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Anemia – tackling a complex problem

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Anemia – tackling a complex problem

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Evolving trends and burden of iron deficiency among children, 1990–2019: a systematic analysis for the global burden of disease study 2019

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Objectives: We aimed to provide a timely, comprehensive, and reliable assessment of the burden of iron deficiency (ID) in children between 1990 and 2019 at the global, regional, and national levels to inform policymakers in developing locally appropriate health policies.

Methods: Data related to ID among children younger than 15 years old were analyzed by sex, age, year, socio-demographic index (SDI), and location according to the Global Burden of Disease Study 2019 (GBD 2019). Age-standardized rates were used to compare the burden between different regions and countries. Furthermore, the Joinpoint regression model was used to assess temporal trends from 1990 to 2019.

Results: In 2019, the number of prevalent cases and disability-adjusted life years (DALYs) for ID in children were 391,491,699 and 13,620,231, respectively. The global age-standardized prevalence and DALY rates for childhood ID in 2019 were 20,146.35 (95% confidence interval: 19,407.85 to 20,888.54) and 698.90 (466.54 to 1015.31) per 100,000, respectively. Over the past 30 years, the global prevalence of ID among children has been highest in low-SDI regions, particularly in Western Sub-Saharan Africa, South Asia, and Eastern Sub-Saharan Africa. Since 1990, the prevalence and DALY of ID in children have been declining in most geographic regions. Nationally, Ecuador, China, and Chile have shown the most significant decreases in prevalence. The greatest decline in age-standardized DALY rate was observed in Ecuador, while Burkina Faso experienced the highest increase. Bhutan had the highest prevalence and DALY rates in 2019. On the age level, the prevalence was relatively higher among the <5 years age group. At the gender dimension, the prevalence of ID in children overall was more pronounced in girls than in boys, as was the case for DALY.

Conclusion: Although the burden of ID in children has been declining, this disease remains a major public health problem, especially in countries with low SDI. Children younger than 5 years of age are an important group for whom targeted measures are needed to reduce the burden of ID.

KEYWORDS

iron deficiency, children, global burden of disease, Joinpoint regression model, epidemiology

Introduction

Iron is the most abundant micronutrient in the body and is involved in numerous physiological processes. Iron balance is critical for maintaining organic health (1, 2). The Sustainable Development Goals (SDGs) released by the United Nations General Assembly in 2015 aim to eliminate all forms of malnutrition by 2030. Iron deficiency (ID) affects billions of people around the world and remains the leading cause of anemia, with profound negative impacts on health (3). According to the Global Burden of Disease (GBD) study 2019, the prevalence of ID varied widely across 204 countries and territories, ranging from 1,990.9 to 32,085.7 per 100,000. ID have an adverse impact on human behavior. Children and adolescents with ID often exhibit sluggishness, poor concentration, and reduced learning ability and memory (4, 5). Iron is also involved in thermoregulation and energy metabolism in the body (6, 7). Additionally, ID leads to a substantial reduction in neutrophils and affects their effector functions, which affects the defense ability and makes it more susceptible to infectious diseases (8).

Unfortunately, children's health has been largely neglected in global public health, as this age group is usually considered healthy. Actually, ID is not only one of the major contributors to the global burden of disease, but it is also the most common nutritional disorder in childhood (9–11). Obviously, children are a key group for ID prevention and treatment (12). However, the prevalence of ID in children varies widely across regions and countries with different economic levels. To our knowledge, no long-term global trends in the epidemiology of childhood ID have been reported. Here, we aimed to comprehensively assess the ID burden in children, including prevalence and DALYs, at global, regional, and national levels.

The GBD Study 2019 is a large database of multinational collaborative research that covers all WHO member countries. It provides a comprehensive assessment of health losses globally between 1990 and 2019 for 204 countries and territories, 369 diseases and injuries, and 87 risk factors (13, 14). It is currently the most extensive and credible database on the burden of disease worldwide and has been widely used in disease burden studies (14, 15). Therefore, in this study, data on the burden of childhood ID, derived from the GBD database, are comprehensive and reliable. In addition, we evaluated temporal trends over the past 30 years. This study will help us to better understand the epidemiology of childhood ID in different geographic regions, so as to formulate appropriate prevention strategies and rationalize the allocation of health resources.

Materials and methods

Data sources

Children under 15 years old were included in our analysis. All data for this study were obtained from the GBD study 2019 database (16). The search was done through the official website of IHME[1] utilizing the GBD result tool. ID in the GBD cause analysis was defined as inadequate iron to meet the body's needs due to inadequate dietary intake of iron, but not due to other causes of absolute or functional ID (16). ID was mapped to the GBD cause list with International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD10) codes D50–D50.9. Detailed descriptions of the raw data and general methods of the GBD 2019 study have been described in previous publications (16, 17). In this study, data stratified by sex and age group (<5, 5–9, 10–14) were collected from the GBD database from 1990 to 2019 at global, regional, and national levels. Firstly, this study focused on the analysis of prevalent cases, age-standardized prevalence rate (ASPR), DALYs, and age-standardized DALY rate for childhood ID globally, in GBD regions, and in 204 countries and territories (18, 19). Secondly, we also analyzed the relationship between SDI and ID burden in children. The SDI ranges from 0 to 1. A location with an SDI of 0 would have a theoretical minimum level of socio-demographic development relevant to health outcomes, and a location with an SDI of 1 would have a theoretical maximum level of socio-demographic development relevant to these health outcomes. Based on the SDI quintiles, countries and territories in this study were categorized into five groups in ascending order: low SDI (0–0.45), low-middle SDI (0.45–0.61), middle SDI (0.61–0.69), high-middle SDI (0.69–0.81), and high SDI (0.81–1) (20).

Joinpoint regression analysis

To assess temporal trends in global prevalence and DALY rates between 1990 and 2019, we performed Joinpoint regression analyses (21). The Joinpoint software (version 4.8.01; National Cancer Institute, Rockville, MD, US) was used to understand temporal trends in a structured way and to test which trends between joinpoints were statistically significant (22). A maximum of six line segments (five joinpoints) were applied in the model. Average Annual Percentage Change (AAPC), Annual Percentage Change (APC), and corresponding 95% confidence intervals (CIs) were calculated for this study.

Moreover, R 4.3.1 was used for data analysis and plotting in this study. A p-value of less than 0.05 was considered statistically significant.

Results

Global level

In 2019, the number of prevalent cases and DALYs for ID in children were 391,491,699 (95% UI: 382,834,301 to 400,499,411) and 13,620,231 (9,174,234 to 19,794,595), respectively (Tables 1, 2). Compared to 1990, prevalence cases and DALYs in 2019 increased by 5.52 and 2.68%, respectively. The global ASPR and age-standardized DALY rate of ID among children have decreased over the past 30 years. More specifically, the ASPR in 1990 was 20,972.41 (95% CI: 20,245.80 to 21,701.94) per 100,000, and the ASPR in 2019 was 20,146.35 per

Abbreviations: AAPC, average annual percentage change; ASPR, age-standardized prevalence rate; ASR, age-standardized rate; CI, confidence interval; DALY, disability-adjusted life year; EAPC, estimated annual percentage change; GBD, Global Burden of Disease; ID, iron deficiency; SDI, socio-demographic index.

1 http://ghdx.healthdata.org/

TABLE 1 Global prevalence of ID among children in 1990 and 2019 and the AAPC from 1990 to 2019.

Prevalence	1990		2019		1990–2019
	Cases no. (95%UI)	ASR/100,000 (95% CI)	Cases no. (95%UI)	ASR/100,000 (95% CI)	AAPC (%) (95%CI)
Global	371021617.39 (362585126.84 to 379230144.11)	20972.41 (20245.80 to 21701.94)	391491699.23 (382834300.61 to 400499411.06)	20146.35 (19407.85 to 20888.54)	−0.14 (−0.15 to −0.12)
Sex					
Boy	186970624.89 (181355712.01 to 192455285.93)	20536.79 (19520.21 to 21623.95)	199097289.22 (192436644.53 to 205993829.95)	19876.25 (18772.73 to 21033.99)	−0.11 (−0.12 to −0.10)
Girl	184050992.50 (178933064.24 to 188967722.99)	21427.59 (20495.42 to 22361.34)	192394410.01 (187056312.84 to 197567478.57)	20433.52 (19553.32 to 21314.45)	−0.16 (−0.18 to −0.14)
SDI					
High	11630759.09 (10600838.92 to 12606533.17)	6845.61 (5886.15 to 7949.87)	7371084.95 (6492145.97 to 8365813.21)	4671.08 (3765.88 to 5786.17)	−1.30 (−1.35 to −1.25)
High-middle	41285179.29 (39345606.43 to 43401737.4)	13681.96 (12561.77 to 14837.86)	22437432.98 (21042722.77 to 23933637.88)	9271.51 (8331.91 to 10291.10)	−1.34 (−1.37 to −1.31)
Middle	104896699.75 (101000282.37 to 108886457.4)	18005.83 (16949.64 to 19111.67)	74903749.56 (71788498.78 to 77947951.98)	13720.54 (12825.23 to 14632.6)	−0.93 (−0.95 to −0.92)
Low-middle	131071853.43 (126533157.28 to 135237914.75)	28464.13 (26922.54 to 29959.92)	131645173.97 (126737773.98 to 136926308.21)	25497.06 (24067.64 to 27050.28)	−0.38 (−0.39 to −0.36)
Low	81935147.88 (79685554.65 to 84239125.34)	32869.73 (31349.02 to 34380.15)	154901334.64 (150630641.37 to 159216170.24)	32516.96 (30993.53 to 34036.61)	−0.04 (−0.06 to −0.02)
GBD regions					
Western Sub-Saharan Africa	30730276.42 (29391762.94 to 32086254.64)	33841.93 (31364.82 to 36261.61)	73558516.47 (69129405.58 to 77971916.44)	36715.50 (33278.85 to 40025.55)	0.28 (0.26 to 0.30)
South Asia	154505860.98 (149259600.43 to 159314925.74)	34723.8 (32939.56 to 36461.51)	160105820.65 (153978399.23 to 166417752.19)	31523.88 (29637.75 to 33458.24)	−0.32 (−0.34 to −0.31)
Eastern Sub-Saharan Africa	26783289.53 (25836793.43 to 27768762.45)	28726.34 (27148.14 to 30379.36)	47854083.91 (45913937.72 to 49880313.35)	26843.23 (25055.24 to 28692.6)	−0.24 (−0.25 to −0.22)
Central Sub-Saharan Africa	6982519.56 (6224702.74 to 7720404.32)	26029.38 (21307.56 to 30808.8)	15180080.69 (13477412.75 to 16882954.38)	26308.13 (21812.73 to 31108.19)	0.01 (−0.04 to 0.06)
Oceania	603969.06 (537450.29 to 674367.56)	22531.89 (18455.82 to 26856.42)	1111810.99 (962617.26 to 1258941.72)	22370.10 (17445.45 to 27781.41)	−0.03 (−0.07 to 0.01)
Caribbean	2339167.85 (2132360.12 to 2545987.37)	20341.59 (17538.42 to 23485.2)	2488561.19 (2256989.54 to 2724735.35)	21502.95 (18148.53 to 25052.68)	0.21 (0.17 to 0.25)
Central Asia	5909378.56 (5481511.36 to 6333784.05)	23090.65 (20283.31 to 26134.89)	5232945.13 (4,653,920 to 5800774.5)	19244.32 (15835.29 to 22913.75)	−0.63 (−0.67 to −0.59)
Southern Sub-Saharan Africa	3480857.51 (3041278.83 to 3945325.87)	17042.33 (13290.04 to 20981.04)	3605687.95 (3152947.92 to 4109775.01)	15304.75 (12325.12 to 18938.37)	−0.36 (−0.43 to −0.30)

(Continued)

TABLE 1 (Continued)

Prevalence	1990		2019		1990–2019
	Cases no. (95%UI)	ASR/100,000 (95% CI)	Cases no. (95%UI)	ASR/100,000 (95% CI)	AAPC (%) (95%CI)
North Africa and Middle East	27374613.92 (25709900.23 to 29186874.75)	18628.01 (16579.13 to 20809.68)	26452064.6 (24566356.7 to 28671263.26)	15187.70 (13391.61 to 17160.04)	−0.71 (−0.75 to −0.66)
Andean Latin America	3267031.63 (2924305.82 to 3683042.76)	21594.63 (17534.67 to 26081.95)	2686720.94 (2298807.32 to 3102904.35)	14834.80 (11554.93 to 18852.94)	−1.30 (−1.34 to −1.25)
Southeast Asia	35886395.92 (33261648.95 to 38777988.56)	20876.28 (18310.71 to 23,622)	20925216.27 (18966205.38 to 22979648.51)	12736.34 (10952.83 to 14777.29)	−1.70 (−1.75 to −1.65)
Tropical Latin America	8811643.93 (7281939.21 to 10611124.08)	16556.42 (11756.46 to 22346.97)	6088968.75 (4840166.53 to 7543103.73)	12536.16 (8376.69 to 17678.4)	−0.95 (−0.97 to −0.94)
Southern Latin America	2167578.37 (1861322.7 to 2523681.77)	14596.41 (11178.44 to 18709.95)	1456907.44 (1113640.66 to 1833745.98)	10144.72 (6595.32 to 14813.74)	−1.25 (−1.31 to −1.19)
Central Europe	3737058.78 (3348518.12 to 4151207.76)	13531.36 (11283.52 to 16029.86)	1664081.59 (1456146.38 to 1911562.33)	9797.95 (7831.49 to 12275.83)	−1.10 (−1.16 to −1.04)
Central Latin America	7918364.25 (7451470.98 to 8451980.79)	12269.9 (11084.05 to 13618.52)	5804863.12 (5379536.02 to 6271630.9)	9114.43 (8124.54 to 10266.49)	−1.01 (−1.03 to −0.99)
Australasia	436887.97 (314627.13 to 599719.08)	9769.42 (5933.53 to 15445.22)	378781.56 (255618.87 to 550578.67)	7152.01 (4056.56 to 12186.55)	−1.07 (−1.09 to −1.05)
High-income Asia Pacific	3427967.90 (2797716.68 to 4168468.3)	10227.36 (7136.79 to 14450.51)	1426249.47 (1050589.26 to 1879055.29)	6350.17 (3884.4 to 10061.75)	−1.63 (−1.64 to −1.61)
Eastern Europe	3651014.64 (3044970.86 to 4315915.64)	7212.68 (5325.01 to 9604.88)	1,799,697 (1330382.64 to 2402253.96)	4951.41 (3124.79 to 7738.71)	−1.30 (−1.39 to −1.22)
Western Europe	4447491.03 (3921527.96 to 5044839.61)	6502.45 (5220.08 to 8021.81)	2784386.85 (2296580.05 to 3337399.75)	4219.94 (3198.71 to 5551.05)	−1.48 (−1.51 to −1.46)
East Asia	36157198.73 (33082772.51 to 39382246.06)	10732.14 (9162.45 to 12413.07)	8555501.51 (7185285.86 to 10167906.68)	3629.73 (2723.62 to 4711.96)	−3.67 (−3.78 to −3.57)
High-income North America	2403050.86 (1934127.33 to 2891510.42)	3911.74 (2686.7 to 5487.18)	2330753.13 (1678762.64 to 3118717.59)	3618.47 (2053.33 to 5826.49)	−0.27 (−0.35 to −0.19)

100,000 (19,407.85 to 20,888.54), with an AAPC of −0.14% (95% CI: −0.15 to −0.12). The age-standardized DALY rate decreased from 751.98 per 100,000 in 1990 (95% CI: 499.20 to 1094.41) to 698.90 per 100,000 in 2019 (466.54 to 1015.31), with an AAPC of −0.25% (95% CI: −0.28% to −0.22%). As shown in Figure 1 and Supplementary Table S1, the global ASPR has slowly declined since 1990, with the most significant decline in ASPR occurring between 2015 and 2019 (APC: −0.52% (−0.56% to −0.48%), $p < 0.05$). Similarly, there was a slight decrease in age-standardized DALY rates for ID in children with different APCs, which declined most significantly between 2017 and 2019 (APC: −1.13% (−1.34% to −0.93%), $p < 0.05$).

Age and sex patterns

Globally, trends in the number of prevalent cases, ASPR, DALYs, and age-standardized DALY rate by sex and age group from 1990 to 2019 are shown in Supplementary Tables S2–S5, Supplementary Figures S1–S3, and Figure 2. With respect to gender, the prevalence of ID in children was more pronounced in girls than in boys, as was the case for DALY. The ASPR for ID has declined over the past three decades in both boys and girls, with an AAPC of −0.11% [95% CI: −0.12% to −0.10%; from 20,536.79 (95% CI: 19,520.21 to 21,623.95) per 100,000 in 1990 to 19,876.25 (18,772.73 to 21,033.99) per 100,000 in 2019] in boys, and −0.16% [−0.18% to −0.14%; from 21,427.59 (20,495.42 to 22,361.34) per 100,000 to 20,433.52 (19,553.32 to 21,314.45) per 100,000] in girls. Similarly, the age-standardized DALY rate declined. The AAPC for males is −0.21% [95% CI: −0.25% to −0.18%; decreasing from 725.74 (95% CI: 480.82 to 1056.29) per 100,000 in 1990 to 681.77 (448.37 to 1002.33) per 100,000 in 2019], and for females is −0.29% [−0.32% to −0.26%; from 779.53 (518.29 to 1125.16) per 100,000 in 1990 to 717.15 (476.56 to 1042.48) per 100,000 in 2019].

In terms of age, the highest prevalence in the last 30 years was in the age group of less than 5 years. The results of the Joinpoint regression analyses showed a decreasing trend in prevalence between 1990 and 2019 in the <5 years age group [AAPC: −0.29% (95% CI: −0.32% to −0.25%), $p < 0.05$], with the most pronounced decrease between 2006 and 2019 [APC: −0.76% (−1.00% to −0.52%), $p < 0.05$]. DALY rates for ID declined in all three age groups during this period,

TABLE 2 Global DALYs of ID among children in 1990 and 2019 and AAPC from 1990 to 2019.

DALY	1990		2019		1990–2019
	Cases no. (95%UI)	ASR/100,000 (95% CI)	Cases no. (95%UI)	ASR/100,000 (95% CI)	AAPC (%) (95%CI)
Global	13265334.81 (8868971.7 to 19146397.21)	751.98 (499.20 to 1094.41)	13620230.64 (9174233.54 to 19794594.83)	698.90 (466.54 to 1015.31)	−0.25 (−0.28 to −0.22)
Sex					
Boy	6586677.11 (4382196.30 to 9587842.03)	725.74 (480.82 to 1056.29)	6847447.67 (4551907.88 to 10070226.66)	681.77 (448.37 to 1002.33)	−0.21 (−0.25 to −0.18)
Girl	6678657.69 (4487320.53 to 9597565.14)	779.53 (518.29 to 1125.16)	6772782.97 (4536543.65 to 9810175.15)	717.15 (476.56 to 1042.48)	−0.29 (−0.32 to −0.26)
SDI					
High SDI	269104.95 (170,046 to 400870.35)	157.31 (96.94 to 241.84)	149955.44 (93839.06 to 231790.76)	93.68 (55.55 to 149.37)	−1.77 (−1.8 to −1.73)
High-middle SDI	1271719.00 (835147.15 to 1882272.61)	421.01 (268.58 to 619.83)	618841.96 (402987.94 to 911054.32)	254.57 (162.91 to 385.17)	−1.73 (−1.76 to −1.69)
Middle SDI	3514211.28 (2316672.84 to 5141554.65)	603.91 (396.74 to 878.07)	2336857.73 (1555153.16 to 3391386.24)	425.65 (282.68 to 624.46)	−1.20 (−1.22 to −1.17)
Low-middle SDI	4965142.94 (3304139.23 to 7163848.93)	1083.47 (713.64 to 1574.43)	4611592.66 (3079567.4 to 6738220.99)	887.81 (585.77 to 1297.56)	−0.68 (−0.69 to −0.67)
Low SDI	3238741.22 (2185104.53 to 4708582.34)	1310.76 (875.23 to 1898.93)	5895716.72 (3944808.94 to 8616020.1)	1240.83 (819.96 to 1813.62)	−0.19 (−0.21 to −0.17)
GBD regions					
Western Sub-Saharan Africa	1222710.22 (813347.31 to 1762107.47)	1354.21 (881.91 to 1991.02)	2847854.97 (1885088.17 to 4113576.07)	1425.30 (921.99 to 2097.08)	0.18 (0.16 to 0.19)
South Asia	6180310.54 (4176161.21 to 8836557.46)	1394.55 (926.82 to 1992.55)	6046176.40 (4004177.84 to 8790538.78)	1180.06 (775.65 to 1732.25)	−0.57 (−0.58 to −0.56)
Eastern Sub-Saharan Africa	1015334.87 (673858.07 to 1469945.44)	1097.35 (723.36 to 1587.21)	1653748.53 (1104166.98 to 2426650.9)	931.01 (613.91 to 1366.16)	−0.57 (−0.61 to −0.53)
Central Sub-Saharan Africa	240571.03 (154332.58 to 358341.65)	910.91 (557.89 to 1403.29)	496116.51 (315481.14 to 735381.39)	863.04 (516.59 to 1321.32)	−0.21 (−0.30 to −0.12)
Oceania	20876.09 (13282.6 to 30499.01)	784.05 (464.61 to 1210.33)	37276.48 (23079.6 to 55039.4)	756.95 (441.32 to 1199.61)	−0.13 (−0.16 to −0.09)
Caribbean	69445.94 (44316.1 to 103816.4)	607.02 (369.47 to 925.28)	75295.50 (48559.93 to 113864.02)	648.64 (391.18 to 1006.31)	0.24 (0.19 to 0.28)
Central Asia	192083.31 (125716.29 to 280014.93)	756.68 (475.62 to 1149.63)	154800.50 (98709.2 to 227226.4)	570.05 (340.27 to 877.65)	−0.98 (−1.01 to −0.94)
Southern Sub-Saharan Africa	104867.32 (66195.05 to 156323.47)	514.16 (301.1 to 813.69)	106635.70 (67522.64 to 159646.54)	451.45 (264.25 to 704.9)	−0.44 (−0.53 to −0.34)
North Africa and Middle East	853493.49 (563829.98 to 1261457.22)	583.27 (376.3 to 866.5)	786372.80 (515429.96 to 1178235.7)	450.83 (283.09 to 684.98)	−0.88 (−0.93 to −0.84)
Andean Latin America	110575.19 (71283.56 to 165935.88)	733.3 (445.26 to 1141.98)	70861.47 (44016.3 to 110157.65)	391.54 (224.06 to 640.84)	−2.15 (−2.22 to −2.07)
Tropical Latin America	301659.79 (184383.07 to 458878.67)	564.04 (301.54 to 939.29)	182219.01 (110308.6 to 283954.34)	372.9 (193.08 to 648.09)	−1.41 (−1.43 to −1.39)
Southeast Asia	1135042.15 (744924.01 to 1714570.04)	659.59 (418.95 to 1006.28)	569630.48 (358447.8 to 864036.43)	343.49 (212.38 to 529.35)	−2.23 (−2.30 to −2.16)
Central Europe	104840.17 (66023.39 to 156111.53)	374.31 (225.5 to 580.94)	39486.66 (24252.71 to 60494.09)	229.62 (133.09 to 368.52)	−1.66 (−1.73 to −1.59)
Central Latin America	225856.01 (146134.5 to 332869.59)	351.26 (225.77 to 523.15)	143777.06 (93522.31 to 210590.62)	224.15 (142.47 to 336.01)	−1.53 (−1.56 to −1.51)

(Continued)

TABLE 2 (Continued)

DALY	1990		2019		1990–2019
	Cases no. (95%UI)	ASR/100,000 (95% CI)	Cases no. (95%UI)	ASR/100,000 (95% CI)	AAPC (%) (95%CI)
Southern Latin America	57386.1 (34,957 to 90704.32)	386.27 (212.73 to 643.7)	31920.77 (18627.47 to 51889.33)	220.66 (109.36 to 413.86)	−1.93 (−2.03 to −1.83)
High-income Asia Pacific	92962.55 (55825.73 to 143062.07)	271.06 (148.56 to 454.08)	31930.17 (18161.51 to 52886.74)	139.62 (65.88 to 258.26)	−2.26 (−2.28 to −2.24)
Australasia	8075.67 (4447.01 to 14469.79)	179.97 (81.63 to 371.61)	6195.33 (3133.51 to 11129.22)	116.21 (47.28 to 244.48)	−1.50 (−1.52 to −1.47)
Eastern Europe	82534.81 (51439.66 to 128088.5)	161.74 (88.67 to 274.67)	35945.10 (20388.75 to 59327.65)	97.8 (47.08 to 183.49)	−1.74 (−1.83 to −1.65)
East Asia	1111198.18 (709307.92 to 1620997.66)	330.69 (198.94 to 504.97)	206133.79 (127438.05 to 317068.12)	87.97 (47.57 to 145.28)	−4.49 (−4.57 to −4.40)
Western Europe	89831.76 (55788.6 to 137744.3)	130.02 (77.32 to 203.8)	52145.66 (31613.08 to 81175.06)	77.99 (44.32 to 129.03)	−1.75 (−1.77 to −1.73)
High-income North America	45679.64 (26652.77 to 71822.76)	74.44 (38.9 to 129.2)	45707.74 (24972.58 to 78926.71)	69.36 (30.06 to 133.63)	−0.24 (−0.29 to −0.18)

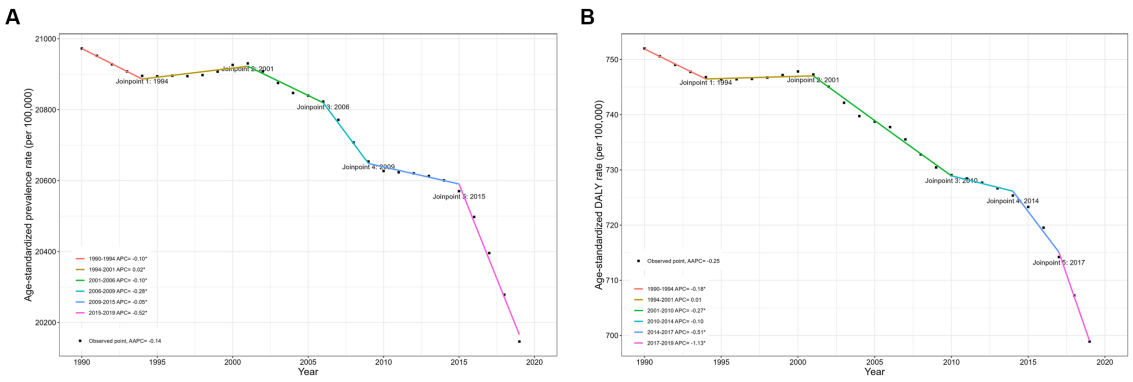

FIGURE 1
Joinpoint regression analysis of global ID prevalence and DALY rates in children younger than 15 years old from 1990 to 2019. **(A)** Prevalence; **(B)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

with the greatest decline in those younger than 5 years of age [AAPC: −0.54% (95% CI: −0.58% to −0.50%)], and the most pronounced decline from 2001 to 2010 [APC: −0.94% (−0.98% to −0.91%), $p < 0.05$]. In 2019, the DALY rate was highest in the 5–9 years age group [829.78 (95% CI: 556.53 to 1203.08) per 100,000].

Association with the socio-demographic index

ID burden among children in different SDI regions is presented in Tables 1, 2 and Figure 3. From 1990 to 2019, the prevalence rates in all five SDI regions showed a decreasing trend, with the largest decrease occurring among those with high-middle SDI [from 13,681.96 (95% CI: 12,561.77 to 14,837.86) per 100,000 in 1990 to 9271.51 (8331.91 to 10,291.10) per 100,000 in 2019; AAPC: −1.34% (95%CI: −1.37% to −1.31%)], followed by high SDI areas. The highest prevalence rates in 2019 were found in the low-SDI quintile countries [32,516.96 (95%CI: 30,993.53 to 34,036.61) per 100,000], and the high-SDI quintile countries [4671.08 (3765.88 to 5786.17) per 100,000] had the lowest estimates.

DALY rates for ID have also declined in all five SDI regions over the past 30 years, with the most pronounced decline in the high SDI region [from 157.31 (95% CI: 96.94 to 241.84) per 100,000 in 1990 to 93.68 (55.55 to 149.37) per 100,000 in 2019; AAPC: −1.77% (95%CI: −1.80% to −1.73%)], followed by the high-middle SDI region. The highest DALY rate in 2019 was found in the low SDI quintile countries [1240.83 (95% CI: 819.96 to 1813.62) per 100,000] and the lowest in the high SDI quintile countries [93.68 (55.55 to 149.37) per 100,000]. The variations in ASPR and DALY rates of ID among children across SDI by 21 GBD regions are shown in Figure 4. Overall, there was a negative correlation between ASPR and SDI across global regions from 1990 to 2019, as well as age-standardized DALY rate.

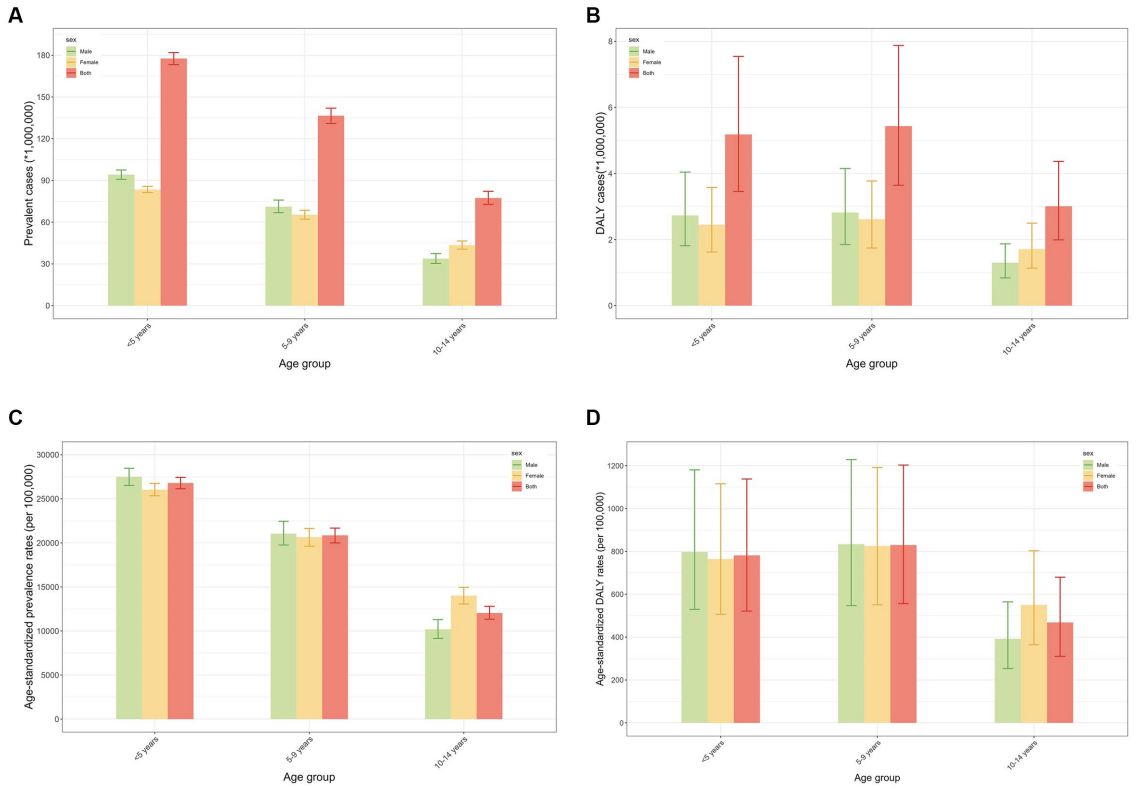

FIGURE 2
Prevalence and DALY of global children with ID in different age groups in 2019. **(A)** Prevalent cases in different age groups in 2019; **(B)** Prevalence rates in different age groups in 2019; **(C)** DALY cases in different age groups in 2019; **(D)** DALY rates in different age groups in 2019. DALY, disability-adjusted life year; ID, iron deficiency.

