE to see if there can be better health perception and patient satisfaction after treatment, if insulin pumps were used by children and adolescents (91). The authors found that it provides more value in terms of treatment and satisfaction, irrespective of the duration of use of the insulin pumps.

Added Value in Using Monitoring Devices That Aid Insulin Control

Another study from the UAE investigated various insulin pump functions and their efficacy in controlling blood glucose. CareLink® Pro 3 software was used for 8–12 weeks (92). They found that if the patient combines the use of Bolus wizard with frequent blood glycemic monitoring, it could help to control blood sugar levels. Another study assessed the benefits of using the flash glucose monitoring system (FGMS) in children and adolescents with T1DM during Ramadan fasting (93). They found that this device could help to fast during Ramadan, without being subject to life-threatening situations that might arise due to hypoglycemia or DKA. Another study from Riyadh, Saudi Arabia, conducted a prospective pilot study of 51 participants with T1DM where they compared the use of the flash glucose monitoring system (FGMS) against the use of continuous subcutaneous insulin infusion (CSII). They did not find much difference among users during Ramadan fasting but found that CSII helped to keep the fluctuations in glucose levels to a minimum (94).

In a study from Qatar, CSII significantly improved glucose control in T1DM children and adolescents who use a standardized protocol. A reduction of HbA1c by 1.6% was achieved after 1 year of CSII initiation (95). A report from Qatar was the first to demonstrate the use of the hybrid closed-loop system in managing a patient with T1DM that resulted in a 1.3% decrease in HBA1c value. The time in range significantly increased to 77% with sensor glucose (SG) values of 139 ± 60 mg/dl, sensor wear of 82% and an auto mode period of 84% per week (96), suggesting that immediate adjustment of the bolus wizard settings such as the ICHR, ISF, and active insulin time should be considered.

DISCUSSION

Childhood DM is a health problem with major health implications in all regions in the Middle East. This review has highlighted the high incidence/prevalence of different types of childhood DM in this region that include NDM, T1DM, T2DM, and syndromic forms of DM. The high incidence of childhood DM in this region imposes a large economic and social burden on the population. We looked at different regions in the Middle East, where the children's population has been affected by DM and its various manifestations. We also discuss the type of studies that we reviewed and discuss the limitations of this study in terms of selection and language bias. Finally, we answer some of the questions for which this study sought answers, in section Aims of this Study.

Our review of the literature suggests that most of the research reported from the Middle East on childhood DM relates to a large number of a retrospective reviews of notes, a few prospective case studies, systemic reviews of the literature, questionnaire-based studies and case reports. A significant number of retrospective studies report on the incidence and prevalence of different types of DM in childhood in the Middle East and as with all retrospective studies these have the potential to be affected (to some degree) by confounding factors and bias. A few prospective studies have reported on the incidence and prevalence of T1DM.

The results of our systemic review did not find any research studies reporting on the establishment of National Childhood Diabetes Registries in any of the Middle Eastern countries. There is a lack of studies focusing on national epidemiology data of different types (such as NDM, T2DM, T2DM, MODY, and syndromic forms) of childhood DM, limited studies on the full complement of autoantibody status (GAD65, Islet, Insulin, and Zinc autoantibodies) in T1DM and HLA haplotype of different populations in the region. Only a few studies report the use of modern technological advances in the management of DM in childhood from the Middle East. Apart from a few research studies in NDM, there is a lack of studies which address the understanding of the molecular basis of rare forms of DM (which are so prevalent in this part of the world) and developing novel therapies or undertaking clinical trials for common or for these rare forms of childhood DM. Finally, it is unclear from our systemic review if there are any national or regional research funding organizations for childhood DM.

National registries hold collective information on diseases of national interest that can be used to plan and regulate healthcare delivery to the population. Childhood DM is one of the major health problems in the Middle East and yet there is no established National Children's Diabetes registry in any of the countries in this region. These registries can influence and improve health outcomes and reduce health care costs. The information in these registries can be used to

competitive advantage by the healthcare providers by adopting best practices. Therefore, establishing National Childhood Diabetes registries is pivotal to the Middle East Region, to advance research and ensure continued health care delivery to the highest standards. National Diabetes registries have been successfully implemented in developed countries such as the United States, Australia, and England (97). Interdisciplinary efforts by registries in these countries, where data is obtained from multiple sources such as physicians, regulatory bodies, national health centers, and other care providers have helped to control and manage the disease and reduce socioeconomic costs (98).

The high prevalence of several different types of childhood diabetes including T1DM, T2DM, NDM, and syndromic forms of DM, provides a unique opportunity to develop research collaborations between the different Middle East countries. However, in our review, there were very few collaborative research studies between the different countries in this region. Government or public health organizations can play a key role in funding and promoting health care programs that will help to reduce the occurrences of chronic illnesses such as the different types of childhood DM. One such implementation program by the national center for chronic disease prevention and the centers for disease control and prevention has helped patients to manage their illnesses better (99). As T1DM is becoming so prevalent in the Middle East the establishment of a reference biochemical/immunology laboratory for measuring diabetes antibodies should be prioritized.

There is very little knowledge that relates to childhood diabetes research-funding opportunities in the Middle East as this information is not freely available. No formal joint funding organizations between different countries have been established which could fund childhood diabetes research in the Middle East. There is a dire need to establish collaborative research funding opportunities for childhood diabetes research in this region. Traditionally funding for registries has been sourced from various stakeholders who might be interested in sharing the data collected, such as foundations interested in the history, progress and therapeutic options available for diabetes, government, insurance and regulatory bodies who are interested in the long-term effects and results of traditional and optional treatments, pharmaceutical and device manufacturing companies, patient groups, private funding, and professional societies. Proactively contacting these institutions or resonding to their request for proposals (RFP) might lead to the discovery of unmet needs that can fulfill the funding requirements (100).

Organizations such as the Diabetes UK, the Juvenile Diabetes Research Foundation (JDRF) provide project grants that support high-quality basic and translational research work on the causes and treatment of diabetes. These funds help to make sure that research is progressive, proper and timely treatments are delivered to the families affected by diabetes and these families are supported and given a voice. The American Diabetes Association works with government and health administrative offices to ensure that enough resources are allocated for diabetes research. Similar funding organizations that are geared to support diabetes studies targeted to the local populations can be set up in the Middle East region.

The Middle East region has an abundant resource of patients with rare and unusual forms (for example NDM and syndromic forms of DM) of childhood DM. Patients with NDM and syndromic forms of DM are rare in the Western world but relatively common in the Middle East region. For example, Saudi Arabia and the UAE have the highest incidences of NDM anywhere in the world. This rich resource of unique patients provides an unprecedented opportunity for undertaking molecular biology research in childhood DM and developing novel therapies for these rare conditions in this region of the world. Understanding the molecular mechanisms of DM in these patients provides fundamental new insights into normal physiological mechanisms involved in the development of DM in the childhood period and for novel disease discovery. More importantly, having a genetic basis for diagnosis can greatly change patient management (for example in some cases of NDM or MODY diabetes).

However, in our review, we were struck by the lack of studies in the Middle East region which focus primarily on understanding the molecular mechanisms of the different forms of childhood DM. Several studies have reported the molecular mechanisms of some types of DM (such as NDM) but the molecular analysis was performed by collaborating with laboratories outside the Middle East region and involved sending blood or DNA samples for analysis to laboratories outside of the region. To address this issue, we suggest that a regional molecular genetics laboratory needs to be established which will serve the needs of all the countries in the Middle East for genetic testing for all forms of childhood DM. A pipeline system should be implemented so that all clinicians can send blood or DNA samples for processing to this regional molecular genetics laboratory (**Figure 4**). This will allow the establishment of a Middle East centralized database and patient registry for all children who are genetically tested for DM.

Lack of Funding Specifically for Pediatric Diabetes Studies

A random sample of 10,501 outcomes reported in the Qatar National Research Fund (QNRF) website (as of February 28th 2019) was extracted. This list consisted of publications or articles (online, journal and conference papers, book chapters, creative work, public report, and patents) that were reported as products of 1,223 unique grant awards over the past years. Of these publications, only 89 grants have the keyword "diabetes" in their title, but none of these 89 titles include the keyword "pediatric." There were only five titles among the list of 1,223 grants, which had the word "pediatric," but none of these studies were related to diabetes. Hence it is reasonable to estimate that there is no specific funding specifically for pediatric diabetic studies and there might be very few exceptions in more recent years, for which publications are yet to be reported under the grants.

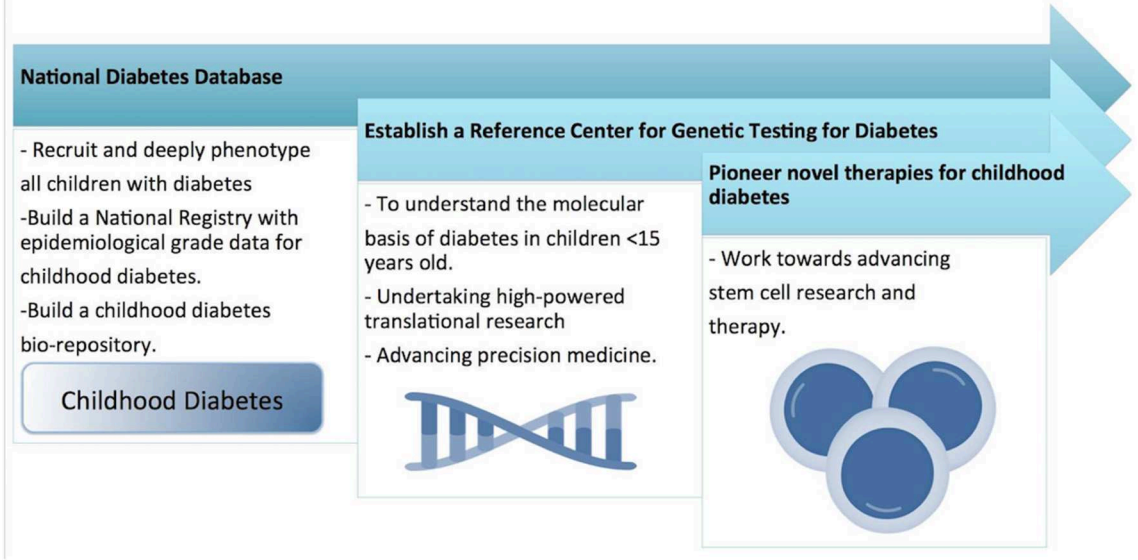

FIGURE 4 | Shows the national research strategy for childhood diabetes, for developing a national wide pediatric diabetes surveillance and intervention system.

Limitations of This Study

This study has several limitations. Firstly, we were not able to establish if there are any local or regional organizations (like Diabetes UK or JDRF) which traditionally dedicate funding for childhood diabetes research in the Middle East. This information was not easily accessible anywhere. It is possible that there are childhood diabetes research funding institutions in the different Middle Eastern countries but we were not able to capture this information. Secondly, we excluded case reports (with one exception) as a measure of research activity and there were a large number of cases published on patients from the Middle East region so this may well be underreported and introduce bias in our analysis. Thirdly there were some publications that report diabetes research outcome measures in both children and adults together. These were again excluded in the final analysis and could represent a source of bias.

Limitations Due to Selection Bias in Using Only English Language Articles

A meta-analysis of 303 studies, has been conducted by Juni et al. (101) to estimate the effect of language bias introduced due to the selection of only English language articles and omission of other language articles published in local journals. Their study found that non-English language articles on trials had a lower number of participants and in some cases reported more significant results. In addition, the quality of the methodology in non-English reviews was lesser than it was in English language articles. In some studies, treatment effects were generally shown to be higher in non-English language publications but were shown to be lesser in other publications. This study concluded that there might be very little difference (as little as 5%) in estimates of treatment effects that were published in English vs. non-English language trials, while there were mixed benefits shown in other studies that were not conclusive. In another study conducted by Egger et al. (102) the authors found that study authors chose English language journals when the results of their studies were more statistically significant [with an odds ratio of 3.75 (95% CI 1.25–11.3)] than when the results were less significant. So, although we recognize that there might be a bias in our review by including only English language articles, there is no clear evidence one way or the other that establishes definite bias if authors select only English language articles. Hence, we opted to include only English language articles.

Limitations Due to Bias in the Selection of Types of Studies

Many of the studies we reviewed were retrospective or prospective observational studies and not interventional or translational. We found that an overwhelmingly large number of papers were publications that were related to non-Arabic or mixed Arabic cohorts related to DM. Most studies were based only on adult populations and comparatively lesser publications were centered on the pediatric populations, which could introduce some population bias in the information obtained. Many of the 43 studies were clinical with only four studies that were considered to be molecular. There were only three studies related to technology. Hence there could be bias in the selection of the type of study due to the non-availability of literature in terms of molecular studies and technology evaluating studies. We also found that there were not many collaborative studies where more than one country was involved. **Figure 5** gives the number of collaborations for each study, which ranges from single country studies to one that has up to seven countries involved in a study.

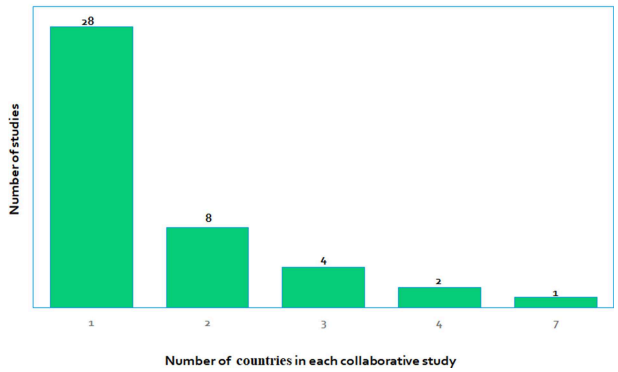

FIGURE 5 | Shows the number of collaborative research studies in the Middle East, with studies involving only one country (28 studies) to some that involve many countries, the highest being seven countries in one of the studies.