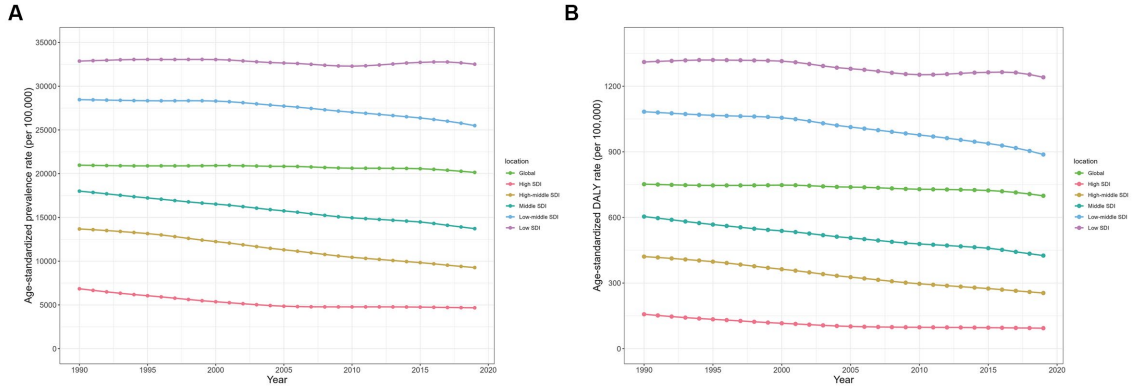

FIGURE 3
Prevalence and DALY rates of children with ID in five SDI regions from1990 to 2019. **(A)** Prevalence; **(B)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

Regional level

At the regional level, from 1990 to 2019, East Asia [from 10,732.14 (95% CI: 9162.45 to 12,413.07) per 100,000 in 1990 to 3629.73 (2723.62 to 4711.96) per 100,000 in 2019; AAPC: −3.67% (95% CI: −3.78% to −3.57%)], Southeast Asia [from 20,876.28 (18,310.71 to 23,622.00) per 100,000 in 1990 to 12,736.34 (10,952.83 to 14,777.29) per 100,000 in 2019; AAPC: −1.70% (−1.75% to −1.65%)], and High-income Asia Pacific [from 10,227.36 (7136.79 to 14,450.51) per 100,000 in 1990 to 6350.17 (3884.40 to 10,061.75) per 100,000 in 2019; AAPC: −1.63% (−1.64% to −1.61%)] had the largest decrease in the prevalence of ID among children. In contrast, ASPR increased in the Caribbean [AAPC: 0.21% (95% CI: 0.17 to 0.25%)] and Western Sub-Saharan Africa [0.28% (0.26 to 0.30%)]. Apparently, in 2019, Western Sub-Saharan Africa [36,715.50 (95% CI: 33,278.85 to 40,025.55) per 100,000], South Asia [31,523.88 (29,637.75 to

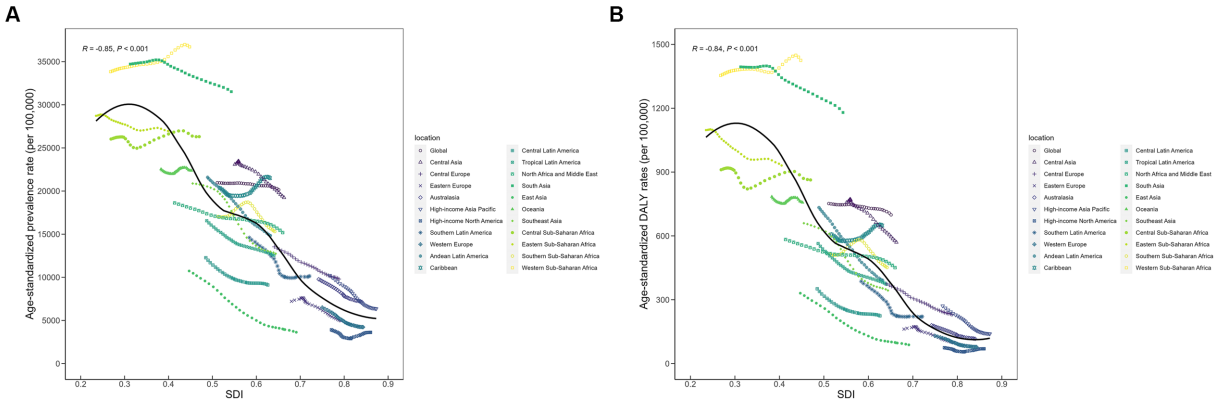

FIGURE 4
Prevalence and DALY rates of children with ID across 21 GBD regions by socio-demographic index. **(A)** Prevalence; **(B)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

33,458.24) per 100,000], and Eastern Sub-Saharan Africa [31,523.88 (29,637.75 to 33,458.24) per 100,000] showed the highest ASPRs, whereas the regions with the lowest ASPRs were High-income North America [3618.47 (2053.33 to 5826.49) per 100,000], East Asia [3629.73 (2723.62 to 4711.96) per 100,000] and Western Europe [4219.94 (3198.71 to 5551.05) per 100,000].

From 1990 to 2019, age-standardized DALY rates tended to decline in the vast majority of geographic regions, especially in East Asia [from 330.69 (95% CI: 198.94 to 504.97) per 100,000 in 1990 to 87.97 (47.57 to 145.28) per 100,000 in 2019; AAPC: −4.49% (95% CI: −4.57% to −4.40%)], Southeast Asia [from 659.59 (418.95 to 1006.28) per 100,000 in 1990 to 343.49 (212.38 to 529.35) per 100,000 in 2019; AAPC: −2.23% (−2.30% to −2.16%)] and High-income Asia Pacific [from 271.06 (148.56 to 454.08) per 100,000 in 1990 to 139.62 (65.88 to 258.26) per 100,000 in 2019; AAPC: −2.26% (−2.28% to −2.24%)]. Notably, in 2019, Western Sub-Saharan Africa [1425.30 (95% CI: 921.99 to 2097.08) per 100,000], South Asia [1180.06 (775.65 to 1732.25) per 100,000], and Eastern Sub-Saharan Africa [931.01 (613.91 to 1366.16) per 100,000] were the regions with the highest DALYs due to ID among children.

National level

Details of ID burden among children in various countries and territories are presented in Supplementary Tables S6, S7 and Figure 5. Nationally, the prevalence of ID in children declined in most countries, with the most pronounced decline in Ecuador [from 18,161.05 (95% CI: 12,274.35 to 25,036.33) per 100,000 in 1990 to 5685.00 (3472.33 to 8622.14) per 100,000 in 2019; EAPC: −4.52 (95%CI: −4.71 to −4.32)], followed by China [from 10,604.68 (9006.75 to 12,325.94) per 100,000 in 1990 to 3415.32 (2478.93 to 4529.54) per 100,000 in 2019; EAPC: −4.26 (−4.51 to −4.01)] and Chile [from 3864.85 (1778.45 to 7758.28) per 100,000 in 1990 to 1543.07 (600.89 to 3446.53) per 100,000 in 2019; EAPC: −3.05 (−3.31 to −2.79)]. Conversely, prevalence rates increased in a few countries, with Burkina Faso [from 29,783.32 (95% CI: 23,608.87 to 36,051.48) per 100,000 in 1990 to 42,571.67 (34,401.56 to 51,755.86) per 100,000 in 2019; EAPC: 1.43 (95% CI: 1.31 to 1.55)], Angola [from 15,982.47 (11,121.66 to 21,501.39) per 100,000 in 1990 to 22,108.10 (15,928.42 to 29,531.69) per 100,000 in 2019; EAPC: 1.55 (1.40 to 1.69)], and Yemen [from 24,791.91 (20,746.81 to 29,176.45) per 100,000 in 1990 to 33,594.07 (28,665.26 to 38,997.44) per 100,000 in 2019; EAPC: 1.36 (1.22 to 1.50)] having the greatest increase. Evidently, in 2019, Bhutan [54,729.81 (95% CI: 46,877.72 to 62,083.13) per 100,000], Mali [47,477.39 (40,291 to 54,123.33) per 100,000] and Gambia [45,473.78 (38,237.83 to 52,214.25) per 100,000] had the highest ASPR, while the countries with the lowest ASPR were Chile [1543.07 (600.89 to 3446.53) per 100,000] and Canada [2023.79 (833.01 to 4157.10) per 100,000].

From 1990 to 2019, a decreasing trend in the age-standardized DALY rate was observed in most of countries, with Ecuador [from 524.27 (95% CI: 273.37 to 899.41) per 100,000 in 1990 to 120.31 (57.91 to 224.17) per 100,000 in 2019; EAPC: −5.61 (95%CI: −5.83 to −5.38)] having the largest decline. On the contrary, Burkina Faso [from 1150.14 (95% CI: 635.05 to 1864.62) per 100,000 in 1990 to 1801.44 (1055.54 to 2822.90) per 100,000 in 2019; EAPC. 1.78 (95% CI: 1.66 to 1.90)] experienced the largest increase during this period. Notably, in 2019, Bhutan [2302.58 (95% CI: 1405.02 to 3478.33) per 100,000], Mali [2151.80 (1316.27 to 3221.81) per 100,000] and Burkina Faso [1801.44 (1055.54 to 2822.9) per 100,000] were the countries with the highest DALY rates.

Discussion

ID is the most common micronutrient deficiency worldwide. ID among children triggers a number of health consequences, such as significantly affecting growth and development, impairing the cognitive development of the brain, diminishing immune function, and affecting the metabolism of other nutrients (23–26). There has been some research showing concern about ID in children (27–29). This study provided a new and comprehensive assessment of the disease burden of ID in children, which will facilitate appropriate preventive measures for childhood ID by regional governments. Strengthening the prevention and treatment of childhood ID has important public health significance for reducing the global burden of disease.

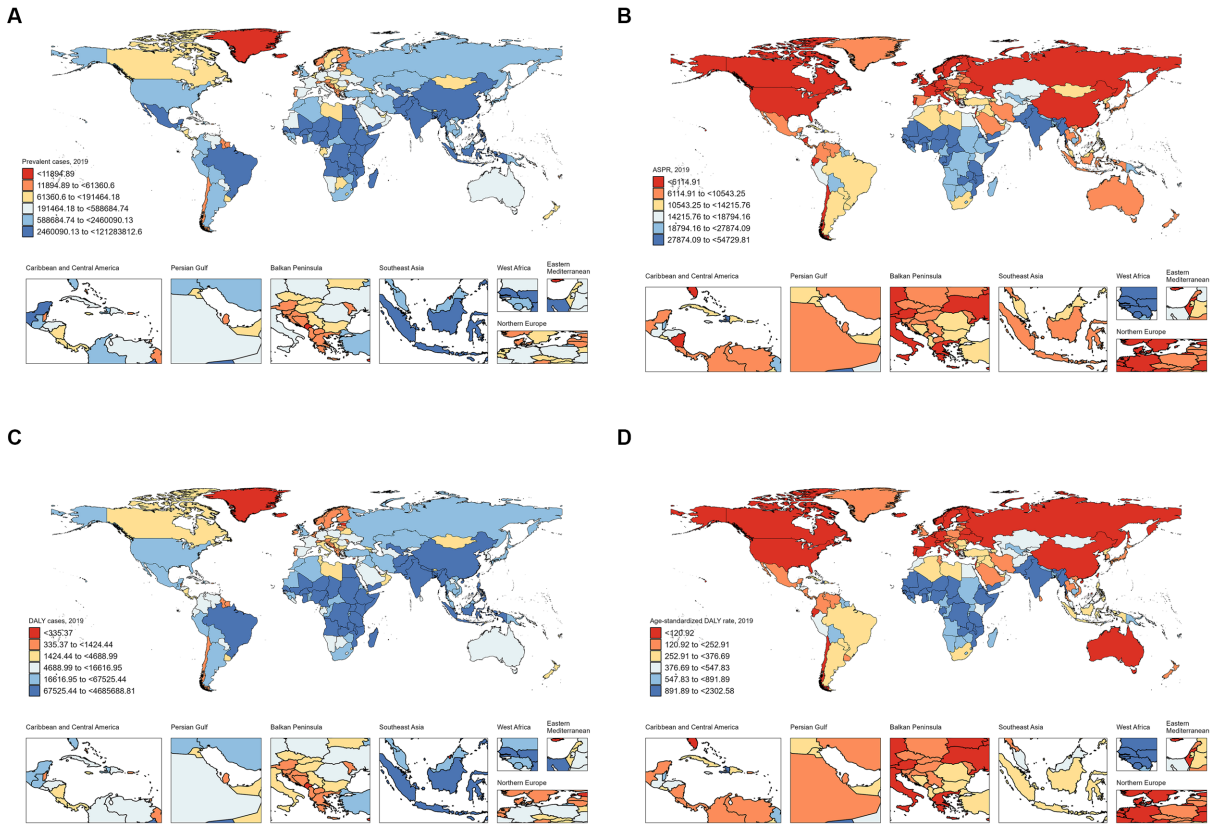

FIGURE 5
Prevalence and DALY of children with ID in different countries and territories in 2019. **(A)** Prevalent cases; **(B)** Prevalence; **(C)** DALY cases; **(D)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

Based on our findings, ID prevalence varied by age and gender, suggesting that age and gender can be used to promote targeted ID screening among children. Since 1990, thanks to worldwide efforts, the global prevalence and DALY rates of ID in children have continued to decline slowly. However, between 1990 and 2019, those younger than five years old were the only age group with a significant decrease in ID prevalence. Despite this, the prevalence is still remarkably higher in children younger than five years old, suggesting that this group is considered to be at high risk for the onset of ID. This may be closely related to increased iron requirements due to rapid growth and development. Given that early childhood is a crucial period for intellectual and psychomotor development, measures to prevent ID should continue to be supported, encouraged and implemented.

Gender differences in ID burden varied across age groups. As a whole, ID prevalence was higher in girls than in boys among children younger than 15 years old. During this period, the prevalence and DALY rates were higher in girls than in boys for children aged 10 to 14 years. Whereas the other two age groups appeared to have a higher prevalence in boys than in girls. In other words, our results indicated that boys under 10 years of age endure a heavier burden of ID than girls worldwide. Consistent with our findings, several studies have reported lower iron stores in male infants and children, which may put them at greater risk for IDA (30, 31). Conversely, other studies have demonstrated girls to have higher rates of anemia than boys (32). That is, public health messages have consistently emphasized that girls are a high-risk group, so mothers of young girls may prioritize their dietary iron needs over those of boys, resulting in higher iron sufficiency rates for girls and greater deficiency in boys (33).

Socio-economic factors also play an important role in childhood ID, with East Asia experiencing the greatest decline in prevalence and DALY rates between 1990 and 2019. Regionally, from 1990 to 2019, age-standardized prevalence DALY rates tended to decline in the vast majority of geographic regions. However, we found an increase in ID prevalence and DALY rates among children in western sub-Saharan Africa, and it is critical to determine the underlying causes of the increased estimates. Western Sub-Saharan Africa, South Asia, and Eastern Sub-Saharan Africa continue to have high prevalence and DALY rates, which may be mainly due to insufficient dietary supply of bioavailable iron to meet children's needs. The causes of ID vary widely at different stages of life, as well as between gender and socio-economic circumstances (34). The scientific feeding of parents, the level of parental knowledge, and the number of children in the family are also associated with the occurrence of children with ID. Notably, micronutrient deficiencies (such as vitamin A, vitamin C, etc.) lead to poor iron absorption, which is particularly evident in children aged 0–6 years. Anemia is one of the many consequences of ID (35). IDA affects cognitive functioning in children, delays motor development, impairs physical functioning and quality of life, and poses a major global health problem and challenge for developing countries (35).

At the national level, India (121,283,813), Nigeria (36,200,362) and Pakistan (27,331,933) had the highest number of prevalent cases in 2019. Iron deficiency anemia (IDA) prevalence is 49.5% in 6–23-month-old and 39.9% in 24–58-month-old children, which has resulted in huge intangible costs and production losses for Indian society (36). The status of literacy and wealth of parents may be an important factor (37). In this regard, educational interventions by school teachers for children or adolescents may be effective in raising their awareness and attitudes towards diseases and their prevention (38). Similarly, Nigeria (39) and Pakistan (40) face the same social problem – ID in children. Bhutan, Mali, and Gambia shouldered the highest ASPR. The prevalence of anemia among preschool children in Bhutan was as high as 42% according to the Bhutan National Nutrition Survey 2015 (41). Notably, we found a more than 35 times difference in the prevalence of ID in children between different countries and territories, ranging from 1,543 to 54,730 per 100,000. It is noteworthy that a few countries in the lower SDI quintile experienced an increase in prevalence during the study period, including Burkina Faso, Angola, and Yemen. More research is needed to understand the reasons for the increase in ID prevalence in these countries. ID burden among children from countries in the lower SDI quintiles requires urgent attention. The country with the largest decrease in prevalence was Ecuador, followed by China and Chile.

There were 7.79 million children with ID in China in 2019, which had decreased by 77.4% since 1990. We found lower age-standardized prevalence and DALY rates in China compared with most of the countries in this study. In 2019, the ASPR for childhood ID in China was 3415.32 per 100,000, which was significantly lower than the estimates for Bhutan (54,729.81 per 100,000) or Mali (47,477.39 per 100,000), but was slightly higher than Italy (3311.53 per 100,000) or Monaco (3270.25 per 100,000). As for the DALY rate of childhood ID, the value for China was 82.6 per 100,000 in 2019, far below worldwide standards (698.9 per 100,000). Overall, the prevalence and DALY rates of childhood ID in China showed a decreasing trend from 1990 to 2019. During this period, the EAPCs of ASPR and DALY rate in China were −4.26 (95% CI, −4.51 to −4.01) and −5.14 (−5.43 to −4.84), respectively. Evidently, the improvement in ID burden among Chinese children was much greater than the global level.

ID is disabling. In 2017, the GBD Study reported that dietary ID remains the fourth and twelfth leading cause of years lived with disability in females and males, respectively (42). Age-standardized DALY rates showed a decreasing trend in most regions, especially in East Asia, Southeast Asia, and High-income Asia Pacific, but countries with lower SDI still had the highest DALY rates in 2019. It is critical to promote effective inter-country interventions and collaboration to improve healthcare for children with ID in countries with low SDI.

Countries and regions with a high ID prevalence among children must take effective preventive measures against ID. First of all, the publicity and prevention of ID should be further improved. In accordance with the relevant regulations, children should be provided with regular health screening and nutritional assessments, and education on nutritional knowledge should be strengthened, so as to ensure that children can have reasonable nutrition and balanced diets, and that bad habits such as picky eating can be positively corrected. In addition, children have rapid growth and development, and the amount of iron contained in food usually fails to meet their iron needs. Oral iron is the first line of treatment for ID in children, and iron supplementation treatment can significantly improve children's lagging growth and development (43).

Limitations

A strength of the study is that we have provided up-to-date and comprehensive estimates of levels and trends associated with ID among children at the global, regional, and national levels. Nevertheless, there are some limitations of this study. First, we assessed ID burden among children based on age, sex, and SDI, but did not assess its other risk factors. In addition, as the data were aggregated across multiple sites globally, there may be limitations of underdiagnosis and underreporting, which may lead to an underestimation of our results and needs to be approached with caution when interpreting the results. Finally, our study was limited by the variable quality of the GBD data and missing data, and we were unable to analyse incidence rates in this study due to the unavailability of data on ID incidence in the GBD database.

Areas for further research

To balance the limitations of GBD study, more international cooperation should be encouraged, including annual searches for available data with national collaborators. The quality of the data used in this study relies on the quality control of the original GBD data collection process, and bias is still inevitable. It is suggested that the findings of this study be further validated with the help of large cohort studies. Moreover, the registration of global epidemiological data should be strengthened. Furthermore, the potential reasons for increased prevalence and DALY rates in a few countries are also worthy of attention. Besides, the incidence of childhood ID needs to be further studied, and Bayesian forecasting model is available for future trends in the burden of disease.

Conclusions and recommendations

Despite the global trend of decreasing prevalence and DALY rates of ID among children between 1990 and 2019, childhood ID still remains an important public health problem in the future due to the rising number of prevalent cases and DALYs, especially in countries with low SDI. Iron fortification remains an essential aspect of childhood health. There is an urgent need for screening and prevention and control of ID for children in economically less developed areas. Children younger than 5 years old are an important population that requires targeted measures to reduce the ID burden. It is imperative to enhance parental awareness of ID and feeding knowledge. Iron-rich foods are recommended to increase the amount of iron available to children through proper dietary intake. In addition, increased food diversity and improved iron bioavailability are also advisable. Moreover, bridging the gap between high-income and low-income countries and carefully defining strategies to reach target populations may facilitate the reduction of ID rates.

Data availability statement

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

Author contributions

DL: Conceptualization, Writing – original draft. CM: Data curation, Writing – original draft. YL: Formal analysis, Writing – review & editing. TZ: Investigation, Writing – review & editing. YX: Project administration, Writing – review & editing. YZ: Project administration, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1275291/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Joinpoint regression analysis of global ID prevalence and DALY rates in children younger than 5 years old from 1990 to 2019 **(A)** Prevalence; **(B)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

SUPPLEMENTARY FIGURE S2
Joinpoint regression analysis of global ID prevalence and DALY rates in children aged 5-9 years from 1990 to 2019 **(A)** Prevalence; **(B)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

SUPPLEMENTARY FIGURE S3
Joinpoint regression analysis of global ID prevalence and DALY rates in children aged 10-14 years from 1990 to 2019 **(A)** Prevalence; **(B)** DALY rates. DALY, disability-adjusted life year; ID, iron deficiency.

References

1. Steinbicker AU, Muckenthaler MU. Out of balance--systemic iron homeostasis in iron-related disorders. *Nutrients*. (2013) 5:3034–61. doi: 10.3390/nu5083034

2. Chen Z, Chen J, Song C, Sun J, Liu W. Association between serum iron status and muscle mass in adults: results from NHANES 2015–2018. *Front Nutr*. (2022) 9:941093. doi: 10.3389/fnut.2022.941093

3. Ning S, Zeller MP. Management of iron deficiency. *Hematology Am Soc Hematol Educ Program*. (2019) 2019:315–22. doi: 10.1182/hematology.2019000034

4. Eden AN. Iron deficiency and impaired cognition in toddlers: an underestimated and undertreated problem. *Paediatr Drugs*. (2005) 7:347–52. doi: 10.2165/00148581-200507060-00003

5. Pivina L, Semenova Y, Doşa MD, Dauletyarova M, Bjørklund G. Iron deficiency, cognitive functions, and neurobehavioral disorders in children. *J Mol Neurosci*. (2019) 68:1–10. doi: 10.1007/s12031-019-01276-1

6. Brigham D, Beard J, Tobin B. Iron and thermoregulation: a review. *Crit Rev Food Sci Nutr*. (1996) 36:747–63. doi: 10.1080/10408399609527748

7. Rosenzweig PH, Volpe SL. Iron, thermoregulation, and metabolic rate. *Crit Rev Food Sci Nutr*. (1999) 39:131–48. doi: 10.1080/10408399908500491

8. Frost JN, Wideman SK, Preston AE, Teh MR, Ai Z, Wang L, et al. Plasma iron controls neutrophil production and function. *Sci Adv*. (2022) 8:eabq5384. doi: 10.1126/sciadv.abq5384

9. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. *Lancet*. (2021) 397:233–48. doi: 10.1016/S0140-6736(20)32594-0

10. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. *Lancet Glob Health*. (2013) 1:e16–25. doi: 10.1016/S2214-109X(13)70001-9

11. Kyu HH, Pinho C, Wagner JA, Brown JC, Bertozzi-Villa A, Charlson FJ, et al. Global and National Burden of diseases and injuries among children and adolescents between 1990 and 2013: findings from the global Burden of disease 2013 study. *JAMA Pediatr*. (2016) 170:267–87. doi: 10.1001/jamapediatrics.2015.4276

12. López-Ruzafa E, Vázquez-López MA, Galera-Martínez R, Lendínez-Molinos F, Gómez-Bueno S, Martín-González M. Prevalence and associated factors of iron deficiency in Spanish children aged 1 to 11 years. *Eur J Pediatr*. (2021) 180:2773–80. doi: 10.1007/s00431-021-04037-8

13. GBD 2019 Demographics. Collaborators. Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950–2019: a comprehensive demographic analysis for the global Burden of disease study 2019. *Lancet*. (2020) 396:1160–203. doi: 10.1016/S0140-6736(20)30977-6

14. Safiri S, Carson-Chahhoud K, Noori M, Nejadghaderi SA, Sullman M, Ahmadian Heris J, et al. Burden of chronic obstructive pulmonary disease and its attributable risk factors in 204 countries and territories, 1990–2019: results from the global Burden of disease study 2019. *BMJ*. (2022) 378:e069679. doi: 10.1136/bmj-2021-069679

15. Long D, Mao C, Liu Y, Zhou T, Xu Y, Zhu Y. Global, regional, and national burden of intestinal obstruction from 1990 to 2019: an analysis from the global Burden of disease study 2019. *Int J Color Dis*. (2023) 38:245. doi: 10.1007/s00384-023-04522-6

16. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global Burden of disease study 2019. *Lancet*. (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9

17. GBD 2019 Viewpoint Collaborators. Five insights from the global Burden of disease study 2019. *Lancet*. (2020) 396:1135–59. doi: 10.1016/S0140-6736(20)31404-5

18. Zhang K, Kan C, Han F, Zhang J, Ding C, Guo Z, et al. Global, regional, and National Epidemiology of diabetes in children from 1990 to 2019. *JAMA Pediatr*. (2023) 177:837–46. doi: 10.1001/jamapediatrics.2023.2029

19. Wu Y, Deng Y, Wei B, Xiang D, Hu J, Zhao P, et al. Global, regional, and national childhood cancer burden, 1990–2019: an analysis based on the global Burden of disease study 2019. *J Adv Res*. (2022) 40:233–47. doi: 10.1016/j.jare.2022.06.001

20. GBD 2019 Cancer Risk Factors Collaborators. The global burden of cancer attributable to risk factors, 2010–19: a systematic analysis for the global Burden of disease study 2019. *Lancet*. (2022) 400:563–91. doi: 10.1016/S0140-6736(22)01438-6

21. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. *Stat Med*. (2000) 19:335–51. doi: 10.1002/(sici)1097-0258(20000215)19:3<335::aid-sim336>3.0.co;2-z

22. Qiu H, Cao S, Xu R. Cancer incidence, mortality, and burden in China: a time-trend analysis and comparison with the United States and United Kingdom based on the global epidemiological data released in 2020. *Cancer Commun (Lond)*. (2021) 41:1037–48. doi: 10.1002/cac2.12197

23. de Deungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK. Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res*. (2000) 48:169–76. doi: 10.1203/00006450-200008000-00009

24. Georgieff MK, Krebs NF, Cusick SE. The benefits and risks of iron supplementation in pregnancy and childhood. *Annu Rev Nutr*. (2019) 39:121–46. doi: 10.1146/annurev-nutr-082018-124213

25. Georgieff MK. Iron assessment to protect the developing brain. *Am J Clin Nutr*. (2017) 106:1588S–93S. doi: 10.3945/ajcn.117.155846

26. Lukowski AF, Koss M, Burden MJ, Jonides J, Nelson CA, Kaciroti N, et al. Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. *Nutr Neurosci*. (2010) 13:54–70. doi: 10.1179/147683010X12611460763689

27. Chouraqui JP. Dietary approaches to iron deficiency prevention in childhood-a critical public health issue. *Nutrients*. (2022) 14:14. doi: 10.3390/nu14081604

28. Paulino C, Nishijima M, Sarti FM. Association of Iron Supplementation Programs with iron-deficiency anemia outcomes among children in Brazil. *Nutrients*. (2021) 13:13. doi: 10.3390/nu13051524

29. Subramaniam G, Girish M. Iron deficiency anemia in children. *Indian J Pediatr*. (2015) 82:558–64. doi: 10.1007/s12098-014-1643-9

30. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. (2014) 123:615–24. doi: 10.1182/blood-2013-06-508325

31. Joo EY, Kim KY, Kim DH, Lee JE, Kim SK. Iron deficiency anemia in infants and toddlers. *Blood Res*. (2016) 51:268–73. doi: 10.5045/br.2016.51.4.268

32. Song Y, Wang HJ, Dong B, Wang Z, Ma J, Agardh A. National Trends in hemoglobin concentration and prevalence of anemia among Chinese school-aged children, 1995–2010. *J Pediatr*. (2017) 183:164–9.e2. doi: 10.1016/j.jpeds.2017.01.012

33. Safiri S, Kolahi AA, Noori M, Nejadghaderi SA, Karamzad N, Bragazzi NL, et al. Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: results from the global Burden of disease study 2019. *J Hematol Oncol*. (2021) 14:185. doi: 10.1186/s13045-021-01202-2

34. Larsen B, Baller EB, Boucher AA, Calkins ME, Laney N, Moore TM, et al. Development of iron status measures during youth: associations with sex, neighborhood socioeconomic status, cognitive performance, and brain structure. *Am J Clin Nutr*. (2023) 118:121–31. doi: 10.1016/j.ajcnut.2023.05.005

35. Bathla S, Arora S. Prevalence and approaches to manage iron deficiency anemia (IDA). *Crit Rev Food Sci Nutr*. (2022) 62:8815–28. doi: 10.1080/10408398.2021.1935442

36. Plessow R, Arora NK, Brunner B, Tzogiou C, Eichler K, Brügger U, et al. Social costs of iron deficiency anemia in 6–59-month-old children in India. *PLoS One*. (2015) 10:e0136581. doi: 10.1371/journal.pone.0136581

37. Bharati S, Pal M, Chakrabarty S, Bharati P. Socioeconomic determinants of iron-deficiency anemia among children aged 6 to 59 months in India. *Asia Pac J Public Health*. (2015) 27:NP1432–43. doi: 10.1177/1010539513491417

38. Salam SS, Ramadurg U, Charantimath U, Katageri G, Gillespie B, Mhetri J, et al. Impact of a school-based nutrition educational intervention on knowledge related to iron deficiency anaemia in rural Karnataka, India: a mixed methods pre-post interventional study. *BJOG*. (2023) 130:113–23. doi: 10.1111/1471-0528.17619

39. Owa OT, Brown BJ, Adeodu OO. Iron deficiency among apparently healthy children aged 6 to 24 months in Ibadan, Nigeria. *Pediatr Hematol Oncol*. (2016) 33:338–46. doi: 10.1080/08880018.2016.1217110

40. Ahmed A, Ahmad A, Khalid N, David A, Sandhu MA, Randhawa MA, et al. A question mark on iron deficiency in 185 million people of Pakistan: its outcomes and prevention. *Crit Rev Food Sci Nutr*. (2014) 54:1617–35. doi: 10.1080/10408398.2011.645087

41. Campbell RK, Aguayo VM, Kang Y, Dzed L, Joshi V, Waid JL, et al. Epidemiology of anaemia in children, adolescent girls, and women in Bhutan. *Matern Child Nutr*. (2018) 14:e12740. doi: 10.1111/mcn.12740

42. GBD 2017 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the global Burden of disease study 2017. *Lancet*. (2018) 392:1859–922. doi: 10.1016/S0140-6736(18)32335-3

43. Mattiello V, Schmugge M, Hengartner H, von der Weid N, Renella R. Diagnosis and management of iron deficiency in children with or without anemia: consensus recommendations of the SPOG pediatric hematology working group. *Eur J Pediatr*. (2020) 179:527–45. doi: 10.1007/s00431-020-03597-5

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Impact of market-based home fortification with micronutrient powder on childhood anemia in Bangladesh: a modified stepped wedge design

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Background: Anemia poses a significant public health problem, affecting 1.6 billion people and contributing to the loss of 68.4 million disability-adjusted life years. We assessed the impact of a market-based home fortification program with micronutrient powder (MNP) called Pushtikona-5 implemented by Bangladesh Rural Advancement Committee (BRAC) on the prevalence of anemia among children aged 6–59 months in Bangladesh.

Methods: We used a modified stepped wedged design and conducted three baseline, two midline, and three endline surveys to evaluate the Pushtikona-5 program implemented through three BRAC program platforms. We interviewed children's caregivers, and collected finger-prick blood samples from children to measure hemoglobin concentration. We also collected data on coverage of Pushtikona-5 and infant and young child feeding (IYCF) practices. We performed bivariate and multivariable analysis and calculated adjusted risk ratios (ARRs) to assess the effect of program outcomes.

Results: A total of 16,936 households were surveyed. The prevalence of anemia was 46.6% at baseline, dropping to 32.1% at midline and 31.2% at endline. These represented adjusted relative reductions of 34% at midline (RR 0.66, 95%CI 0.62 to 0.71, value of p <0.001) and 32% at endline (RR 0.68, 95%CI 0.64 to 0.71, value of p <0.001) relative to baseline. Regarding MNP coverage, at baseline, 43.5% of caregivers surveyed had heard about MNP; 24.3% of children had ever consumed food with MNP, and only 1.8% had

consumed three or more sachets in the 7 days preceding the survey. These increased to 63.0, 36.9, and 4.6%, respectively, at midline and 90.6, 68.9, and 11.5%, respectively, at endline.

Conclusion: These results show evidence of a reduction in the prevalence of anemia and an improvement in coverage. This study provides important evidence of the feasibility and potential for impact of linking market-based MNP distribution with IYCF promotion through community level health workers.

KEYWORDS

home fortification, micronutrient powder, market-based program, anemia, children, Bangladesh

Introduction

Anemia poses a significant public health problem, affecting 1.6 billion people and contributing to the loss of 68.4 million disability-adjusted life years (1). Childhood anemia is widespread across regions and countries. In South Asia (including Bangladesh and India), preschool children are the most affected. More than half of Bangladeshi children under age five (51%) are estimated to be anemic (2), and prevalence has consistently been high in the past several decades. Although multiple factors can result in anemia, iron deficiency is considered the most common cause (1). Anemia can have severe effects on children, including impaired cognitive, mental and motor development (3, 4) and several chronic diseases (5), and results in fatigue and reduced work productivity among adult women (6).

Multiple interventions are available to address childhood anemia. Home fortification of foods with micronutrient powders (MNPs), managed through the primary healthcare setting using community vendors, has the potential to reduce anemia and iron deficiency in children (5, 7). The World Health Organization (WHO) recommends the use of MNPs in areas where diverse foods are unavailable or unaffordable and where industrial fortification of food with multiple micronutrients is inadequate to fill nutrient gaps (8–10). Despite these recommendations, few countries have scaled up home fortification to a national level. A global assessment published in 2013 (11) found that only 12 out of 63 home fortification programs in low-income countries were scaled up nationally, the recent evidence specifically focusing on the effect of interventions distributing home fortification products (HFP) on infant and young child feeding (IYCF) practices instead of evaluating home fortification with MNP implementation at global scale (12).

Most of these nationally scaled-up programs were implemented with the support of external funding agencies and distributed MNPs to caregivers at no cost (11). This dependence on external funding creates concerns about the programs' long-term sustainability (13). Market-based MNP promotion has been identified potentially as a sustainable approach to home fortification (14, 15) because it generates revenue to subsidize operational costs. Having local health workers sell MNPs to caregivers as part of the promotion of home fortification can help programs last even after external funding is phased out. Market-based home fortification with micronutrient powder (MNP) was kick started through the efforts of several organizations including Bangladesh Rural Advancement Committee (BRAC) and the Global Alliance for Improved Nutrition (GAIN) in several low- and middle-income countries including in Bangladesh and Kenya (15, 16). However, there is limited evidence on whether market-based MNP interventions implemented nationally have a demonstrated impact on the reduction of anemia among children under age five (15, 16).

From 2014 to 2018, BRAC implemented a market-based home fortification, named the Maternal Infant and Young Child Nutrition (MIYCN) program (17), to reduce anemia among children 6–59 months old. BRAC used an MNP, called Pushtikona-5, which contained five micronutrients (iron, zinc, folic acid, vitamin A, and vitamin C). The micronutrient composition in the Pushtikona-5 includes 12.5 mg of iron (as ferrous fumarate), 5 mg of zinc, 160 µg of folic acid, 300 µg RE of vitamin A, and 30 mg of vitamin C. BRAC's Shasthya Shebikas (SSs)—female volunteer community health workers—sold MNP sachets to the caregivers of children under age five. The MIYCN program was designed to transform the MNP approach into a well-accepted, large-scale, high-quality, and sustainable intervention, using three mechanisms. First, the program built the capacity of BRAC's community health workers and incentivized them to increase sales of Pushtikona-5 and promote household compliance with the recommended MNP regimen. Second, the program built stakeholder acceptance of home fortification as a critical component of the MIYCN package and supported policies to enable the distribution of Pushtikona-5 through public and private channels. Third, it created public awareness of and demand for Pushtikona-5 through a mass media campaign. The Pushtikona-5 program was delivered in a phased rollout across three existing program platforms (detailed in Supplementary Table S1) over five years:

1. Phase 1 was delivered to 68 sub-districts in 10 districts and 2 urban slums through the Maternal, Neonatal, and Child Health (MNCH) program platform in October 2014.
2. Phase 2 was delivered to additional 48 sub-districts in 15 districts and 2 urban slums through the Alive & Thrive (A&T) program platform beginning in April 2015.

Abbreviations: BRAC, Bangladesh Rural Advancement Committee; CI, Confidence Interval; Hb, Hemoglobin; IYCF, Infant and young child feeding; MIYCN, Maternal Infant and Young Child Nutrition; MNCH, Maternal, Neonatal, and Child Health; MNP, micronutrient powders; RR, Relative risk; SS, Shasthya Shebikas; A&T, Alive & Thrive.

3. Phase 3 was delivered to additional 48 sub-districts in 9 districts through the BRAC Nutrition program platform in addition to 2 urban slums which began in May 2016.

We evaluated this program concurrently—evaluation activities were carried out alongside the program implementation based on a mixed-methods approach, including qualitative assessments and coverage surveys conducted according to a modified stepped wedged design and including measurement of hemoglobin concentration (17). Several evaluation results have been published elsewhere (18–22). In this paper, we analyze coverage survey data to assess the impact of the program on the prevalence of anemia and hemoglobin concentration among children aged 6–59 months in Bangladesh, on indicators of the coverage of Pushtikona-5, and on the coverage of infant and young child feeding (IYCF) practices. We also consider the outcomes against prevailing secular trends.

Materials and methods

Study design

We employed a modified stepped-wedge design to assess the Pushtikona-5 interventions, as the implementation of these interventions commenced at varying times across different program platforms. Several factors informed our choice of this evaluation design. Firstly, it enabled us to evaluate the interventions within the context of routine implementation (23). Secondly, due to resource constraints, it was not feasible to administer interventions simultaneously to all targets (24). Furthermore, this design proved to be pertinent when there was a requirement to detect or control for any time trend effects on the effectiveness of the Pustikona-5 program strategy (25). The program was implemented between September 2014 and May 2018 in the three BRAC program platforms (MNCH, A&T and BRAC Nutrition) in Bangladesh. We conducted three baseline (before implementation), two midline (during implementation), and three endline surveys (post-implementation) to evaluate the outcomes of MIYCN program. All eight surveys were population-based conducted in a cross-sectional nature. Figure 1 describes the evaluation timelines across the three platforms. There were three survey waves in the MNCH and A&T platforms: baseline, midline and endline. However, the BRAC nutrition program undertook only two surveys, one at baseline and another at endline. The study protocol has been prospectively registered with RIDIE (Registry for International Development Impact Evaluation) and registration number is RIDIE-STUDY-ID-553a7c4db7267. The protocol number was PR-14048.

Participants

The study participants included children aged 6–59 months and caregivers of those children. Children aged 6–59 months were included in the study due to the nutritional transition that occurs after 6 months of age. It is recognized that exclusive breastfeeding is sufficient to meet the nutritional needs of the child before six months. The study centers on a market-based home fortification program with the primary objective of assessing the impact of MNP on anemia. This age group was deliberately chosen as the study population because children within this range are more vulnerable. Understanding the effects of MNP on anemia in this critical developmental stage is of paramount importance. A caregiver was defined as either the child's biological mother or the person who took care of or looked after the child and gave the child most meals on most days. Households having at least one child aged 6–59 months were selected. The child and the caregiver were eligible to participate if they were not sick on the day of the interview and had no physical or mental challenges that would impede their ability to provide informed consent and respond to questions.

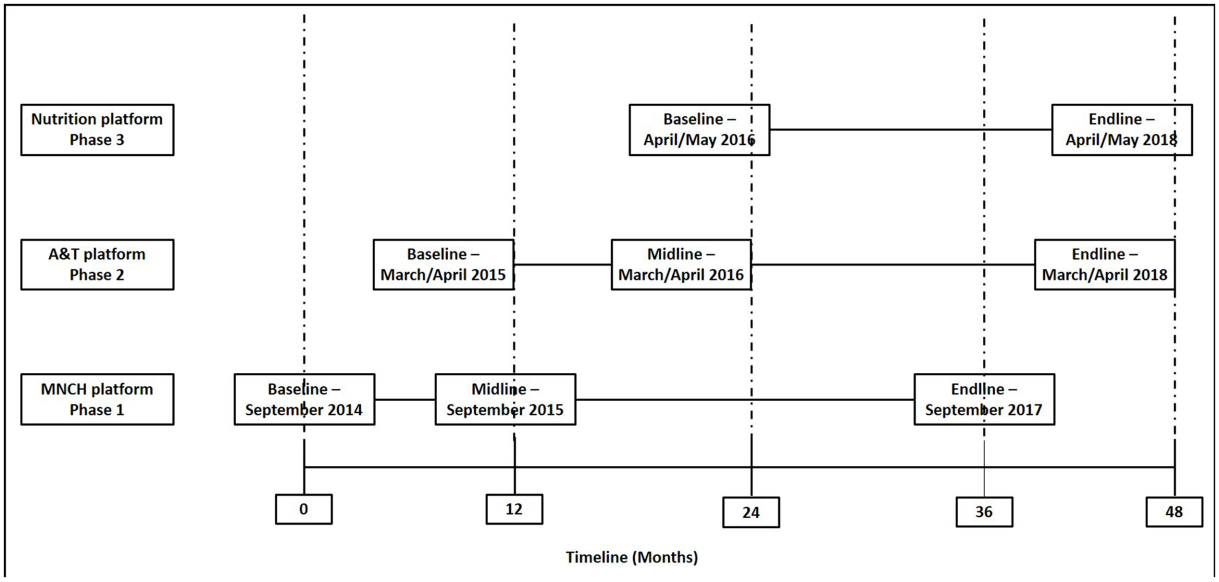

FIGURE 1
Survey design and timeline of baseline, midline, and endline surveys in three program platforms.

Sample size estimation

The sample size for the baseline and midline surveys was predicated on estimating coverage of the intervention, which was one of the outcomes of the first phase of the MIYCN program. Limited information was available on coverage of MNP at baseline, because before the implementing the MIYCN program, no large-scale evaluation survey was conducted in Bangladesh; it was therefore assumed to be 50% as this gave the most conservative sample size. At least 192 households, each with one eligible child, would be sufficient to estimate baseline coverage in each district with a precision of 10% of the 95% confidence interval, assuming a design effect of 2.0. A total of 16 clusters were identified from each district and 12 households selected from each cluster.

The sample size calculation for the endline survey was based on detecting a reduction in prevalence of anemia. Using data from the five baseline and midline surveys, the prevalence of anemia was determined to be 42%. In the endline surveys, it was determined that 969 households each with at least 1 eligible child would be sufficient to detect a 10% percentage point decrease in the prevalence of anemia with 90% power at the 5% level of significance assuming a design effect of 2.0 due to clustering. Seven households were selected from each of 22 clusters in each of nine districts included in the endline surveys.

Selection of study participants

Eligible households were identified using a two-stage cluster sampling procedure. Clusters were defined as the catchment area of a BRAC community health worker, which ranged from 250 to 400 households, with a population ranging from 1,250 to 2,000. At the first stage, the primary sampling units were selected from the list of each district provided by BRAC, using systematic random sampling. For the second stage, we used a physical map segmented sampling and followed WHO's EPI-5 sampling procedure (26) to identify eligible households. We selected twelve households from each BRAC community in the baseline and midline survey and increased the number of BRAC community along with an additional seven households from each BRAC community in the endline survey. Further details on the sampling process and selection of study participants are published elsewhere (27).

Data collection

We collected data through interviews with children's caregivers and collected finger-prick blood samples from the children to measure hemoglobin concentration. For interviews with caregivers, we used a structured questionnaire initially adapted from the WHO's Food and Nutrition Technical Assistance methodology (28). Trained interviewers administered the questionnaire to the caregivers. The interviewers underwent a comprehensive two-week training period prior to each survey. To gauge their proficiency, a pre-test assessing knowledge of data collection methodologies, study objectives, and research ethics was administered before the final training session. Following the training, a post-test was conducted to further evaluate their performance. Throughout the training, mock interviews were conducted among the interviewers, and constructive feedback was provided to enhance their skills. Additionally, practical sessions were held to practice drawing blood samples. From the pool of trained interviewers, approximately 95% were selected to participate in the final data collection from households. This rigorous training and selection process aimed to ensure the competence and reliability of the interviewers in carrying out the surveys effectively. The trained medical technologists took blood samples from the children by gently pricking either the middle finger or ring finger with a lancet. Using a dry gauze pad, the medical technologist wiped away the first drop of blood to stimulate spontaneous blood flow, gently pressed the finger until the second drop of blood appeared, and ensured that it was big enough to fill the microcuvette completely in one attempt. Hemoglobin concentration was then measured using a HemoCue machine (Hb 301, HemoCue AB, Angelholm, Sweden). We shared the anemia status with the caregiver of the participant child, and any child found to be severely anemic was referred to the nearest health facility (Hb of <7.0 g/dL). A total of 33 severe anemic children were referred to the nearest health facility. This disclosure did not affect the child's participation in the study.

Outcomes and covariate measures

The primary outcome was prevalence of anemia defined as hemoglobin concentration less than 11.0 g/dL. Secondary outcomes included infant and young child feeding practices defined aligned with WHO recommendations (28). Coverage levels draw on the Tanahashi model of coverage (29). Message coverage of the home-fortification program was defined as whether the caregiver of a child had heard about MNP. Contact coverage was defined as whether a child had ever consumed food with MNP, and effective coverage, defined as whether a child had consumed three or more sachets of MNP in three or more of the seven days preceding data collection. Total number of MNP sachets consumed by the index child prior to the day of interview were calculated then categorized them into three: none, 1–30 sachets, and >30 sachets.

The main explanatory variable was survey round as a proxy measure of exposure to the intervention. This given that the intervention to promote home fortification with MNP was rolled out immediately after the baseline survey.

Variables that characterized households, parents and children surveyed include: household size, number of children, income and expenditure; parental age, occupation, level of education and religion; child age, sex and birthweight.

Derived variables included a binary variable derived from total household income to indicate whether it was above or below the median household income and a binary household food security indicator based on the Household Food Insecurity Access Scale (30). Households were then grouped as moderately or severely food insecure for comparison against those classified as food secure or mildly food insecure. Household wealth quintiles were obtained from principal components analysis of indicators of household assets followed by extraction of scores from the first principal component and grouping of these scores into quintiles (31).

Ethical consideration

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Board of International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) which consists of two committees, the Research Review Committee and the

Ethical Review Committee. Written informed consent was obtained from all subjects/patients' legal guardians, especially for children.

Statistical analysis

The proportions of children with anemia, children whose feeding met the criteria of good infant and young child feeding, and of each of the three measured of coverage were calculated at the baseline, midline and endline surveys. Unadjusted risk ratios (RR) comparing these outcomes at midline relative to baseline and endline relative to baseline were estimated using generalized estimating equations with fixed effects for platform and robust standard errors to adjust for clustering of observations within BRAC program districts and survey communities. Risk ratios adjusting for child's age, child's sex, household wealth quintile and platform were also estimated. Subgroup analyses exploring the effect of the intervention on anemia and coverage among children who had ever consumed none, up to 30 or more than 30 sachets, and within each of the three platforms, were conducted. Only complete observations were included for all outcomes and covariates. Unadjusted and adjusted RR with 95% confidence intervals were used to present the regression estimates.

Results

Sample characteristics

A total of 16,936 households were surveyed. There were 6,892 households in the baseline survey, 4,807 at midline and 5,237 at endline. Table 1 shows the distributions of characteristics of households, parents and children surveyed across the three time-points. Households did not differ significantly with a median size of five members, median household incomes and expenditures of about 90 USD (1 Taka = 0.0089 USD), and a median of one child between the ages of six to 59 months. Household food insecurity varied over time: about a quarter of households were moderately or severely food insecure at baseline (24.8%), rising to about a third at midline (30.2%) then dropping to a fifth at endline (20.4%). Parent and child characteristics did not differ significantly over time. Mothers had a mean age of around 27 years and fathers approximately 33 years. About 76% of mothers reported having at least 5 years of formal education, but only about 64% of fathers reported the same amount of education. A majority, more than 90% of mothers, were housewives, and a similar proportion were Muslims. Fathers earned about 80–90% of the reported household income (Table 1).

Anemia

The prevalence of anemia was 46.6% at baseline, dropping to 32.1% at midline and 31.2% at endline. The mean hemoglobin concentration was 10.96 g/dL at baseline, increased to 11.31 g/dL at midline and 11.20 g/dL at endline (Table 2). These represented adjusted relative reductions of 34% at midline (RR 0.66, 95%CI 0.62 to 0.71, value of p <0.001) and 32% at endline (RR 0.68, 95%CI 0.64 to 0.71, value of p <0.001) relative to baseline (Table 2). There was no evidence of a difference in reduction of prevalence of anemia comparing children who had consumed none, up to 30 sachets, or greater than 30 sachets of MNP at midline (interaction p value 0.999) or endline (interaction p value 0.639) relative to baseline (Table 3). There was also no evidence of a difference in the post-baseline reduction in prevalence of anemia across platforms (interaction p values 0.593 and 0.283 for reductions at midline and endline, respectively). There was no evidence of a difference in the post-baseline reduction in prevalence of anemia comparing the surveys conducted in the first year of the program to those conducted between the second and fourth year (interaction p value 0.834) (Table 4).

Coverage of micronutrient powder (Pushtikona-5)

At baseline, 43.5% of caregivers surveyed had heard about MNP (message coverage); 24.3% of children had ever consumed food with MNP (contact coverage), and only 1.8% had consumed three or more sachets in the 7 days preceding the survey (effective coverage) (Table 2). These increased to 63.0, 36.9, and 4.6%, respectively, at midline and 90.6, 68.9, and 11.5%, respectively, at endline. The adjusted relative changes were 42% increase in message coverage (RR 1.42, 95%CI 1.34 to 1.50, value of p <0.001), 48% increase in contact coverage (RR 1.48, 95%CI 1.37 to 1.61, value of p <0.001) and 169% increase effective coverage (RR 2.69, 95%CI 2.03 to 3.58, value of p <0.001) at midline and 104% increase in message coverage (RR 2.04, 95%CI 1.95 to 2.13, value of p <0.001), 176% increase in contact coverage (RR 2.76, 95%CI 2.59 to 2.94, value of p <0.001) and 515% increase in effective coverage (RR 6.15, 95%CI 4.82 to 7.85, value of p <0.001) at endline (Table 2).

There was no significant difference in the post-baseline increases in message coverage (interaction p value 0.597) or contact coverage (interaction p value 0.553) across platforms at midline, but strong evidence of a significant difference in the post-baseline increases effective coverage across platforms at midline (interaction p value <0.001) (Table 3). At endline, there was strong evidence of between-platform differences in post-baseline significantly increases in all forms of coverage (all interaction p values 0.001 or less).

There was also evidence of a difference in post-baseline increases in message coverage and contact coverage comparing the surveys conducted in the first year of the program to those conducted between the second and fourth year (interaction p value <0.001) but no evidence of a difference in the increase in effective coverage over the same time periods (interaction p value 0.363) (Table 4).

Infant and young child feeding practices

There was considerable variation in prevalence of the various components of IYCF practices at baseline (Table 5). Practices such as continued breastfeeding, introduction of complimentary foods, and age-appropriate breastfeeding were highly prevalent, practised by 88% or more of caregivers at baseline. Others, including minimum meal frequency and continued breastfeeding at two years were also highly prevalent at baseline, 80 and 84%, respectively. However, only around half of children received diets of at least the recommended minimum dietary diversity (53%) or minimum acceptable diet (49%).

Feeding practices were mostly static over time with no discernible pattern to any observed changes. There was a 4%

TABLE 1 Characteristics of households and children included in the analysis.

Characteristics	Baseline (*N* = 6,892)	Midline (*N* = 4,807)	Endline (*N* = 5,237)	Overall (*N* = 16,936)
Platform				
Maternal, Neonatal and Child Health (MNCH), *n* (%)	1,927 (28.0)	1,924 (40.0)	1,540 (29.4)	5,391 (31.8)
Alive and Thrive Infant and Young Child Feeding (A&T), *n* (%)	2,887 (41.9)	2,883 (60.0)	2,310 (44.1)	8,080 (47.7)
BRAC Nutrition Program (Nutrition), *n* (%)	2,078 (30.2)	0 (0)	1,387 (26.5)	3,465 (20.5)
Household				
Household size, median (range)	5 (2–38)	5 (2–27)	5 (2–25)	5 (2–38)
Household income in Taka, median (range)	10,400 (0–300,000)	10,000 (0–300,000)	14,000 (0–300,000)	12,000 (0–300,000)
Household expenditure in Taka, median (range)	10,000 (0–450,000)	10,000 (0–360,000)	10,000 (1000–150,000)	10,000 (0–450,000)
Household food security, *n* (%)[1]				
Food secure or mildly insecure	3,733 (75.2)	3,356 (69.8)	4,171 (79.6)	11,260 (75.0)
Moderately or severely insecure	1,232 (24.8)	1,451 (30.2)	1,066 (20.4)	3,749 (25.0)
Household wealth quintile, *n* (%)[1]				
1 (lowest)	1,989 (28.9)	844 (17.6)	572 (10.9)	3,405 (20.1)
2	1,259 (18.3)	1,109 (23.1)	1,004 (19.2)	3,372 (19.1)
3	1,244 (18.1)	1,067 (22.2)	1,104 (21.1)	3,415 (20.2)
4	1,169 (17.0)	982 (20.4)	1,213 (23.2)	3,364 (19.9)
5 (highest)	1,231 (17.9)	805 (16.8)	1,344 (25.7)	3,380 (20.0)
Children 6–59 months, median (range)	1 (1–5)	1 (1–6)	1 (1–5)	1 (1–6)
Mother/caregiver				
Age in years, mean (SD)	26.6 (6.0)	27.0 (6.2)	26.5 (5.8)	26.7 (6.0)
Has 5+ years of education, *n* (%)	5,108 (74.1)	3,643 (75.8)	4,186 (79.9)	12,937 (76.4)
Housewife[2], *n* (%)	1,900 (91.4)	2,725 (94.5)	5,035 (96.1)	9,660 (94.7)
Income in Taka, median (range)	1,200 (0–30,000)	1,000 (0–15,300)	2,000 (0–30,000)	1,500 (0–30,000)
Muslim[2], *n* (%)	6,241 (90.6)	4,249 (88.4)	4,720 (90.1)	15,210 (89.8)
Father				
Age in years, mean (SD)	33.4 (6.9)	33.9 (7.2)	33.3 (6.5)	33.5 (6.9)
Has 5+ years of education, *n* (%)	4,256 (76.0)	3,057 (69.4)	3,536 (77.3)	10,849 (74.4)
Occupation, *n* (%)				
Unemployed	62 (3.4)	95 (3.5)	213 (4.4)	370 (3.9)
Service sector worker	276 (14.9)	361 (13.4)	662 (13.7)	1,299 (13.8)
Farmer	343 (18.5)	625 (23.1)	983 (20.3)	1,951 (20.8)
Businessman	479 (25.9)	628 (23.2)	1,253 (23.9)	2,360 (25.1)
Migrant worker	23 (1.2)	138 (5.1)	102 (2.1)	263 (2.8)
Day laborer	476 (25.7)	619 (22.9)	1,122 (23.2)	2,217 (23.6)
Other	194 (10.5)	239 (8.8)	498 (10.3)	931 (9.9)
Income in Taka[3], median (range)	9,000 (0–300,000)	8,000 (0–100,000)	10,000 (0–150,000)	10,000 (0–300,000)
Child				
Age in months, mean (SD)	29.9 (14.7)	30.8 (15.0)	29.0 (14.1)	29.9 (14.6)
Sex, *n* (%)				
Male	3,584 (52.0)	2,523 (52.5)	2,718 (51.9)	8,825 (52.1)
Female	3,308 (48.0)	2,284 (47.5)	2,519 (48.1)	8,111 (47.9)
Reported Birthweight in grams, mean (SD)	2,924 (660.7)	2,952 (642.6)	2,980 (620.6)	2,963 (634.1)

[1]Not included in baseline survey of MNCH platform. [2]This was the predominant response to this question; all other categories treated as 'other'. [3]1 Taka = 0.0089 USD.

TABLE 2 Effect of the intervention on anemia and MNP coverage.

Outcome	Baseline (*N* = 6,892)	Midline (*N* = 4,807)	Endline (*N* = 5,237)	Risk ratio (95% CI) and value of *p*			
				Midline vs. Baseline		Endline vs. Baseline	
	n (%) [x̄]	*n* (%) [x̄]	*n* (%) [x̄]	Unadjusted	Adjusted*	Unadjusted	Adjusted*
Anemia [x̄ hemoglobin g/dL]	3,196 (46.6) [10.96]	1,538 (32.1) [11.31]	1,630 (31.2) [11.20]	0.69 (0.64–0.74) $p < 0.001$	0.66 (0.62–0.71) $p < 0.001$	0.67 (0.64–0.71) $p < 0.001$	0.68 (0.64–0.71) $p < 0.001$
Coverage							
Message coverage – child's caregiver ever heard about MNP	3,000 (43.5)	3,028 (63.0)	4,746 (90.6)	1.44 (1.37–1.52) $p < 0.001$	1.42 (1.34–1.50) $p < 0.001$	2.07 (1.98–2.16) $p < 0.001$	2.04 (1.95–2.13) $p < 0.001$
Contact coverage – child ever consumed food with MNP	1,671 (24.3)	1,773 (36.9)	3,609 (68.9)	1.51 (1.40–1.64) $p < 0.001$	1.48 (1.37–1.61) $p < 0.001$	2.80 (2.63–2.98) $p < 0.001$	2.76 (2.59–2.94) $p < 0.001$
Effective coverage – consumed 3 or more sachets of MNP in past 7 days	123 (1.8)	217 (4.6)	603 (11.5)	2.51 (1.89–3.34) $p < 0.001$	2.69 (2.03–3.58) $p < 0.001$	6.38 (5.02–8.11) $p < 0.001$	6.15 (4.82–7.85) $p < 0.001$

*Adjusting for child's age and sex, platform, and household wealth quintile.

TABLE 3 Effect of intervention on anemia and coverage according to number of MNP sachets consumed and within platform.

Outcome	Adjusted* Midline vs. Baseline risk ratio (95% CI)				Adjusted* Endline vs. Baseline risk ratio (95% CI)			
	MNP sachets consumed			Value of *p*	MNP sachets consumed			Value of *p*
	None	1–30	> 30		None	1–30	> 30	
Anemia	0.68 (0.63–0.73)	0.68 (0.55–0.83)	0.68 (0.44–1.06)	0.999	0.76 (0.71–0.81)	0.72 (0.61–0.83)	0.67 (0.46–0.99)	0.639
	Platform			Value of *p*	Platform			Value of *p*
	MNCH	A&T	Nutrition		MNCH	A&T	Nutrition	
Anemia	0.65 (0.59–0.71)	0.67 (0.61–0.74)	_	0.593	0.65 (0.60–0.71)	0.71 (0.65–0.78)	0.64 (0.57–0.72)	0.283
Message coverage	1.34 (1.22–1.47)	1.38 (1.28–1.49)	_	0.597	1.84 (1.71–1.98)	2.02 (1.90–2.16)	2.32 (2.11–2.54)	0.001
Contact coverage	1.36 (1.18–1.56)	1.43 (1.28–1.61)	_	0.553	2.17 (1.94–2.44)	2.83 (2.58–3.11)	3.32 (2.92–3.78)	<0.001
Effective coverage	1.42 (0.90–2.25)	6.25 (3.99–9.81)	_	< 0.001	2.86 (1.87–4.37)	16.61 (10.96–25.18)	4.18 (2.88–6.07)	<0.001

*Adjusting for child's age and sex, platform, and household wealth quintile. **Not applicable because the definition of subgroups and the outcome are both dependent on MNP awareness or consumption value of ps for test of interaction (difference of effect) by subgroup.

TABLE 4 Comparing post- vs. pre-intervention surveys, adjusting for and investigating interaction with time.*

Outcome	Pre- (*N* = 6,892)	Post- (*N* = 10,044)	Risk ratio (95% CI) and value of *p*			
	n (%)	*n* (%)	Unadjusted	Adjusted**	Year 1**	Years 2–4**
Anemia	3,196 (46.6)	3,168 (31.6)	0.77 (0.73–0.82) $p < 0.001$	0.78 (0.73–0.82) $p < 0.001$	_	_
Coverage						
Message coverage – child's caregiver ever heard about MNP	3,000 (43.5)	7,774 (77.4)	1.66 (1.57–1.74) $p < 0.001$	1.65 (1.57–1.74) $p < 0.001$	1.35 (1.26–1.45)	2.03 (1.85–2.21)
Contact coverage – child ever consumed food with MNP	1,671 (24.3)	5,382 (53.9)	1.92 (1.79–2.06) $p < 0.001$	1.91 (1.78–2.04) $p < 0.001$	1.38 (1.25–1.52)	2.62 (2.30–2.99)
Effective coverage – consumed 3 or more sachets of MNP in past 7 days	123 (1.8)	820 (8.2)	3.01 (2.31–3.91) $p < 0.001$	3.03 (2.33–3.94) $p < 0.001$	_	_

*Binary time variable distinguishing between surveys conducted in the first year versus those in the second through to fourth year. **Adjusting for child's age and sex, and household wealth quintile. Effects in first vs. later years reported separately where there is evidence of interaction between pre/post-intervention effect and time (value of *p*s < 0.001). No adjustment for platform because there is no data for the nutrition platform in the first year.

adjusted increase in continued breastfeeding at midline relative to baseline (RR 1.04, 95%CI 1.01 to 1.08, value of *p* 0.017) but no adjusted difference at endline (Table 5). Similarly, there was a 5% increase in prevalence of introduction of complimentary foods (RR 1.05, 95%CI 1.01 to 1.10, value of *p* 0.013) but no adjusted difference at endline. The adjusted prevalence of both minimum meal frequency and minimum acceptable diet declined by 7 and 8%, respectively, at endline relative to baseline (RR 0.93, 95%CI 0.90 to 0.96, value of *p* <0.001 and RR 0.92, 95%CI 0.86 to 0.97, value of *p* 0.004 respectively). There was some evidence of increases in the adjusted prevalence of continued breastfeeding at two years (RR 1.07, 95%CI 1.01 to 1.13, value of *p* 0.020 at midline and RR 1.06, 95%CI 1.00 to 1.11, value of *p* 0.056 at endline) and age-appropriate breastfeeding (RR 1.06, 95%CI 1.04 to 1.07, value of *p* <0.001 at midline and RR 1.05, 95%CI 1.02 to 1.09, value of *p* <0.001 at endline).

Discussion

The results of this study show evidence of a reduction in the prevalence of anemia from baseline to endline and of increased

TABLE 5 Effect of the intervention on IYCF practices.

Outcome	Baseline	Midline	Endline	Risk ratio (95% CI) and value of *p*			
				Midline vs. Baseline		Endline vs. Baseline	
	n/N* (%)	n/N (%)	n/N (%)	Unadjusted	Adjusted**	Unadjusted	Adjusted**
IYCF practices (age of children)							
Continued breastfeeding (12–15 months)	538/580 (92.8)	359/375 (95.7)	440/468 (94.0)	1.03 (1.00–1.06) $p = 0.037$	1.04 (1.01–1.08) $p = 0.017$	1.01 (0.98–1.05) $p = 450$	1.02 (0.99–1.06) $p = 0.206$
Introduction of complimentary foods (6–8 months)	379/408 (92.9)	278/291 (95.5)	240/254 (94.5)	1.03 (0.99–1.07) $p = 0.133$	1.05 (1.01–1.10) $p = 0.013$	1.02 (0.98–1.06) $p = 0.386$	1.03 (0.99–1.06) $p = 0.163$
Minimum dietary diversity (6–23 months)	1,389/2,633 (52.8)	977/1,762 (55.5)	1,134/2,084 (54.4)	1.05 (0.99–1.12) $p = 0.123$	1.04 (0.98–1.11) $p = 0.195$	1.03 (0.97–1.09) $p = 0.327$	0.96 (0.91–1.02) $p = 0.175$
Minimum meal frequency (6–23 months)	2,106/2,622 (80.3)	1,469/1,749 (84.0)	1,583/2,076 (76.3)	1.05 (1.01–1.08) $p = 0.007$	0.99 (0.96–1.03) $p = 0.654$	0.95 (0.92–0.98) $p = 0.002$	0.93 (0.90–0.96) $p < 0.001$
Minimum acceptable diet (6–23 months)	1,272/2,622 (48.5)	908/1,749 (51.9)	995/2,076 (47.9)	1.07 (1.00–1.14) $p = 0.052$	1.02 (0.96–1.09) $p = 0.519$	0.98 (0.92–1.05) $p = 0.601$	0.92 (0.86–0.97) $p = 0.004$
Continued breastfeeding at 2 years (20–23 months)	459/550 (83.5)	348/390 (89.2)	396/461 (85.9)	1.07 (1.02–1.12) $p = 0.008$	1.07 (1.01–1.13) $p = 0.020$	1.03 (0.98–1.09) $p = 0.244$	1.06 (1.00–1.11) $p = 0.056$
Age-appropriate breastfeeding (0–23 months)	2,310/2,622 (88.1)	1,617/1,749 (92.5)	1,894/2,076 (91.2)	1.05 (1.03–1.07) $p < 0.001$	1.06 (1.04–1.09) $p < 0.001$	1.04 (1.01–1.06) $p = 0.003$	1.05 (1.02–1.09) $p < 0.001$

*Denominators depend on number of children in age range. **Adjusting for child's age and sex, platform, and household wealth quintile.

message and contact coverage of Pushtikona-5 from baseline to midline and to endline surveys. However, additional analysis suggested that the observed reduction in anemia prevalence was not due to message and contact coverage and to ever consumption of ≥30 sachets of Pushtikona-5. There was also no evidence of a midline-baseline or endline-baseline difference in the prevalence of anemia according to the effective coverage of Pushtikona-5. This may be because of very low effective coverage of Pushtikona-5 in all platforms in the all three survey time-points.