Key Questions That Were Answered in This Study

a) What types (basic, clinical, and translational) of research has been reported in childhood DM?
There were no basic or translational research studies. Mostly there were clinical studies, with a few molecular and technological studies.

b) What impact does this research have on the local population of children in the Middle East?
These studies were very relevant to the local population but the emphasis was on the management of the disease rather than on offering better and improved treatment options.

c) What research strategies are in place to tackle the burden of childhood DM in the Middle East?
An increasing number of molecular studies are taking place that identifies factors pertaining to the local population. This can improve the standard of health care for the local population.

d) What funding opportunities are available for childhood DM research in the Middle East?
Only six of the 44 projects were funded in this review. Increasing funding opportunities for childhood DM research is imperative.

e) What collaborations exist between different Middle Eastern countries in childhood DM research?
We found that most studies (28) were stand-alone and performed in one country only, whereas there were other studies where several countries were involved (**Figure 5**), where the number of collaborating countries ranges from 2 to 7.

CONCLUSIONS AND RECOMMENDATIONS

Childhood DM is a major health burden for the Middle East region which needs to be addressed urgently. The incidences of both T1DM and T2DM in childhood are increasing rapidly in the Middle East region and urgent research efforts are needed to be focus on understanding the reasons behind this. Comprehensive national and regional epidemiological data on all types of childhood DM needs to be collected and databases set up. National and regional funding schemes for basic and translational childhood diabetes research should be established with support from central governments. The challenges of childhood DM can only be tackled by undertaking focused research which addresses the issues of regional collaboration, establishing a regional molecular genetics laboratory, building comprehensive epidemiology data, focusing on understanding disease mechanism/s and pathophysiology and establishing a regional childhood diabetes research funding organization. As the field of childhood DM advances and new treatments come on board, the Middle East region should be prepared to embrace and implement the new state of the art research [such as possible islet transplantation, stem cell-based therapies, induced pluripotent stem cell (iPSC) based treatments and immunomodulation therapies] that will benefit patients and undertake clinical trials of potential new therapies for childhood DM. The rich resources of the Middle East need to be channeled so that all children with DM in the Middle East will benefit from such translational research. A new generation of scientists, beta-cell physiologists, epidemiologists, diabetologists, and physicians looking after children with DM need to engage, collaborate, and develop a strategic vision so that they can make strides in this extremely important research area. These initiatives have the potential to manage the burden of childhood DM in the Middle East and improve the quality of lives of all children with diabetes. Investing in and funding basic and translational childhood diabetes research will bring enormous benefits financially, economically, and socially for the whole of the Middle East region.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SS and SA-K carried out the search and screened the titles and abstracts to retrieve papers. KH and NE selected articles of interest. All authors were involved in the writing and editing 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/fendo.2019.00805/full#supplementary-material

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Intermittent Fasting Could Be Safely Achieved in People With Type 1 Diabetes Undergoing Structured Education and Advanced Glucose Monitoring

Ebaa Al-Ozairi[1,2], Abeer El Samad[1], Jumana Al Kandari[1,3] and Ali M. Aldibbiat[4,5]*

[1] DAFNE Unit, Dasman Diabetes Institute, Kuwait City, Kuwait, [2] Faculty of Medicine, Kuwait University, Kuwait City, Kuwait, [3] Ministry of Health, Kuwait City, Kuwait, [4] Department of Diabetes and Endocrinology, James Cook University Hospital, Middlesbrough, United Kingdom, [5] Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Background: Fasting during Ramadan is a form of intermittent fasting in which a person abstains from oral intake between the hours of sunrise and sunset. The fasting month of Ramadan is observed by Muslims worldwide. People with type 1 diabetes (T1DM) who choose to fast during Ramadan are at a particularly high risk of acute diabetes complications including hypoglycemia and significant hyperglycemia. We hypothesized that people with uncomplicated T1DM would be able to fast safely during Ramadan following structured education and with daily advanced glucose monitoring.

Methods: People with stable and uncomplicated T1DM treated with multiple daily injections (MDIs) or continuous subcutaneous insulin infusion (CSII) who chose to fast during Ramadan were recruited for the study. Participants attended Dose Adjustment for Normal Eating (DAFNE) structured education training, and basal insulin was reduced in a controlled fashion. Participants were assigned a sensor-augmented insulin pump or FreeStyle Libre for advanced glucose monitoring. The primary endpoint was the rate of hypoglycemia during Ramadan compared to before Ramadan. Secondary endpoints were percentage time spent <4 mmol/L, >10 mmol/L (range, 4–10 mmol/L), episodes of diabetic ketoacidosis (DKA), and acute kidney injury or hospitalization for any cause.

Results: Rates of hypoglycemia were significantly reduced during Ramadan compared with rates before Ramadan (0.53 ± 0. 49 vs. 0.81 ± 0.69 episodes/day, $p = 0.0015$). No episodes of severe hypoglycemia, DKA, acute kidney injury, or hospitalization occurred during Ramadan period. Percentage time spent >10 mmol/L (46.7 ± 17.7% vs. 42.5 ± 16.4%, $p = 0.03$) was significantly increased, and percentage time [range, 4–10 mmol/L (48.8 ± 15.9% vs. 50.9 ± 15.9%, $p = 0.13$)] and percentage time spent <4 mmol/L (4.7 ± 5.4.7% vs. 5.7 ± 6.3%, $p = 0.09$) were reduced, but these differences were not significant.

Conclusions: People with uncomplicated T1DM could safely participate in intermittent fasting similar to Ramadan fasting if equipped with structured education and advanced glucose monitoring systems.

Keywords: intermittent fasting, Ramadan, CSII, CGM, DAFNE

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Suhad Maatoug Bahijri, King Abdulaziz University, Saudi Arabia

****Correspondence:***
Ebaa Al-Ozairi
ebaa.alozairi@dasmaninstitute.org

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INTRODUCTION

Intermittent fasting has increased in popularity in recent years because of accumulating evidence regarding its favorable metabolic impact on various aspects of human health. Intermittent fasting can reduce cardiovascular risks and improve cardiovascular health (1–3), improve insulin sensitivity and lead to weight reduction (4), mitigate hypertension (5, 6), and reduce inflammation (7). Fasting during the holy month of Ramadan is practiced by Muslims worldwide and involves abstaining from all oral intakes between sunrise and sunset. Typically, patients with underlying disease or illness are exempted from fasting unless it is performed in a controlled environment where risks and complications are minimal or avoided. Certain individuals with T2DM who have a controlled diet or are managed using medications that do not increase the risk of hypoglycemia can fast in a safe manner. However, fasting is not recommended in individuals with T1DM because of their particularly high risk of hypoglycemia. Some individuals with T1DM feel that they are sufficiently healthy to fast during Ramadan despite the safety concerns of their health care professionals. These cases require measured and tailored support in order to minimize potential risks (8, 9). Poorly managed fasting could cause kidney damage, including acute kidney injury secondary to dehydration, which is compounded by the concomitant use of nephrotoxic medications, and hypoglycemia when endogenous counter-regulatory mechanisms fail to match the energy expenditure. Although the benefits of planned and well-supported fasting outweigh the risks in a select group of individuals with T2DM, the primary challenge that patients with T1DM face when attempting to fast is the complete lack of glucose regulation, and thereby, the unacceptable risk of hypoglycemia and significant hyperglycemia. Because of the absence of endogenous insulin production, individuals with T1DM require exogenous insulin to prevent diabetic ketoacidosis (DKA), even in situations wherein glucose levels are not elevated (10). Although glycogenolysis, gluconeogenesis, and negative feedback of insulin secretion are protective against fasting-induced hypoglycemia in normal physiology, they may not have the same impact in individuals with T1DM. Individuals with T1DM have an inherently higher risk of hypoglycemia due to the hypoglycemic effect of unregulated exogenous insulin therapy. Other potentially predisposing risks for hypoglycemia include impaired or loss of glucagon response to hypoglycemia in long-standing T1DM (11), increased risk of celiac disease (12), and increased risk of hypoadrenalism due to the predisposition of a shared autoimmune background (13). A previous study on fasting in patients with poorly controlled T1DM reported wide fluctuations in glucose levels with increased hyperglycemia, more so during the eating period, and significant incidence of hypoglycemia during the fasting period (14). Kadiri and colleagues demonstrated that the rate of hypoglycemia was halved and 2-h postprandial glucose was lower after Iftar (fast-breaking meal) when using insulin lispro compared with regular human insulin in individuals with T1DM treated with basal isophane insulin who fasted during Ramadan (15); albeit risk of hypoglycemia persisting. An assessment of adolescents with T1DM who were treated with either basal-bolus or pre-mixed insulin and who attempted to fast during Ramadan reported that 61.5% of those on basal-bolus and 44% on pre-mixed insulin broke fasting due to hypoglycemia, whereas 7.6% on basal-bolus and 55% on pre-mixed insulin broke fasting due to significant hyperglycemia (16). The current standard therapy approach for T1DM involves basal-bolus insulin treatment with multiple daily injections (MDI) using analog insulin preparations; however, some patients with hypoglycemia or poor glycemic control may require continuous subcutaneous insulin infusion (CSII) via an insulin pump (17, 18). An observational study by Benbarka and colleagues reported a much lower rate of hypoglycemia in patients with T1DM treated with CSII, although one patient (2%) was admitted to the hospital with significant hyperglycemia (19). Khalil and colleagues reported similar observations in patients with T1DM who fasted Ramadan while using CSII, with no severe hypoglycemia and less than half of the minor hypoglycemic episodes occurring during the fasting periods (20). In another study, Alamoudi and colleagues recruited patients with T1DM who were treated with either MDI or CSII who wanted to fast Ramadan. Both groups achieved similar outcomes with under 10% experiencing mild hypoglycemia and around 1% experiencing severe hypoglycemia in both groups (21).

Advanced glucose monitoring systems including flash glucose monitoring (Flash) and continuous glucose monitoring (CGM) have increased in popularity in recent years because of the high level of details provided on glucose monitoring, enhanced level of accuracy, and a growing evidence base for impact and clinical utility provided by clinical trials (22–24). Flash and CGM systems measure interstitial fluid glucose which is intrinsically delayed from blood glucose. The primary difference between Flash and CGM is that CGM provides real-time glucose readings and alarms when glucose is low or getting low, while Flash is user-dependent and provides a glucose reading only when the user actively scans the sensor. Both systems provide trend arrows indicating predicted glucose change.

CURRENT GAPS IN THE LITERATURE

Experts from various countries proposed a consensus guideline for fasting in Ramadan in people with diabetes mellitus (25), which was followed by two subsequent updates (26, 27). These guidelines point out the limited and weak evidence from randomized controlled trials (RCTs) especially in patients with T1DM and Ramadan fasting. The recommendations in the guideline were based on opinion and personal experiences rather than being rigorously tested or evaluated in clinical trials.

The Epidemiology of Diabetes and Ramadan (EPIDIAR) study group, reported a large observational survey of individuals with diabetes who fasted during Ramadan. There was increased rate of acute complications in both T1DM and T2DM groups (28). The T1DM group had a 4.7-fold increase in severe hypoglycemia incidence (with or without ketoacidosis). However, 78% of patients were treated with twice-daily pre-mixed insulin. The standard of care varied significantly between participating centers with 10.3% not receiving regular diabetes

care and only 68% of those in the T1DM group received Ramadan specific treatment recommendations with 67% were self-monitoring their blood glucose.

The National Institute for Health and Care Excellence (NICE) has recommended the implementation of structured educational programs to enhance individuals' knowledge regarding diabetes and improve their management (29). Educational programs train individuals to safely cope with specific situations, such as exercise or illness; however, there is a lack of education on fasting and insulin therapy. One of the most effective educational programs is the Dose Adjustment for Normal Eating (DAFNE) course, a 5-day structured educational program for adults with T1DM to empower them to adjust their insulin dose according to grams of carbohydrates consumed in their food. Several studies have reported improved HbA_{1C} and quality of life without an increased risk of hypoglycemia (30, 31). This UK-based educational program was adopted and adapted for use in the Kuwait region. Dasman Diabetes Institute has been endorsed by DAFNE UK to be the training center for DAFNE in the Middle East. In addition to the DAFNE standard curriculum, a fasting-specific educational session on safe fasting during Ramadan is conducted annually for all DAFNE graduates. The session seeks to gauge participants' views and preferences on fasting and reviews common foods consumed during Ramadan along with structured therapy recommendations for both MDI and insulin pump users.

STUDY HYPOTHESIS

In the present study, we hypothesized that structured education and advanced glucose monitoring could facilitate safe intermittent fasting during Ramadan without increasing the risk of hypoglycemia or hyperglycemia in individuals with uncomplicated T1DM.

METHODS

Design

This non-randomized open-label study included patients with type 1 diabetes (T1DM) who were treated with CSII or MDI, who were DAFNE graduates and were either on, or provided with, CGM (CSII participants) or Flash (MDI and CSII participants). The primary endpoint was the rate of moderate-to-severe hypoglycemia or clinically significant hyperglycemia with or without DKA. Secondary endpoints included the rate of hypoglycemia, percentage time spent <4 mmol/L, >10 mmol/L, or in the range of 4–10 mmol/L, episodes of DKA, acute kidney injury, or hospitalization for any cause during Ramadan compared to the preceding and following months.

Setting

Participants were recruited, and the study was conducted at Central DAFNE Kuwait, with approval from the Institutional Review Board at the Kuwaiti Ministry of Health (2016/435). All included participants provided informed written consent.

Study Cohort and Inclusion/Exclusion Criteria

All Kuwaiti residents (Arabs) with T1DM aged $\geq$18 years attending Dasman Diabetes Institute for their diabetes care who chose to fast during Ramadan (May 17, 2018 through June 15, 2018) were assessed for suitability by the diabetes care multi-disciplinary team (MDT) at Central DAFNE Kuwait. Exclusion criteria included $\geq$1 episodes of severe hypoglycemia in the previous 12 months, poor diabetes control defined as HbA_{1C} $>$ 75 mmol/mol (9%), established kidney disease defined as estimated glomerular filtration rate (eGFR) $<$ 60 mL/min/1.73 m^2 with or without albuminuria, T1DM diagnosed $<$1 year ago, $\geq$1 episodes of DKA in the previous 12 months, inability to monitor capillary glucose for a minimum of 4 times a day, not being DAFNE graduate, inability to attend Ramadan-specific DAFNE workshop before beginning of Ramadan 2018, and treatment with pre-mixed insulin preparations. Patients with any of these conditions were advised against fasting.