Given the level of reduction in the prevalence of anemia in the MIYCN program area from baseline to endline, it is possible that the combined interventions (promotion of feeding practice combined with MNP) contributed to the reduction of anemia. Because we did not have an appropriate comparison group, we are unable to attribute the reduced prevalence of anemia in the program districts solely to the interventions. However, home fortification with MNP has shown mixed results in its effects on anemia in previous studies (32–37). Some studies have found that home fortification with MNPs can reduce anemia prevalence and improve hemoglobin levels (32–34). Other studies have found that low-dose iron-containing MNPs did not improve iron status or reduce anemia prevalence (35). The efficacy of MNPs in reducing anemia may depend on factors such as the dose of iron in the MNP, the prevalence of infections, and the frequency of MNP administration (36–38).

There is strong evidence that coverage of Pushtikona-5, particularly message and contact coverage, improved over time. This result suggests that this market-based model can be an appropriate delivery mechanism for home fortification at the community level. In the existing literature, there is limited evidence on the effectiveness of market-based MNP interventions at scale (14, 15, 27); thus this evaluation of a large-scale program using a sales model is a strong addition to the literature. The successful implementation of MNP program may be dependent on the effective operation, quality of training and commitment of community-level health workers. For example, the analyses showed that Pushtikona-5 coverage (including effective coverage) was significantly associated with home visits made by the BRAC community health workers (27). Households of the caregivers who received more visits from the community health workers were more likely to feed Pushtikona-5-fortified foods to their children (27). This finding is consistent with a study conducted in Madagascar (32) showing that interaction with health workers increased consumption of ≥30 sachets of MNP and was associated with a significant reduction in the prevalence of anemia among children. This evaluation also identified a range of individual, household, community, and program-level factors associated with low home visits by the BRAC community health workers, which may be critical for BRAC to consider when implementing similar programs in the future (18, 19, 22).

Our analysis revealed that the prevalence of IYCF practices increased from baseline to midline but remained steady from midline to endline. Several indicators of infant feeding practices were already very high at baseline, potentially leaving little room for further increase. For others, qualitative analysis of this evaluation published elsewhere provides several reasons for low prevalence of other IYCF practices (18, 22). One reason may be that BRAC community health workers were responding to new incentives to promote home fortification with Pushtikona-5, which may have led them to reduce their focus on promoting IYCF (20). When integrating MNP and IYCF interventions, program implementers and stakeholders should be careful to prevent programmatic changes from having unintended negative consequences on program outcomes and should take course-correcting actions as needed during implementation (17, 19). A previous study had recommended that MNP interventions implemented at scale be integrated with IYCF programs because community-based MNP interventions would likely increase community health workers' contacts with households, thereby strengthening IYCF counseling and support (20, 39, 40).

The evaluation has several limitations and strengths. The evaluation did not have a comparison group, which limited our ability to determine causality regarding the change in prevalence of anemia. The results of the study may not be representative of Bangladesh as a whole or of the districts where the surveys were

implemented. We assessed anemia with a drop of blood through capillary finger prick. This process allowed us to measure children's hemoglobin but limited our ability to measure serum iron and ferritin, which help distinguish the proportion of anemia due to iron deficiency. Single drop capillary hemoglobin assessment is also highly subject to random error (41–43), which may reduce the potential to detect small changes. Additionally, there might have been recall bias, as caregivers may have trouble recalling the use of Pushtikona-5 in the past. Other limitations include the potential for confounding by temporal trends, where comparisons of outcomes between earlier and later periods may be influenced by background changes that affect anemia, irrespective of the program. Furthermore, if temporality was a significant confounder, our sample size might have been compromised by these temporal effects. The Pushtikona-5 program was implemented at large scale in three phases using existing delivery platforms. The use of a stepped wedged survey design, and collected data from all program districts is an important strength, ensuring feasibility for evaluation in real-world program settings (18).

Conclusion

These results show evidence of a reduction in the prevalence of anemia and an improvement in Pushtikona coverage and some infant and young child feeding practices across the three surveys. However, we found no evidence that any observed improvements in anemia were different from the prevailing secular trends. Results for message and contact coverage for which improvements were greater in the latter years than the initial years of the program suggest positive effects of the intervention. This study provides important evidence of the feasibility and potential for impact of linking market-based MNP distribution with IYCF promotion through community level health workers. Anemia reduction however, will likely require additional actions that address the multi-causality of the condition.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Institutional Review Board of icddr,b, which consists of two committees, the Research Review Committee and the Ethical Review Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

HS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MR: Data curation, Methodology, Writing – review & editing. MT: Methodology, Writing – review & editing, Conceptualization, Formal analysis, Funding acquisition. MI: Methodology, Writing – review & editing, Data curation, Investigation, Project administration. MM: Conceptualization, Methodology, Writing – review & editing. GA: Conceptualization, Writing – review & editing, Methodology. SA: Conceptualization, Funding acquisition, Writing – review & editing. CH: Conceptualization, Methodology, Writing – review & editing, Funding acquisition. RK: Methodology, Writing – review & editing, Conceptualization. MB: Data curation, Methodology, Writing – review & editing. SS: Data curation, Writing – review & editing, Methodology. MAR: Data curation, Methodology, Writing – review & editing. SAS: Writing – review & editing, Data curation. MC: Writing – review & editing. KA: Writing – review & editing, Conceptualization. SG: Writing – review & editing. CB: Formal analysis, Methodology, Writing – review & editing, Data curation. CD'E: Formal analysis, Methodology, Writing – review & editing. MS: Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review & editing, Investigation. LN: Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review & editing. TA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

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References

1. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low-and middle-income countries. *Ann N Y Acad Sci*. (2019) 1450:15–31. doi: 10.1111/nyas.14092

2. National Institute of Population Research and Training (NIPORT), Mitra and Associates, and ICF International. *Bangladesh demographic and health survey 2011*. Dhaka, Bangladesh and Calverton, MD: NIPORT, Mitra and Associates, and ICF International (2013).

3. Sachdev HP, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public Health Nutr*. (2005) 8:117–32. doi: 10.1079/PHN2004677

4. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr*. (2007) 85:931–45. doi: 10.1093/ajcn/85.4.931

5. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. *Lancet*. (2016) 387:907–16. doi: 10.1016/S0140-6736(15)60865-0

6. Haas JD, Fairchild MW. Summary and conclusions of the international conference on iron deficiency and Behavioral development, October 10-12. *Am J Clin Nutr*. (1988) 50:703–5.

7. Kassebaum NJ. The global burden of anemia. *Hematol Oncol Clin North Am*. (2016) 30:247–308. doi: 10.1016/j.hoc.2015.11.002

8. Schauer C, Zlotkin S. Home fortification with micronutrient sprinkles–a new approach for the prevention and treatment of nutritional anemias. *Paediatr Child Health*. (2003) 8:87–90. doi: 10.1093/pch/8.2.87

9. World Health Organization. *Guideline: Daily iron supplementation in infants and children*. Geneva: World Health Organization (2016).

10. Osei A, Septiari A, Suryantan J, Hossain MM, Chiwile F, Sari M, et al. Using formative research to inform the design of a home fortification with micronutrient powders (MNP) program in Aileu District. *Timor-Leste Food Nutri Bull*. (2014) 35:68–82. doi: 10.1177/156482651403500109

11. Jefferds ME, Irizarry L, Timmer A, Tripp K. UNICEF—CDC global assessment of home fortification interventions 2011: current status, new directions, and implications for policy and programmatic guidance. *Food Nutri Bull*. (2013) 34:434–43. doi: 10.1177/156482651303400409

12. Locks LM, Newell KB, Imohe A, Moloney GM, Shaker-Berbari L, Paudyal N, et al. The effect of interventions distributing home fortification products on infant and young child feeding (IYCF) practices: a systematic narrative review. *Matern Child Nutr*. (2023) 19:e13488. doi: 10.1111/mcn.13488

13. Iwelunmor J, Blackstone S, Veira D, Nwaozuru U, Airhihenbuwa C, Munodawafa D, et al. Toward the sustainability of health interventions implemented in sub-Saharan Africa: a systematic review and conceptual framework. *Implementat Sci*. (2015) 11:43. doi: 10.1186/s13012-016-0392-8

14. Pluye P, Potvin L, Denis JL. Making public health programs last: conceptualizing sustainability. *Eval Program Plann*. (2004) 27:121–33. doi: 10.1016/j.evalprogplan.2004.01.001

15. Suchdev PS, Shah A, Jefferds ME, Eleveld A, Patel M, Stein AD, et al. Sustainability of market-based community distribution of sprinkles in western Kenya. *Matern Child Nutr*. (2013) 9:78–88. doi: 10.1111/j.1740-8709.2012.00450.x

16. Olney DK, Rawat R, Ruel MT. Identifying potential programs and platforms to deliver multiple micronutrient interventions. *J Nutr*. (2012) 142:178S–85S. doi: 10.3945/jn.110.137182

17. Sarma H, Uddin F, Islam MA, Rahman M, Aaron GJ, Harbour C, et al. Use of concurrent evaluation to improve implementation of a home-fortification programme in Bangladesh: a methodological innovation. *Public Health Nutr*. (2020) 24:s37–47. doi: 10.1017/S1368980020000439

18. Sarma H, Jabeen I, Luies SK, Uddin MF, Ahmed T, Bossert TJ, et al. Performance of volunteer community health workers in implementing home-fortification interventions in Bangladesh: a qualitative investigation. *PLoS One*. (2020) 15:e0230709. doi: 10.1371/journal.pone.0230709

19. Sarma H, Tariqujjaman M, Mbuya MN, Askari S, Banwell C, Bossert TJ, et al. Factors associated with home visits by volunteer community health workers to implement a home-fortification intervention in Bangladesh: a multilevel analysis. *Public Health Nutr*. (2020) 24:s23–36. doi: 10.1017/S1368980019003768

20. Tariqujjaman M, Rahman M, Luies SK, Karmakar G, Ahmed T, Sarma H. Unintended consequences of programmatic changes to infant and young child feeding practices in Bangladesh. *Matern Child Nutr*. (2020) 17:e13077. doi: 10.1111/mcn.13077

21. Ahmed S, Sarma H, Hasan Z, Rahman M, Ahmed MW, Islam MA, et al. Cost-effectiveness of a market-based home fortification of food with micronutrient powder programme in Bangladesh. *Public Health Nutr*. (2020) 24:s59–70. doi: 10.1017/S1368980020003602

22. Sarma H, Uddin MF, Harbour C, Ahmed T. Factors influencing child feeding practices related to home fortification of micro-nutrient powder among caregivers of Under-5 children in Bangladesh. *Food Nutri Bull*. (2016) 37:340–52. doi: 10.1177/0379572116645916

23. Matthews JN, Forbes AB. Andrew, Forbes. Stepped wedge designs: insights from a design of experiments perspective. *Stat Med*. (2017) 36:3772–90. doi: 10.1002/SIM.7403

24. Hemming K, Girling A, Martin J, Bond SJ. Stepped wedge cluster randomized trials are efficient and provide a method of evaluation without which some interventions would not be evaluated. *J Clin Epidemiol*. (2013) 66:1058–9. doi: 10.1016/J.JCLINEPI.2012.12.020

25. Keriel-Gascou M, Buchet-Poyau K, Rabilloud M, Duclos A, Colin C. A stepped wedge cluster randomized trial is preferable for assessing complex health interventions. *J Clin Epidemiol*. (2014) 67:831–3. doi: 10.1016/J.JCLINEPI.2014.02.016

26. Henderson RH, Sundaresan T. Cluster sampling to assess immunization coverage: a review of experience with a simplified sampling method. *Bull World Health Organ*. (1982) 60:253–60.

27. Sarma H, Mbuya MN, Tariqujjaman M, Rahman M, Askari S, Khondker R, et al. Role of home visits by volunteer community health workers: to improve the coverage of micronutrient powders in rural Bangladesh. *Public Health Nutri*. (2020) 24:s48–58. doi: 10.1017/S1368980020000038

28. World Health Organization. *Indicators for assessing infant and young child feeding practices: Part 1: Definitions: Conclusions of a consensus meeting held 6–8 November 2007 in Washington DC, USA*. Geneva: World Health Organization (2008).

29. Tanahashi T. Health service coverage and its evaluation. *Bull World Health Organ*. (1978) 56:295–303.

30. Coates J, Swindale A, Bilinsky P (2007). Household food insecurity access scale (HFIAS) for measurement of food access: indicator guide. Available at: http://www.fao.org/fileadmin/user_upload/eufao-fsi4dm/doc-training/hfias.pdf (Accessed December 12, 2018)

31. The DHS Program. (2018) Wealth index construction. Available at: https://www.dhsprogram.com/topics/wealth-index/Wealth-Index-Construction.cfm (Accessed December 12, 2018)

32. Locks L, Reerink I, Tucker BA, Gnegne S, Ramalanjaona N, Nanama S, et al. The impact of integrated infant and young child feeding and micronutrient powder intervention on feeding practices and anemia in children aged 6–23 months in Madagascar. *Nutrients*. (2017) 9:581. doi: 10.3390/nu9060581

33. Serdula M, Lundeen E, Nichols E, Imanalieva C, Minbaev M, Mamyrbaeva T, et al. Effects of a large-scale micronutrient powder and young child feeding education program on the micronutrient status of children 6–24 months of age in the Kyrgyz Republic. *Eur J Clin Nutr*. (2013) 67:703–7. doi: 10.1038/ejcn.2013.67

34. Nikooyeh B, Neyestani TR. Effectiveness of various methods of home fortification in under-5 children: where they work, where they do not. A systematic review and meta-analysis. *Nutr Rev*. (2021) 79:445–61. doi: 10.1093/NUTRIT/NUAA087

35. Das JK, Salam RA, Mahmood SB, Moin A, Kumar R, Mukhtar K, et al. Food fortification with multiple micronutrients: impact on health outcomes in general population. *Cochrane Database Syst Rev*. (2019) 2020:CD011400. doi: 10.1002/14651858.CD011400.PUB2

36. Barth-Jaeggi T, Moretti D, Kvalsvig J, Holding PA, Njenga J, Mwangi A, et al. In-home fortification with 2.5 mg iron as NaFeEDTA does not reduce anaemia but increases weight gain: a randomised controlled trial in K enyan infants. *Matern Child Nutr*. (2015) 11:151–62. doi: 10.1111/MCN.12163

37. Kupka R. The role of folate in malaria–implications for home fortification programmes among children aged 6–59 months. *Matern Child Nutr*. (2015) 11:1–5. doi: 10.1111/MCN.12102

38. Kejo D, Petrucka P, Martin H, Mosha TCE, Kimanya ME. Efficacy of different doses of multiple micronutrient powder on haemoglobin concentration in children aged 6–59 months in Arusha District. *Scientifica*. (2019) 2019:1–7. doi: 10.1155/2019/8979456

39. Siekmans K, Bégin F, Situma R, Kupka R. The potential role of micronutrient powders to improve complementary feeding practices. *Mater Child Nutri*. (2017) 13:e12464. doi: 10.1111/mcn.12464

40. Sarma H, D'Este C, Ahmed T, Bossert TJ, Banwell C. Developing a conceptual framework for implementation science to evaluate a nutrition intervention scaled-up in a real-world setting. *Public Health Nutri*. (2021) 24:s7–s22. doi: 10.1017/S1368980019004415

41. Neufeld LM, Larson LM, Kurpad A, Mburu S, Martorell R, Brown KH. Hemoglobin concentration and anemia diagnosis in venous and capillary blood: biological basis and policy implications. *Ann N Y Acad Sci*. (2019) 1450:172–89. doi: 10.1111/nyas.14139

42. Larson LM, Braat S, Hasan MI, Mwangi MN, Estepa F, Hossain SJ, et al. Preanalytic and analytic factors affecting the measurement of haemoglobin concentration: impact on global estimates of anaemia prevalence. *BMJ Glob Health*. (2021) 6:e005756. doi: 10.1136/bmjgh-2021-005756

43. Rappaport AI, Karakochuk CD, Hess SY, Whitehead RD, Namaste SML, Dary O, et al. Variability in haemoglobin concentration by measurement tool and blood source: an analysis from seven countries. *J Clin Pathol*. (2020) 74:657–63. doi: 10.1136/jclinpath-2020-206717

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Association between dietary inflammatory index and anemia in US adults

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Background and aims: Anemia is a widespread global health concern, and recent research has unveiled a link between anemia and inflammation. The Dietary Inflammation Index (DII) is a novel tool used to assess the overall inflammatory potential of an individual's diet. However, until now, there have been no studies demonstrating a connection between DII and anemia. This study aimed to explore the relationship between DII and the risk of anemia among Americans, as well as to examine the influence of other risk factors on this association.

Methods: Data from 32,244 patients were collected from the National Health and Nutrition Examination Survey (NHANES) database spanning from 1999 to 2018. Using multivariable logistic regression, we examined the correlation between DII and anemia. Subgroup analyses and smoothed curve analyses were conducted to further investigate the association between DII and anemia.

Results: The analysis revealed a significant positive association between higher DII scores and increased anemia risk in the American population (Odds Ratio [OR] = 1.06, 95% Confidence Interval [CI] = 1.03 to 1.09, $p < 0.0001$). This association remained consistent in subgroup analyses, encompassing various age groups, distinct Body Mass Index (BMI) categories, varying diabetes mellitus statuses, histories of hypertension, females, individuals with a RIP <3.5, and Non-Hispanic Black individuals. Notably, the association was particularly significant among non-smokers. Smoothed curve fitting analysis demonstrated a linear relationship between DII and the prevalence of anemia.

Conclusion: Our findings underscore a positive correlation between the inflammatory potential of one's diet and the risk of anemia, especially when coupled with other risk factors. Consequently, reducing the consumption of pro-inflammatory foods may serve as one of the effective measures against the development of anemia. Given the variations in gender, age, BMI, and chronic diseases observed in our study, tailored policies could better cater to the specific needs of diverse populations.

KEYWORDS

dietary inflammatory index, anemia, NHANES, cross-sectional study, adult

1 Introduction

Anemia is characterized by a condition in which the concentration of hemoglobin or red blood cells in the peripheral blood falls below the normal range, leading to the development of various symptoms. This condition represents a significant global public health challenge, with an estimated 32.9% of the world's population being affected by anemia

(1). Notably, anemia is associated with adverse pregnancy outcomes in women (2), serves as an independent prognostic factor for mortality among congestive heart failure patients (3), and contributes to weakness and fatigue, impacting the productivity of adults (4). The prevalence of anemia among the older adult is steadily increasing each year (5) making it a critical concern with far-reaching implications for both human health and socioeconomic development. Therefore, addressing strategies to reduce its prevalence is of paramount importance.

In the context of anemia, inflammation assumes a pivotal role, and anemia of inflammation (AI) stands as the second most common cause of anemia, following iron deficiency anemia (IDA) (6). Research has found that the impact of inflammation on iron homeostasis and the shortening of red blood cell lifespan may exacerbate anemia by inhibiting the differentiation of the erythrocyte lineage (7, 8). Remarkably, dietary components can exert a direct influence on inflammation. Healthy diets, rich in vegetables, whole grains, and fruits, are associated with reduced levels of inflammatory mediators (9); Conversely, Western-style, high-calorie diets coupled with unhealthy lifestyles can induce chronic metabolic inflammation (10). Therefore, quantifying the overall inflammatory potential of an individual's diet could offer valuable insights for tailoring disease-specific dietary recommendations.

DII is a novel tool used to assess the overall inflammatory potential of an individual's diet. Elevated DII scores correspond to stronger pro-inflammatory effects, while lower scores indicate more potent anti-inflammatory effects (11). Previous studies have provided initial evidence of associations between DII and an increased risk of number of chronic diseases such as cancer (12), diabetes and cardiovascular risk (13). Furthermore, In recent years, it has become increasingly evident that the spectrum of diseases in which inflammation contributes to anemia has expanded (8). Quantifying the link between dietary inflammatory potential and anemia is crucial for effective prevention and management. Surprisingly, prior research has not delved into this specific relationship. To address this knowledge gap, our study aimed to investigate the association between DII and anemia, making use of publicly accessible data from the National Health and Nutrition Examination Survey (NHANES).

2 Materials and methods

2.1 Study population and design

NHANES is a continuous cross-sectional survey conducted biennially to assess the dietary and health status of the civilian noninstitutionalized U.S. population and is a research project conducted by the National Center for Health Statistics at the Centers for Disease Control and Prevention (14, 15). NHANES utilizes a "stratified multistage probability sampling" approach to ensure a representative sample. In this study, 10 consecutive NHANES cycles between 1999/2000 and 2017/2018 were included. Exclusion criteria for participants in this study were (1) age < 20 years, (2) cancer, and (3) lack of complete data on HBG and DII. Our final analysis included 32,244 eligible participants aged ≥20 years (Figure 1). This study received approval from the Ethical Review Board of the National Center for Health Statistics. All participants provided written informed consent.

2.2 Assessment of anemia

Anemia was defined in accordance with the World Health Organization (WHO) criteria (16), with Hgb levels below 12 g/dL for women and below 13 g/dL for men.

2.3 Dietary inflammatory index

The DII is now a widely recognized parameter for assessing overall dietary inflammation, and its structural validity and calculation methodology have been published (11). Refer to Supplementary Table S1 for the food parameter-specific overall inflammatory effect score. Dietary data for this study were collected from 24-h dietary recall interviews conducted at a mobile health center as part of the NHANES survey. We computed DII scores based on the 24-h dietary data. First, we calculated various dietary parameters and their respective z-scores for each participant. Then, the values were then converted to median percentiles. For each median percentile, we calculated a standardized overall inflammatory impact score, considering a range of dietary factors. By summing the DII scores for each participant, we obtained an "overall DII score" that reflects the individual's dietary inflammatory potential. The dietary parameters included in this study encompassed a wide range of factors, including energy, protein, total fat, fiber, carbohydrate, cholesterol, alcohol, vitamins (B12, B6, A, C, E), β-carotene, caffeine, monounsaturated fatty acids (MUFA), n-3 fatty acids, folic acid, iron (Fe), magnesium (Mg), niacin, riboflavin, polyunsaturated fatty acids (PUFA), saturated fat, selenium (Se), thiamin, and zinc (Zn). Participants were categorized into quartiles based on their DII values. This comprehensive approach allowed us to assess the relationship between dietary inflammatory potential and anemia prevalence.

2.4 Covariates

In our study, we analyzed several relevant variables, including: Age, Gender, Race (Mexican American, Other Hispanic, Non-Hispanic White, Other Race, Non-Hispanic Black), Academic level (Less Than high school, High School or Equivalent, College or above), Marital Status (Married, Separated, Never married), Ratio of family income to poverty (RIP)(<1.3, 1.3–3.5, >3.5), drinking (alcohol consumption was obtained through the question: "Had at least 12 alcohol drinks in 1 year?"), Smoking (smoking status was obtained through the question: "Smoked at least 100 cigarettes in life?"), Body Mass Index (BMI) (<25, 25 ≤ BMI < 30, ≥30), White Blood Cell, Red Blood Cell, Platelet Count, Hypertension, High Cholesterol, Diabetes, Congestive Heart Failure, Coronary Heart Disease. Detailed information about the measurement processes for these variables is publicly available at www.cdc.gov/nchs/nhanes/.

2.5 Statistical analysis

All data were analyzed using R Statistics (version 4.2.0) and Empower Stats software.[1] Data were weighted and analyzed

1 http://www.empowerstats.net

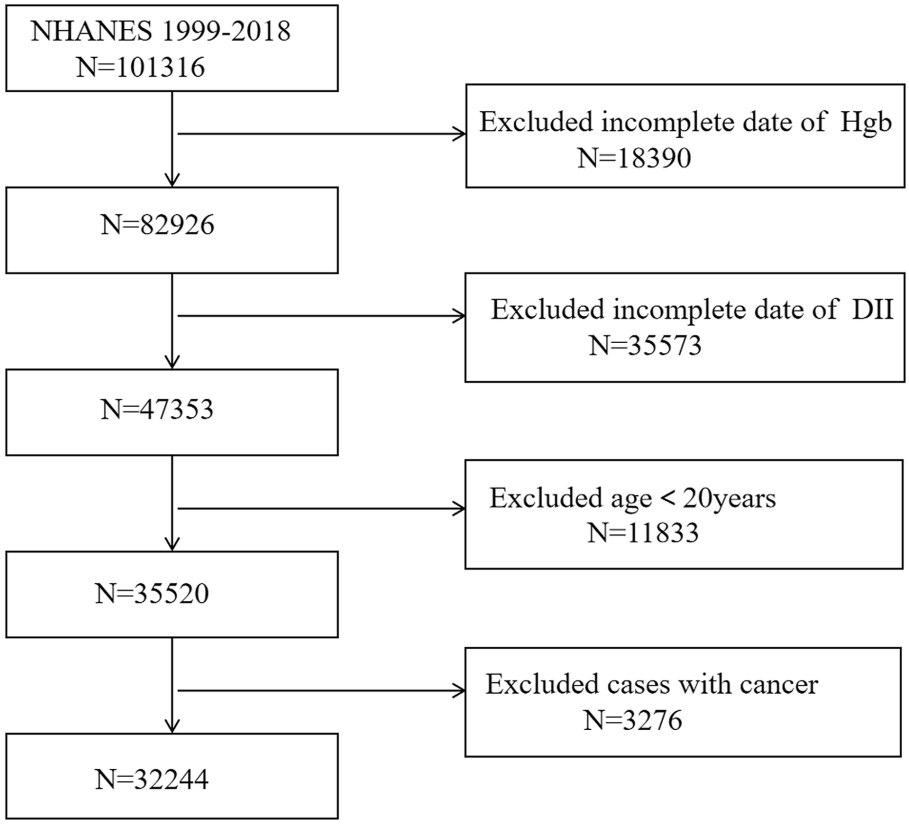

FIGURE 1
Flow chart of participants selection. NHANES, National Health and Nutrition Examination Survey; DII, dietary inflammatory index.

following the available NHANES guidelines. Normally distributed continuous variables are presented as mean ± standard deviation, while categorical variables are expressed as frequencies or percentages. Statistical methods employed in this study included logistic regression to examine the impact of exposure factors on the disease. To account for the influence of other factors on anemia and to isolate the independent effect of DII on anemia, we conducted multiple regression analysis in three distinct models. In Model 1, no covariates were adjusted. Model 2 incorporated adjustments for gender, age, and race. Model 3 included adjustments for all covariates. Subgroup analyses were also carried out. As DII was considered a continuous variable, we explored potential nonlinear relationships using smooth curve fitting and generalized additive modeling (GAM). A significance level of $p < 0.05$ was employed to determine statistical significance in all analyses.

3 Results

3.1 Baseline characteristics

A total of 32,244 participants were included in the study, with a mean age of 48.20 ± 17.78 years, consisting of 48.53% males and 51.47% females. Among the participants, 8.85% had anemia.

Table 1 presents the clinical characteristics of individuals with anemia. In comparison to the non-anemic group, participants with anemia exhibited significantly higher DII values (1.30 ± 1.83 vs. 1.83 ± 1.75, $p < 0.001$), were older (45.07 ± 16.07 vs. 49.27 ± 18.28, $p < 0.001$), and had a higher mean BMI (28.64 ± 6.56 vs. 29.69 ± 7.75 kg/m2, $p < 0.001$). Additionally, anemic participants had lower levels of education and annual household income. There were also differences in the racial composition between the two groups. Furthermore, the prevalence of hypertension, diabetes, heart failure, and coronary heart disease was higher among individuals in the anemia group compared to the non-anemic group.

Table 2 displays the characteristics of the participants categorized by DII quartiles. The mean DII was 1.45 ± 1.82, with quartiles 1 to 4 having DII ranges of −5.28 to −0.20, 0.20 to 1.67, 1.67 to 2.87, and 2.87 to 5.79, respectively. Significant differences were observed among different quartiles of DII for factors such as gender, race, academic level, marital status, RIP, drinking habits, smoking habits, BMI, white blood cell count, red blood cell count, platelet count, C-reactive protein (CRP), hypertension, high cholesterol, diabetes, congestive heart failure, and coronary heart disease. Moreover, the proportion of individuals with anemia increased with higher DII quartiles (Quartile 1: 4%, Quartile 2: 4.97%, Quartile 3: 6.23%, Quartile 4: 8.30%; $p < 0.0001$). Similarly, the average hemoglobin (Hgb) level for all participants was 14.18 ± 1.56. The average hemoglobin level for the highest quartile was 14.08 ± 1.54, while for the lowest quartile, it was 14.61 ± 1.39 ($p<0.05$). Importantly, white blood cell counts and C-reactive protein (CRP) levels also increased with the rising quartiles of DII ($p<0.05$).

TABLE 1 Weighted characteristics of the study population based on anemia.

	Non-anemia	Anemia	*p*-value
DII	1.30 ± 1.83	1.83 ± 1.75	<0.0001
Age	45.07 ± 16.07	49.27 ± 18.28	<0.0001
Gender (%)			<0.0001
Male	50.65	25.03	
Female	49.35	74.97	
Race (%)			<0.0001
Mexican American	8.54	9.36	
Other Hispanic	5.73	7.12	
Non-Hispanic White	69.77	44.86	
Non-Hispanic Black	9.51	32.21	
Other Race	6.45	6.45	
Academic level (%)			<0.0001
Less than high school	16.94	24.61	
High School or Equivalent	25.02	25.24	
College or above	58.04	50.16	
Marital status (%)			<0.0001
Married	71.10	72.61	
Separated	2.47	4.36	
Never married	26.43	23.03	
RIP (%)			<0.0001
<1.3	19.76	28.74	
1.3–3.5	36.25	41.62	
>3.5	43.99	29.64	
Mean ± SD	3.06 ± 1.62	2.53 ± 1.60	
Drinking (%)			<0.0001
No	14.09	24.00	
Yes	46.14	36.35	
Missing	39.78	39.66	
Smoking (%)			<0.0001
No	53.15	63.59	
Yes	46.85	36.41	
BMI (kg/m^2) (%)			<0.0001
<25	31.50	31.37	
25 ≤ BMI < 30	33.71	27.92	
≥30	34.79	40.70	
Mean ± SD	28.64 ± 6.56	29.69 ± 7.75	
White blood cell (10^3 cells/uL)	7.31 ± 2.16	7.15 ± 2.50	0.0024
Red blood cell (10^6 cells/uL)	4.78 ± 0.46	4.12 ± 0.52	<0.0001
Platelet (10^3 cells/uL)	258.21 ± 63.98	280.53 ± 91.69	<0.0001
CRP (mg/dL)	0.39 ± 0.72	0.68 ± 1.33	<0.0001
Hypertension (%)			<0.0001
NO	72.92	61.23	
YES	27.08	38.77	
High cholesterol (%)			0.0026

(Continued)

TABLE 1 (Continued)

	Non-anemia	Anemia	*p*-value
NO	48.77	52.08	
YES	28.10	28.02	
Missing	23.13	19.91	
Diabetes (%)			<0.0001
NO	92.72	82.30	
YES	7.28	17.70	
Congestive heart failure (%)			<0.0001
NO	98.37	94.60	
YES	1.63	5.40	
Coronary heart disease (%)			<0.0001
NO	97.19	93.53	
YES	2.81	6.47	

Mean ± SD for continuous variables; % for categorical variables; BMI, body mass index; DII, dietary inflammatory index; RIP, ratio of family income to poverty; CRP, C-reactive protein.

3.2 Association between DII and anemia

We performed a multivariable logistic regression analysis to investigate the association between DII and anemia. Both unadjusted and adjusted models are detailed in Table 3. When compared to the lowest quartile of DII, a higher prevalence of anemia was observed in the fourth quartile in Model 1 (OR = 1.90, 95% CI = 1.69–2.12, $p < 0.01$), Model 2 (OR = 1.36, 95% CI = 1.21–1.53, $p < 0.01$), and Model 3 (OR = 1.35, 95% CI = 1.17–1.57, $p < 0.01$). Across all models, there was a significant association between higher DII levels and an increased prevalence of anemia (p for trend <0.01). Furthermore, even after adjusting for all covariate confounders, smoothed curve fitting analysis (Figure 2) revealed a linear relationship between DII and anemia.

3.3 Subgroup analysis

Subgroup analyses were conducted to further explore the relationship between DII and anemia in different populations, as shown in Table 4. In subgroup analyses according to Age, Gender, Race, RIP, Drinking, Smoking, BMI, Diabetes and Hypertension. The results showed a positive association between DII and anemia in different age, BMI, alcohol, diabetes, and hypertension groups. Specifically, in individuals aged 60 or younger, the prevalence of anemia increased by 4% with each unit increase in DII, while in those over 60, the prevalence increased by 9% per unit increase in DII. For non-diabetic patients, each unit increase in DII was associated with a 5% increase in the prevalence of developing anemia, compared to an 11% increase in diabetic patients.