Patients treated with CSII were provided with NovoRapid for the insulin pump (Novo Nordisk, Bagsværd, Denmark), whereas those treated with MDI were administered insulin glargine U100 (Sanofi, Gentilly, France) and NovoRapid insulin. All patients underwent advanced glucose monitoring. Those treated with CSII were either on Medtronic MiniMed 640G with Guardian Link and Enlite 2 sensor or Medtronic Paradigm Veo insulin pump with either MiniLink and Enlite-2 sensor (Medtronic, Northridge, CA, USA) or Abbott FreeStyle Libre Flash system (Abbott Diabetes Care Inc., Alameda, CA, USA). Participants treated with MDIs where either existing users of FreeStyle Libre or they were started on it after dedicated training in preparation for fasting. All participants received standard capillary glucose monitoring using the Contour Next glucose meter (Ascensia Diabetes Care, Basel, Switzerland). Subjects were provided with ketone testing strips to use with the FreeStyle Libre or Optium Neo ketone monitoring system (Abbott Diabetes Care Inc., Alameda, USA), if they were not on Flash, to use when feeling unwell or when glucose was $>$14 mmol/L (250 mg/dL).

Structured Education and Diabetes Management During Ramadan

DAFNE course and Ramadan-specific DAFNE workshop were delivered in Arabic, the participants' mother tongue. Decisions on fasting during Ramadan and support during the fasting period were made using a stepped approach. One-on-one consultations were scheduled within 2 weeks before the start of Ramadan with each subject who fulfilled the inclusion/exclusion criteria and planned to fast. Expectations were set and full instructions on safe fasting were discussed. Participants deciding to proceed agreed to follow an insulin modification plan during the fasting month. They were directed to use the highest pre-Ramadan insulin-to-carbohydrate ratio (ICR) for Iftar and to use the lowest meal ICR for Suhoor (pre-dawn meal). Those on MDIs were instructed to reduce their basal insulin by 20% if they were on a once-daily dose or reduce the evening-morning dose by 20% if they were on a twice-daily dose. Participants on CSII had a Ramadan-specific basal profile programmed into their devices with a 20% reduction

in basal rates from dawn until 2 h before sunset, followed by a 30% reduction over the next 2 h (the time of breaking the fast) when the basal rate was increased by 10% for 2 h before returning to the standard basal rate until dawn. Furthermore, participants were trained to use a temporary basal tool to manage hypoglycemia or hyperglycemia when necessary. All patients treated with CSII were given insulin pens (NovoRapid FlexPen and Lantus SoloStar pen) to use in case of pump malfunction or failure. Continuous use of glucose sensors (CGM or Flash) was emphasized and encouraged. SMBG check was requested upon waking, at midday, at 3 p.m., before Iftar, before Suhoor, at midnight, and before going to sleep in addition to other relevant times including before driving and when feeling unwell or hypoglycemia is suspected. All included participants were invited to a 2-day educational workshop conducted by the MDT before the start of Ramadan. The workshop consisted of a carbohydrate counting refresher session with emphasis on the most commonly consumed foods and drinks during Ramadan. Participants were advised to break their fast when they developed hypoglycemia (SMBG < 4.0 mmol/L, 72 mg/dL), when their glucose sensor indicated hypoglycemia (glucose < 4.0 mmol/L, 72 mg/dL) or when hypoglycemia was impending (downward arrow with expected glucose to reach <4.0 mmol/L, 72 mg/dL in 15 min), when glucose was >14 mmol/L (252 mg/dL) with an upward arrow, when ketones were >1.5 mmol/L with or without hyperglycemia, or when the patient felt unwell. All participants were provided ketone testing strips to use when glucose was >13 mmol/L (234 mg/dL) or when they felt unwell regardless of glucose level. All participants were contacted daily via phone to check on their well-being and to collect information on any hypoglycemic episodes and the number of days fasted. They were also provided with a 24-h helpline to contact the study team when urgent advice is required. Participants were asked to ensure family members are aware of their participation in the study and to call for an ambulance if they feel very unwell. They were also invited to DAFNE clinics during Ramadan whenever their presence was considered clinically necessary.

Standard demographic data were collected for all participants. A baseline assessment was performed on all participants within 1 month before Ramadan including HbA_{1C}, weight, and body mass index assessment, current insulin regimen, hypoglycemic history, and diabetes-related complications. These assessments were repeated at the end of Ramadan and 1 month after Ramadan.

Statistical Analysis

All devices were downloaded before Ramadan, at the end of Ramadan, and 1 month after Ramadan. Data were separated for the periods before, during, and after Ramadan, and the Ramadan period was separated into periods of fasting and eating. Participants not including a minimum of 1 complete day of glucose data in their profiles were excluded. Average glucose was calculated for all glucose values in each captured period divided by the number of days of valid data collected. Time spent >10 mmol/L (180 mg/dL), time spent in the range of 4–10 mmol/L (72–180 mg/dL), time spent <4 mmol/L (72 mg/dL), and time spent <3.0 mmol/L (54 mg/dL) were calculated as percentages of overall captured glucose data for the time periods outlined. All continuous episodes of hypoglycemia were assessed when glucose values were <4 mmol/L for a minimum of 3 consecutive logged readings. The rate of hypoglycemia was calculated as the total number of hypoglycemic episodes divided by the total number of days in each period. Glucose variability (GV) was evaluated with the coefficient of variation (CV) and the slope index (SI). The CV was calculated as the standard deviation divided by average glucose for the calculated period then multiplied by 100. SI was calculated according to the following formula:

$$SI = \frac{\sum_{t0}^{tn} \left| \frac{dG}{dT} \right|}{total\ days}$$

Where SI is slope index, t is time point, dG is change between consecutive glucose values, and dT is change in time interval for each glucose value. SI measures the total intensity of glucose changes during the examined period.

Percentage of area under the curve for time spent >10 mmol/L (180 mg/dL) was calculated according to the following formula:

$$\%AUCt > 10 = \frac{\sum_{t0}^{tn} \left(\frac{g1+g2}{2} \right) * (t2 - t1)}{total\ days}$$

Where AUCt > 10 is area under the curve for time spent >10.0 mmol/L (180 mg/d/L), t is time point, and g is glucose values >10 mmol/L.

Similarly, area above the curve for time spent <4.0 mmol/L (72 mg/dL) was calculated according to the following formula:

$$\%AACt < 4 = \frac{\sum_{t0}^{tn} \left(\frac{4}{t2-t1} \right) - \left(\left(\frac{g1+g2}{2} \right) * (t2 - t1) \right)}{total\ days}$$

Where AACt < 4 is the area above the curve for time spent <4.0 mmol/L, t is time point, and g is glucose when glucose <4 mmol/L.

The paired Student t-test was used to measure significant differences between means of parameters in different periods and groups. Differences were considered significant when p values were <0.05.

RESULTS

A total, 43 participants were enrolled in the study, their characteristics are summarized (**Table 1**). All subjects educational level was of high school and above. Pre-existing diabetes-related complications were present in 5 (12%), all of which were diabetic retinopathy except 1 case related to cardiovascular disease (CSII group). All participants were graduates of the DAFNE structured education program. In the CSII group, 12 participants were managed with Medtronic MiniMed 640G and 9 were managed with Medtronic Paradigm Veo insulin pump (CSII). All CSII subjects had augmentation with an Enlite-2 sensor through the Guardian Link and MiniLink CGM system except 3 with Paradigm Veo who used Abbott FreeStyle Libre (FSL) Flash. Participants were on CSII therapy for 4.2 ± 2.4 (mean ± SD)

TABLE 1 | Summary of participants' characteristics.

	All	CSII	MDI	*P* value
Number	43	21	22	
Gender F (%)	21 (49)	10 (48)	11 (50)	
Age	31.7 ± 82	34.6 ± 8.6	30.3 ± 7.7	NS
BMI	23.6 ± 10	25.9 ± 7.5	21.5 ± 11.7	NS
Duration of Diabetes	16 ± 6.5	15 ± 6	16.7 ± 8.5	NS
HbA1c %	7.7 ± 1.1	7.2 ± 0.8	8.2 ± 1.2	0.002
Diabetes related complications	5	2	3	
Enlite-2 CGM (%)	18 (42)	18 (86%)	0 (0%)	
FreeStyle Libre (%)	25 (58)	3 (14)	22 (100%)	

Data presented as absolute numbers or means ± SD as relevant. Comparison was made between the CSII group and MDI group. P value < 0.05 was considered statistically significant. NS, not significant.

TABLE 2 | Comparison between subjects according to the number of days fasted in Ramadan.

	Days fasted out of 29 days duration of Ramadan		
	20–29 days	10–19 days	<10 days
Total number	23 (54%)	16 (37%)	4 (9%)
Number of females	9 (39%)	10 (63%) (NS)	2 (50%)
Duration of diabetes (years)	16 ± 7.5	16 ± 8.3 (NS)	11.5 ± 3.5
HbA1C (%)	7.4 ± 1.01	7.8 ± 1.1 (NS)	8.6 ± 0.9
BMI kg/M^2	23 ± 1.3	24 ± 11.2 (NS)	23.3 ± 2.9
CSII/MDI %	78/22%	19/81%*	0/100%

*NS, not significant; *indicates statistically significant difference (p < 0.05) between CSII and MDI groups. Data presented as mean ± SD.*

years prior to the study. The MDI group included 22 participants, all of whom had concomitant use of Flash. The average duration of Flash use was 5.4 ± 4.6 (mean ± SD) months before the start of the study. Participants fasted for a median of 23 days (range, 4–29). More than half of participants (54%) fasted between 20 and 29 days out of 29 fasting days in Ramadan 2018, while 37% fasted between 10 and 19 days and 9% fasted <10 days. There were significantly more subjects treated with CSII (78%) in those who fasted 20–29 days while other parameters including duration of diabetes, number of females, HbA1C, and BMI were comparable with those who fasted 10–19 days (**Table 2**). Reasons for not fasting included menstrual period in female participants, work related-causes, not feeling well (not related to diabetes), and glucose disturbance during the eating period.

Diabetes Control and Hypoglycemia

Total daily dose (TDD) of insulin was 8.3% lower during Ramadan than before Ramadan and was accompanied by a small but significant rise in glucose by 0.5 mmol/L (9 mg/dL) during Ramadan compared with before Ramadan (**Table 3**). There was a non-significant drop in HBA_{1c} after Ramadan compared to before Ramadan [61 ± 8.7 vs. 58 ± 8.5 mmol/mol (7.7 ± 1.1% vs. 7.5 ± 1.1%)] ($p = 0.2$). CGM and Flash profiles showed a significant 4% increase in percentage time spent >10 mmol/L (180 mg/dL) with no significant difference for percentage time spent in the range of 4–10 mmol/L (72–180 mg/dL) or time spent <4.0 mmol/L (72 mg/dL) (**Figure 1**). No DKA or hospital admission occurred during Ramadan.

None of the participants experienced severe hypoglycemia during the fasting period. The number of hypoglycemic episodes detected on glucose monitoring was significantly reduced during Ramadan compared with rates before Ramadan (0.53 ± 0.48 vs. 0.81 ± 0.69 hypoglycemic episodes/day, mean ± SD, $p = 0.0014$) (**Figure 2**). Moreover, reduction in time spent <3.0 mmol/L (54 mg/dL) and time spent <4.0 mmol/L (72 mg/dL) as well as the area above the curve for time spent <4.0 mmol/L (72 mg/dL) were also observed but was only significant in the CSII + CGM group (**Table 3**).

Glucose Variability

In the overall cohort, no difference was observed in glucose CV during Ramadan compared with before or after Ramadan; however, it was significantly lower in the CSII + CGM group during Ramadan compared to before Ramadan (**Table 3**). Interestingly, the reduction in glucose CV in the CSII + CGM group was maintained after Ramadan (**Figure 3A**). Similarly, SI was smaller during Ramadan than before Ramadan, an effect that was maintained after Ramadan in the whole cohort (**Figure 3B**), denoting a reduction in GV.

Fasting vs. Non-fasting Periods During Ramadan

Average glucose was significantly lower during the fasting period compared with the eating period, although the difference was small [10.0 ± 1.9 vs. 10.8 ± 2.9 mmol/L (180 ± 34.2 vs. 194.4 ± 52.2 mg/dL), respectively, $p = 0.01$]. The overall rate of hypoglycemia was lower during Ramadan compared to before or after Ramadan, however, a small but significant increase in the rate of hypoglycemia occurred during the fasting period compared to the eating period (0.31 ± 0.28 vs. 0.18 ± 0.19 hypoglycemic episode/patient/day, respectively, $p = 0.003$). During the fasting period, participants spent significantly more time in the range of 4–10 mmol/L [72–180 mg/dL (percentage time spent was 50.7 ± 16.3 vs. 45.3 ± 8.5%, respectively, $P = 0.017$)] and less time spent >10 mmol/L (180 mg/dL) compared to the eating period (percentage time spent was 44.6 ± 17.9 vs. 50.5 ± 21.4%, $p = 0.019$), with similar time spent <4 mmol/L (72 mg/dL) in both periods (percentage time spent 5.0 ± 5.2 vs. 4.5 ± 4.1%, $p = 0.66$) (**Figure 4**).

DISCUSSION

The present study met its primary and secondary endpoints and showed that it was possible for a group of individuals with uncomplicated T1DM who received structured education and used advanced glucose monitoring to safely observe intermittent fasting during the month of Ramadan without an increase in hypoglycemia and no incidence of severe hypoglycemia or significant hyperglycemia while maintaining reduced GV during the fasting month. While the EPIDIR study, demonstrated increased risk of hypoglycemia, including severe hypoglycemia,

TABLE 3 | Summary of fasted days, insulin, and glucose data analysis from CGM or Flash for the periods before, during, and after Ramadan.

Parameters	Patients groups								
	Total (no. 43)			CSII + CGM (no. 18)			MDI + Flash (no. 22)		
	Before Ramadan	During Ramadan	After Ramadan	Before Ramadan	During Ramadan	After Ramadan	Before Ramadan	During Ramadan	After Ramadan
Days fasted	–	20 ± 8	–	–	25 ± 5	–	–	15 ± 8‡	–
Days included in glucose analysis	15 ± 9	19 ± 8	20 ± 11	15 ± 8	21 ± 6	16 ± 9	17 ± 8	18 ± 9	22 ± 11
TDD	51.2 ± 25.7	47.0 ± 23.7*	48.8 ± 23.7*	48.8 ± 20.7	45.0 ± 20.7	47.8 ± 20.1	54.6 ± 31.0	49.9 ± 27.4	51.0 ± 28.1
TBI	22.0 ± 8.5	20.2 ± 8.4	21.0 ± 8.4	21.1 ± 8.2	18.8 ± 7.9	20.7 ± 8.5	23.2 ± 9.1	21.7 ± 9.1	21.7 ± 8.6
Average glucose	9.8 ± 1.9	10.3 ± 2.0*§	9.7 ± 1.6	9.0 ± 0.7	9.6 ± 1.1*§	9.1 ± 0.8	10.4 ± 2.3	10.7 ± 2.4§	10.0 ± 1.7
HbA1c	7.7 ± 1.1	–	7.5 ± 1.1	7.1 ± 0.8	–	7.1 ± 0.8	8.2 ± 1.2	–	7.8 ± 1.2
% time spent <3 mmol/L	2.19 ± 4.4	1.5 ± 2.3	2.0 ± 2.7	1.1 ± 1.3	0.6 ± 0.8*	0.6 ± 0.9	3.6 ± 6.3	2.4 ± 3.0‡	3.1 ± 3.2‡
% time spent <4 mmol/L	5.7 ± 6.3	4.7 ± 4.7	8.4 ± 12.3	4.8 ± 3.4	2.8 ± 3.0*§	3.0 ± 3.0*	7.2 ± 8.7	6.5 ± 5.4	12.6 ± 15.5
% time spent 4–10 mmol/L	50.9 ± 16.0	48.8 ± 15.9	49.7 ± 18.7	60.1 ± 8.1	56.1 ± 10.6§	61.3 ± 9.3	45.6 ± 14.7‡	43.1 ± 15.6‡	42.8 ± 19.6‡
% time spent >10 mmol/L	42.5 ± 16.4	46.7 ± 17.7*§	42.0 ± 15.4	35.1 ± 7.8	41.1 ± 12.2*§	35.7 ± 9.5	47 ± 18.6	50.8 ± 18.9	44.6 ± 15.8
No. Hypo per day	0.81 ± 0.69	0.53 ± 0.48*	0.61 ± 0.48	1.21 ± 0.65	0.60 ± 0.49*§	0.70 ± 0.54*	0.49 ± 0.56‡	0.46 ± 0.49	0.58 ± 0.47
AUC for time spent >10 mmol/L	132.2 ± 65.2	146.6 ± 73.1§	126.6 ± 56.5*	106.12 ± 24.3	124.5 ± 43.3*§	104.3 ± 30.1	150.0 ± 76.3‡	161.3 ± 81.8	135.3 ± 55.1*‡
AAC for time spent <4.0 mmol/L	1.10 ± 1.83	0.74 ± 0.94	0.86 ± 1.02	0.74 ± 0.65	0.43 ± 0.51*	0.43 ± 0.50	1.52 ± 2.67	1.06 ± 1.16	1.20 ± 1.22
CV	39.7 ± 6.2	39.8 ± 8.2	38.6 ± 7.1	38.5 ± 4.5	35.6 ± 5.2*	34.6 ± 5.2*	42.3 ± 6.5	42.1 ± 9.6‡	41.3 ± 6.7‡
Slope index	8.29 ± 5.19	7.22 ± 4.47*	6.66 ± 4.25*	13.55 ± 1.85	11.89 ± 1.86*	12.04 ± 0.94*	3.68 ± 0.65‡	3.40 ± 0.91‡	3.44 ± 0.61‡

*Data presented for all cohort (total) and for sub-groups CSII + CGM and MDI + Flash. TDD, total daily dose; TBI, total basal insulin; AUC, area under the curve; AAC, area above the curve; CV, coefficient of variation. *Indicates statistically significant differences ($p < 0.05$) between Ramadan and before the Ramadan periods within same group. §Indicates statistically significant differences between Ramadan and after the Ramadan periods within same group. ‡Indicates statistically significant differences between CSII + CGM group and MDI + Flash groups for the corresponding period. Data presented as mean ± SD.*

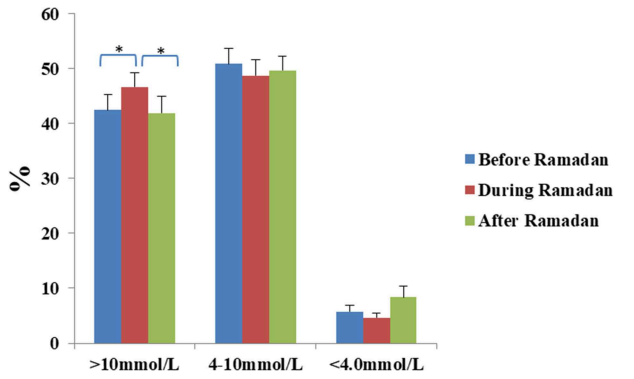

FIGURE 1 | Percentage time spent >10 mmol/L, between 4 and 10 mmol/L, and <4 mmol/L for the periods before, during and after Ramadan in the whole cohort. Data represents mean ± SEM, *represent statistically significant differences ($p < 0.05$).

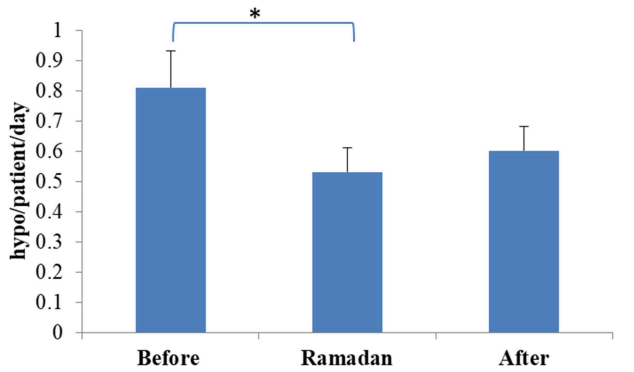

FIGURE 2 | Rate of hypoglycaemia before, during, and after Ramadan. Data represents mean ± SEM, *represent statistically significant differences ($p < 0.05$).

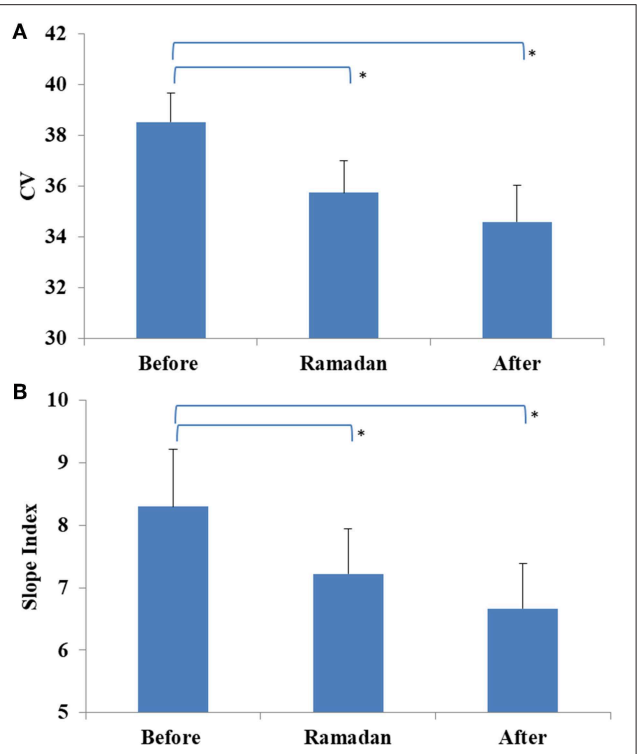

FIGURE 3 | Glucose variability profiles measured before, during, and after Ramadan, including Coefficient of variation (CV) for CSII + CGM group **(A)** and Slope Index (SI) for the whole cohort **(B)**. Data represents mean ± SEM, *represent statistically significant differences ($p < 0.05$).

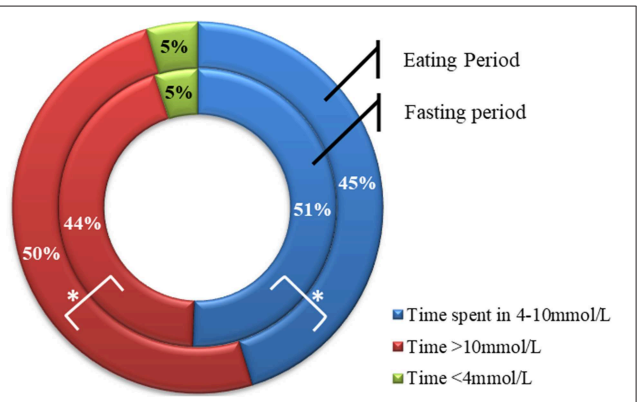

FIGURE 4 | Percentage time spent >10.0 mmol/L, between 4 and 10 mmol/L, and <4 mmol/L during fasting (inner circle) and eating (outer circle) periods. Data represents means. *represent statistically significant differences ($p < 0.05$).

and hyperglycemia/DKA in those with diabetes mellitus who fasted Ramadan (28), other studies reported better outcomes in those with T1DM who fasted Ramadan following Ramadan specific education (32), used analog insulin compared to human insulin (15, 33), or used insulin pumps (19, 20, 34). Alamoudi recently reported similar outcomes in those on CSII compared to those on MDI using analog insulin preparations during Ramadan fasting, though severe hypoglycaemia was reported in 1.3% (21).

Several factors contributed to the favorable outcome of our study. First, participants with uncomplicated T1DM were carefully selected for inclusion in the study, as metabolic stress and the relative state of dehydration anticipated during the fasting period could aggravate pre-existing complications. This is particularly important in those with diabetic nephropathy with reduced GFR because of the increased risk of hypoglycemia in the present cohort (35) as well as the risk of pre-renal acute kidney injury secondary to dehydration. Moreover, previous severe hypoglycemia is the strongest predictor of recurrence (36), and those with poor diabetes control may not engage safely in glucose monitoring and diabetes management during the fasting period, which increases their risk of deterioration. Second, providing all participants with DAFNE structured education and emphasizing food options commonly consumed during Ramadan empowered them to manage their insulin in light of the dietary changes experienced during Ramadan. Third, providing all participants with advanced glucose monitoring sensors enabled them to gain deeper insight into their glucose changes, and therefore,

mitigate hypoglycemia and hyperglycemia the majority of the time. Finally, proactively modifying insulin regimens led to effective therapy with minimal hypoglycemia.

The findings in the present study are relevant to people with T1DM who choose to observe fasting during Ramadan as well as to other individuals with T1DM who intend to fast for other reasons. The study demonstrated in our cohort of people with uncomplicated T1DM who underwent structured diabetes education and used advanced glucose monitoring that they can fast safely whether treated with MDI or CSII. Although the number of participants was small and the study was not a randomized trial, the safety outcomes were novel and quite encouraging in this cohort. Further validation in larger randomized controlled trials is required. In the meantime, the protocol adopted in this study could serve as a safe guiding blueprint for individuals with T1DM who intend to observe fasting.

ETHICS STATEMENT

The study protocol was reviewed and approved by the Institutional Review Board at the Kuwaiti Ministry of Health (Approval No. 2016/435). Written informed consent was given by all participants. This was in both Arabic, the mother tongue of all participants, and English in case that was required. All subjects were adults and able to consent. Subjects were aware and able to exit the study whenever they wished so. No animals were used in this study.

AUTHOR CONTRIBUTIONS

EA-O developed the study design and concept, conceived the study, acquired the data, and wrote the manuscript. JA contributed to the recruitment of the participants, acquired the data, handled nutrition analysis, and participated in the critical revision of the manuscript. AE contributed to the recruitment of the participants, acquired the data, handled the data, and drafted the manuscript. AA handled data analysis, interpretation, and writing the manuscript. All authors have read and given their approval to the final manuscript.

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Patterns of Obesity and Overweight in the Iranian Population: Findings of STEPs 2016

Shirin Djalalinia[1,2], *Sahar Saeedi Moghaddam*[2], *Ali Sheidaei*[2,3], *Nazila Rezaei*[2], *Seyed Sina Naghibi Iravani*[2], *Mitra Modirian*[2], *Hossein Zokaei*[2], *Moein Yoosefi*[2,3], *Kimiya Gohari*[2,4], *Ahmad Kousha*[5], *Zhaleh Abdi*[6], *Shohreh Naderimagham*[2], *Ahmad Reza Soroush*[7], *Bagher Larijani*[8] *and Farshad Farzadfar*[2]*

[1] Deputy of Research and Technology, Ministry of Health and Medical Education, Tehran, Iran, [2] Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran, [3] Department of Biostatistics, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4] Department of Biostatistics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, [5] Department of Health Education and Promotion, Faculty of Health Sciences, Tabriz University of Medical Sciences, Tabriz, Iran, [6] Department of Research and Education, National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran, [7] Obesity and Eating Habits Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran, [8] Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

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Mohamed Abu-Farha, Dasman Diabetes Institute, Kuwait

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***Correspondence:**
Farshad Farzadfar
f-farzadfar@tums.ac.ir

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Background: Obesity has become a common health problem all over the world. Benefiting from a national representative sample, the present study aimed to estimate the prevalence of overweight/obesity and the distribution of Body Mass Index (BMI) levels in the Iranian adult population, by sex, age, and geographical distribution.

Methods: This was a large-scale national cross-sectional study of Non-communicable Diseases risk factor surveillance in Iran. Through a systematic random sampling cluster, 31,050 Iranian adult participants aged 18 years and over were enrolled in the study. The main research tools were used to assess three different levels of data, namely: (1) demographic, epidemiologic, and risk-related behavioral data, (2) physical measurements, and (3) lab measurements. Anthropometric measurements were taken using standard protocols and calibrated instruments.

Results: In 2016, the national prevalence rates of normal weight, obesity, and overweight/obesity among Iranian adults were, 36.7% (95% CI: 36.1–37.3), 22.7% (22.2–23.2), and 59.3% (58.7–59.9), respectively. There was a significant difference between the prevalence of obesity among males [15.3% (14.7–15.9)] and females [29.8% (29.0–30.5)] ($p < 0.001$). The 55–64 [31.5% (30.1–33.0)] and the 18–24 [8.3% (7.3–9.4)] year-old age groups had the highest and lowest prevalence of obesity, respectively. The results show a geographical pattern at provincial level, where the level of BMI increases among populations ranging from the southeastern to the northwestern regions of the country. The highest provincial prevalence of obesity was almost 2.5-fold higher than the lowest provincial prevalence.