Among ethnic groups, there was no significant association between DII and anemia in Mexican American, Other Hispanic, and Non-Hispanic White groups. However, in the Non-Hispanic Black group, a strong correlation was observed between DII and anemia (OR = 1.07, 95% CI 1.02–1.12, $p = 0.0069$). Subsequently, we found that when RIP <1.3, DII had an effect on the prevalence of developing anemia, with each unit increase in DII increased the prevalence of developing anemia by 6%, and when the RIP was 1.3–3.5, the prevalence of developing anemia increased by 7% for each additional unit of DII. There was also a correlation between DII and anemia when the gender was female (OR = 1.07, 95% CI 1.03–1.10, $p = 0.0003$). In the male population, no significant association was observed between DII and anemia (Figure 3). Simultaneously, in the adjusted model, we observed a significant interaction between smoking and DII (p for interaction <0.05). A positive correlation between DII and anemia was evident in non-smokers, who comprised 60.5% women, (OR = 1.09, 95% CI 1.05–1.13, $p < 0.0001$), while no such correlation was found in smokers (OR = 1.00, 95% CI 0.96–1.05, $p = 0.9011$). Therefore, this nonlinear relationship was described by smooth curve fitting and generalized additive modeling (Figure 4).

4 Discussion

In this cross-sectional study involving 32,244 participants, the results consistently demonstrated a positive association between anemia and DII, both when adjusted and unadjusted for covariates. These findings suggest that an elevation in DII may indeed contribute to a heightened prevalence of anemia. In light of the findings presented, we propose that integrating dietary modifications aimed at lowering the DII may serve as a complementary strategy in the multifactorial management of anemia.

Recent decades of research have increasingly highlighted a critical link: the connection between dietary patterns, types of food, inflammation, and the risk of diseases (17, 18). In this context, the Dietary Inflammation Index emerges as a valuable tool for understanding the interplay between diet and inflammation. A systematic review and meta-analysis revealed that diets primarily based on plant foods are associated with reduced levels of serum CRP, an indicator of inflammation, and total white blood cell (WBC) counts, markers of innate immunity (19). Moreover, randomized controlled trials have shown that vegetarian diets result in lower DII scores compared to meat-based diets (20). Correspondingly, in our study population, higher DII scores were associated with increased WBC and CRP levels. These results underscore the importance of

TABLE 2 Weighted characteristics of the study population based on DII quartiles.

	DII Quartiles				
	Quartile1 −5.28–0.20	Quartile2 0.20–1.67	Quartile3 1.67–2.87	Quartile4 2.87–5.79	*p*-value
Age	45.37 ± 15.52	45.13 ± 15.82	44.89 ± 16.53	45.90 ± 17.16	0.0010
Gender (%)					<0.0001
Male	62.01	52.13	44.54	35.63	
Female	37.99	47.87	55.46	64.37	
Race (%)					<0.0001
Mexican American	8.65	9.42	8.45	7.71	
Other Hispanic	5.25	6.44	5.77	5.80	
Non-Hispanic White	71.80	67.52	67.80	65.70	
Non-Hispanic Black	7.31	9.92	11.98	14.74	
Other Race	6.99	6.69	5.99	6.05	
Academic level (%)					<0.0001
Less than high school	12.94	16.66	18.65	22.10	
High School or Equivalent	20.28	24.39	26.22	30.10	
College or above	66.78	58.95	55.14	47.80	
Marital status (%)					<0.0001
Married	73.10	72.53	70.15	68.59	
Separated	1.85	2.50	2.80	3.28	
Never married	25.05	24.97	27.05	28.13	
RIP (%)					<0.0001
<1.3	15.39	18.36	21.31	27.06	
1.3–3.5	32.06	36.31	38.66	39.89	
>3.5	52.56	45.33	40.03	33.05	
Mean ± SD	3.36 ± 1.61	3.13 ± 1.61	2.92 ± 1.60	2.64 ± 1.60	
Drinking (%)					<0.0001
No	10.70	13.12	15.33	20.35	
Yes	46.13	44.15	45.89	46.15	
Missing	43.17	42.73	38.79	33.50	
Smoking (%)					<0.0001
No	56.29	55.27	52.18	50.74	
Yes	43.71	44.73	47.82	49.26	
BMI (kg/m^2) (%)					<0.0001
<25	34.64	32.12	29.82	28.82	
25 ≤ BMI < 30	34.94	34.59	33.10	30.47	
≥30	30.43	33.28	37.08	40.71	
Mean ± SD	28.00 ± 6.23	28.54 ± 6.46	28.98 ± 6.75	29.39 ± 7.09	
White blood cell (10^3 cells/uL)	7.01 ± 1.96	7.26 ± 2.22	7.44 ± 2.12	7.54 ± 2.40	<0.0001
Red blood cell (10^6 cells/uL)	4.79 ± 0.48	4.76 ± 0.48	4.73 ± 0.49	4.68 ± 0.49	<0.0001
Hgb (g/dL)	14.61 ± 1.39	14.47 ± 1.45	14.33 ± 1.50	14.08 ± 1.54	<0.0001
Platelet (10^3 cells/uL)	250.25 ± 61.43	257.70 ± 63.89	263.64 ± 66.74	268.01 ± 71.49	<0.0001
CRP (mg/dL)	0.32 ± 0.74	0.38 ± 0.67	0.43 ± 0.74	0.49 ± 0.91	<0.0001
Hypertension (%)					<0.0001

(Continued)

TABLE 2 (Continued)

	DII Quartiles				
	Quartile1 −5.28–0.20	Quartile2 0.20–1.67	Quartile3 1.67–2.87	Quartile4 2.87–5.79	*p*-value
NO	74.46	73.57	71.92	68.49	
YES	25.54	26.43	28.08	31.51	
High cholesterol (%)					<0.0001
NO	50.63	49.58	47.91	47.41	
YES	29.07	27.42	27.78	28.04	
Missing	20.30	23.01	24.32	24.54	
Diabetes (%)					<0.0001
NO	93.15	92.59	91.85	90.64	
YES	6.85	7.41	8.15	9.36	
Congestive heart failure (%)					<0.0001
NO	98.69	98.42	97.82	97.58	
YES	1.31	1.58	2.18	2.42	
Coronary heart disease (%)					0.0379
NO	97.09	97.36	96.74	96.66	
YES	2.91	2.64	3.26	3.34	
Anemia (%)					<0.0001
NO	96.00	95.03	93.77	91.70	
YES	4.00	4.97	6.23	8.30	

Mean ± SD for continuous variables; % for categorical variables; BMI, body mass index; RIP, ratio of family income to poverty; Hgb, hemoglobin; DII, dietary inflammatory index; CRP, C-reactive protein.

TABLE 3 The odds ratio for the relationship between DII and anemia.

	MODEL 1 OR (95%CI) *p*-value	MODEL 2 OR (95%CI) *p*-value	MODEL 3 OR (95%CI) *p*-value
DII	1.15 (1.12, 1.17) <0.0001	1.07 (1.04, 1.09) <0.0001	1.06 (1.03, 1.09) <0.0001
DII Quintiles			
Quartile 1	Reference	Reference	Reference
Quartile 2	1.27 (1.12, 1.43) 0.0001	1.12 (0.99, 1.27) 0.0620	1.17 (1.01, 1.36) 0.0422
Quartile 3	1.51 (1.34, 1.70) <0.0001	1.23 (1.09, 1.38) 0.0008	1.20 (1.04, 1.40) 0.0158
Quartile 4	1.90 (1.69, 2.12) <0.0001	1.36 (1.21, 1.53) <0.0001	1.35 (1.17, 1.57) <0.0001
p for trend	<0.0001	<0.0001	<0.0001

MODEL 1: No covariates were adjusted. MODEL 2: Age, Gender, Race were adjusted. MODEL 3: Age, Gender, Race, Academic level, Marital status, RIP, drinking, Smoking, BMI, White blood cell, Red blood cell, Platelet, Hypertension, High cholesterol, Diabetes, Congestive heart failure, Coronary heart disease were adjusted. 95% CI, 95% confidence interval; OR, odds ratio; DII, dietary inflammatory index.

dietary choices in controlling inflammation and reducing the risk of related diseases.

Anemia represents a widespread global health concern, and recent studies have unveiled a significant link between anemia and inflammation (21, 22). In animal models of AI and in patients with inflammatory diseases, elevated hepcidin levels are linked to decreased ferroportin expression in duodenal enterocytes and macrophages, resulting in impaired dietary iron absorption (23). Furthermore, even low-grade inflammation can hinder intestinal iron absorption (24), which consequently leads to a reduced iron supply for erythropoiesis (25), and thus negatively impacting anemia. Inflammatory cytokines additionally play a role in reducing the lifespan of red blood cells, possibly by activating macrophages (6). Given this backdrop, dietary patterns, especially pro-inflammatory diets, play a crucial role. Pro-inflammatory diets, by increasing the body's inflammatory responses, may heighten the risk of anemia. A prospective cohort study (26) on anemia in pregnant women has shown that higher pro-inflammatory diet scores correlate with lower hemoglobin (HGB) levels in expectant mothers. Including an anti-inflammatory diet may help prevent maternal anemia in women with

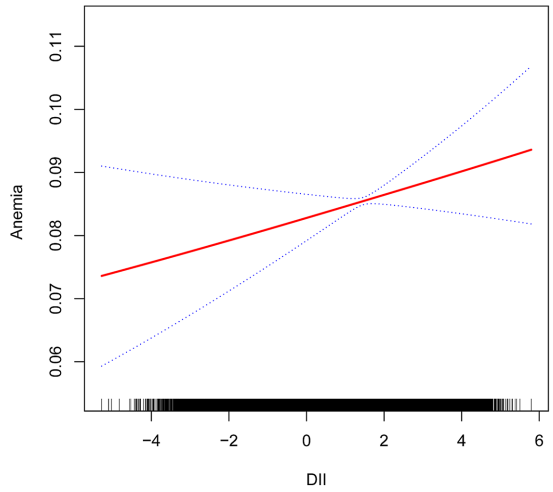

FIGURE 2
Relationship between dietary inflammatory index and anemia. The vertical axis represents log OR of anemia. DII, dietary inflammatory index.

gestational diabetes. Thus, it appears that focusing on dietary factors, especially in reducing pro-inflammatory diets, may play a significant role in managing anemia. It is important to note that while adhering to a vegetarian diet may help reduce the DII, individuals prone to anemia need to carefully manage their diet and supplements to maintain optimal health. High levels of phytates in grains and legumes can interfere with the absorption of essential minerals like calcium, zinc, iron, iodine, and magnesium (27). Consequently, diets rich in these foods may lead to mineral deficiencies (28). Although plant foods are high in iron, studies show that strict vegetarians are at a higher risk of iron deficiency (29). Plant-based iron, being non-heme, is less bioavailable compared to the heme iron found in animal products (28, 30). Therefore, a practical approach could be the moderate inclusion of nutrient-rich animal-sourced foods for a more balanced dietary intake.

Our results highlights that factors such as gender, age, ethnicity, RIP, body mass index, and diabetes, exhibit similar directional effects on the relationship between the prevalence of anemia and DII. The findings related to age, ethnicity, and economic status align with the results obtained from the world population Anemia risk survey (16). Subgroup analysis revealed a stronger DII impact on anemia in individuals over 60, females, non-smokers, those of Non-Hispanic Black ethnicity, economically disadvantaged groups, and patients with hypertension, diabetes mellitus, or heart failure. The overlapping effects of pro-inflammatory foods and these risk factors on anemia prevalence are noteworthy.

Numerous studies have revealed disparities in anemia across different regions and ethnic groups (1, 16), which may be closely related to the dietary cultures of different ethnicities. For instance, research reports indicate that the intake of fruits and vegetables is generally lower among the Black/African American population (31). Furthermore, compared to white individuals with anemia, Black individuals exhibit a higher incidence rate of inflammation related to anemia (32). Our cross-sectional study results also emphasize the importance of the relationship between inflammatory diets and the prevalence of anemia. How to integrate traditional dietary habits of different ethnic groups with anti-inflammatory principles requires further exploration. Understanding the dietary differences between ethnic groups and their impact on food choices is crucial in formulating effective and culturally adaptive public health strategies.

Notably, anemia tends to be more prevalent in women compared to men (16), partly due to factors like menstrual cycles, pregnancy, and higher susceptibility to iron deficiency in high-DII environments (33). The interaction between female physiological differences, including sex hormone variations, and DII is complex and merits deeper exploration. For instance, initial studies suggest a link between DII and sex hormones in female adolescents (34) and the role of estrogen in inflammatory responses has been recognized (35). These findings indicate that estrogen and DII may jointly influence anemia development in women. Furthermore, gender-specific dietary habits (36), including food choices and intake, could contribute to the varied impact of DII on anemia between men and women.

Smoking, a key factor linked to inflammation and various diseases (37), necessitates detailed examination for its potential role in modulating the relationship between dietary inflammatory indices and anemia. The presence of harmful chemicals like tar, nicotine, and carbon monoxide in tobacco smoke could impact the immune system, possibly suppressing or altering its inflammatory response (38–40). While smoking itself triggers inflammation, it may also weaken the immune response in high-inflammatory settings, potentially reducing susceptibility to the effects of a pro-inflammatory diet. Compared to non-smokers, smokers have been found to consume fewer fruits and vegetables while having a higher intake of fats and alcohol (41, 42). These dietary discrepancies could potentially affect the relationship between the DII and anemia. The particular effects of these dietary habits on DII in smokers, however, remain under-researched. Additional studies are needed to investigate the influence of smoking on DII and iron status. Consequently, comprehending the complex role of smoking in this relationship is vital, necessitating further research to decode the underlying mechanisms.

Age, chronic diseases, and dietary inflammatory indices are interconnected in anemia development. Diseases often associated with anemia, like myelodysplastic syndromes (MDS), chronic kidney disease (CKD), and gastrointestinal (GI) conditions, are prevalent in older individuals (43). A pro-inflammatory state, common in the older adult, is increasingly recognized as a contributor to anemia (5). Additionally, anemia is frequently associated with a higher BMI, which not only indicates elevated inflammatory cytokine levels (44) but also tends to correlate with diets high in calories and fats, yet low in fiber (45). This dietary pattern raises DII, and, when combined with the heightened risk of metabolic and cardiorespiratory diseases associated with high BMI (46), can intensify anemia through inflammation. Furthermore, recent evidence indicates a potential link between pro-inflammatory diets and an increased incidence of chronic diseases such as diabetes (47) and hypertension (48). Our stratified analysis indicating a heightened risk of anemia in those with these chronic conditions. Therefore, it appears that adopting a low-inflammatory dietary pattern might be beneficial in potentially reducing factors associated with anemia. This approach aims to address not only the direct

TABLE 4 Subgroup analysis of the association between dietary inflammatory index with anemia.

	OR (95%CI) *p*-value	*p* for interaction
Age		0.1442
≤60	1.04 (1.00, 1.08) 0.0395	
>60	1.09 (1.03, 1.14) 0.0009	
Gender		0.3033
Male	1.03 (0.98, 1.09) 0.2443	
Female	1.07 (1.03, 1.10) 0.0003	
Race		0.4108
Mexican American	1.06 (0.99, 1.14) 0.1023	
Other Hispanic	1.10 (0.98, 1.23) 0.0999	
Non-Hispanic White	1.01 (0.95, 1.07) 0.7373	
Non-Hispanic Black	1.07 (1.02, 1.12) 0.0069	
Other Race	1.10 (1.00, 1.21) 0.0472	
RIP		0.7894
<1.3	1.06 (1.01, 1.11) 0.0285	
1.3–3.5	1.07 (1.02, 1.12) 0.0039	
>3.5	1.04 (0.99, 1.10) 0.1315	
BMI		0.9939
<25	1.06 (1.01, 1.11) 0.0284	
25 ≤ BMI < 30	1.06 (1.01, 1.12) 0.0311	
≥30	1.06 (1.01, 1.11) 0.0117	
Drink		0.8934
NO	1.06 (1.00, 1.12) 0.0462	
Yes	1.05 (1.00, 1.10) 0.0338	
Smoking		0.0110
NO	1.09 (1.05, 1.13) <0.0001	
YES	1.00 (0.96, 1.05) 0.9011	
Diabetes		0.1237
NO	1.05 (1.02, 1.08) 0.0033	
YES	1.11 (1.04, 1.20) 0.0028	
Hypertension		0.1647
NO	1.04 (1.01, 1.08) 0.0233	
YES	1.09 (1.04, 1.14) 0.0005	

Adjusted for all covariates except effect modifier.

dietary contributors to anemia but also the chronic conditions that may exacerbate its occurrence. However, it is important to note that this hypothesis requires further investigation. In conclusion, effective management of anemia necessitates acknowledging and addressing these interconnected factors. A comprehensive approach, tailored to individual needs and considering the intricate interplays among DII, gender-specific factors, smoking, age, chronic diseases, and BMI, is essential for the precise prevention and treatment of anemia.

However, it's essential to acknowledge certain limitations in the current study that require consideration. Firstly, the cross-sectional design of this study inherently limits its ability to establish causality. Secondly, it is important to note that our study faced limitations in directly linking DII with specific types of anemia, primarily due to sample size constraints regarding laboratory tests related to anemia (e.g., iron deficiency, vitamin deficiencies, etc.) within the database. This limitation may have influenced the precision of our results. Thirdly, the study's results may not be generalizable to younger patients, as some participants under the age of 20 were not included in the analysis. Finally, the anemia data used in this study were based on laboratory diagnoses and may not encompass patients with a historical record of anemia, potentially leading to incomplete results.

5 Conclusion

Our study reveals an association between an inflammatory diet and a heightened prevalence of anemia, with a more

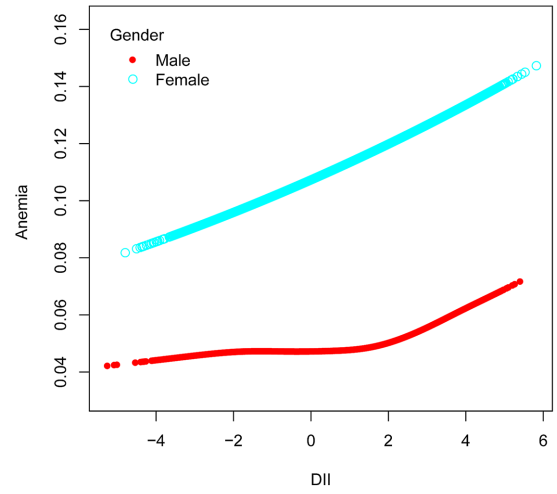

FIGURE 3
Association between anemia and DII stratified by gender. The vertical axis represents log OR of anemia. DII, dietary inflammatory index.

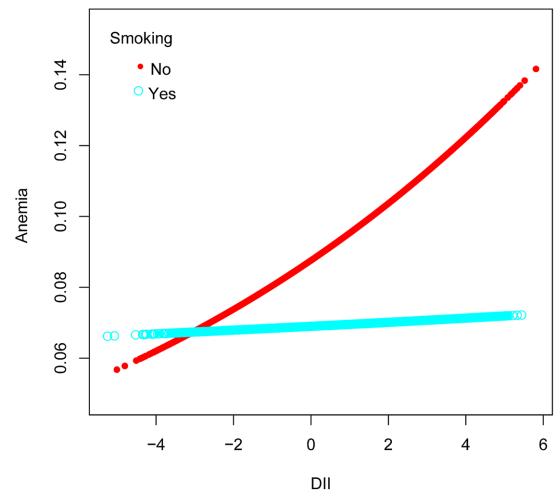

FIGURE 4
Association between anemia and DII stratified by smoking, respectively. The vertical axis represents log OR of anemia. DII, dietary inflammatory index.

pronounced effect observed in women, older individuals, those with higher BMI, non-smokers, Non-Hispanic Black individuals, and those with less favorable family conditions. These findings offer valuable insights for public health authorities and healthcare providers, enabling the development of more precise strategies for anemia screening and prevention. The potential role of dietary modification, specifically reducing inflammatory dietary patterns, warrants further investigation. While these preliminary observations suggest a direction for public health interventions, robust clinical trials are required to establish causative links and to develop effective, targeted dietary guidelines for anemia prevention, particularly in demographically diverse populations.

Data availability statement

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

Ethics statement

The studies involving humans were approved by National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HM: Conceptualization, Formal analysis, Methodology, Supervision, Writing – original draft. WD: Data curation, Formal analysis, Software, Supervision, Writing – original draft. HC: Formal Analysis, Software, Writing – review & editing. XD: Conceptualization, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1310345/full#supplementary-material

References

1. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global Anemia burden from 1990 to 2010. *Blood*. (2014) 123:615–24. doi: 10.1182/blood-2013-06-508325

2. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ*. (2013) 346:f3443. doi: 10.1136/bmj.f3443

3. Ezekowitz JA, McAlister FA, Armstrong PW. Anemia is common in heart failure and is associated with poor outcomes: insights from a cohort of 12 065 patients with new-onset heart failure. *Circulation*. (2003) 107:223–5. doi: 10.1161/01.cir.0000052622.51963.fc

4. Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *J Nutr*. (2001) 131:676S–90S; discussion 88S-90S. doi: 10.1093/jn/131.2.676S

5. Stauder R, Valent P, Theurl I. Anemia at older age: Etiologies, clinical implications, and management. *Blood*. (2018) 131:505–14. doi: 10.1182/blood-2017-07-746446

6. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and Etiology in low- and middle-income countries. *Ann N Y Acad Sci*. (2019) 1450:15–31. doi: 10.1111/nyas.14092

7. Nairz M, Theurl I, Wolf D, Weiss G. Iron deficiency or Anemia of inflammation? Differential diagnosis and mechanisms of Anemia of inflammation. *Wien Med Wochenschr*. (2016) 166:411–23. doi: 10.1007/s10354-016-0505-7

8. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood*. (2019) 133:40–50. doi: 10.1182/blood-2018-06-856500

9. Di Giosia P, Stamerra CA, Giorgini P, Jamialahamdi T, Butler AE, Sahebkar A. The role of nutrition in Inflammaging. *Ageing Res Rev*. (2022) 77:101596. doi: 10.1016/j.arr.2022.101596

10. Christ A, Lauterbach M, Latz E. Western diet and the immune system: an inflammatory connection. *Immunity*. (2019) 51:794–811. doi: 10.1016/j.immuni.2019.09.020

11. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr*. (2014) 17:1689–96. doi: 10.1017/s1368980013002115

12. Syed Soffian SS, Mohammed Nawi A, Hod R, Ja'afar MH, Isa ZM, Chan HK, et al. Meta-analysis of the association between dietary inflammatory index (dii) and colorectal cancer. *Nutrients*. (2022) 14:1555. doi: 10.3390/nu14081555

13. Hariharan R, Odjidja EN, Scott D, Shivappa N, Hébert JR, Hodge A, et al. The dietary inflammatory index, obesity, type 2 diabetes, and cardiovascular risk factors and diseases. *Obes Rev*. (2022) 23:e13349. doi: 10.1111/obr.13349

14. Xiang S, Wang Y, Qian S, Li J, Jin Y, Ding X, et al. The association between dietary inflammation index and the risk of rheumatoid arthritis in Americans. *Clin Rheumatol*. (2022) 41:2647–58. doi: 10.1007/s10067-022-06217-9

15. Wu L, Shi Y, Kong C, Zhang J, Chen S. Dietary inflammatory index and its association with the prevalence of coronary heart disease among 45, 306 US adults. *Nutrients*. (2022) 14:4553. doi: 10.3390/nu14214553

16. Safiri S, Kolahi AA, Noori M, Nejadghaderi SA, Karamzad N, Bragazzi NL, et al. Burden of Anemia and its underlying causes in 204 countries and territories, 1990–2019: results from the global burden of disease study 2019. *J Hematol Oncol*. (2021) 14:185. doi: 10.1186/s13045-021-01202-2

17. Grant WB. Diet, inflammation, and infectious diseases. *Nutrients*. (2023) 15:2891. doi: 10.3390/nu15132891

18. Wang P, Song M, Eliassen AH, Wang M, Fung TT, Clinton SK, et al. Optimal dietary patterns for prevention of chronic disease. *Nat Med*. (2023) 29:719–28. doi: 10.1038/s41591-023-02235-5

19. Craddock JC, Neale EP, Peoples GE, Probst YC. Vegetarian-based dietary patterns and their relation with inflammatory and immune biomarkers: a systematic review and meta-analysis. *Adv Nutr*. (2019) 10:433–51. doi: 10.1093/advances/nmy103

20. Turner-McGrievy GM, Wirth MD, Shivappa N, Wingard EE, Fayad R, Wilcox S, et al. Randomization to plant-based dietary approaches leads to larger short-term improvements in dietary inflammatory index scores and macronutrient intake compared with diets that contain meat. *Nutr Res*. (2015) 35:97–106. doi: 10.1016/j.nutres.2014.11.007

21. Marques O, Weiss G, Muckenthaler MU. The role of iron in chronic inflammatory diseases: from mechanisms to treatment options in Anemia of inflammation. *Blood*. (2022) 140:2011–23. doi: 10.1182/blood.2021013472

22. Namaste SM, Aaron GJ, Varadhan R, Peerson JM, Suchdev PS. Methodologic approach for the biomarkers reflecting inflammation and nutritional determinants of Anemia (Brinda) project. *Am J Clin Nutr*. (2017) 106:333s–47s. doi: 10.3945/ajcn.116.142273

23. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. (2005) 352:1011–23. doi: 10.1056/NEJMra041809

24. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, et al. Regulation of iron homeostasis in Anemia of chronic disease and iron deficiency Anemia: diagnostic and therapeutic implications. *Blood*. (2009) 113:5277–86. doi: 10.1182/blood-2008-12-195651

25. Lanser L, Fuchs D, Kurz K, Weiss G. Physiology and inflammation driven pathophysiology of iron homeostasis-mechanistic insights into Anemia of inflammation and its treatment. *Nutrients*. (2021) 13:3732. doi: 10.3390/nu13113732

26. Hayashi I, Sakane N, Suganuma A, Nagai N. Association of a pro-Inflammatory Diet and Gestational Diabetes Mellitus with maternal Anemia and Hemoglobin levels during pregnancy: a prospective observational case-control study. *Nutr Res*. (2023) 115:38–46. doi: 10.1016/j.nutres.2023.05.003

27. Agnoli C, Baroni L, Bertini I, Ciappellano S, Fabbri A, Papa M, et al. Position paper on vegetarian diets from the working Group of the Italian Society of human nutrition. *Nutr Metab Cardiovasc Dis*. (2017) 27:1037–52. doi: 10.1016/j.numecd.2017.10.020

28. O'Keefe JH, O'Keefe EL, Lavie CJ, Cordain L. Debunking the vegan myth: the case for a plant-forward omnivorous whole-foods diet. *Prog Cardiovasc Dis*. (2022) 74:2–8. doi: 10.1016/j.pcad.2022.08.001

29. Bakaloudi DR, Halloran A, Rippin HL, Oikonomidou AC, Dardavesis TI, Williams J, et al. Intake and adequacy of the vegan diet. A systematic review of the evidence. *Clin Nutr*. (2021) 40:3503–21. doi: 10.1016/j.clnu.2020.11.035

30. Bothwell TH, Baynes RD, Mac Farlane BJ, Mac Phail AP. Nutritional iron requirements and food iron absorption. *J Intern Med*. (1989) 226:357–65. doi: 10.1111/j.1365-2796.1989.tb01409.x

31. Bennett G, Bardon LA, Gibney ER. A comparison of dietary patterns and factors influencing food choice among ethnic groups living in one locality: a systematic review. *Nutrients*. (2022) 14:941. doi: 10.3390/nu14050941

32. Zakai NA, McClure LA, Prineas R, Howard G, McClellan W, Holmes CE, et al. Correlates of Anemia in American blacks and whites: the regards renal ancillary study. *Am J Epidemiol*. (2009) 169:355–64. doi: 10.1093/aje/kwn355

33. Benson CS, Shah A, Stanworth SJ, Frise CJ, Spiby H, Lax SJ, et al. The effect of iron deficiency and anaemia on Women's health. *Anaesthesia*. (2021) 76:84–95. doi: 10.1111/anae.15405

34. Ma Y, Li R, Zhan W, Huang X, Zhou Y, Sun Y, et al. Associations between dietary inflammatory index and sex hormones among 6- to 19-year-old children and adolescents in NHANES 2015–2016. *Front Endocrinol*. (2021) 12:792114. doi: 10.3389/fendo.2021.792114

35. Sha Q, Achtyes E, Nagalla M, Keaton S, Smart L, Leach R, et al. Associations between Estrogen and progesterone, the kynurenine pathway, and inflammation in the post-partum. *J Affect Disord*. (2021) 281:9–12. doi: 10.1016/j.jad.2020.10.052

36. Razaz JM, Balam FH, Karimi T, Rahmani J, Kalantari N, Shariatpanahi SP, et al. Sex differences in healthy eating: investigating the moderating effect of self-efficacy. *J Nutr Educ Behav*. (2022) 54:151–8. doi: 10.1016/j.jneb.2021.05.011

37. Johannsen A, Susin C, Gustafsson A. Smoking and inflammation: evidence for a synergistic role in chronic disease. *Periodontol*. (2014) 64:111–26. doi: 10.1111/j.1600-0757.2012.00456.x

38. Chen H, Cowan MJ, Hasday JD, Vogel SN, Medvedev AE. Tobacco smoking inhibits expression of Proinflammatory cytokines and activation of Il-1r-associated kinase, P 38, and Nf-Kappab in alveolar macrophages stimulated with Tlr 2 and Tlr 4 agonists. *J Immunol*. (2007) 179:6097–106. doi: 10.4049/jimmunol.179.9.6097

39. Parkes GC, Whelan K, Lindsay JO. Smoking in inflammatory bowel disease: impact on disease course and insights into the aetiology of its effect. *J Crohns Colitis*. (2014) 8:717–25. doi: 10.1016/j.crohns.2014.02.002

40. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha 7 subunit is an essential regulator of inflammation. *Nature*. (2003) 421:384–8. doi: 10.1038/nature01339

41. Subar AF, Harlan LC, Mattson ME. Food and nutrient intake differences between smokers and non-smokers in the us. *Am J Public Health*. (1990) 80:1323–9. doi: 10.2105/ajph.80.11.1323

42. McClure JB, Divine G, Alexander G, Tolsma D, Rolnick SJ, Stopponi M, et al. A comparison of Smokers' and Nonsmokers' fruit and vegetable intake and relevant psychosocial factors. *Behav Med*. (2009) 35:14–22. doi: 10.3200/bmed.35.1.14-22

43. Stauder R, Thein SL. Anemia in the elderly: clinical implications and new therapeutic concepts. *Haematologica*. (2014) 99:1127–30. doi: 10.3324/haematol.2014.109967

44. Ma Y, Li R, Zhan W, Huang X, Zhang Z, Lv S, et al. Role of Bmi in the relationship between dietary inflammatory index and depression: an intermediary analysis. *Front Med*. (2021) 8:748788. doi: 10.3389/fmed.2021.748788

45. Harbury C, Collins CE, Callister R. Diet quality is lower among adults with a Bmi ≥40kgm(−2) or a history of weight loss surgery. *Obes Res Clin Pract*. (2019) 13:197–204. doi: 10.1016/j.orcp.2018.10.003

46. Madssen E, Skaug EA, Wisløff U, Ellingsen Ø, Videm V. Inflammation is strongly associated with cardiorespiratory fitness, sex, Bmi, and the metabolic syndrome in a self-reported healthy population: hunt 3 fitness study. *Mayo Clin Proc*. (2019) 94:803–10. doi: 10.1016/j.mayocp.2018.08.040

47. Denova-Gutiérrez E, Muñoz-Aguirre P, Shivappa N, Hébert JR, Tolentino-Mayo L, Batis C, et al. Dietary inflammatory index and type 2 diabetes mellitus in adults: the diabetes mellitus survey of Mexico City. *Nutrients*. (2018) 10:385. doi: 10.3390/nu10040385

48. Vissers LET, Waller M, van der Schouw YT, Hébert JR, Shivappa N, Schoenaker D, et al. A pro-inflammatory diet is associated with increased risk of developing hypertension among middle-aged women. *Nutr Metab Cardiovasc Dis*. (2017) 27:564–70. doi: 10.1016/j.numecd.2017.03.005

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Does millet consumption contribute to raising blood hemoglobin levels compared to regular refined staples?: a systematic review and meta-analysis

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Millets are recognized for their health and nutritional values, and the United Nations declared 2023 the International Year of Millets. Among the several health and nutritional benefits of millets, their impact on hemoglobin concentration is important since anemia is a major public health issue in many countries. To investigate the effect of millet (including sorghum) consumption on hemoglobin concentration in the blood, a systematic review and meta-analysis were conducted. Thirteen published studies featuring randomized control trials involving 590 individuals in the intervention group and 549 control individuals were eligible for the meta-analysis. The difference-in-differences analysis revealed highly significant ($p < 0.01$) positive effects of millet consumption on hemoglobin concentration, with an effect size of +0.68 standardized mean difference units. The change in hemoglobin concentration observed in the intervention group was +13.6%, which is statistically significant ($p < 0.0005$), compared to that in the control group, which was +4.8% and not statistically significant ($p = 0.1362$). In four studies, the consumption of millets in the intervention group demonstrated a change from mild anemia to normal status among children, whereas there was no change in the control group. The findings provide evidence that the consumption of millets can improve blood hemoglobin concentration, likely resulting from increased iron intake. Further research is needed involving the assessment of iron content and bioavailability to better understand the effect variation among millet types and the mechanisms involved.