Conclusion: We found a significant difference between the prevalence of obesity in males and females. Moreover, there was a considerable difference in the geographical pattern of the prevalence of obesity and overweight. Further evidence is warranted to promote strategies and interventions related to prevention and control of factors that are associated with weight gain.

Keywords: obesity, overweight, BMI, STEPs, Iran

EBM RATINGS WILL BE BASED ON A SCALE OF 1 TO 5, AS FOLLOWS

Level I: Evidence obtained from at least one properly designed randomized controlled trial, systematic review and meta-analysis, and experimental study.

Level II: Evidence obtained from well-designed controlled trials without randomization.

Level III: Evidence obtained from well-designed cohort or case-control analytic studies.

Level IV: Evidence obtained from multiple time series with or without intervention, such as case studies. Dramatic results in uncontrolled trials might also be regarded as this type of evidence.

Level V: Opinions of respected authorities, based on descriptive studies, narrative reviews, clinical experience, or reports of expert committees.

The present paper reveals the most updated results of overweight/obesity and BMI at national and subnational levels in 2016. Using proportional to size sampling, one of the main strengths of the study was its comprehensive protocols focused on standards and regulatory guidelines.

INTRODUCTION

Obesity has become a common health problem, and its prevalence as a global pandemic continues to increase in many developed and developing countries (1–3). According to recent analyses, obesity is probably the most important of the four prominent global risk factors that fulfill the criteria for being governmental health priorities.

It has been estimated that worldwide, 603.7 million adults (overall prevalence of 12.0%) were obese in 2015 (4). Compared to 1980, this prevalence has doubled in 73 countries (4). In 2015, globally, a burden of about 4.0 million deaths (7.1% of all deaths) and 120 million disability-adjusted life years (DALYs) (4.9% of all DALYs) were attributed to overweight and obesity (4).

In 2005, the estimated mean number of deaths attributable to excess Body Mass Index (BMI) in Iranian males and females were 13,000 and 17,000, respectively (5).

Based on the results of a systematic review which included evidence from January 2005 to January 2014, the range of overweight and obesity prevalence among the Iranian adult population at subnational level was between 12.8–76.4 and 2.4–35.4%, respectively (6).

The STEPs survey in 2011 showed that, overall, 22.3% of Iranian adults aged ≥15 years were obese (14% of males and 27.7% of females) (7). In 2011, Population Attributable Fraction (PAF) analysis showed that at least 33.78, 10.25, and 30.56% of the prevalent diabetes mellitus (DM) could be attributed to overweight (BMI ≥ 25 kg/m^2), general obesity (BMI ≥ 30 kg/m^2), and central obesity (waist circumference ≥ 90 cm), respectively (8). However, we have no new information following the STEPs survey of 2011 (9).

The World Health Organization (WHO) Action Plan for the control and prevention of Non-communicable Diseases (NCDs) and Sustainable Development Goals (SDGs target 3.4) requests member states to reduce the unconditional probability of deaths due to NCDs for people aged 30–70 years by modifying lifestyle and metabolic risk factors, including obesity and overweight. Based on the global agenda of risk reduction, having reliable information on the level, trend, and distribution of NCD risk factors—including overweight and obesity—is crucial for designing, implementing, and evaluating National Action Plans at country level (1, 10, 11).

Benefiting from national and subnational representative samples of STEPs survey 2016 (9), the present study estimates the prevalence of overweight/obesity and the distribution of BMI levels among the Iranian population, by sex, age, and geographical distribution.

METHODS

Following the WHO STEPwise approach to NCD risk factor surveillance, we designed and conducted the national STEPs survey 2016, with representative samples from urban and rural areas of Iran (12). The details of the procedures and methods of STEPs 2016 are discussed elsewhere (9). Here, we only point out some essential requirements.

Sampling

We used the national postal code database, which includes addresses of all residential buildings in the country, as the sampling frame. Through systematic proportional to size cluster sampling, we selected proportional to size samples from rural and urban areas within each province. In sum, 3,105 clusters and 31,050 participants were selected from 18 ≤ year-old Iranian adults. The main research tools were used to assess three different levels of data, namely: (1) demographic, epidemiologic, and risk–related behavioral data, (2) physical measurements, (3) lab measurements (12–14). After collecting information from each household regarding age, sex, and other demographic

characteristics of every member, we included the individuals of each household whose age was 18 years and above. We randomly excluded one or more individuals if a household had two or more individuals in the same age-sex group (age groups were 18–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70, and above). We also excluded those with severe physical and mental conditions, which made them unable to be interviewed. For all included participants, we filled a questionnaire, measured anthropometric indices, and took blood and urine samples for lab tests (lab tests were done for those aged 25 years and above). The full details of the sampling protocol as well as sample size calculation have been fully described elsewhere (9).

Questionnaire

Following the WHO STEPwise approach to risk factor surveillance, trained experts were involved in the processes of sampling and examinations. Information was recorded through validated questionnaires containing: demographic characteristics, lifestyle information (e.g., nutrition, physical activity, smoking, and alcohol consumption), history of metabolic risk factors and treatment, history of injuries and their risk factors, health care utilization, and screening programs (9).

Physical Measurements

A team of trained healthcare experts performed the examinations under standard protocols using calibrated instruments (9). Systolic and diastolic blood pressures were measured thrice at a time interval of 5 min, and the mean values of the second and third instances were used for further analyses. Weight was measured in light clothing to the nearest 100 g, and height was measured to the nearest 1 cm while the participants were standing, without shoes, with shoulders in a normal position. The BMI of each individual was calculated as weight (kg) divided by squared height (m^2). The WHO criteria were used, so that individuals with BMI $<$ 18.5, 18.5 $\leq$ BMI $<$ 25, 25 $\leq$ BMI $<$ 30, 30 $\leq$ BMI were considered underweight, normal, overweight, and obese, respectively. Obese individuals were divided into class I obese (30 $\leq$ BMI $<$ 35), class II obese (35 $\leq$ BMI $<$ 40), and class III obese (40 $\leq$ BMI) (1, 15).

Laboratory Measurements

The collected blood and urine samples were stored under a temperature of 4°C in vaccine transfer boxes. They were transferred in $<$18 h as the shortest possible time—to the central processing and archiving laboratory of study in the Non-Communicable Diseases Research Center (NCDRC) of the Endocrinology and Metabolism Population Sciences Institute of Tehran University of Medical Sciences. All the collected samples were examined by unique brands of devices and kits in the NCDRC laboratory (9).

Definitions of Variables

Education was defined as the number of successfully completed years of schooling and was categorized into four subgroups [0 (Illiterate), 1–6, 7–12, and $>$12 years]. Principal component analysis was used to calculate the participants' wealth index from household asset data. The participants' calculated wealth indices were categorized into five quintiles from the poorest (first quintile) to the richest (fifth quintile). Diabetes based on Fasting Plasma Glucose (FPG) was defined as FPG $\geq$ 126 mg/dl or self-reported [based on the intake of Oral Hypoglycemic Agents) OHA and/or insulin injection]. Diabetes based on HbA1c was defined as HbA1c $\geq$ 6.4% or self-reported (OHA and/or insulin injection). Pre-diabetes based on FPG was defined as 100 $\leq$ FPG $<$ 126 mg/dl among those who were not recognized as diabetic. Pre-diabetes based on HbA1c was defined as 5.7 $\leq$ HbA1c $<$ 6.4% among those who were not recognized as diabetic. Hypertriglyceridemia was defined as serum triglycerides $\geq$ 150 mg/dl. LDL–C was considered as a level of low-density lipoprotein cholesterol $\geq$ 100 mg/dl. Hypercholesterolemia was defined as total cholesterol $\geq$ 200 mg/dl or self-reported drug intake. Hypertension was defined as a systolic blood pressure $\geq$ 140 mmHg, or a diastolic blood pressure $\geq$ 90 mmHg, or self-reported drug intake. Pre-hypertension was defined as 120 $\leq$ systolic blood pressure $<$ 140 mmHg or 80 $\leq$ diastolic blood pressure $<$ 90 mmHg among those who were not recognized as hypertensive. Participants were considered as "ever tobacco smokers" if they reported an experience of any tobacco derivatives during their lifetime. "Ever daily cigarette smokers" and "current daily cigarette smokers" were defined, respectively, as those with an experience of cigarette smoking on a daily basis during their lifetime, and currently smoking on a daily basis. The incidence of heart attack and stroke were defined as self-reported histories of events within the past year.

Statistical Analyses

The data were used for presenting the descriptive statistics of interested variables, by sex and age subgroups at national and subnational levels. Moreover, the specifications of geographical data sampling enabled us to provide the interest outcomes at provincial level.

The national prevalence rates and means (for all ages) have been presented with 95% Confidence Intervals (95% CI) in the tables. Age-standardization of provincial mean BMI and prevalence of each BMI for defined categories was achieved according to the 2016 National Population and Housing Census conducted by Iran's Statistical Center[1]. The aforementioned results have been presented in maps, with a combination of area of residence and sex.

While assessing laboratory indices with different BMI categories, individuals who were 25 years and older were taken into consideration. The age-adjusted Odds Ratio (OR) of BMI categories with respect to smoking status, anthropometry (pre-hypertension and hypertension), and laboratory (pre-diabetes, diabetes, LDL-C, hypertriglyceridemia, hypercholesterolemia) and self-reported incidence of cardiovascular diseases (heart attack and stroke) variables were calculated by logistic regression at three significance levels. These analyses were performed by Stata software (version 11) and R software (version 3.0.2).

[1]https://www.amar.org.ir/english/Population-and-Housing-Censuses

TABLE 1 | Demographic characteristics of participants.

Variable	Characteristics	Sex		
		Female $n = 15,044$	Male $n = 14,080$	Both $n = 29,124$
Area of residency	Rural	4,467 (14.9%)	4,118 (14.0%)	8,585 (28.8%)
	Urban	10,577 (36.6%)	9,962 (34.6%)	20,539 (71.2%)
Age category	18–24 years	1,420 (4.9%)	1,231 (4.5%)	2,651 (9.4%)
	25–34 years	3,529 (12.1%)	3,328 (11.6%)	6,857 (23.7%)
	35–44 years	3,203 (11.0%)	3,048 (10.5%)	6,251 (21.5%)
	45–54 years	2,867 (9.7%)	2,571 (8.7%)	5,438 (18.4%)
	55–64 years	2,198 (7.5%)	2,025 (6.9%)	4,223 (14.4%)
	65–69 years	759 (2.6%)	616 (2.1%)	1,375 (4.7%)
	70 years and more	1,068 (3.6%)	1,261 (4.3%)	2,329 (7.9%)
Education	Illiterate	2,959 (9.9%)	1,400 (4.7%)	4,359 (14.6%)
	1–6 years	3,938 (13.3%)	3,390 (11.5%)	7,328 (24.8%)
	7–12 years	5,392 (18.8%)	6,202 (21.7%)	11,594 (40.5%)
	More than 12 years	2,755 (9.5%)	3,088 (10.7%)	5,843 (20.2%)
Marital status	Never married	1,937 (6.8%)	2,411 (8.8%)	4,348 (15.6%)
	Married	11,008 (37.7%)	11,338 (38.9%)	22,346 (76.6%)
	Divorced/separated	401 (1.4%)	116 (0.4%)	517 (1.8%)
	Widowed	1,619 (5.6%)	147 (0.5%)	1,766 (6.1%)
Basic insurance	No	998 (3.5%)	1,271 (4.6%)	2,269 (8.1%)
	Yes	14,011 (48.0%)	12,784 (44.0%)	26,795 (91.9%)
Type of basic insurance	Health insurance	5,599 (19.1%)	5,265 (18.2%)	10,864 (37.3%)
	Social insurance	6,307 (21.8%)	5,769 (19.9%)	12,076 (41.7%)
	Army insurance	611 (2.1%)	563 (2.0%)	1,174 (4.1%)
	Imam Khomeini Relief Foundation insurance	131 (0.5%)	47 (0.2%)	178 (0.6%)
	Other insurances	1,363 (4.5%)	1,140 (3.8%)	2,503 (8.2%)
	No insurance	998 (3.5%)	1,271 (4.6%)	2,269 (8.1%)
Complementary insurance	No	11,715 (40.4%)	11,117 (38.7%)	22,832 (79.0%)
	Yes	3,174 (11.0%)	2,860 (10.0%)	6,034 (21.0%)
Wealth index quintile	Poorest	3,019 (10.4%)	2,616 (9.2%)	5,635 (19.6%)
	2	2,988 (10.6%)	2,644 (9.4%)	5,632 (19.9%)
	3	2,919 (10.3%)	2,757 (9.7%)	5,676 (20.0%)
	4	2,868 (10.1%)	2,818 (10.0%)	5,686 (20.1%)
	Richest	2,825 (10.1%)	2,906 (10.4%)	5,731 (20.4%)

Data presented as number and percent (%).
All values were calculated among those with a non-missing variable.

Ethical Considerations

Ethical approval for the study was obtained from the Ethical Committee of the National Institute for Medical Research Development (NIMAD) (ID: IR.NIMAD.REC.1394.032). Participation in the study was voluntary. Regarding ethical considerations, the objectives and methods of the study were described for all eligible individuals and written informed consent was obtained from all those who accepted to participate.

RESULTS

In the STEPs study, of the 30,541 participants who completed the questionnaires (step 1), 30,042 individuals were anthropometrically measured (step 2). In the current study, we included 29,124 participants (15,044 females and 14,080 males) who had non-missing BMI values. Most of the participants were urban residents (71.2%), aged 25–34 years (23.7%), educated (14.6% were illiterate), married (76.6%), and were covered by basic insurance (91.9%). The demographic characteristics of the participants are presented in **Table 1**.

In 2016, the national prevalence of normal weight, obesity, and overweight/obesity in 18≤ year-old Iranian adults was estimated at, 36.7% (95% CI: 36.1–37.3), 22.7% (22.2–23.2), and 59.3% (58.7–59.9), respectively. In the obese group, the distribution of the three obesity categories were estimated at 16.9% (16.4–17.3) in class I obese, 4.6% (4.3–4.9) in class II obese, and 1.3% (1.1–1.4) in class III obese (**Table 2**). With respect to differences in sex, there was a significant difference between the prevalence of obesity in males [15.3% (14.7–15.9)] and females [29.8% (29.0–30.5)] ($p < 0.001$). This difference was also detected in the national prevalence rate of overweight/obesity [M: 53.6% (52.7–54.4), F: 64.7% (63.9–65.5) ($p < 0.001$)] (**Table 2**). Compared to females, Iranian males had a lower mean BMI [25.6 (25.5–25.7) vs. 27.4

TABLE 2 | Distribution of different categories of BMI prevalence (%) according to selected characteristics of Iranian adults.

Variable	Characteristics	Underweight (BMI < 18.5)	Normal (18.5 ≤ BMI <25)	Overweight (25 ≤ BMI <30)	Overweight/ obesity (25 ≤ BMI)	Obesity (30 ≤ BMI)	Class I obesity (30 ≤ BMI < 35)	Class II obesity (35 ≤ BMI < 40)	Class III obesity (40 ≤ BMI)
Overall		4.0 (3.8–4.2)	36.7 (36.1–37.3)	36.6 (36.0–37.1)	59.3 (58.7–59.9)	22.7 (22.2–23.2)	16.9 (16.4–17.3)	4.6 (4.3–4.9)	1.3 (1.1–1.4)
Area of residency	Rural	6.2 (5.7–6.8)	42.2 (41.1–43.2)	32.1 (31.1–33.1)	51.6 (50.5–52.7)	19.5 (18.6–20.4)	14.5 (13.7–15.3)	4.0 (3.6–4.5)	1.0 (0.8–1.2)
	Urban	3.1 (2.8–3.3)	34.5 (33.8–35.2)	38.4 (37.7–39.1)	62.4 (61.7–63.1)	24.1 (23.4–24.7)	17.9 (17.3–18.4)	4.8 (4.5–5.1)	1.4 (1.2–1.5)
Sex	Female	3.7 (3.4–4.0)	31.6 (30.8–32.4)	35.0 (34.2–35.8)	64.7 (63.9–65.5)	29.8 (29.0–30.5)	20.9 (20.2–21.6)	6.8 (6.4–7.3)	2.0 (1.8–2.2)
	Male	4.3 (4.0–4.6)	42.1 (41.3–43.0)	38.3 (37.4–39.1)	53.6 (52.7–54.4)	15.3 (14.7–15.9)	12.6 (12.0–13.2)	2.2 (2.0–2.5)	0.4 (0.3–0.6)
Age category	18–24 years	10.8 (9.6–12.0)	59.2 (57.3–61.2)	21.6 (20.0–23.3)	30.0 (28.2–31.8)	8.3 (7.3–9.4)	6.5 (5.6–7.5)	1.6 (1.1–2.1)	0.2 (0.1–0.4)
	25–34 years	5.4 (4.8–5.9)	46.7 (45.4–47.9)	34.1 (32.9–35.3)	48.0 (46.7–49.2)	13.9 (13.0–14.7)	10.9 (10.2–11.7)	2.3 (1.9–2.6)	0.7 (0.5–0.9)
	35–44 years	2.3 (1.9–2.6)	33.0 (31.8–34.3)	39.4 (38.1–40.6)	64.7 (63.5–66.0)	25.4 (24.2–26.5)	19.1 (18.1–20.1)	4.9 (4.4–5.5)	1.3 (1.0–1.6)
	45–54 years	1.9 (1.5–2.3)	25.9 (24.7–27.0)	41.1 (39.7–42.4)	72.2 (71.0–73.5)	31.2 (29.9–32.4)	22.1 (21.0–23.3)	7.0 (6.2–7.7)	2.1 (1.7–2.5)
	55–64 years	2.5 (2.0–3.0)	26.4 (25.0–27.7)	39.6 (38.1–41.1)	71.2 (69.7–72.6)	31.5 (30.1–33.0)	22.3 (21.0–23.6)	7.6 (6.7–8.4)	1.7 (1.3–2.1)
	65–69 years	2.4 (1.6–3.2)	27.9 (25.4–30.4)	40.2 (37.5–42.9)	69.7 (67.2–72.2)	29.5 (27.0–32.0)	22.6 (20.3–24.9)	4.9 (3.7–6.0)	2.0 (1.3–2.8)
	70 years and more	5.0 (4.1–5.9)	39.5 (37.4–41.5)	35.9 (33.9–37.9)	55.5 (53.4–57.6)	19.6 (17.9–21.2)	15.6 (14.1–17.2)	3.1 (2.4–3.9)	0.8 (0.4–1.2)
Education	Illiterate	4.6 (3.9–5.2)	33.5 (32.1–35.0)	34.3 (32.8–35.8)	61.9 (60.4–63.4)	27.6 (26.2–29.0)	19.4 (18.2–20.6)	6.6 (5.8–7.4)	1.6 (1.2–2.0)
	1–6 years	3.6 (3.2–4.1)	31.3 (30.2–32.4)	36.6 (35.4–37.7)	65.1 (63.9–66.2)	28.5 (27.4–29.6)	20.5 (19.5–21.4)	6.2 (5.6–6.8)	1.8 (1.5–2.1)
	7–12 years	3.9 (3.5–4.3)	38.0 (37.0–38.9)	37.1 (36.1–38.0)	58.2 (57.2–59.1)	21.1 (20.3–21.9)	16.0 (15.3–16.7)	4.0 (3.6–4.4)	1.1 (0.9–1.3)
	More than 12 years	4.2 (3.7–4.7)	43.1 (41.8–44.5)	37.2 (36.0–38.5)	52.7 (51.3–54.0)	15.4 (14.5–16.4)	12.5 (11.6–13.4)	2.3 (1.9–2.7)	0.6 (0.4–0.8)
Marital status	Never married	9.2 (8.3–10.1)	56.0 (54.4–57.5)	25.8 (24.4–27.1)	34.8 (33.3–36.3)	9.0 (8.1–9.9)	7.3 (6.5–8.1)	1.4 (1.1–1.8)	0.3 (0.1–0.4)
	Married	2.9 (2.7–3.1)	33.5 (32.9–34.1)	39.0 (38.3–39.7)	63.6 (62.9–64.2)	24.6 (24.0–25.2)	18.3 (17.8–18.9)	4.9 (4.6–5.2)	1.3 (1.2–1.5)
	Divorced/ separated	4.9 (3.1–6.8)	36.7 (32.4–41.0)	33.4 (29.1–37.6)	58.4 (54.0–62.8)	25.0 (21.1–28.9)	18.3 (14.8–21.8)	5.8 (3.8–7.9)	0.9 (0.0–1.8)
	Widowed	3.8 (2.9–4.7)	27.6 (25.3–29.9)	34.9 (32.6–37.2)	68.6 (66.2–71.0)	33.7 (31.4–36.0)	22.6 (20.6–24.7)	8.3 (6.9–9.8)	2.7 (1.9–3.5)
Basic insurance	No	4.0 (3.2–4.9)	43.2 (41.0–45.3)	33.2 (31.2–35.2)	52.8 (50.6–54.9)	19.6 (17.9–21.3)	14.7 (13.2–16.2)	3.8 (3.0–4.6)	1.1 (0.6–1.5)
	Yes	4.0 (3.7–4.2)	36.1 (35.5–36.7)	36.9 (36.3–37.5)	59.9 (59.3–60.5)	23.0 (22.5–23.5)	17.1 (16.6–17.5)	4.7 (4.4–4.9)	1.3 (1.1–1.4)
Type of basic insurance	Health insurance	5.5 (5.1–6.0)	39.2 (38.3–40.2)	34.3 (33.4–35.3)	55.2 (54.3–56.2)	20.9 (20.1–21.7)	15.4 (14.7–16.2)	4.4 (4.0–4.8)	1.1 (0.9–1.3)
	Social insurance	2.7 (2.4–3.0)	34.0 (33.2–34.9)	38.8 (37.9–39.7)	63.3 (62.4–64.2)	24.5 (23.7–25.2)	18.2 (17.5–18.9)	4.9 (4.5–5.3)	1.3 (1.1–1.5)
	Army insurance	2.6 (1.7–3.5)	32.2 (29.5–35.0)	39.7 (36.8–42.5)	65.2 (62.4–68.0)	25.5 (23.0–28.1)	18.3 (16.0–20.5)	5.2 (3.9–6.5)	2.0 (1.2–2.9)
	Imam Khomeini Relief Foundation insurance	6.7 (2.9–10.5)	39.0 (31.6–46.4)	27.3 (20.5–34.1)	54.3 (46.7–61.9)	27.0 (20.0–33.9)	20.4 (14.0–26.7)	4.0 (1.0–7.0)	2.6 (0.1–5.1)

(Continued)

TABLE 2 | Continued

Variable	Characteristics	Underweight (BMI < 18.5)	Normal (18.5 ≤ BMI <25)	Overweight (25 ≤ BMI <30)	Overweight/ obesity (25 ≤ BMI)	Obesity (30 ≤ BMI)	Class I obesity (30 ≤ BMI < 35)	Class II obesity (35 ≤ BMI < 40)	Class III obesity (40 ≤ BMI)
	Other insurances	3.9 (3.1–4.7)	34.3 (32.3–36.2)	38.2 (36.2–40.1)	61.8 (59.9–63.8)	23.7 (22.0–25.4)	17.8 (16.3–19.3)	4.5 (3.7–5.3)	1.4 (0.9–1.9)
	No insurance	4.0 (3.2–4.9)	43.2 (41.0–45.3)	33.2 (31.2–35.2)	52.8 (50.6–54.9)	19.6 (17.9–21.3)	14.7 (13.2–16.2)	3.8 (3.0–4.6)	1.1 (0.6–1.5)
Complementary insurance	No	4.5 (4.2–4.8)	38.6 (37.9–39.2)	35.5 (34.9–36.2)	56.9 (56.3–57.6)	21.4 (20.9–22.0)	16.0 (15.5–16.5)	4.4 (4.1–4.6)	1.1 (0.9–1.2)
	Yes	2.1 (1.7–2.5)	29.5 (28.3–30.7)	40.7 (39.5–42.0)	68.4 (67.2–69.6)	27.7 (26.5–28.8)	20.3 (19.3–21.4)	5.4 (4.8–5.9)	2.0 (1.6–2.4)
Wealth index quintile	Poorest	8.7 (8.0–9.5)	45.9 (44.6–47.3)	30.4 (29.2–31.6)	45.3 (44.0–46.7)	14.9 (14.0–15.9)	10.9 (10.1–11.8)	3.2 (2.7–3.6)	0.9 (0.6–1.1)
	2	3.8 (3.3–4.3)	37.7 (36.4–39.0)	35.0 (33.7–36.3)	58.5 (57.1–59.8)	23.5 (22.3–24.7)	17.2 (16.2–18.2)	4.8 (4.2–5.4)	1.5 (1.2–1.9)
	3	2.9 (2.5–3.3)	35.1 (33.8–36.4)	36.6 (35.3–37.9)	62.0 (60.7–63.4)	25.4 (24.2–26.6)	18.7 (17.6–19.7)	5.5 (4.9–6.1)	1.3 (1.0–1.6)
	4	2.8 (2.4–3.3)	33.4 (32.2–34.7)	38.3 (37.0–39.6)	63.7 (62.4–65.0)	25.4 (24.2–26.6)	19.1 (18.0–20.1)	5.1 (4.5–5.7)	1.2 (0.9–1.5)
	Richest	2.0 (1.7–2.4)	31.4 (30.1–32.6)	42.3 (40.9–43.6)	66.6 (65.3–67.8)	24.3 (23.2–25.5)	18.4 (17.4–19.4)	4.5 (3.9–5.0)	1.4 (1.1–1.8)

Data in parentheses are 95% Confidence Intervals (CI).

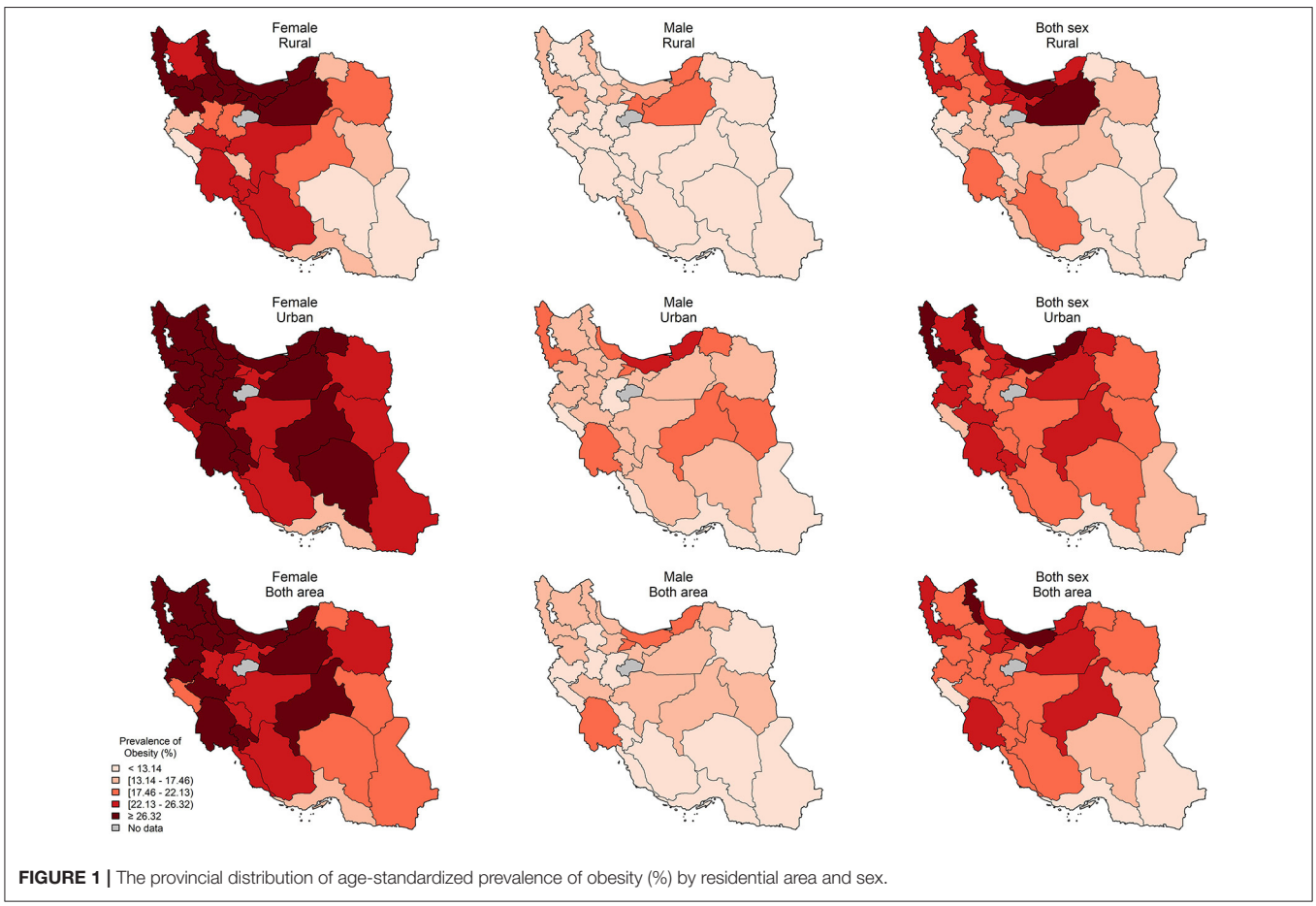

FIGURE 1 | The provincial distribution of age-standardized prevalence of obesity (%) by residential area and sex.