KEYWORDS

iron deficiency anemia, millets, hemoglobin, dietary iron, difference-in-differences

1 Introduction

Iron deficiency anemia (IDA) is a global public health issue that affects children and young women in particular. According to the World Health Organization (WHO) estimates for 2019, global anemia prevalence in women of reproductive age and children was 29.9 and 39.8%, respectively, accounting for half a billion women and 269 million children (1). A deficit in dietary iron intake remains a significant challenge, exacerbated by the growing global consumption of refined and highly processed foods, leading to micronutrient deficiency in vulnerable populations. Staple cereals continue to dominate food consumption in developing countries; these mainly include refined wheat, rice, and maize, while other nutrient-rich crops such as millets and sorghum are underutilized (2–4) Supplementation with iron, widespread fortification of foods with iron, and dietary diversity are evidence-based approaches to combating anemia. Interventions aimed at enhancing dietary diversity also facilitate the intake of a wide range of micronutrients, rather than focusing on just one. Studies in different parts of the world have shown that enhancing dietary diversity has resulted in improved hemoglobin levels (5, 6). In this context, millets play an important role in promoting dietary diversity, which in turn ensures the consumption of a wide spectrum of essential vitamins, minerals, and other nutrients. Regions in which millets historically constituted a significant part of the diet have seen their prominence in the dietary landscape gradually diminish over time (7). Whole grain millets have advantages over refined cereals, as they have higher levels of nutrients such as iron, zinc, and protein, to name a few (2). The effect of consuming millets that are rich in iron on blood hemoglobin concentration and anemia has been studied by many researchers. However, this is the first time that evidence on the impact of consuming millet on hemoglobin levels compared to the consumption of other common staples has been collated.

A previous systematic review and meta-analysis showed that millets reduce fasting blood glucose levels (8) and hyperlipidemia (9) and improve growth in children (10). In terms of their effects on anemia, Anitha et al. (11) conducted a systematic review of the potential of millets in raising blood hemoglobin levels, showing that the levels increased from the baseline to the endline. However, the study did not account for changes in the control group. The current systematic review and meta-analysis extends the study conducted by Anitha et al. (11) by accounting for the control group.

The review question, therefore, is, "Does the consumption of millets have positive effects on blood hemoglobin concentration in the controlled assessment design as well, where the control group consumes common staples?

2 Materials and methods

2.1 Study period and protocol

The systematic review and meta-analysis were conducted from October 2017 to September 2022. The PRISMA checklist (12, 13) was used to write the protocol, which was registered with the unique identification number "reviewregistry1114" in the online platform "research registry." Figure 1 describes the process of the systematic review.

2.2 Search

Studies published in English until September 2022 were obtained through major search engines, namely, Google Scholar, Scopus, Web of Science, PubMed, and CAB abstracts.

2.3 Search strategy

The search was conducted using predetermined terminology such as "hemoglobin level AND millets," "Anemia AND millets," "millet consumption AND hemoglobin level," and "millet consumption and anemia." The search was repeated by replacing millet with the specific type of millet.

2.4 Inclusion criteria

The review included randomized controlled trials conducted on the effect of the consumption of millets on blood hemoglobin level, where the control group consumed a regular diet, and studies conducted on any age group (children, adolescents, and adults) or gender of any geographical region. Only human studies were considered as were peer-reviewed journal articles and completed MSc or PhD theses that were available online.

2.5 Exclusion criteria

Review articles, animal studies, publications with incomplete data on hemoglobin levels, and papers using only an intervention group were excluded.

2.6 Data items

From each eligible study, information on the authors, year of publication, age group and gender of the study participants, country details, sample size in both intervention and control groups, and study methodology were recorded in an Excel sheet (14, 15) along with the mean and standard deviation difference in hemoglobin levels in g/dl.

2.7 GRADE assessment

A GRADE assessment was conducted to evaluate the quality of the published articles included in the meta-analysis, based on criteria described by Cochrane (16). Two co-authors of this systematic review independently conducted the GRADE assessment and had no disagreement, obviating the need for another co-author's input. Quality was assessed based on the ranking of eight criteria, namely, risk of bias, inconsistency, indirectness, imprecision, publication bias, large magnitude of effects, dose–response, and effects of all plausible confounding factors. Ratings were given to downgrade the first five criteria and/or upgrade the sixth to eighth criteria according to the assessment for each criterion. Publication bias was assessed using a funnel plot (17).

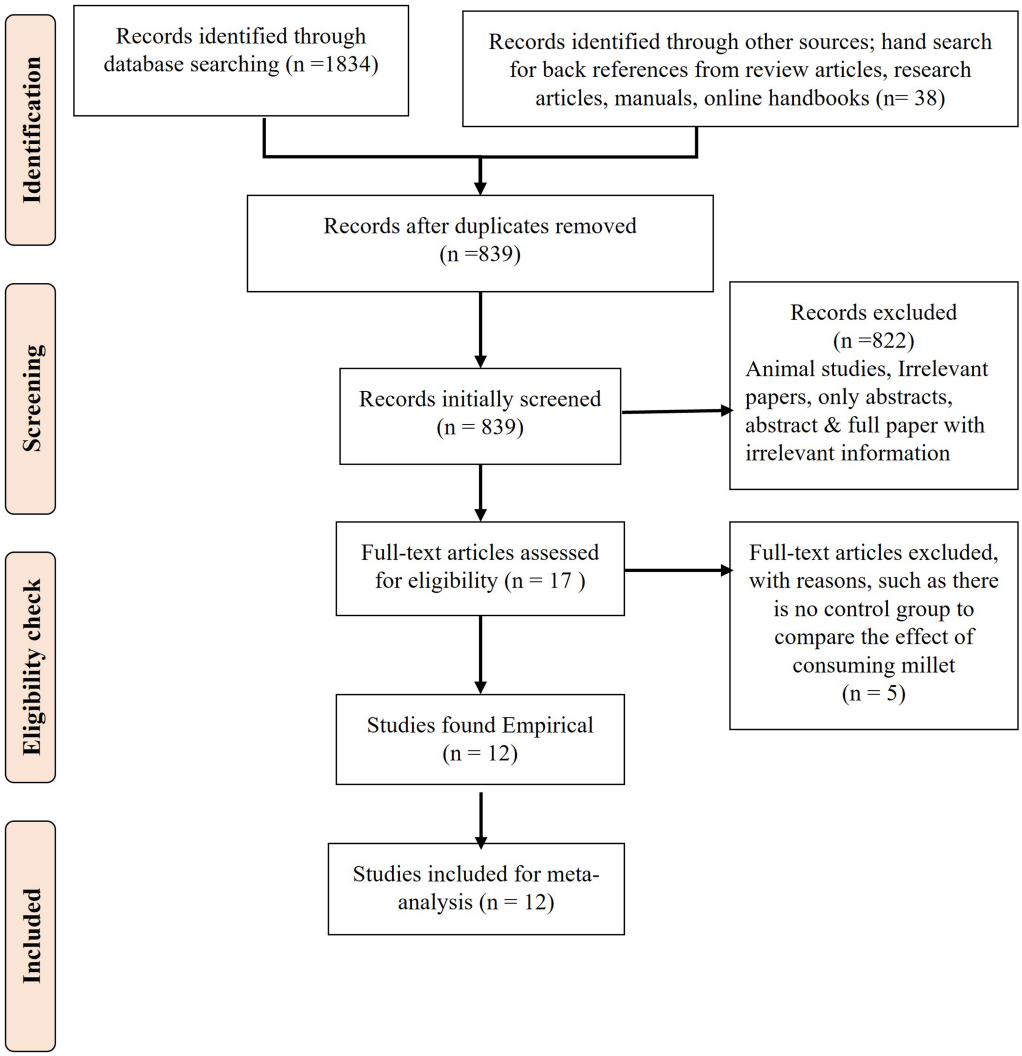

FIGURE 1
PRISMA flow diagram of the study process.

2.8 Data analysis

A total of 12 published articles with 13 studies were found eligible for inclusion in the meta-analysis. The mean change and standard deviation of hemoglobin levels were observed in the intervention and control groups consuming a normal diet (i.e., refined rice and/or wheat). The effects of the treatment were evaluated based on the difference-in-differences (DID) method to address the bias arising from changes in the control group and any initial difference between the two groups. The statistical significance of the DID was examined by the Wilcoxon matched-pairs signed rank (WSR) test. Furthermore, the treatment effect was formally estimated by the panel DID regression analysis. Standard errors (SEs) were clustered at the individual level to obtain the SE robust to heteroscedasticity. The Hausman specification test was used to choose between the fixed effect and random effect models for the panel regression. The meta-analysis was conducted using R Studio version 4.2.1 (18) to obtain forest plots to determine intervention effects and funnel plots to determine publication bias. The mean change in hemoglobin level (g/dl) before and after the intervention was shown for each group, along with the standard deviation and sample size, to determine the standardized mean difference (SMD) (19–21). The results of the fixed and random effect models were obtained to describe the effect size (22, 23). In addition, the participants were subgrouped into children, adolescents, and adults to determine the intervention effects by age.

Expected outcome: Impact of millet consumption on hemoglobin levels compared to other staple consumption in any geographical location.

3 Results

The meta-analysis of the 13 studies from 12 publications (23–35) (Figure 2) shows high heterogeneity ($I^2 = 80\%$). The random effect model used to interpret the results showed significant ($p < 0.01$) effects of millet consumption on blood hemoglobin concentration, with an average standard mean difference (SMD) of +0.68 and a confidence interval of [+0.33; +1.02].

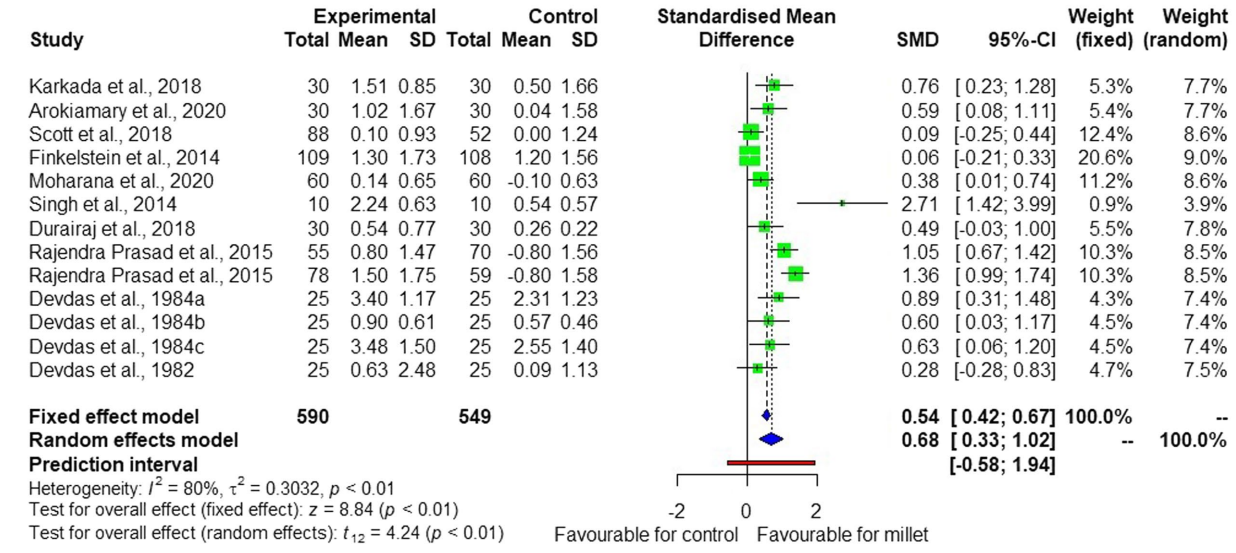

FIGURE 2
Forest plot showing the effects of millet consumption on blood hemoglobin level.

TABLE 1 Descriptive statistics for blood hemoglobin concentration (g/dl).

	Treatment group (n = 13)		Control group (n = 13)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Mean	9.95	11.31	10.20	10.66
Standard deviation	1.07	1.26	0.99	1.09

TABLE 2 Changes and difference-in-differences (DID) in blood hemoglobin concentration using the Wilcoxon matched-pairs signed rank (WSR) test.

Treatment group (n = 13)		Control group (n = 13)		Effect of treatment	
Mean % change	WSR p-value	Mean % change	WSR p-value	DID	WSR p-value
+13.6***	0.000	+4.8	0.1362	+8.8	0.017**

*** and ** indicate $p < 0.01$ and 0.05, respectively.

Descriptive statistics (Table 1) show that the mean hemoglobin concentration in the intervention group was 9.95 ± 1.07 g/dL before consuming non-refined millet-based food, which increased to 11.31 ± 1.09 g/dL after the intervention. In the control group, it was 10.20 ± 0.99 before the intervention and 10.66 ± 1.09 after the intervention.

The WSR test shows that the percentage change in the hemoglobin concentration in the treatment group was +13.6% ($p = 0.000$), whereas the change in the control group was not statistically significant ($p = 0.136$) (Table 2). The DID result suggests that the average treatment effect of millet-based diets on blood hemoglobin concentration was +8.8%.

Table 3 provides a formal estimation of the treatment effects on blood hemoglobin concentration. The Hausman test was not significant, suggesting the use of the random effect model. The DID estimator indicates that the effect of millet-based diets on hemoglobin concentration was +0.862 g/dL on average.

Interestingly, the subgroup analysis showed that the treatment effects were significant only in the children's group but not in the adolescent and adult groups (Figure 3). The changes in hemoglobin concentration differed among the three subgroups.

4 Discussion

The studies included 297 adolescents, 268 children, and 25 adults in the intervention groups who consumed millet-based food. The control groups had a total of 260 adolescents, 264 children, and 25 adults. Four studies used pearl millet, two used finger millet, one used mixed millet, and one used sorghum (Supplementary Table S1). The millets, specifically finger millet, pearl millet, and sorghum, are generally not refined in India; therefore, the current study assumes that these are whole grain or whole grain flour. However, rice is consumed in refined form in India. One study was conducted for 25 days and another for 45 days, while the rest of the studies were conducted from 100 days to 4.5 years. The mean hemoglobin concentration in the intervention group before the intervention

TABLE 3 Effect of the treatment on hemoglobin concentration using random effect DID regression.

	Coeff.	Robust SE	*p*-value
Treatment (1 if treated, 0 if control)	−0.209	0.668	0.754
Timing (1 for post-treatment, 0 for pre-treatment)	0.489	0.285	0.086
Difference-in-differences (DID)	0.862**	0.419	0.040
Constant	10.167	0.495	0.000
Dependent variable =: Blood hemoglobin concentration (g/dl) Hausman test $\chi^2 = 0.00$ ($p = 1.000$) Number of observations = 52; number of individuals = 26 $\sigma_u = 1.49$; $\sigma_e = 0.75$; Wald χ^2 (d.f. = 3)			

Robust SE = The standard errors robust to individual-level clustering. ** = $p < 0.05$.

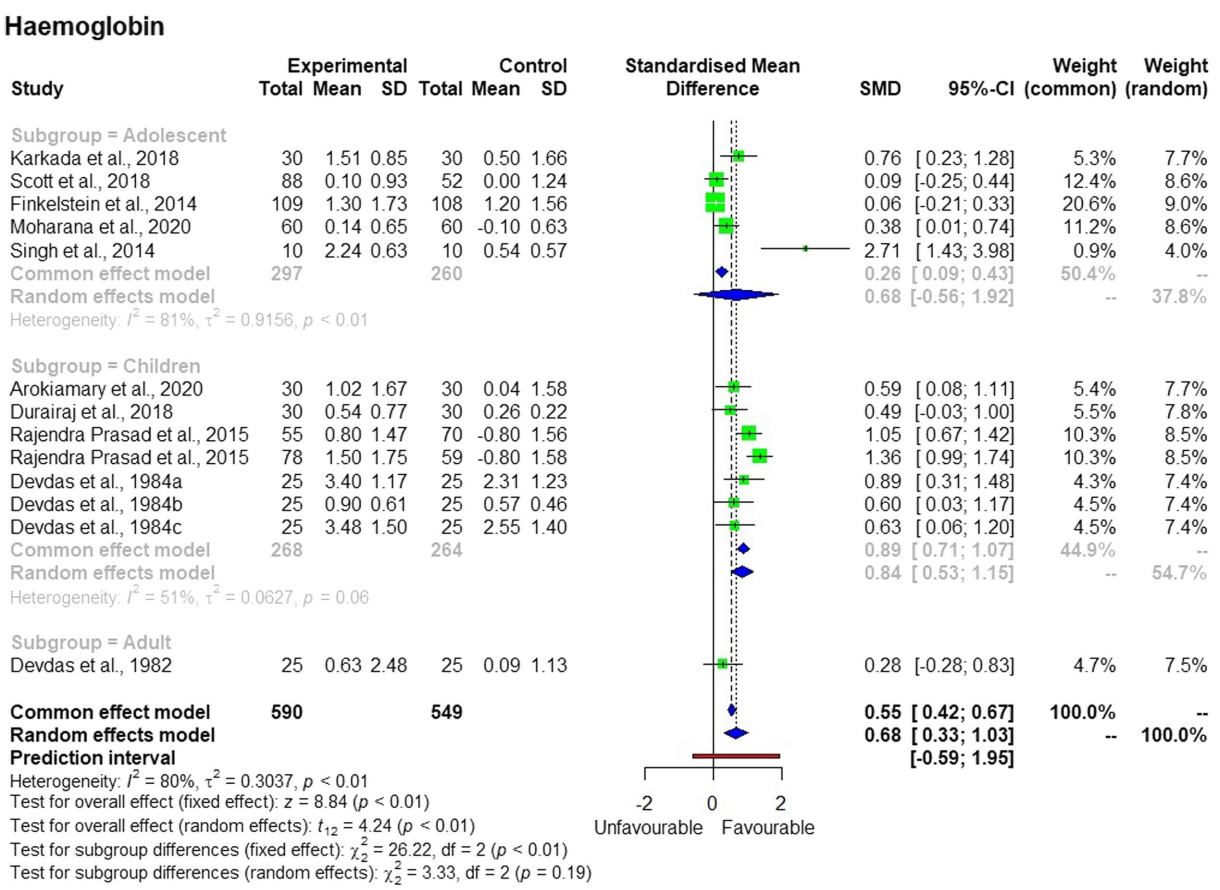

FIGURE 3
Subgroup analysis of the effects of millet consumption on different age groups.

was 9.95 ± 1.07 g/dL, which increased to 11.3 ± 1.09 g/dL, indicating that, overall, individuals with mild anemia saw an improvement to normal levels. In particular, four studies conducted on children reported an increase in hemoglobin levels, demonstrating a shift from mild anemia to a normal status. In the control group, the mean hemoglobin concentration was 10.2 ± 0.99 g/dL before the treatment, which increased to 10.6 ± 1.09 g/dL after the consumption of their regular staple. This also shows that the anemia status in the control group did not change.

The difference between children and adults in the subgroup analysis could be due to the limited number of studies, especially on adults. The low heterogeneity in the children's group could have arisen from three studies that used the same type of millet, the small sample size, the same geographical location, and the same population.

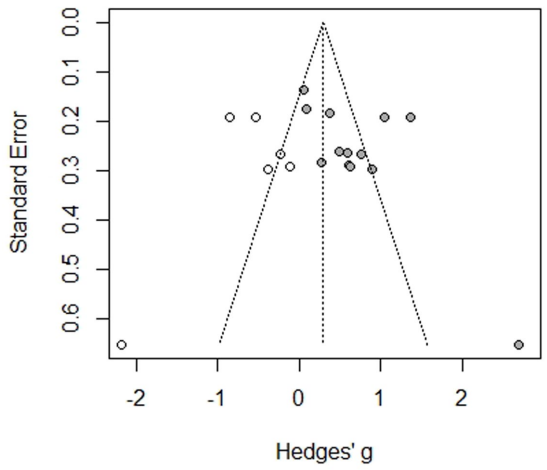

FIGURE 4
Funnel plot of the studies included in the meta-analysis.

The interventions in all the studies reviewed in this study were through controlled trials. However, the studies did not provide information on whether they were randomized in terms of selection and assigning samples. Furthermore, the blinding of the intervention was generally not conducted, which is understandable due to the obvious distinction between millet-based meals and common staple foods in terms of texture and appearance. One study noted the attrition of the participants, which was not justified by the study. The heterogeneity of the studies was high (80%), which may be due to the difference in the age group of participants. Only one study was conducted on adults.

The funnel plot (Figure 4) shows that there is less publication bias, which is evident from the studies that are not scattered in the middle or at the base of the triangle, with the exception of one study. The small sample size in the studies would have made a difference, and therefore, the quality of the obtained evidence was rated as moderate. Although the evidence generated is valuable, it is important to conduct similar studies across various geographical regions with various age groups to strengthen the evidence.

5 Conclusion

This study conducted a meta-analysis of 13 studies involving 1,139 participants in the intervention and control groups. Results show that the consumption of millet-based food had a significant positive effect on blood hemoglobin levels. Yet, it is worth noting that these effects were significant only among the children's group. More robust findings would have been possible if the studies had confirmed the intake of iron in the intervention and control groups.

The limitations of this study include the limited number of studies reviewed in each age group, the limited types of millets tested, and the lack of geographical diversity. Similar studies across diverse geographical regions and sociocultural groups should be conducted to corroborate the evidence of the beneficial effects of millet consumption in addressing anemia.

Data availability statement

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

Author contributions

SA: Conceptualization, Data curation, Formal analysis, Validation, Writing – original draft. TT: Formal analysis, Methodology, Writing – review & editing. DG: Writing – review & editing. JK-P: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. RoB: Data curation, Formal analysis, Validation, Writing – review & editing. NS: Methodology, Writing – review & editing. SU: Writing – review & editing. MV: Methodology, Writing – review & editing. AR: Writing – review & editing. DP: Writing – review & editing. TL: Writing – review & editing. KS: Writing – review & editing. RaB: Writing – review & editing.

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Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1305394/full#supplementary-material

References

1. WHO Global anaemia estimates eds (2021). Available at: https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children (Accessed August 17, 2023).

2. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K. *Indian food composition table*, National Institute of Nutrition; Indian Council of Medical Research: Hyderabad, India. (2017). pp. 1–578.

3. Vaidyanathan G. Healthy diets for people and the planet. *Nature*. (2021) 600:22–5. doi: 10.1038/d41586-021-03565-5

4. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT-lancet commission on healthy diets from sustainable food systems. *Lancet*. (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4

5. Shinde S, Madzorera I, Fawzi WW. Association of iron supplementation and dietary diversity with nutritional status and learning outcomes among adolescents: results from a longitudinal study in Uttar Pradesh and Bihar, India. *J Glob Health*. (2021) 11:04037. doi: 10.7189/jogh.11.04037

6. Saaka M, Galaa SZ. How is dietary diversity related to haematological status of preschool children in Ghana? Food. *Nutr Res*. (2017) 61:1333389. doi: 10.1080/16546628.2017.1333389

7. Kane-Potaka J, Kumar P. "Smart food – food that is good for you, the planet and the farmer," In *State of India's livelihoods report* (2019) (New Delhi: Access Development Services), 71–82. Available at: SOIL-Smart-Foods.pdf (smartfood.org).

8. Anitha S, Tsusaka TW, Botha R, Givens DI, Rajendran A, Parasannanavar DJ, et al. Impact of regular consumption of millets on fasting and post prandial blood glucose level: a systematic review and meta-analysis. *Front Sustain Food Syst*. (2023) 7:1226474. doi: 10.3389/fsufs.2023.1226474

9. Anitha S, Tsusaka TW, Botha R, Kane-Potaka J, Givens DI, Rajendran A, et al. Are millets more effective in managing hyperlipidaemia and obesity than major cereal staples? A systematic review and meta-analysis. *Sustainability*. (2022) 14:6659. doi: 10.3390/su14116659

10. Anitha S, Givens DI, Subramaniam K, Upadhyay S, Kane-Potaka J, Vogtschmidt YD, et al. Can feeding a millet-based diet improve the growth of children?—a systematic review and meta-analysis. *Nutrients*. (2022) 14:225. doi: 10.3390/nu14010225

11. Anitha S, Kane-Potaka J, Botha R, Givens DI, Sulaiman NLB, Upadhyay S, et al. Millets can have a major impact on improving iron status, haemoglobin level and in reducing iron deficiency anaemia—a systematic review and meta-analysis. *Front Nutr*. (2021) 8:725529. doi: 10.3389/fnut.2021.725529

12. Mohar D, Liberati A, Tetzlaff J, Altman DG PRISMA Group. Preferred reporting items for systematic reviews and meta-analysis: the PRISMA statement. *Open Med*. (2009) 3:8. doi: 10.1136/bmj.b2535

13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 88:105906. doi: 10.1371/journal.pmed.1003583

14. Harrer M, Cuijpers P, Furukawa TA, Ebert DD. *Doing meta-analysis with R: a hands-on guide*. (2019). Available at: https://bookdown.org/MathiasHarrer/Doing_Meta_Analysis_in_R/ (Accessed February 28, 2021).

15. Atkinson LZ, Cipriani A. How to carry out a literature search for a systematic review: a practical guide. *BJPsych Adv*. (2018) 24:74–82. doi: 10.1192/bja.2017.3

16. Ryan R, Hill S. *How to GRADE the quality of the evidence? Cochrane consumers and communication group. Version 3.0*. (2016). Available at: http://cccrg.cochrance.org/author-resources (Accessed December 7, 2021).

17. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ. *Cochrane handbook for systematic reviews of interventions version 6.2 (updated February 2021); Welch, V.A., Ed.; Cochrane: London, UK*, (2021). Available at: www.training.cochrane.org/handbook (Accessed April 13, 2021).

18. R Core Team. *R: a language and environment for statistical computing*; R Foundation for Statistical Computing: Vienna, Austria, (2022). Available at: https://www.R-project.org/ (Accessed September 4, 2022).

19. Ahn E, Kang H. Introduction to systematic review and meta-analysis. *Korean J Anaesthesiol*. (2019) 71:103–12. doi: 10.4097/kjae.2018.71.2.103

20. Hak T, Van Rhee HJ, Suurmond R. *How to interpret results of meta-analysis; Erasmus Rotterdam Institute of Management: Rotterdam, the Netherlands*, (2016); pp. 1–2. Available at: https://www.erim.eur.nl/research-support/metaessentials/downloads (Accessed April 26, 2021).

21. Isreal H, Richter RP. A guide to understanding meta-analysis. *J Orthop Sports Phys Ther*. (2011) 41:496–504. doi: 10.2519/jospt.2011.3333

22. Bell A, Fairbrother M, Jones K. Fixed and random effects models: making an informed choice. *Qual Quant*. (2019) 53:1051–74. doi: 10.1007/s11135-018-0802-x

23. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial? *Evid Based Ment Health*. (2019) 22:153–60. doi: 10.1136/ebmental-2019-300117

24. Karkada S, Upadhya S, Upadhya S, Bhat G. Beneficial effect of ragi (finger millet) on haematological parameters, body mass index, and scholastic performance among anemic adolescent high-school girls (AHSG). *Compr Child Adolesc Nurs*. (2018) 42:141–50. doi: 10.1080/24694193.2018.1440031

25. Arokiamary S, Senthilkumar R, Kanchana S. Impact of pearl millet based complementary food on biochemical and cognitive profiles of school children (5-6 year old). *European J Nutr Food Saf*. (2020) 12:24–34. doi: 10.9734/ejnfs/2020/v12i730245

26. Scott SP, Murray-Kolb LE, Wenger MJ, Udipi SA, Ghugre PS, Boy E, et al. Cognitive performance in Indian school going adolescents is positively affected by consumption of iron-biofortified pearl millet: a 6-month randomized controlled efficacy trial. *J Nutr*. (2018) 148:1462–71. doi: 10.1093/jn/nxy113

27. Finkelstein JL, Mehta S, Udipi SA, Ghugre PS, Luna SV, Wenger MJ, et al. A randomized trial of iron-biofortified pearl millet in school children in India. *J Nutr*. (2015) 145:1576–81. doi: 10.3945/jn.114.208009

28. Moharana A, Khosla P, Nayak D, Tripathy P. Effect of finger millet [ragi] ladoo consumption on the level of hemoglobin. *Eur J Mol Clin Med*. (2020) 7:1018–22.

29. Singh TS, Goyal M, Sheth M. Intervention trials with pearl millet-based iron rich ladoo and iron folic acid (IFA) tablets on hemoglobin status of adolescent females in Bikaner city. *Stud Ethno-Med*. (2014) 8:77–82. doi: 10.1080/09735070.2014.11886475

30. Durairaj M, Gurumurthy G, Nachimuthu V, Muniappan K, Balasubramanian S. Dehulled small millets: the promising nutri cereals for improving the nutrition of children. *Matern Child Nutr*. (2019) 15:e12791. doi: 10.1111/mcn.12791

31. Rajendra Prasad MP, Senhur B, Kommi K, Madhari R, Rao MV, Patil JV. Impact of sorghum supplementation on growth and micronutrient status of school going children in southern India–a randomized trial. *Indian J Pediatr*. (2015) 83:9–14. doi: 10.1007/s12098-015-1782-7

32. Devdas RP, Chandrasekhar U, Bhooma N. Nutritional outcomes of a rural diet supplemented with low-cost locally available foods – V impact on pre-schoolers followed over a period of four and a half years. *Indian J Nutr Diet*. (1984) 21:153–64.

33. Devdas RP, Chandrasekhar U, Bhooma N. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – VIII impact on preschool children from a tribal community. *Indian J Nutr Diet*. (1984) 21:315–21.

34. Devdas RP, Chandrasekhar U, Bhooma N. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – VI impact on school children. *Indian J Nutr Diet*. (1984) 21:187–93.

35. Devdas RP, Chandrasekhar U, Bhooma N. Nutritional outcomes of a rural diet supplemented with low cost locally available foods – I impact on expectant women. *Indian J Nutr Diet*. (1982) 19:111–6.

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Association of soluble transferrin receptor/log ferritin index with all-cause and cause-specific mortality: National Health and Nutrition Examination Survey

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Background: Soluble transferrin receptor (sTfR)/log ferritin index (sTfR Index) can be used to assess the entire spectrum of iron status, and is valuable in evaluating iron status in population studies. There is still a lack of evidence on the association between sTfR index and all-cause mortality.

Object: To explore the association between sTfR index and all-cause mortality, as well as mortality due to cardiovascular disease (CVD) and cancer.

Method: Data were from the National Health and Nutrition Examination Survey (NHANES) between 2003 to 2020. Participants aged 16 years and older who had complete data of serum ferritin and sTfR were included. Pregnant individuals or those with ineligible data on death or follow-up were excluded from the analysis. Baseline sTfR index was calculated by baseline sTfR/log (ferritin) and classified as three tertile. We performed the Cox proportional hazard regression to assess the association of sTfR index (both continuous and categorical scale) with all-cause and cause-specific mortality and further assess the non-linear relationship between sTfR index and the outcomes with restricted cubic spline.

Result: In this study, 11,525 participants, a total of 231 (2.0%) all-cause deaths occurred during a median follow-up of 51 months. The risk of all-cause mortality, CVD-related mortality, and cancer-related mortality was higher in participants with highest tertile of sTfR index. After confounding factors adjustment, participants with highest tertile of sTfR index were associated with an increased risk of all-cause mortality (HR: 1.71, 95% CI: 1.14–2.57) as compared with lowest tertile. Additionally, sTfR index per SD increment was associated with a 25% increasing risk of all-cause mortality (HR: 1.25, 95% CI: 1.08–1.45, $p = 0.003$) and a 38% cancer-related mortality (HR: 1.38, 95% CI: 1.07–1.77, $p = 0.018$). These associations remained robust after adjusting for the serum ferritin as well as in various subgroups stratified by age, sex, smoking statue, hypertension, diabetes, and CVD. Spline analysis showed that there is approximately linear relationship between sTfR index with all-cause mortality (p for non-linear = 0.481). Moreover, ferritin was not a predictor of all-cause death after adjustment for confounding factors.

Significance: This cohort study demonstrated a significant association between sTfR index increment and an increased risk of all-cause and cancer-related mortality, independent of ferritin levels.

KEYWORDS

soluble transferrin receptor, sTfR index, all-cause mortality, cardiovascular disease, cancer soluble transferrin receptor, cancer

1 Introduction

The soluble transferrin receptor (sTfR) in serum is derived from the proteolysis of surface receptors on early erythrocytes, especially under conditions of low iron levels (1). It has significant application value in diagnosing iron deficient anemia (IDA) and serves as a sensitive and reliable indicator of functional iron deficiency (2). Ferritin is a cellular storage protein for iron, consisting of 24 subunits and its spherical cavity can store up to 4,500 iron atoms (3). However, the source of circulating ferritin and the pathway through which cells secrete ferritin are mostly unclear (4). Factors that affect serum ferritin expression include acute infection with concurrent inflammation (5), hemophilic lymphohistiocytosis (6) and cellular iron status, etc. Studies show that relying solely on ferritin level may delay diagnosis of combined IDA and anemia of chronic disease (7).