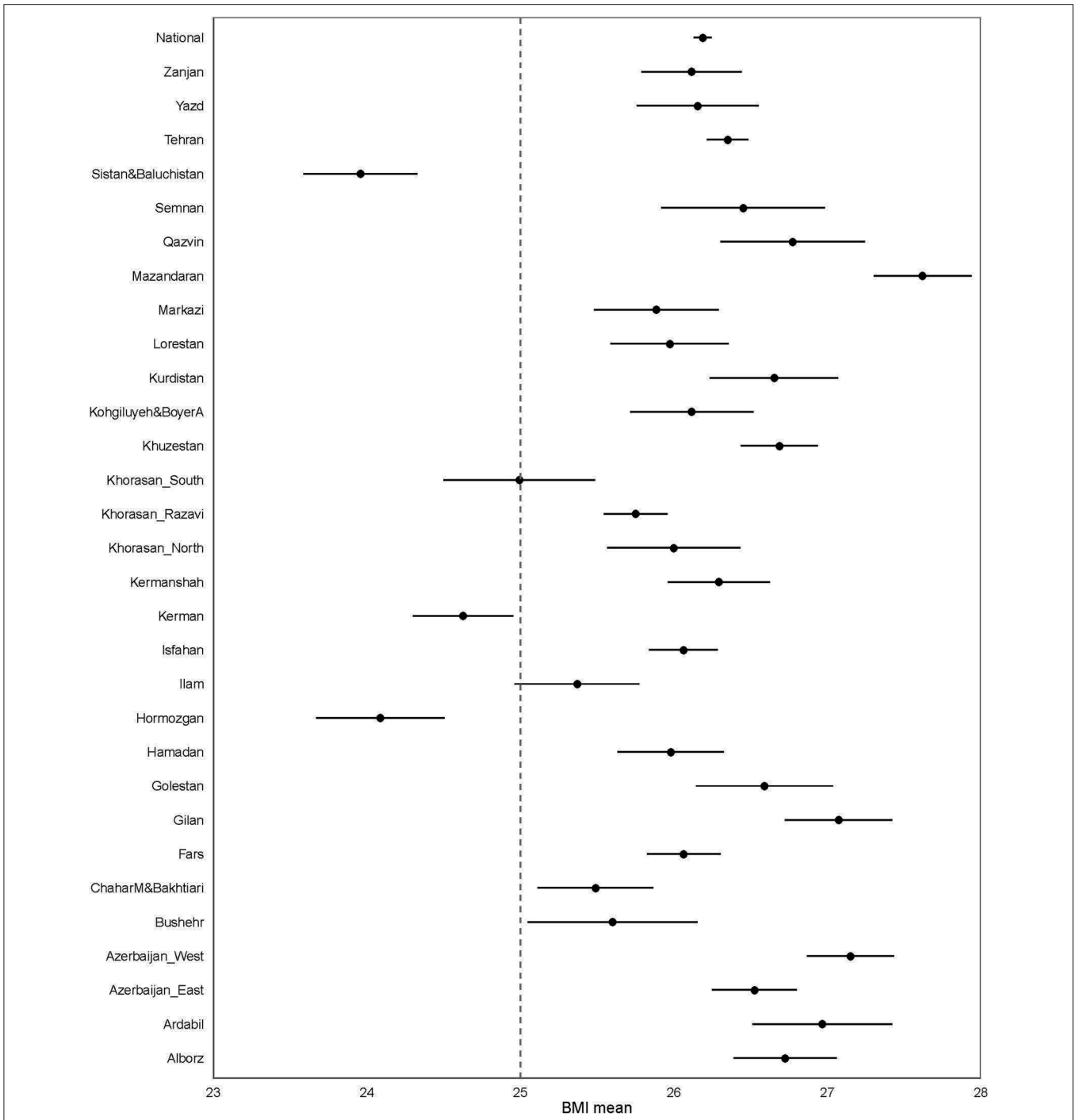

FIGURE 2 | The national and provincial age-standardized mean BMI.

(27.3–27.5) kg/m^2] ($p < 0.001$). Another noticeable point is that 4.3% (4.0–4.6) of males and 3.7% (3.4–4.0) of females were underweight (**Table 2**).

Given the comparative results of the age groups, the highest and lowest prevalence of obesity belonged to the 55–64 [31.5% (30.1–33.0)] and 18–24 [8.3% (7.3–9.4)] year-olds, respectively. In the overweight/obese group, the highest and the lowest estimates belonged to the age groups of 45–54 [72.2% (71.0–73.5)] and 18–24 [30.0% (28.2–31.8)], respectively (**Table 2**).

The analysis of results showed that participants with >12 years of schooling had a significantly lower prevalence of obesity and overweight ($p < 0.001$). With regards to the marriage status, the never married population had the lowest prevalence of both obesity [9.0% (8.1–9.9)]

and overweight [34.8% (33.3–36.3)] among all the age groups (**Table 2**).

Based on provincial patterns, the highest prevalence of being underweight was seen in the southeastern provinces (**Data Sheets 1**, **2**). On the other hand, the highest prevalence of obesity belonged to the northeastern and central provinces (**Figure 1** and **Data Sheet 3**).

The difference in age-standardized prevalence of obesity and overweight/obesity between the provinces were 17.9% (lowest: 11.7–highest: 29.6) and 28.1% (lowest: 38.8–highest: 66.9), respectively. The level of BMI increases as we move from the southeast to the northwest of the country (**Data Sheet 4**).

Considering the distribution of different categories of BMI across the provinces, similar to the national estimated level, the age-standardized mean BMI was >25 kg/m^2 (**Figure 2**).

Sistan and Baluchistan had the highest underweight prevalence for both males (15.7%) and females (14.8%). It is noteworthy that in many provinces such as South Khorasan there is a double burden of obesity, especially among females (underweight prevalence is 12.1% and obesity prevalence is 19.5% for females) (**Data Sheet 5**).

Compared to rural areas, the mean BMI was significantly higher among individuals from urban areas for all age groups, in both males (Rural: 24.62, Urban: 26.02) and females (Rural: 26.74, Urban: 27.69) (**Data Sheet 6**).

The mean BMI was significantly higher in participants suffering from metabolic and lifestyle risk factors. A noteworthy point is that compared to the normal population, the mean BMI was significantly higher in patients with a positive history of heart attack and stroke over the past year (**Table S1**).

Among females, pre-diabetes based on FPG (OR: 1.71 [1.47–1.99]) or HbA1c (OR: 1.59 [1.37–1.83]), diabetes based on FPG (OR: 1.80 [1.44–2.26]) or HbA1c (OR: 1.90 [1.53–2.37]), LDL–C (OR: 1.48 [1.33–1.65]), hypertriglyceridemia (OR: 2.37 [2.05–2.75]), hypercholesterolemia (OR: 1.48 [1.27–1.71]), pre-hypertension (OR: 1.30 [1.20–1.41]), hypertension (OR: 1.96 [1.77–2.18]), and heart attack (OR: 1.81 [1.30–2.60]), increased the age-adjusted OR of overweight/obesity. Among males, pre-diabetes based on FPG (OR: 1.48 [1.29–1.71]) or HbA1c (OR: 1.31 [1.16–1.49]), diabetes based on FPG (OR: 2.01 [1.64–2.48]) or HbA1c (OR: 2.32 [1.90–2.84]), LDL–C (OR: 1.29 [1.16–1.43]), hypertriglyceridemia (OR: 3.33 [2.94–3.77]), hypercholesterolemia (OR: 1.90 [1.65–2.19]), pre-hypertension (OR: 1.31 [1.22–1.41]), hypertension (OR: 2.34 [2.12–2.57]), and heart attack (OR: 1.81 [1.30–2.60]), increased the age-adjusted OR of overweight/obesity. Ever tobacco smoking, ever daily cigarette smoking, and current daily cigarette smoking increased the age-adjusted OR of underweight and normal weight in both males and females and decreased the age-adjusted OR of overweight, overweight/obesity, and obesity (**Table 3**).

DISCUSSION

We estimated the prevalence of overweight/obesity and distribution of BMI levels in the Iranian population, by sex, age, and geographical distribution. Our findings show that, in 2016, approximately 22.7% (95% CI: 22.2–23.2) of the 18≤ year-old Iranian adults were obese and 59.3% (58.7–59.9) were overweight. We found a significant difference between the prevalence of obesity in males and females. The highest provincial prevalence of obesity (29.6% for Mazandaran) was almost 2.5 times higher than the lowest provincial prevalence of obesity (11.7% for Hormozgan).

The number of obese and overweight people has become an important health concern in many developing countries, however, the predisposing factors and affiliated adverse health outcomes follow different patterns in different populations (1, 15). In 2014, in Iran, the global age-standardized mean BMI in males and females was estimated at 24.2 (24.0–24.4) kg/m^2 and 24.4 (24.2–24.6) kg/m^2, respectively (1). Age-standardized prevalence of obesity was estimated at 10.8% (9.7–12.0) in males, and 14.9% (13.6–16.1) in females. At the same time, 2.3% (2.0–2.7) of males and 5.0% (4.4–5.6) of females were severely obese (BMI ≥ 35 kg/m^2) at global level. The age-standardized global prevalence of underweight was 8.8% (7.4–10.3) in males and 9.7% (8.3–11.1) in females (1).

Several studies have provided reports on obesity trends in Iran and these trends mostly match our findings (1, 3, 6, 15). The Global Burden of Diseases (GBD) study has reported the obesity prevalence in Iranian adult females and males (≥20 years) at 29.3 and 13.6%, respectively; significantly lower than our estimates (3). Considering the multifactorial nature of obesity, in order to investigate the causes of change and reported values, both medical and non-medical predisposing factors such as age, gender, race/ethnicity, socioeconomic status, and-understandably- lifestyle patterns have been discussed (1, 16). Moreover, given the effects of epidemiological changes, many sources of differences can be extracted from data quality and our approaches toward applying data-driven estimates or relying on the estimates that were derived from different modeling methods (1, 9).

Considering gender differences, studies in many regions of the world have shown that, compared to males, females are at greater risk of obesity (15, 17). Evidence has confirmed that males and females have differences in anatomical fat distribution, fat utilization, and obesity co-morbidities (18). These may be rooted in differences in genetics, sex hormones, and even unknown molecular mechanisms (17, 18).

Geographically, the highest levels of BMI were detected in the northwestern and central provinces. Earlier studies have shown that these regions have a mostly higher economic status (5). Results from other relevant research indicate that during recent decades, there has been a slower increase in BMI in high-income populations (1, 19). On the other hand, some studies have shown otherwise. For instance, in a comprehensive analysis of global data, the largest increase in BMI occurred in high-income English-speaking countries (1). In this regard, numerous components such as variations in lifestyle and geographical factors have been discussed in the literature (1, 17, 20).

The current paper brings to light the most updated results of overweight/obesity and BMI at national and subnational

TABLE 3 | The Odds Ratio of BMI categories according to metabolic and lifestyle risk factors of Iranian adults by sex.

Variable	Sex	Status	Underweight (BMI < 18.5)	Normal (18.5 ≤ BMI < 25)	Overweight (25 ≤ BMI < 30)	Overweight/obesity (25 ≤ BMI)	Obesity (30 ≤ BMI)	Class I obesity (30 ≤ BMI < 35)	Class II obesity (35 ≤ BMI < 40)	Class III obesity (40 ≤ BMI)
Pre-diabetes based on FPG (100 ≤ FPG < 126 mg/dl among who did not recognize as diabetic)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.74 (0.51–1.08)	0.59 (0.50–0.69)***	0.93 (0.82–1.06)	1.71 (1.47–1.99)***	1.61 (1.41–1.83)***	1.29 (1.11–1.49)**	1.60 (1.30–1.98)***	2.05 (1.46–2.89)***
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.58 (0.40–0.82)**	0.72 (0.62–0.83)***	1.18 (1.04–1.34)*	1.48 (1.29–1.71)***	1.40 (1.20–1.64)***	1.32 (1.11–1.56)**	1.61 (1.12–2.32)*	1.70 (0.79–3.65)
Pre-diabetes based on HbA1c (5.7 ≤ HbA1c < 6.4% among who did not recognize as diabetic)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.78 (0.54–1.14)	0.63 (0.55–0.73)***	0.80 (0.70–0.90)***	1.59 (1.37–1.83)***	1.77 (1.57–2.01)***	1.42 (1.24–1.62)***	1.71 (1.38–2.12)***	1.96 (1.37–2.79)***
	Male	No	1	1	1	1	1	1	1	1
		Yes	1.11 (0.82–1.52)	0.74 (0.65–0.84)***	1.07 (0.95–1.21)	1.31 (1.16–1.49)***	1.38 (1.19–1.60)***	1.28 (1.09–1.51)**	1.64 (1.14–2.36)**	2.02 (0.91–4.49)
Diabetes based on FPG [FPG ≥ 126 mg/dl or self-report (OHA and/or insulin taking)]	Female	No	1	1	1	1	1	1	1	1
		Yes	0.10 (0.04–0.29)***	0.62 (0.49–0.78)***	0.95 (0.81–1.12)	1.80 (1.44–2.26)***	1.57 (1.33–1.85)***	1.41 (1.18–1.67)***	1.36 (1.05–1.75)*	1.40 (0.92–2.14)
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.28 (0.14–0.55)***	0.56 (0.45–0.69)***	1.18 (0.98–1.42)	2.01 (1.64–2.48)***	1.99 (1.63–2.44)***	1.89 (1.52–2.34)***	1.77 (1.10–2.84)*	3.06 (1.22–7.70)*
Diabetes based on HbA1c [HbA1c ≥ 6.4% or self-report (OHA and/or insulin taking)]	Female	No	1	1	1	1	1	1	1	1
		Yes	0.10 (0.04–0.29)***	0.59 (0.47–0.73)***	0.98 (0.84–1.15)	1.90 (1.53–2.37)***	1.56 (1.33–1.83)***	1.38 (1.16–1.63)***	1.35 (1.06–1.73)*	1.57 (1.04–2.37)*
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.19 (0.09–0.37)***	0.50 (0.41–0.61)***	1.31 (1.10–1.56)**	2.32 (1.90–2.84)***	2.04 (1.69–2.47)***	1.87 (1.53–2.29)***	1.98 (1.29–3.05)**	4.38 (1.94–9.86)***
LDL–C (low-density lipoprotein cholesterol ≥ 100 mg/dl)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.46 (0.33–0.62)***	0.73 (0.65–0.81)***	1.11 (1.01–1.23)*	1.48 (1.33–1.65)***	1.26 (1.14–1.41)***	1.16 (1.04–1.31)*	1.22 (1.01–1.46)*	1.52 (1.11–2.09)*
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.51 (0.39–0.67)***	0.85 (0.76–0.94)**	1.15 (1.03–1.27)*	1.29 (1.16–1.43)***	1.21 (1.06–1.39)**	1.21 (1.05–1.40)**	1.11 (0.79–1.56)	1.34 (0.67–2.68)
Hypertriglyceridemia (Triglyceride ≥ 150 mg/dl)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.10 (0.05–0.21)***	0.47 (0.41–0.55)***	0.92 (0.82–1.04)	2.37 (2.05–2.75)***	2.04 (1.82–2.30)***	1.60 (1.41–1.82)***	2.08 (1.71–2.52)***	1.56 (1.11–2.19)*
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.18 (0.11–0.28)***	0.35 (0.31–0.40)***	1.78 (1.59–1.98)***	3.33 (2.94–3.77)***	2.30 (2.01–2.63)***	2.19 (1.90–2.53)***	2.02 (1.48–2.77)***	2.93 (1.47–5.84)**

(Continued)

TABLE 3 | Continued

Variable	Sex	Status	Underweight (BMI < 18.5)	Normal (18.5 ≤ BMI < 25)	Overweight (25 ≤ BMI < 30)	Overweight/obesity (25 ≤ BMI)	Obesity (30 ≤ BMI)	Class I obesity (30 ≤ BMI < 35)	Class II obesity (35 ≤ BMI < 40)	Class III obesity (40 ≤ BMI)
Hypercholesterolemia (total Cholesterol ≥ 200 mg/dl or self-report of drug taking)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.34 (0.22–0.53)***	0.73 (0.63–0.85)***	1.08 (0.96–1.22)	1.48 (1.27–1.71)***	1.25 (1.11–1.41)***	1.26 (1.11–1.44)**	0.97 (0.79–1.19)	1.37 (0.93–2.03)
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.30 (0.19–0.47)***	0.59 (0.51–0.68)***	1.28 (1.12–1.45)***	1.90 (1.65–2.19)***	1.72 (1.48–2.01)***	1.60 (1.36–1.88)***	1.78 (1.20–2.62)**	3.08 (1.47–6.46)**
Pre-hypertension (120 ≤ systolic blood pressure < 140 mmHg or 80 ≤ diastolic blood pressure < 90 mmHg among who did not recognize as hypertensive individual)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.58 (0.47–0.73)***	0.83 (0.76–0.90)***	1.18 (1.09–1.27)***	1.30 (1.20–1.41)***	1.08 (0.99–1.17)	1.09 (1.00–1.19)	0.98 (0.84–1.13)	1.08 (0.85–1.39)
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.59 (0.49–0.71)***	0.83 (0.77–0.89)***	1.30 (1.21–1.39)***	1.31 (1.22–1.41)***	1.03 (0.94–1.14)	1.06 (0.95–1.18)	0.98 (0.77–1.23)	0.67 (0.38–1.16)
Hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or self-report of drug taking)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.43 (0.32–0.58)***	0.54 (0.49–0.60)***	0.89 (0.81–0.97)*	1.96 (1.77–2.18)***	1.93 (1.76–2.12)***	1.53 (1.38–1.69)***	1.75 (1.50–2.05)***	2.49 (1.89–3.29)***
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.38 (0.29–0.49)***	0.48 (0.44–0.53)***	1.34 (1.22–1.46)***	2.34 (2.12–2.57)***	2.34 (2.09–2.63)***	2.04 (1.81–2.31)***	2.57 (1.98–3.32)***	6.29 (3.44–11.52)***
Ever tobacco smoking	Female	No	1	1	1	1	1	1	1	1
		Yes	1.80 (1.34–2.43)***	1.18 (1.01–1.39)*	0.86 (0.74–0.99)*	0.76 (0.65–0.89)**	0.89 (0.76–1.04)	0.87 (0.74–1.03)	0.94 (0.69–1.27)	1.19 (0.78–1.83)
	Male	No	1	1	1	1	1	1	1	1
		Yes	1.83 (1.55–2.16)***	1.25 (1.16–1.35)***	0.79 (0.74–0.86)***	0.72 (0.67–0.78)***	0.83 (0.74–0.91)***	0.79 (0.71–0.88)***	1.08 (0.85–1.38)	0.86 (0.46–1.62)
Ever daily cigarette smoking	Female	No	1	1	1	1	1	1	1	1
		Yes	2.24 (1.29–3.87)**	1.56 (1.11–2.2)*	0.70 (0.52–0.94)*	0.57 (0.41–0.79)**	0.82 (0.61–1.12)	0.88 (0.64–1.21)	0.83 (0.41–1.66)	0.75 (0.30–1.87)
	Male	No	1	1	1	1	1	1	1	1
		Yes	1.89 (1.58–2.25)***	1.38 (1.28–1.50)***	0.75 (0.69–0.81)***	0.65 (0.60–0.70)***	0.76 (0.68–0.85)***	0.73 (0.65–0.82)***	1.01 (0.78–1.31)	0.90 (0.45–1.82)
Current daily cigarette smoking	Female	No	1	1	1	1	1	1	1	1
		Yes	3.24 (1.70–6.18)***	1.86 (1.27–2.73)**	0.70 (0.48–1.02)	0.44 (0.30–0.64)***	0.60 (0.40–0.91)*	0.71 (0.45–1.12)	0.43 (0.18–1.07)	0.96 (0.30–3.08)
	Male	No	1	1	1	1	1	1	1	1
		Yes	1.95 (1.61–2.36)***	1.57 (1.44–1.72)***	0.70 (0.64–0.77)***	0.57 (0.52–0.62)***	0.65 (0.57–0.74)***	0.61 (0.53–0.70)***	0.94 (0.71–1.26)	0.89 (0.40–1.98)

(Continued)

TABLE 3 | Continued

Variable	Sex	Status	Underweight (BMI < 18.5)	Normal (18.5 ≤ BMI < 25)	Overweight (25 ≤ BMI < 30)	Overweight/obesity (25 ≤ BMI)	Obesity (30 ≤ BMI)	Class I obesity (30 ≤ BMI < 35)	Class II obesity (35 ≤ BMI < 40)	Class III obesity (40 ≤ BMI)
Heart attack incidence within the last year (self-report)	Female	No	1	1	1	1	1	1	1	1
		Yes	1.23 (0.60–2.60)	0.50 (0.30–0.70)**	0.87 (0.60–1.20)	1.81 (1.30–2.60)**	1.93 (1.40–2.60)***	1.45 (1.00–2.10)*	2.00 (1.20–3.20)**	2.32 (1.10–4.90)*
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.51 (0.20–1.20)	0.53 (0.40–0.70)***	1.22 (0.90–1.60)	2.03 (1.50–2.70)***	2.07 (1.50–2.80)***	1.73 (1.30–2.40)**	3.24 (1.90–5.50)***	0.94 (0.10–6.80)
Stroke incidence within the last year (self-report)	Female	No	1	1	1	1	1	1	1	1
		Yes	0.91 (0.30–2.50)	0.65 (0.40–1.10)	0.71 (0.40–1.10)	1.51 (1.00–2.40)	2.00 (1.30–3.00)**	1.69 (1.10–2.60)*	1.37 (0.70–2.80)	3.12 (1.30–7.30)**
	Male	No	1	1	1	1	1	1	1	1
		Yes	0.49 (0.10–2.00)	0.97 (0.60–1.50)	0.86 (0.60–1.30)	1.12 (0.70–1.70)	1.53 (0.90–2.50)	1.09 (0.60–2.00)	2.36 (0.90–5.90)	7.04 (2.10–23.30)**

Data in parentheses are 95% Confidence Intervals (CI).

**Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$.*

levels in 2016. Using proportional to size sampling, one of the main strengths of the study was its comprehensive protocols focused on standards and regulatory guidelines. Moreover, the digitalized online study provided the most reliable data that could be used as practical evidence for better policymaking.

We also faced many limitations. The main limitation of this study is its cross-sectional nature, which limits us in the inferential analysis. Data from previous rounds of the STEPs surveys are available and might be the subject of another study using meta-regression to arrive at better estimates of the BMI or obesity trends at national and subnational levels (21, 22).

The present study has many important implications. Based on other countries' experiences, the current ongoing interventions and policies are not enough to stop the rise in BMI (1, 11, 17, 23).

The global NCD target of obesity cleared the path for policies on the worldwide management of the problem. Based on evidence, we must exactly follow our defined national goals (11, 24). As the next step, monitoring and evaluation of the implemented programs must be investigated carefully. For better planning and more effective interventions, we need comprehensive approaches benefiting from all national resources and capacities. In this regard, literature confirms that primary care systems with trained community health-care workers together with well-developed guidelines can be effective in preventing and managing non-communicable diseases and risk factors (2, 23, 25). Moreover, a wide range of interventions for prevention or treatment may be selected based on comprehensive plans that involve different aspects of individual and population interventions aimed at specific target groups (16, 26). Given the gaps in the relevant evidence needed, more research on individual and social obesity-related behaviors should be conducted through complementary studies (16, 26).

Another important point is that, like many other countries that have focused on obesity and its adverse health outcomes, the issue and consequences of being underweight in many subpopulations has largely been overshadowed in Iran too (27, 28). The alarming burden caused by malnutrition in females of reproductive age, pregnant females, and children, must lead to interventions focused on prompt problem-based solutions (27, 28). In order to manage the problem, social and food policies must include practical policies and strategies that enhance food security, especially among poor households. Furthermore, the prevention of overconsumption of unhealthy foods such as processed carbohydrates must also be borne in mind (29). Along with these considerations, the screening of at-risk populations, detection of possible comorbidities (such as anorexia nervosa), nutritional supplementation in pregnant females and students, and using the most effective and safe treatment for weight restoration in inpatient target groups, should be undertaken as appropriate population-based interventions (19, 30).

Following the global agenda on risk reduction, with the participation of all stakeholders, Iran developed a comprehensive national non-communicable diseases action plan through which prevention and control of overweight and obesity were targeted as the main risk factors. Benefiting from a multisectoral

approach, supplementary agreements were signed for the implementation of obligations on behalf of other collaborating organizations (24, 31).

It is also remarkable that, in spite of considerable earlier efforts, there are still noticeable gaps and limitations in the evidence required for policy making. The possible causes of patterns of risk factors as well as the epidemiological transition should be investigated for various metabolic risks and all risks in more detail. Moreover, behavioral changes in smoking, physical activity, alcohol intake, and psychosocial factors according to demographic specifications such as sex, age, and ethnicity, must be addressed further as a complex set of predisposing factors (1, 6, 15, 28). These should be followed through complementary researches in relevant and specific fields.

CONCLUSION

In conclusion, to the best of our knowledge, the present study is the first comprehensive experience of a systematic and fully digitalized national survey in Iran. Given the evidence on national and subnational requirements for the promotion of strategies and interventions to prevent and control weight gain, and to achieve SDG 3.4 and the WHO Action Plan for NCD control and prevention, we propose comprehensive programs that meet the needs of all stakeholders. We found a significant difference between the prevalence of obesity in males and females. Moreover, there was a considerable difference in the geographical pattern of the prevalence of obesity and overweight. Further focus needs to be laid on trends analyses of BMI risk factors to identify the priority interventions.

DATA AVAILABILITY STATEMENT

The data used in the current study are available upon request from the corresponding author. Aggregated reports are published online on https://vizit.report and are freely available to public for non-commercial use.

AUTHOR CONTRIBUTIONS

FF and SD: general design of paper. FF, SS, AS, and SD: design of methods. FF, AS, SS, MY, and KG: analysis. SD, SS, AS, and SSN: primary drafting of the manuscript. BL, MM, HZ, MY, KG, NR, SN, AK, ZA, and ARS: manuscript revision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00042/full#supplementary-material

Data Sheet 1 | The provincial distribution of age-standardized prevalence of underweight (%) by residential area and sex.

Data Sheet 2 | The provincial distribution of age-standardized prevalence of normal weight (%) by residential area and sex.

Data Sheet 3 | The provincial distribution of age-standardized prevalence of overweight/obesity (%) by residential area and sex.

Data Sheet 4 | The provincial distribution of age-standardized mean BMI by residential area and sex.

Data Sheet 5 | Distribution of the BMI categories' percentage by sex and province.

Data Sheet 6 | Distribution of the mean BMI and its categories by sex, residential area, and age groups.

Table S1 | The BMI mean according to metabolic and lifestyle risk factors by sex in Iranian adults.

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