There are several ways to express the ratio of sTfR to serum ferritin (SF) as indicator of iron status (8): the logarithm of the ratio of sTfR to SF, the simple ratio of sTfR to SF, the ratio of sTfR to the logarithm of SF. The logarithm of the ratio of sTfR to SF concentrations, expressed as mg/kg body weight, known as the body iron index, is linearly related to total body iron stores (9, 10). It merely indicates the severity of the iron deficit at the low end of the spectrum and the magnitude of the iron surplus at the high end of the spectrum. The utility of the simple ratio of sTfR to SF concentrations (expressed in μg/L) is limited due to large differences in sTfR assays, and can only be used with data generated by an assay that performs equivalently to the Ramco or Roche assay (11, 12). Finally, the sTfR index, calculated as the ratio of sTfR to the logarithm of SF, was introduced as an indicator to identify persons with depleted iron stores (13, 14).

The sTfR index, which is superior to sTfR, improves detection of IDA, particularly in situations where routine markers provide equivocal results (7). sTfR index is calculated by dividing baseline sTfR by/log (ferritin). The sTfR index can be used to assess the entire spectrum of iron status, ranging from positive iron stores to negative iron balance, and is particularly valuable in evaluating iron status in population studies (15). The latest research indicates that sTfR index has greater diagnostic utility than sTfR in detecting iron deficiency anemia in the presence of chronic inflammation or discriminating iron deficiency without anemia (16, 17). sTfR index can also identify healthy subjects with subclinical iron deficits (18).

Maintaining iron homeostasis is essential for proper cardiac function and plays a crucial role in cancer and other diseases. An increasing body of research indicates that an iron imbalance is a common factor in many cardiovascular disease subtypes (1, 19). The elevated level of sTfR was linked to the prevalence of cardiovascular disease (20). It has also been shown that the expression of sTfR correlates with the occurrence of cancers (21) and tumor differentiation in breast, lung, and lymphoma cancers (22). Additionally, elevated sTfR levels are associated with an increased risk of developing T2DM in obese individuals (23), and represent an additional risk factor in systolic hypertension (24). Furthermore, sTfR index were significantly associated with 28 days mortality in sepsis patients admitted to the ICU (25).

However, the relationship between sTfR index and all-cause mortality, as well as mortality related to cardiovascular disease (CVD) and cancer patients, remains unclear. Therefore, we conducted this retrospective cohort study using data from the National Health and Nutrition Examination Survey 2003–2020 to investigate whether sTfR index can serve as an indicator for predicting the prognosis of diverse populations.

2 Methods

2.1 Study design and population

This is a prospective cohort study of a nationally representative sample of US adults using National Health and Nutrition Examination Survey (NHANES) from 2003–2010. NHANES is a comprehensive nationwide survey conducted by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC). This survey involves home interview and collects data on various aspects including socioeconomic status, medical examinations, dietary habits, health information, as well as physical and physiological measurements of the U.S. population. NHANES is specifically designed to evaluate the health and nutritional status of both adults and children.

Figure 1 depicts the selection process of this study. Briefly, participants aged 16 years and older from the NHANES datasets spanning from 2003 to 2020 were included. We excluded participants with missing ferritin and sTFR data. Also, pregnant individuals and those who were not covered by the NDI were excluded from the analysis.

2.2 Exposure measurement

The primary exposure in this study was the sTfR index, calculated as baseline sTfR/log (ferritin) (3). sTfR index tertile assignment was as follows: tertile 1 (sTfR index ≤0.63), tertile 2 sTfR index (0.64–0.94), and tertile 3 (sTfR index >0.94). Ferritin was used as a measure of iron (Fe) stores, while serum soluble transferrin receptor (sTfR) was served as an indicator of Fe deficiency. The method measurement of Ferritin was performed using immuno-turbidimetry on the Roche/Hitachi 912 clinical analyzer. Latex-bound ferritin antibodies reacted with the antigen in the sample to form an antigen/antibody complex, and turbidimetric measurement was conducted after agglutination. The formed complexes were proportional to the ferritin concentration and were measured at a primary wavelength of 700 nm. The measurement of sTfR was conducted using a particle-enhanced immunoturbidimetric assay with Roche kits on the Cobas® c501 clinical analyzer. Latex particles coated with anti-sTfR antibodies reacted with the antigen in the sample, leading to the formation of an

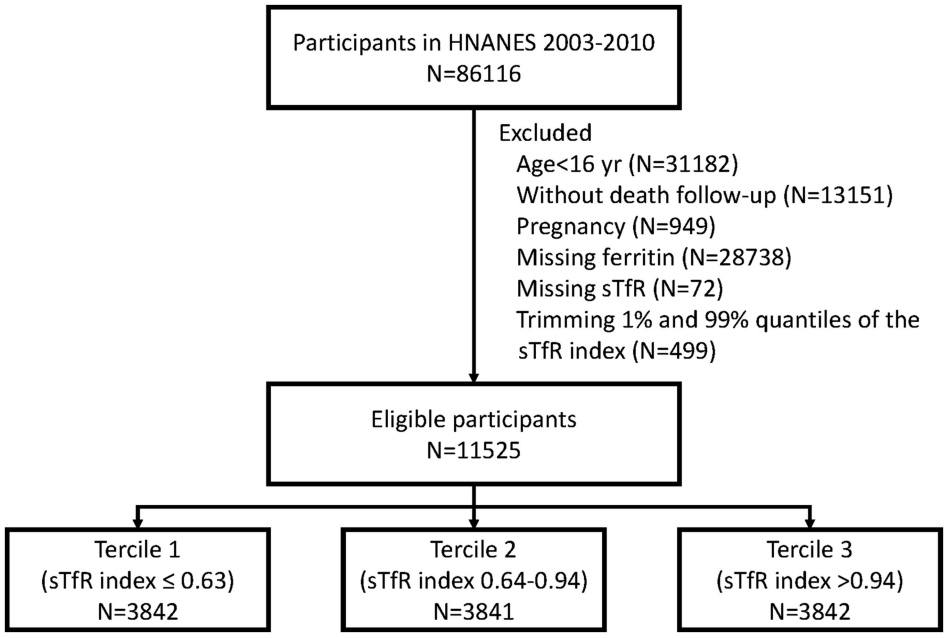

FIGURE 1
Study flowchart. sTFR, soluble transferrin receptor.

antigen/antibody complex. After agglutination, the precipitate was photometrically determined.

2.3 Primary and secondary outcomes

Primary outcome was all-cause mortality; secondary outcomes included CVD-related mortality and cancer-related mortality. Mortality records, including death date and cause, were extracted from the National Death Index (NDI) for participants. Baseline data from NHANES 2003 to 2020 were linked to mortality data from the NDI death certificate records until December 31, 2019 to identify mortality status. Cause-specific deaths were determined using the International Statistical Classification of Diseases, 10th Revision (ICD-10). Cardiovascular death included rheumatic heart disease, hypertensive heart and renal disease, ischemic heart disease, heart failure, and cerebrovascular disease (054-064 in NCHS code). Cancer deaths included all malignant neoplasms (019-043 in NCHS code). The follow-up period was defined from the date of participants' inclusion in the survey until the earliest of the last follow-up time (Dec 31, 2019) or the date of death. Participants not matched with a death record were considered alive through the entire follow-up period.

2.4 Covariates assessment

Baseline characteristics, including socioeconomic conditions (age, sex, ethnicity, and education), behavior, and history of diseases, were obtained through questionnaires. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) and categorized into five levels (<18.5, 18.5–24.9, 25.0–29.9, 30.0–34.9, and ≥35.0) (26). Drinking status was classified as ≥4 drinks per day, non-drinker or missing; smoking status was classified as current smoker, past smoker, non-smoker or missing. Laboratory results included serum albumin, total cholesterol, serum creatinine, serum potassium, hemoglobin, mean corpuscular volume (MCV), and ferritin. A history of hypertension or diabetes was defined based on self-reported physician diagnosis. The history of CVD was determined using self-reported questionnaires. Participants were asked five questions: "Has a doctor or other health professional ever told you that you have congestive heart failure, coronary heart disease, angina pectoris, heart attack, or stroke?." Participants with the answer of "yes" to any question were considered to have CVD.

2.5 Statistical analyses

Baseline characteristics and laboratory measurements were presented as median (IQR) and frequency (%) for continuous and categorical variables. Group comparison was performed using Kruskal–Wallis and chi-square test, as appropriate. We conducted multivariable hazard proportional regression. Kaplan–Meier survival curves were plotted to calculate cumulative mortality using three tertile categories of sTfR index, and compared using the log-rank test. We utilized Cox proportional hazards regression models to assess the associations of sTfR index (both continuous and tertiles) with all-cause, CVD-related, and cancer-related mortality. To adjust for confounding factors, three model as fitted: Model 1 was adjusted for age (continuous), sex, and race/ethnicity; model 2 further adjusted for smoking status, drinking status, and education. In model 3 (primary analysis model), we additionally adjusted for BMI, SBP, DBP, albumin, total cholesterol, serum creatinine, serum potassium, Hemoglobin, MCV, diabetes, hypertension, CVD, and ferritin. The results of Cox regression were reported as hazard ratios (HRs) and their corresponding 95% confidence interval (CIs). Considering the potential non-linear relationship between sTfR index and all-cause

mortality, we further performed a Cox regression with restricted cubic spline. We compared CVD-related and cancer-related mortality using Fine and Gray competing risks regression, with the creation of a cumulative incidence function. Non-cardiovascular and non-cancer mortality during the follow-up period were considered as competing risks, and patients lost to follow-up were censored. Sub-distribution hazard ratios (sHRs) and their corresponding 95% CIs were reported. Furthermore, we estimated the HRs of all-cause mortality, CVD-related mortality, and cancer-related mortality within 1, 5, and 10 years associated with sTfR index to examine the potential impact of sTfR index on short-term and long-term mortality. With respected to the cause-specific mortality in this study, we conducted an additional analysis to detect the associations between sTfR index (per SD increment) and mortality among patients with CVD and cancer.

Additionally, we classified patients into three groups based on the three tertiles of ferritin and examine the association between ferritin and all-cause mortality, CVD-related mortality, and cancer-related mortality using the same confounders adjustment mentioned above. For subgroups analyses, we assessed the association of sTfR index with all-cause mortality stratified by different effect modifiers, including age (≥40 and <40 years), sex, smoking status, hypertension, diabetes, CVD, and cancer. Interaction between TFR and pre-defined effect modifiers was fitted using the product term, and $p < 0.05$ indicated the significant effect modification. Considering the reverse causality, we conducted a sensitivity analysis to assess the sTfR index and study outcomes after excluding participants with baseline CVD or cancer.

Missing value were imputed by multiple imputation under the assumption of missing at random. Additionally, we conducted a sensitivity analysis after eliminating patients with missing values. A *p*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.1.1).

3 Results

3.1 Baseline characteristics of the study population

Overall, 28,738 and 13,151 participants were excluded due to the missing serum ferritin measurement and without death follow-up. Given to the potential selection bias, we further compared the characteristics among participants with and without serum ferritin measurement (Supplementary Table S1) and those with and without death follow-up (Supplementary Table S2). Finally, a total of 11,525 eligible participants were included in this study [median (IQR) age: 38 yr. (27–48); 80.4% were male] (Figure 1). The baseline characteristics of participants across three tertiles of sTfR index are presented in Table 1. The distribution of serum ferritin was showed in Supplementary Figure S1. Participants in tertile 3 (>0.94) of sTfR index were more likely to be younger, female, Non-Hispanic Black, and have a higher BMI.

3.2 Association of sTfR index with all-cause and cause-specific mortality

During a median follow-up of 51 months, 231 (2.0%) all-cause deaths occurred, including 53 (0.5%) CVD deaths and 60 (0.5%) cancer-related deaths. The risk of all-cause mortality, CVD-related mortality, and cancer-related mortality was higher in patients with higher sTfR index (Table 1 and Figure 2). After adjusting for confounders (Table 2), the HR of all-cause and cancer-related mortality associated with each SD increment in sTfR index was 1.25 [95% confidence interval (CI), 1.08–1.45] and 1.38 (1.07–1.77). Compared to participants in the lowest tertile, those in highest tertile of sTfR index had a 71% higher risk of all-cause mortality [hazard ratio (HR), 1.71, 95% CI, 1.14–2.57] after adjustment for all potential covariates (Table 2). A similar trend toward an increased risk of cancer-related mortality was observed. However, no significant association was found between sTfR index and CVD mortality (HR, 1.18; 95% CI, 0.83–1.68). There were approximately linear associations between sTfR index with all-cause mortality (*p* for non-linear association >0.05, Figure 2). That is the risk of all-cause mortality increased linearly as sTfR index increased. Regarding the mortality within 1 year (Supplementary Table S3), only 62 (0.5%) participants died and no significant association was found between the sTfR index and mortality. However, when considering the 10 years mortality, participants in the highest tertile of sTfR index were significantly associated with a higher risk of all-cause mortality compared to those in the lowest tertile (HR, 1.30; 95% CI, 1.11–1.53). Moreover, we performed separate analyses of patients with CVD or cancer at baseline and similar associations between sTfR index and mortality were showed (Table 3).

In different subgroups of the population stratified by baseline characteristics and co-morbidities (Figure 3), the association between higher sTfR index and increased risk of all-cause mortality was consistent. Hence, baseline characteristics including age, sex, smoking status, hypertension, diabetes, CVD, and cancer did not affect the relationship between sTfR index and mortality (all *p* for interaction >0.05).

For additional analysis (Table 4), the HR for all-cause mortality, CVD-related mortality, and cancer-related mortality across three tertiles of ferritin were 1.09 (0.84–1.43), 1.00 (0.55–1.83), and 0.71 (0.44–1.15), respectively. Similarly, participants with iron deficiency (≤15 μg/L) were not significantly associated with study outcome as compared with those with normal levels of ferritin (Table 5), indicating that there was no significant association between ferritin levels and all-cause mortality, CVD-related mortality, and cancer-related mortality. In addition, we further excluded 1,326 patients who diagnosed with CVD or cancer at baseline to preclude the reverse causality, and consistent associations were found between sTfR index and study outcomes (Supplementary Table S4). For missing values, we excluded patients with missing values at baseline characteristics and repeated the analysis. The results of the sensitivity analysis were consistent to the primary analysis (Supplementary Table S5).

4 Discussion

Based on a nationally representative sample of US participants, this study found that sTfR index was independently associated with an increased risk of all-cause death and cancer related death, regardless of ferritin levels. After adjusting for confounding factors, each SD increment in sTfR index was associated with a 25% increasing risk of all-cause death. The association between sTfR index and all-cause mortality remained consistent in different subgroups stratified by age,

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TABLE 1 Baseline characteristics by three tertile of sTfR index.

Characteristics	Overall (*N* = 11,525)	Tertile 1 (*N* = 3,842)	Tertile 2 (*N* = 3,841)	Tertile 3 (*N* = 3,842)	*p*-value
sTfR index	0.27–7.39	0.27–0.63	0.64–0.94	0.94–7.39	—
Demographic					
Age, year	38 [27, 48]	43 [31, 57]	37 [26, 48]	35 [24, 44]	<0.001
Gender, female (%)	9,147 (79.4)	2,445 (63.6)	3,151 (82.0)	3,551 (92.4)	<0.001
Ethnicity (%)					<0.001
Mexican American	2068 (17.9)	678 (17.6)	670 (17.4)	720 (18.7)	
Other Hispanic	1,110 (9.6)	383 (10.0)	347 (9.0)	380 (9.9)	
Non-Hispanic White	4,344 (37.7)	1,600 (41.6)	1,505 (39.2)	1,239 (32.2)	
Non-Hispanic Black	2,562 (22.2)	569 (14.8)	860 (22.4)	1,133 (29.5)	
Other Race	1,441 (12.5)	612 (15.9)	459 (12.0)	370 (9.6)	
Education (%)					<0.001
Less than high school	2,188 (19.0)	781 (20.3)	698 (18.2)	709 (18.5)	
High school or equivalent	2,325 (20.2)	809 (21.1)	756 (19.7)	760 (19.8)	
College or above	5,979 (51.9)	2074 (54.0)	2016 (52.5)	1889 (49.2)	
Missing	1,033 (9.0)	178 (4.6)	371 (9.7)	484 (12.6)	
BMI, kg/m^2					<0.001
<18.5	274 (2.4)	92 (2.4)	97 (2.5)	85 (2.2)	
18.5–24.9	3,440 (29.8)	1,155 (30.1)	1,222 (31.8)	1,063 (27.7)	
25.0–29.9	3,243 (28.1)	1,196 (31.1)	1,047 (27.3)	1,000 (26.0)	
30.0–34.9	2,177 (18.9)	739 (19.2)	704 (18.3)	734 (19.1)	
≥35.0	2,250 (19.5)	614 (16.0)	726 (18.9)	910 (23.7)	
Missing	141 (1.2)	46 (1.2)	45 (1.2)	50 (1.3)	
Smoking status (%)					<0.001
Never/past	1872 (16.2)	767 (20.0)	622 (16.2)	483 (12.6)	
Current	2,199 (19.1)	883 (23.0)	687 (17.9)	629 (16.4)	
Missing	7,454 (64.7)	2,192 (57.1)	2,532 (65.9)	2,730 (71.1)	
Drinking status (%)					<0.001
Nondrinker	2,371 (20.6)	793 (20.6)	819 (21.3)	759 (19.8)	
Drinker	4,450 (38.6)	1778 (46.3)	1,434 (37.3)	1,238 (32.2)	
Missing	4,704 (40.8)	1,271 (33.1)	1,588 (41.3)	1845 (48.0)	
Laboratory					
sTfR, mg/L	3.10 [2.57, 3.94]	2.41 [2.11, 2.73]	3.10 [2.80, 3.50]	4.30 [3.70, 5.26]	<0.001
Ferritin, μg/L	57 [28, 116]	124 [75.10, 210]	58.60 [38, 95]	22 [12, 36.90]	<0.001
Albumin, g/dL	4.10 [3.90, 4.40]	4.20 [3.90, 4.40]	4.20 [3.90, 4.40]	4.10 [3.90, 4.30]	<0.001
Total cholesterol, mg/dL	184 [160, 211]	187 [161, 214]	183 [160, 211.50]	182 [159, 208]	<0.001
Serum creatinine, μmoI/L	67.18 [58.34, 78.68]	69.84 [59.23, 82.21]	67.18 [58.34, 79.56]	63.65 [56.58, 73.37]	<0.001
Serum potassium, mmol/L	3.90 [3.73, 4.20]	4 [3.80, 4.20]	3.90 [3.71, 4.17]	3.90 [3.70, 4.10]	<0.001
Hemoglobin, mg/dL	13.60 [12.80, 14.40]	14.10 [13.30, 15]	13.70 [13, 14.43]	13 [12.20, 13.80]	<0.001
MCV, f/L	88.70 [85.10, 91.90]	90.10 [87.30, 92.90]	89.20 [86.10, 92.10]	86.20 [81.80, 89.90]	<0.001
Comorbidity (%)					
Diabetes	1,011 (8.8)	377 (9.8)	322 (8.4)	312 (8.1)	0.019
Hypertension	2,798 (24.3)	1,043 (27.1)	906 (23.6)	849 (22.1)	<0.001
CVD	743 (6.4)	263 (6.8)	256 (6.7)	224 (5.8)	0.154
Cancer	728 (6.3)	277 (7.2)	265 (6.9)	186 (4.8)	<0.001

(Continued)

TABLE 1 (Continued)

Characteristics	Overall (*N* = 11,525)	Tertile 1 (*N* = 3,842)	Tertile 2 (*N* = 3,841)	Tertile 3 (*N* = 3,842)	*p*-value
Outcomes					
All-cause mortality (%)	231 (2.0)	70 (1.8)	65 (1.7)	96 (2.5)	0.026
CVD mortality (%)	53 (0.5)	15 (0.4)	14 (0.4)	24 (0.6)	0.744
Cancer mortality (%)	60 (0.5)	17 (0.4)	17 (0.4)	26 (0.7)	0.002
Follow-up, months	51 [27, 145]	33 [23, 116]	55 [26, 149]	123 [35, 163]	<0.001

CVD, cardiovascular diseases; MCV, mean corpuscular volume.

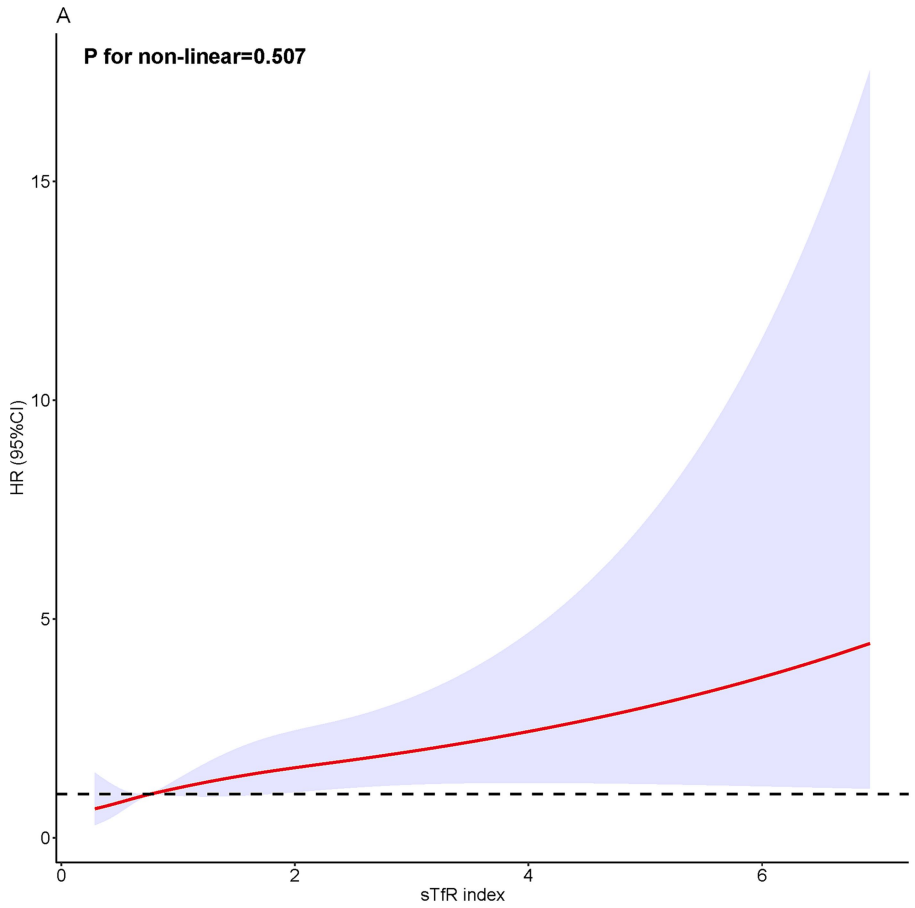

FIGURE 2
Restricted cubic spline analysis.

sex, smoking status, hypertension, diabetes, and CVD. However, ferritin was not a predictor of all-cause death after adjusting for confounding factors. This study might provide additional insights into the implications of the sTfR index in assessing mortality risk and future research directions of investigating the underlying pathways linking chronic inflammation, functional iron deficiency, and adverse health outcomes, which could provide valuable insights into novel therapeutic targets and interventions.

A growing body of evidence suggests that iron imbalance plays a crucial role in various subtypes of cardiovascular disease, including atherosclerosis, drug-induced heart failure, myocardial ischaemia-reperfusion injury, sepsis-induced cardiomyopathy, arrhythmia and diabetic cardiomyopathy (1, 19). Some studies have demonstrated that increased sTfR levels are associated with higher mortality in patients with heart failure (19, 27–30). High serum sTfR accurately reflect depleted iron stores in the bone marrow of heart failure patients and identify those at higher risk of 3 years mortality (29). However, in this study, after adjusting for variables, there was no significant correlation between sTfR index and CVD-related mortality.

The main reasons for this lack of significant correlation may be attributed to several factors. First, the number of CVD-related deaths in this cohort was relatively small, which could potentially affect statistical efficiency. Second, the complexity of the CVD population in this study, which includes congestive heart failure,

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TABLE 2 The association between sTfR index and all-cause, CVD, and cancer mortality.

	All-cause mortality			CVD-related mortality			Cancer-related mortality		
	No. event	HR (95% CI)	*p*-value	No. event	sHR (95% CI)[a]	*p*-value	No. event	sHR (95% CI)[a]	*p*-value
Model 1									
Per SD increment	231	1.19 (1.07–1.32)	0.001	53	1.17 (0.92–1.49)	0.21	60	1.22 (1.01–1.48)	0.043
Tertile 1	70	1.00 (Ref.)	—	15	1.00 (Ref.)	—	17	1.00 (Ref.)	—
Tertile 2	65	0.97 (0.69–1.37)	0.870	14	1.10 (0.53–2.30)	0.800	17	0.96 (0.49–1.89)	0.906
Tertile 3	96	1.62 (1.17–2.25)	0.004	24	2.46 (1.25–4.83)	0.012	26	1.60 (0.84–3.03)	0.157
Model 2									
Per SD increment	231	1.20 (1.07–1.34)	0.002	53	1.06 (0.79–1.41)	0.721	60	1.25 (1.03–1.51)	0.032
Tertile 1	70	1.00 (Ref.)	—	15	1.00 (Ref.)	—	17	1.00 (Ref.)	—
Tertile 2	65	0.96 (0.68–1.36)	0.816	14	1.02 (0.48–2.16)	0.966	17	1.00 (0.50–2.02)	0.992
Tertile 3	96	1.55 (1.11–2.16)	0.010	24	1.73 (0.86–3.51)	0.139	26	1.80 (0.93–3.48)	0.090
Model 3									
Per SD increment	231	1.25 (1.08–1.45)	0.003	53	1.18 (0.83–1.68)	0.375	60	1.38 (1.07–1.77)	0.018
Tertile 1	70	1.00 (Ref.)	—	15	1.00 (Ref.)	—	17	1.00 (Ref.)	—
Tertile 2	65	1.08 (0.74–1.57)	0.689	14	1.19 (0.54–2.64)	0.674	17	1.19 (0.56–2.53)	0.654
Tertile 3	96	1.71 (1.14–2.57)	0.011	24	2.48 (1.05–5.87)	0.053	26	2.29 (1.02–5.14)	0.053

Model 1: unadjusted; model 2: adjusted for age, sex, ethnicity, smoking status, drinking status, and education; and model 3: further adjusted for BMI, SBP, DBP, albumin, total cholesterol, serum creatinine, serum potassium, hemoglobin, MCV, diabetes, hypertension, CVD, cancer, and ferritin. [a]Sub-distribution hazard ratio accounting for the competing event.

TABLE 3 The association between sTfR index and all-cause mortality among patients with CVD and cancer.

	All-cause mortality		CVD-related mortality		Cancer-related mortality	
	HR (95%CI)	*p*-value	sHR (95% CI)[a]	*p*-value	sHR (95% CI)[a]	*p*-value
Patients with CVD (N = 743)						
Model 1	1.20 (0.97–1.48)	0.103	1.27 (0.96–1.70)	0.113	1.35 (0.87–2.10)	0.272
Model 2	1.23 (0.95–1.58)	0.126	1.37 (0.92–2.03)	0.498	1.74 (0.93–3.25)	0.475
Model 3	1.27 (0.91–1.77)	0.168	1.53 (0.96–2.42)	0.468	1.41 (0.77–2.56)	0.578
Patients with cancer (N = 728)						
Model 1	1.11 (0.83–1.49)	0.481	1.19 (0.73–1.95)	0.515	1.01 (0.54–1.87)	0.983
Model 2	1.00 (0.67–1.49)	0.994	0.48 (0.09–2.44)	0.631	1.32 (0.70–2.47)	0.638
Model 3	1.12 (0.67–1.88)	0.683	0.38 (0.07–2.13)	0.580	1.46 (0.48–4.48)	0.697

Model 1: unadjusted; model 2: adjusted for age, sex, ethnicity, smoking status, drinking status, and education; and model 3: further adjusted for BMI, SBP, DBP, albumin, total cholesterol, serum creatinine, serum potassium, hemoglobin, MCV, diabetes, hypertension, CVD, cancer, and ferritin. [a]Sub-distribution hazard ratio accounting for the competing event.

coronary heart disease, angina pectoris, heart attack, or stroke, might have implications on the results. However, the mortality rate of heart failure patients could not be separately obtained, which could lead to differences in the outcome.

Iron (Fe) has been indicated to play a critical role in leukemia cell growth (31). The expression of soluble transferrin receptor (sTfR) has also been identified in many malignant tumours (2). In lung cancer, lymphoma, and breast cancer, the expression of sTfR has been shown to correlate with tumor differentiation, suggesting a potential prognostic value (22). Consistent with these findings, our study also demonstrated that each SD increment in sTfR index was associated with a 38% increased risk of cancer-related death.

Several reasons may explain why sTfR index increment, independent of ferritin levels, is associated with an increased risk of all-cause death. First, sTfR index is valuable in diagnosing iron-deficiency anemia (1), and anemia itself reflects a person's overall health status and disease severity (32). Second, iron deficiency is a health-related condition in which iron availability is insufficient to meet the body's needs and which also can be present without anemia (33–35). Iron deficiency in patients with chronic heart failure can worsen the underlying condition and negatively impact clinical outcomes and quality of life (33, 34). Iron deficiency is also common in patients with idiopathic pulmonary arterial hypertension and is associated with disease severity and poor clinical outcome (35). Third, a confirmed relationship exists between reduced iron concentration and the occurrence of frailty syndrome (36). Patients with diagnosed frailty syndrome represent a unique group of individuals with chronic illnesses. In the classic definition, frailty syndrome includes parameters such as reduced muscle strength, subjective fatigue, unintentional weight loss, slow gait, and low physical activity, which increase the

Subgroups	Event	N	adjusted HR (95%CI)	P value	P for interaction
Age < 40					
Tercile 1	9	1649	1.00(Ref.)	-	
Tercile 2	15	2114	1.27(0.55-2.94)	0.569	0.756
Tercile 3	21	2350	1.56(0.7-3.5)	0.280	
Age ≥ 40					
Tercile 1	61	2193	1.00(Ref.)	-	
Tercile 2	50	1727	1.04(0.69-1.57)	0.837	
Tercile 3	75	1492	1.65(1.07-2.54)	0.024	
Sex, Male					
Tercile 1	26	1397	1.00(Ref.)	-	0.436
Tercile 2	21	690	1.46(0.79-2.69)	0.225	
Tercile 3	17	291	1.79(0.89-3.61)	0.105	
Sex, Female					
Tercile 1	44	2445	1.00(Ref.)	-	
Tercile 2	44	3151	0.92(0.59-1.42)	0.705	
Tercile 3	79	3551	1.56(1.01-2.4)	0.044	
Smoke, Never					
Tercile 1	22	767	1.00(Ref.)	-	0.704
Tercile 2	15	622	0.71(0.36-1.41)	0.332	
Tercile 3	21	483	1.34(0.68-2.61)	0.398	
Smoke, Current					
Tercile 1	25	883	1.00(Ref.)	-	
Tercile 2	25	687	1.38(0.78-2.46)	0.270	
Tercile 3	33	629	1.80(1.01-3.22)	0.048	
Drink, Never					
Tercile 1	17	793	1.00(Ref.)	-	0.678
Tercile 2	11	819	0.67(0.31-1.44)	0.302	
Tercile 3	17	759	1.27(0.62-2.61)	0.509	
Drink, Current					
Tercile 1	25	1778	1.00(Ref.)	-	
Tercile 2	23	1434	1.31(0.72-2.36)	0.375	
Tercile 3	25	1238	1.65(0.89-3.05)	0.113	
Without Hypertension					
Tercile 1	34	2799	1.00(Ref.)	-	0.977
Tercile 2	33	2935	1.02(0.62-1.68)	0.939	
Tercile 3	48	2993	1.62(0.99-2.67)	0.056	
Hypertension					
Tercile 1	36	1043	1.00(Ref.)	-	
Tercile 2	32	906	1.11(0.67-1.86)	0.677	
Tercile 3	48	849	1.68(0.99-2.85)	0.057	
Without diabetes					
Tercile 1	56	3465	1.00(Ref.)	-	0.760
Tercile 2	48	3519	1.01(0.67-1.52)	0.976	
Tercile 3	70	3530	1.56(1.02-2.41)	0.042	
Diabetes					
Tercile 1	14	377	1.00(Ref.)	-	
Tercile 2	17	322	1.31(0.63-2.74)	0.469	
Tercile 3	26	312	2.02(0.97-4.19)	0.060	
Without CVD					
Tercile 1	56	3579	1.00(Ref.)	-	0.663
Tercile 2	48	3585	1.00(0.66-1.52)	0.989	
Tercile 3	71	3618	1.54(1.00-2.37)	0.050	
CVD					
Tercile 1	14	263	1.00(Ref.)	-	
Tercile 2	17	256	1.32(0.64-2.75)	0.454	
Tercile 3	25	224	2.11(1.02-4.33)	0.043	
Without cancer					
Tercile 1	54	3565	1.00(Ref.)	-	0.347
Tercile 2	51	3576	1.09(0.72-1.65)	0.687	
Tercile 3	85	3565	1.84(1.19-2.85)	0.006	
Cancer					
Tercile 1	16	277	1.00(Ref.)	-	
Tercile 2	14	265	0.98(0.47-2.05)	0.953	
Tercile 3	11	186	1.02(0.45-2.29)	0.968	

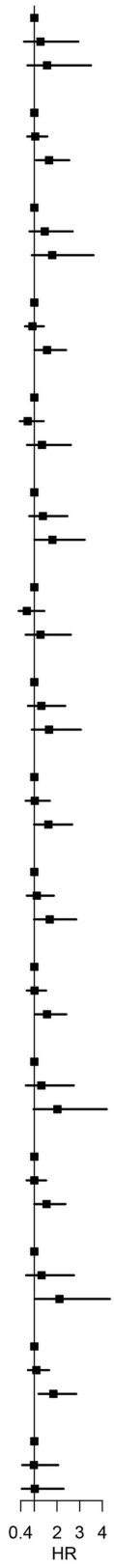

FIGURE 3
Subgroup analysis. HR was adjusted for age, sex, ethnicity, smoking status, drinking status, education, BMI, SBP, DBP, albumin, total cholesterol, serum creatinine, serum potassium, Hemoglobin, MCV, diabetes, hypertension, CVD, and ferritin.

TABLE 4 The association between three tertiles of ferritin and all-cause, CVD, and cancer mortality.

	All-cause mortality			CVD-related mortality			Cancer-related mortality		
	No. event	HR (95% CI)	*p*-value	No. event	sHR (95% CI)[a]	*p*-value	No. event	sHR (95% CI)[a]	*p*-value
Model 1									
Per SD increment	231	1.02 (0.88–1.18)	0.793	53	1.04 (0.77–1.41)	0.804	60	0.94 (0.71–1.24)	0.667
Tertile 1	53	1.00 (Ref.)	—	10	1.00 (Ref.)	—	17	1.00 (Ref.)	—
Tertile 2	78	1.24 (0.87–1.77)	0.228	19	1.36 (0.63–2.97)	0.438	16	0.83 (0.42–1.65)	0.601
Tertile 3	100	1.29 (0.89–1.87)	0.173	24	1.09 (0.49–2.44)	0.839	27	1.27 (0.65–2.49)	0.482
Model 2									
Per SD increment	231	1 (0.87–1.16)	0.961	53	1.09 (0.8–1.49)	0.581	60	0.89 (0.68–1.18)	0.439
Tertile 1	53	1.00 (Ref.)	—	10	1.00 (Ref.)	—	17	1.00 (Ref.)	—
Tertile 2	78	1.14 (0.8–1.63)	0.477	19	1.24 (0.56–2.74)	0.595	16	0.82 (0.41–1.64)	0.574
Tertile 3	100	1.2 (0.83–1.73)	0.334	24	1.15 (0.52–2.57)	0.727	27	1.1 (0.56–2.17)	0.781
Model 3									
Per SD increment	231	1.09 (0.84–1.43)	0.519	53	1.00 (0.55–1.83)	0.989	60	0.71 (0.44–1.15)	0.178
Tertile 1	53	1.00 (Ref.)	—	10	1.00 (Ref.)	—	17	1.00 (Ref.)	—
Tertile 2	78	1.32 (0.91–1.92)	0.15	19	1.19 (0.52–2.70)	0.681	16	0.9 (0.43–1.87)	0.781
Tertile 3	100	1.55 (0.96–2.51)	0.077	24	0.94 (0.33–2.65)	0.907	27	1.33 (0.54–3.32)	0.540

Model 1: unadjusted; model 2: adjusted for age, sex, ethnicity, smoking status, drinking status, and education; and model 3: further adjusted for BMI, SBP, DBP, albumin, total cholesterol, serum creatinine, serum potassium, Hemoglobin, MCV, diabetes, hypertension, CVD, cancer, and ferritin. [a]Sub-distribution hazard ratio accounting for the competing event.

TABLE 5 The association between iron deficiency and all-cause, CVD, and cancer mortality.[a]

	All-cause mortality		CVD-related mortality		Cancer-related mortality	
	HR (95% CI)	*p*-value	sHR (95% CI)[b]	*p*-value	sHR (95% CI)[b]	*p*-value
Model 1	1.20 (0.77–1.85)	0.424	0.83 (0.25–2.74)	0.758	1.71 (0.82–3.56)	0.16
Model 2	1.24 (0.80–1.92)	0.341	0.86 (0.26–2.84)	0.801	1.74 (0.83–3.65)	0.151
Model 3	1.16 (0.74–1.82)	0.528	0.80 (0.24–2.74)	0.731	1.69 (0.80–3.57)	0.179

Model 1: unadjusted; model 2: adjusted for age, sex, ethnicity, smoking status, drinking status, and education; and model 3: further adjusted for BMI, SBP, DBP, albumin, total cholesterol, serum creatinine, serum potassium, Hemoglobin, MCV, diabetes, hypertension, CVD, and cancer. [a]Iron deficiency was defined as serum ferritin ≤15 μg/L.
[b]Sub-distribution hazard ratio accounting for the competing event.

incidence of adverse events, such as falls, hospitalizations and even death (36). Moreover, iron deficiency commonly co-occurs with depressive symptoms in older individuals (21). Lastly, elevated sTfR levels are characteristic of functional iron deficiency, a condition defined by tissue iron deficiency despite adequate iron stores (2).

Furthermore, sTfR may have other functions beyond detecting iron deficiency that merit further investigation. First, apart from erythrocytes, activated lymphocytes also release a soluble form of the human transferrin receptor *in vitro* (37). Second, iron deficiency frequently co-occurs with chronic inflammatory diseases (34). The sTfR has been found to significantly and positively correlate with CRP concentration (36) and antioxidant status, independently of covariates such as serum ferritin and hepcidin (38). Mild elevation of sTfR levels in multiple sclerosis patients may indicate active inflammation with ongoing oxidative damage that is not detectable through history or examination (39). Third, the frequency of the G allele at the position 210 of the transferrin receptor gene was significantly higher in type 2 diabetes patients (40). The sTfR levels could be spuriously elevated in subjects with insulin resistance (41). Both insulin sensitivity and glucose tolerance status are significantly associated with serum sTfR concentrations, with insulin sensitivity mainly predicts circulating sTfR in subjects with normal glucose tolerance (NGT). The implications of the interrelationships between iron and glucose metabolism warrant further investigation (42). Finally, patients with *H. pylori* infection showed higher sTfR concentration and higher sTfR index levels (43).

Several limitations of this study should be noted in this study. First, like all retrospective studies, there may be other potential and unknown confounders that were not considered. However, we made efforts to adjust for as many confounders as possible and achieved good balance in the PSM cohorts. Second, given the established link between inflammation, iron metabolism, and mortality outcomes, it may not be fully considered that inflammation markers were not included as covariates in the analysis. Third, the relatively small number of CVD-related and cancer-related deaths in the cohort could potentially affect statistical efficiency to some extent. Fourth, as the data we analyzed was obtained from an observational database, the results reported in our study need further validation through additional randomized trials. Fifth, although the median follow-up was 51 months, the shortest follow-up duration is less than 1 year, therefore, the accuracy of the relationship between sTfR index and death needs to be interpreted with caution.

In conclusion, our study revealed that higher sTfR index was significantly and linearly associated with higher risks of all-cause and cancer-related mortality. Adding sTfR index to assessments of overall health may identify more individuals at risk for mortality and thus has the potential to improve decisions to implement preventative or treatment approaches.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the NHANES Research Ethics Review Committee granted approval for the NHANES research protocols for 2003–2020, with all participants providing written informed consent. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YY: Conceptualization, Data curation, Investigation, Writing – original draft. DL: Formal analysis, Methodology, Software, Writing – original draft. ZZ: Funding acquisition, Project administration, Resources, Visualization, Writing – review & editing. LT: Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1275522/full#supplementary-material

References

1. Speeckaert MM, Speeckaert R, Delanghe JR. Biological and clinical aspects of soluble transferrin receptor. *Crit Rev Clin Lab Sci*. (2010) 47:213–28. doi: 10.3109/10408363.2010.550461

2. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. *Clin Chim Acta*. (2003) 329:9–22. doi: 10.1016/s0009-8981(03)00005-6

3. Arosio P, Levi S. Cytosolic and mitochondrial ferritins in the regulation of cellular iron homeostasis and oxidative damage. *Biochim Biophys Acta*. (2010) 1800:783–92. doi: 10.1016/j.bbagen.2010.02.005

4. Truman-Rosentsvit M, Berenbaum D, Spektor L, Cohen LA, Belizowsky-Moshe S, Lifshitz L, et al. Ferritin is secreted via 2 distinct nonclassical vesicular pathways. *Blood*. (2018) 131:342–52. doi: 10.1182/blood-2017-02-768580

5. Annous Y, Manning S, Khoujah D. Ferritin, fever, and frequent visits: hyperferritinemic syndromes in the emergency department. *Am J Emerg Med*. (2021) 48:249–54. doi: 10.1016/j.ajem.2021.04.088

6. Lehmberg K, McClain KL, Janka GE, Allen CE. Determination of an appropriate cut-off value for ferritin in the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. (2014) 61:2101–3. doi: 10.1002/pbc.25058

7. Skikne BS, Punnonen K, Caldron PH, Bennett MT, Rehu M, Gasior GH, et al. Improved differential diagnosis of anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the sTfR/log ferritin index. *Am J Hematol*. (2011) 86:923–7. doi: 10.1002/ajh.22108

8. Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. *Am J Clin Nutr*. (2017) 106:1606s–14s. doi: 10.3945/ajcn.117.155887

9. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*. (1990) 75:1870–6. doi: 10.1182/blood.V75.9.1870.1870

10. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. *Blood*. (2003) 101:3359–63. doi: 10.1182/blood-2002-10-3071

11. Ronnenberg AG, Wood RJ, Wang X, Xing H, Chen C, Chen D, et al. Preconception hemoglobin and ferritin concentrations are associated with pregnancy outcome in a prospective cohort of Chinese women. *J Nutr*. (2004) 134:2586–91. doi: 10.1093/jn/134.10.2586

12. Grant FK, Martorell R, Flores-Ayala R, Cole CR, Ruth LJ, Ramakrishnan U, et al. Comparison of indicators of iron deficiency in Kenyan children. *Am J Clin Nutr*. (2012) 95:1231–7. doi: 10.3945/ajcn.111.029900

13. Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood*. (1997) 89:1052–7. doi: 10.1182/blood.V89.3.1052

14. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. (2005) 352:1011–23. doi: 10.1056/NEJMra041809

15. Skikne BS. Serum transferrin receptor. *Am J Hematol*. (2008) 83:872–5. doi: 10.1002/ajh.21279

16. Oustamanolakis P, Koutroubakis IE. Soluble transferrin receptor-ferritin index is the most efficient marker for the diagnosis of iron deficiency anemia in patients with IBD. *Inflamm Bowel Dis*. (2011) 17:E158–9. doi: 10.1002/ibd.21881

17. Krawiec P, Pac-Kożuchowska E. Soluble transferrin receptor and soluble transferrin receptor/log ferritin index in diagnosis of iron deficiency anemia in pediatric inflammatory bowel disease. *Dig Liver Dis*. (2019) 51:352–7. doi: 10.1016/j.dld.2018.11.012

18. Suominen P, Punnonen K, Rajamäki A, Irjala K. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. *Blood*. (1998) 92:2934–9. doi: 10.1182/blood.V92.8.2934.420k07_2934_2939

19. Fang X, Ardehali H, Min J, Wang F. The molecular and metabolic landscape of iron and ferroptosis in cardiovascular disease. *Nat Rev Cardiol*. (2022) 20:7–23. doi: 10.1038/s41569-022-00735-4

20. Zhu S, Liu C, Zhao C, Chen G, Meng S, Hong M, et al. Increased serum soluble transferrin receptor levels were associated with high prevalence of cardiovascular diseases: insights from the National Health and Nutrition Examination Survey 2017–2018. *Front Cell Dev Biol*. (2022) 10:874846. doi: 10.3389/fcell.2022.874846

21. Zhang Y, Xue N, Jia W, Chen X, Chen X, Li H, et al. Associations between serum soluble transferrin receptor and the prevalence of cancers. *Front Oncol*. (2022) 12:1039930. doi: 10.3389/fonc.2022.1039930

22. Dowlati A, Loo M, Bury T, Fillet G, Beguin Y. Soluble and cell-associated transferrin receptor in lung cancer. *Br J Cancer*. (1997) 75:1802–6. doi: 10.1038/bjc.1997.307

23. Fernández-Cao JC, Arija V, Aranda N, Basora J, Diez-Espino J, Estruch R, et al. Soluble transferrin receptor and risk of type 2 diabetes in the obese and nonobese. *Eur J Clin Investig*. (2017) 47:221–30. doi: 10.1111/eci.12725

24. Wang H, Qi Q, Song S, Zhang D, Feng L. Association between soluble transferrin receptor and systolic hypertension in adults: National Health and Nutrition Examination Survey (2007–2010 and 2015–2018). *Front Cardiovasc Med*. (2022) 9:1029714. doi: 10.3389/fcvm.2022.1029714

25. Jiang Y, Jiang FQ, Kong F, An MM, Jin BB, Cao D, et al. Inflammatory anemia-associated parameters are related to 28-day mortality in patients with sepsis admitted to the ICU: a preliminary observational study. *Ann Intensive Care*. (2019) 9:67. doi: 10.1186/s13613-019-0542-7

26. Gao M, Piernas C, Astbury NM, Hippisley-Cox J, O'Rahilly S, Aveyard P, et al. Associations between body-mass index and COVID-19 severity in 6·9 million people in England: a prospective, community-based, cohort study. *Lancet Diabetes Endocrinol*. (2021) 9:350–9. doi: 10.1016/s2213-8587(21)00089-9

27. Dhaliwal S, Kalogeropoulos AP. Markers of iron metabolism and outcomes in patients with heart failure: a systematic review. *Int J Mol Sci*. (2023) 24:5645. doi: 10.3390/ijms24065645

28. Grammer TB, Scharnagl H, Dressel A, Kleber ME, Silbernagel G, Pilz S, et al. Iron metabolism, hepcidin, and mortality (the Ludwigshafen risk and cardiovascular health study). *Clin Chem*. (2019) 65:849–61. doi: 10.1373/clinchem.2018.297242

29. Sierpinski R, Josiak K, Suchocki T, Wojtas-Polc K, Mazur G, Butrym A, et al. High soluble transferrin receptor in patients with heart failure: a measure of iron deficiency and a strong predictor of mortality. *Eur J Heart Fail*. (2021) 23:919–32. doi: 10.1002/ejhf.2036

30. Weidmann H, Bannasch JH, Waldeyer C, Shrivastava A, Appelbaum S, Ojeda-Echevarria FM, et al. Iron metabolism contributes to prognosis in coronary artery disease: prognostic value of the soluble transferrin receptor within the AtheroGgene study. *J Am Heart Assoc*. (2020) 9:e015480. doi: 10.1161/jaha.119.015480

31. Taetle R, Rhyner K, Castagnola J, To D, Mendelsohn J. Role of transferrin, Fe, and transferrin receptors in myeloid leukemia cell growth. Studies with an antitransferrin receptor monoclonal antibody. *J Clin Invest*. (1985) 75:1061–7. doi: 10.1172/jci111768

32. Corazza F, Beguin Y, Bergmann P, André M, Ferster A, Devalck C, et al. Anemia in children with cancer is associated with decreased erythropoietic activity and not with inadequate erythropoietin production. *Blood*. (1998) 92:1793–8. doi: 10.1182/blood.V92.5.1793

33. Lam CSP, Doehner W, Comin-Colet J. Iron deficiency in chronic heart failure: case-based practical guidance. *ESC Heart Fail*. (2018) 5:764–71. doi: 10.1002/ehf2.12333

34. Cappellini MD, Comin-Colet J, de Francisco A, Dignass A, Doehner W, Lam CS, et al. Iron deficiency across chronic inflammatory conditions: international expert opinion on definition, diagnosis, and management. *Am J Hematol*. (2017) 92:1068–78. doi: 10.1002/ajh.24820

35. Rhodes CJ, Howard LS, Busbridge M, Ashby D, Kondili E, Gibbs JSR, et al. Iron deficiency and raised hepcidin in idiopathic pulmonary arterial hypertension: clinical prevalence, outcomes, and mechanistic insights. *J Am Coll Cardiol*. (2011) 58:300–9. doi: 10.1016/j.jacc.2011.02.057

36. Zawadzki B, Mazur G, Butrym A. Iron dysregulation and frailty syndrome. *J Clin Med*. (2021) 10:5596. doi: 10.3390/jcm10235596

37. Woith W, Nüsslein I, Antoni C, Dejica DI, Winkler TH, Herrmann M, et al. A soluble form of the human transferrin receptor is released by activated lymphocytes *in vitro*. *Clin Exp Immunol*. (1993) 92:537–42. doi: 10.1111/j.1365-2249.1993.tb03434.x

38. Suárez-Ortegón MF, Arbeláez A, Moreno-Navarrete JM, Ortega-Ávila JG, Mosquera M, Fernández-Real JM. Soluble transferrin receptor, antioxidant status and cardiometabolic risk in apparently healthy individuals. *Antioxidants*. (2022) 12. doi: 10.3390/antiox12010019

39. Sfagos C, Makis AC, Chaidos A, Hatzimichael EC, Dalamaga A, Kosma K, et al. Serum ferritin, transferrin and soluble transferrin receptor levels in multiple sclerosis patients. *Mult Scler*. (2005) 11:272–5. doi: 10.1191/1352458505ms1171oa

40. Fernández-Real JM, Mercader JM, Ortega FJ, Moreno-Navarrete JM, López-Romero P, Ricart W. Transferrin receptor-1 gene polymorphisms are associated with type 2 diabetes. *Eur J Clin Investig*. (2010) 40:600–7. doi: 10.1111/j.1365-2362.2010.02306.x

41. Suárez-Ortegón MF, McLachlan S, Wild SH, Fernández-Real JM, Hayward C, Polašek O. Soluble transferrin receptor levels are positively associated with insulin resistance but not with the metabolic syndrome or its individual components. *Br J Nutr*. (2016) 116:1165–74. doi: 10.1017/s0007114516002968

42. Fernández-Real JM, Moreno JM, López-Bermejo A, Chico B, Vendrell J, Ricart W. Circulating soluble transferrin receptor according to glucose tolerance status and insulin sensitivity. *Diabetes Care*. (2007) 30:604–8. doi: 10.2337/dc06-1138

43. Enko D, Wagner H, Kriegshäuser G, Wögerer J, Halwachs-Baumann G, Schnedl WJ, et al. Iron status determination in individuals with *Helicobacter pylori* infection: conventional vs. new laboratory biomarkers. *Clin Chem Lab Med*. (2019) 57:982–9. doi: 10.1515/cclm-2018-1182

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Prospective changes in anemia are associated with the incidence and persistence of sarcopenia among older Mexican adults

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Background: Low hemoglobin levels are a significant biomarker in the prognosis of sarcopenia. Anemia and sarcopenia are frequent and disabling conditions in the older adult population, but little is known about the role of anemia in the onset and progression of sarcopenia. This study aimed to determine whether prospective changes in anemia are associated with the incidence and persistence of sarcopenia.

Methods: Data come from the second and third waves (2014, 2017) of the World Health Organization (WHO) Study on global AGEing and adult health (SAGE) in Mexico. SAGE-Mexico is a dynamic cohort with national representativeness, including a follow-up sample and new enrollments. For this study, 1,500 older adults (aged 50 or above) with measurements in both waves were included. Sarcopenia was defined as having low muscle quantity and either/both slow gait speed and weak handgrip strength. Anemia was defined according to hemoglobin concentrations, adjusted for altitude, as recommended by the WHO, <120 g/L for women and <130 g/L for men. Multinomial logistic regression was used to estimate the association between anemia and prospective changes in sarcopenia.

Results: The baseline prevalence of anemia was 17.4%, and that of sarcopenia was 12.1%. The incidence and persistence of anemia were 10.6% (95% CI: 7.3–15.0%) and 6.9% (95% CI: 4.7–9.8%), respectively, and for sarcopenia, they were 5.3% (95% CI: 3.7–7.7%) and 9.2% (95% CI: 6.4–13.0%), respectively. Incident anemia was associated with incident (RRR = 3.64, 95% CI: 1.18–11.19) but not with persistent (RRR = 0.75, 95% CI: 0.18–3.20) sarcopenia. Persistent anemia was significantly associated with persistent (RRR = 3.59, 95% CI: 1.14–11.27) but not incident (RRR = 1.17, 95% CI: 0.30–4.54) sarcopenia.

Conclusion: Changes in anemia are significantly associated with incident and persistent sarcopenia. Primary actions to promote a healthy diet rich in antioxidants, high-quality proteins, and micronutrients, as well as moderate physical activity and maintaining a healthy weight, are crucial for the aging population to delay the deleterious effects of anemia and sarcopenia.

KEYWORDS

anemia, sarcopenia, incidence, prevalence, older adults

1 Introduction

The decline in physical function in aging is one of the main drivers of the onset of sarcopenia, a geriatric syndrome characterized by poor physical performance, low strength, and low mass muscle (1), with function loss accelerating three times faster than muscle loss and increasing the appearance of disability falls and frailty (2). Among older adults, sarcopenia is a frequent condition, with prevalence rates ranging from 0–15% in healthy older adults and 2–34% in geriatric outpatients (3). For Mexico, the prevalence ranges from 9.3 to 33.6% (4). Empirical evidence has shown that sarcopenia negatively affects older people's cognitive function, quality of life, and survival rates (5–7). Anemia is also a frequent condition in older adults. The global prevalence of anemia for this age group has been estimated at 23.9 and 28.8% for Mexico (8, 9). Like sarcopenia, anemia has been identified as a risk factor for critical health indicators such as poor physical performance, quality of life, disability, and mortality (10).

Previous studies have analyzed the association between anemia and sarcopenia with inconclusive results. A recent meta-analysis with 98,502 community-dwelling participants aged 60+ years that aimed to identify the factors associated with sarcopenia reported that anemia was significantly associated with sarcopenia. However, for this last association, only two Japanese studies with 2,408 older adults were included (11). Meanwhile, studies on the specific association between anemia and sarcopenia have mixed results. One study with Taiwanese older adults reported a significant association (12), but for another two studies (Japan and Taiwan), the association was no longer significant (13, 14). Two additional prospective cohort studies with older Australian men and older American adults, have shown that anemia increased the risk in the decline of physical performance and sarcopenia (15, 16).

Despite this incipient prior evidence, knowledge gaps still must be addressed. This is mainly because the results have been generated either by cross-sectional or longitudinal studies that have yet to explore the influence that changes in anemia have on changes in sarcopenia prospectively. Moreover, whether anemia has a role in the onset or persistence of sarcopenia has yet to be explored, and the evidence from the Latin American population is null, even though structural risk factors might be higher than those in high-income countries (HIC). Particularly, anemia rates in populations from low- and middle-income countries are higher and also have different causes compared to those in HIC (17). In fact, it remains uncertain whether the accumulated risk of persistent anemia affects sarcopenia over time.

Anemia and sarcopenia are frequent conditions in the older adult population, and both are modifiable risk factors. In contexts where the double burden of malnutrition exists (particularly in low- and middle-income countries such as Mexico), early identification of biomarkers as a prognosis of the onset of sarcopenia is crucial to promote early actions and delay their pervasive consequences. This study aimed to evaluate whether prospective changes in anemia were associated with an increasing risk of sarcopenia over a 3 years follow-up in a representative national sample of older Mexican adults.

2 Materials and methods

2.1 Population and sample

We used data from the World Health Organization (WHO) Study on global AGEing and adult health (SAGE) in Mexico. A multicountry, longitudinal study, SAGE, was based on nationally representative samples of individuals aged 50+ years in six countries: China, Ghana, India, Mexico, Russia, and South Africa. Details of the study design have been published elsewhere (18). The SAGE-Mexico study and sample (cross-sectional and longitudinal) have been previously described (19, 20). Briefly, SAGE-Mexico included a sample of follow-up respondents from SAGE Wave 0, a baseline cohort created during the 2002–2004 World Health Survey, and new respondents. Proxy respondents were identified for respondents who were unable to provide reliable responses or due to poor health. Also, SAGE-Mexico is a nationally-representative sample of older Mexican adults (50+ years) collected using a stratified multistage cluster sample design. In-person interviews were used to collect household and individual level data for each wave. Specifically, two strata were defined by dwelling area (urban and rural). Within these strata, the Basic Geo-Statistical Areas (AGEB by its Spanish acronym) defined by the Mexican National Institute of Statistics were used as primary sampling units (PSU). Households within PSU were randomly selected and constituted the secondary sampling units. Household weights were post-stratified by AGEB according to population census projections. Finally, individuals within households made up tertiary sampling units. Individual weights were post-stratified by sex and age-groups (18–34, 35–49, 50–59, 60–105) according to the census projections. Sample size and date for each wave were: Wave 1 (baseline data) was collected in 2009 with 2,306 respondents. Wave 2 was carried out in 2014 with 2,033 interviews, and Wave 3 was carried out in 2017 with 1,791 participants (plus 618 newly enrolled individuals). For this study, we use data from the most recent waves of SAGE-Mexico (2014 and 2017). The analytical sample consisted of 1,500 older adults who had measurements of anemia and sarcopenia in both waves, with an overall response rate of 84% (Figure 1). Baseline differences between the final sample and excluded participants were observed. Older adults without follow-up measurements were older, had a lower prevalence of sarcopenia and smoking, were mostly women and were mainly from rural areas ($p < 0.05$).

2.2 Outcome

2.2.1 Sarcopenia

The presence of sarcopenia was defined, according to previous studies using the Mexico-SAGE data, as having low skeletal muscle mass (SMM), reflected by lower skeletal muscle mass index (SMI), and either or both slow gait speed and weak handgrip strength. We recently provided a detailed description of this measurement (21). We observed four groups according to changes in sarcopenia: no sarcopenia in both measurements (none), sarcopenia at baseline and not in follow-up (recovery), sarcopenia only in follow-up (incident), and sarcopenia in both measures (persistent). Since the proportion of individuals in the recovery category was low (≈2%), the none and recovery categories were joined, leaving the following categories for sarcopenia: none/recovery, incident, and persistent.

2.3 Main exposure

2.3.1 Anemia

Dried blood spot (DBS) samples were extracted with a finger lancet and collected on standard Whatman 903 filter paper. The

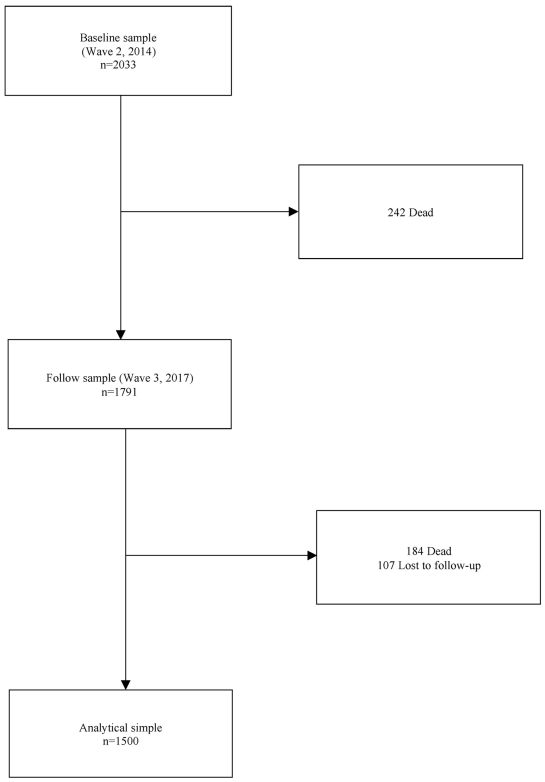

FIGURE 1
Study population and analytical sample.

samples were analyzed after 24 h of drying at room temperature. A 6 mm spot was punctured from the filter paper and eluted for 14 h in 400 µL of MULTIAGEN Hemoglobin Denaturant. Hemoglobin (Hb) was run via blood using the Abbott Architect CI8200 chemistry analyzer. The total Hb was determined by measuring absorbance at 604 nm. Anemia was defined according to Hb concentrations, as recommended by the WHO, <120 g/L for women and <130 g/L for men. Four groups were also defined in relation to observed changes in anemia: none, recovery, incident and persistent.

2.4 Covariates

The following health, socioeconomic and lifestyle baseline variables were used as potential confounders: sex (female = 1), age, number of years of formal education, and dwelling area (rural = 1). The socioeconomic status (SES) of the household was derived using the WHO standard approach to estimate permanent income from household ownership of durable goods, dwelling characteristics (type of floors, walls, and cooking stove), and access to services such as water, sanitation, and electricity (22). SES was included as a continuous variable, with higher values indicating better SES. Multimorbidity was included as a dichotomous variable defined as the presence of two or more chronic noncommunicable conditions from the list of 12 chronic diseases included in the SAGE study. The operational definitions of these diseases have been published elsewhere (23). The body mass index (BMI) was calculated using weight (kg) and height (cm) [BMI = Weight (kg)/Height (m^2)] and was incorporated into the analysis as a continuous variable. The C-reactive protein (mg/L) DBS values were included as a potential inflammation marker. Physical activity was assessed with the Global Physical Activity Questionnaire (GPAQ), which classifies older adults into three categories (low, moderate, and high physical activity) based on reported time spent in moderate or vigorous activities during work, recreational/leisure time, and transportation. As for tobacco use and alcohol consumption, respondents were asked if they had ever used tobacco or consumed alcohol, and if participants answered affirmatively the frequency of use was recorded (24). With this information tobacco use was categorized as *never*; *ever smoked*, *no longer*; *current smoker*, *not daily*; *current smoker*, *daily*; and alcohol consumption as *never*; *ever drinker*, *no longer*; current drinker, low risk; *current drinker*, *high risk*. Fruit and vegetable consumption (servings per day), and sedentary behavior (daily sitting hours) were self-reported. Food insecurity (FI) was operationalized using items adapted from similar items in food security questionnaires of the US Household Food Security Survey Module and National Health and Nutrition Examination Survey (NHANES) Food Security module. In line with previous SAGE studies, FI was coded as severely food insecure, moderately food insecure, and food secure (25).

2.5 Statistical analyses

Baseline characteristics are presented in percentages and means (standard deviation) as appropriate. Health and sociodemographic characteristics related to sarcopenia groups were compared using

chi-square or ANOVA tests. We used a multinomial logistic regression model to estimate the association between changes in anemia and sarcopenia. Relative risk ratios (RRRs) and 95% confidence intervals were reported. Associations were considered significant if $p < 0.05$. All statistical analyses considered the sampling weights and were performed using STATA version 18.0 software (StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC.). This study was conducted following the STROBE guidelines for reporting cohort studies (STROBE checklist is reported in Supplementary material).

2.6 Ethics

This investigation was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (as revised in 1983). The study was approved by the research and ethics committees of the National Institute of Public Health, Cuernavaca, Mexico (CI/2020/550). All subjects gave written informed consent.

3 Results

The baseline study sample included 1,500 older adults, with a mean age of 61.2 years (SE = 0.49). A total of 54.5% were female, with a mean of 6.2 years of formal education (SE = 0.38), 13.8% had severe food insecurity, and 61.7% had multimorbidity. For lifestyle variables, 45.8% performed a low level of physical activity and had a mean daily sitting hours of 2.7 (SE = 0.14). Finally, 37.5% had never smoked, and 71.4% had never consumed alcohol (Table 1).

The proportions of sarcopenia and anemia for each transition group are shown in Table 2. The cumulative incidence of sarcopenia was 5.3% (3.7–7.7%), and persistence was 9.2% (6.4–13.0%). Regarding anemia, the recovery rate was 12.0% (8.6–16.4%), the cumulative incidence was 10.6% (7.3–15.0%), and the persistence was 6.9% (4.7–9.8%).

Table 1 shows the exposure and covariate distribution by sarcopenia groups. Older adults with incident or persistent sarcopenia were older ($p < 0.01$), with a higher prevalence of food insecurity ($p < 0.01$), fewer years of schooling ($p < 0.01$), and poorer ($p = 0.03$) than individuals in the group with no/recovery sarcopenia. No significant differences were observed in health and lifestyle variables (Table 1).

Table 3 depicts the results of the multinomial logistic regression model. The cumulative incidence of anemia was significantly associated with incident sarcopenia (RRR = 3.66; 95% CI 1.18–11.19; $p = 0.02$) and persistent anemia with persistent sarcopenia (RRR = 3.59; 95% CI 1.14–11.27; $p = 0.03$). No significant associations were observed for recovery from anemia. Incident anemia was not significantly related to persistent sarcopenia, nor was persistent anemia related to incident sarcopenia.

Figure 2 shows the conditional probabilities of incident and persistent sarcopenia, given the observed transitions in anemia. Compared with the incident anemia group, older adults with persistent anemia were four times more likely to have persistent sarcopenia. At the same time, older adults with incident anemia had a 4.7 times greater chance of incident sarcopenia than individuals with persistent anemia.

4 Discussion

The results of this study provide evidence of the prospective changes in anemia and their association with incident and persistent sarcopenia in a representative sample of older adults in Mexico with longitudinal data that encompasses a 3 years follow-up. Incident anemia was consistently associated with a 4.7 times risk of incident sarcopenia, and persistent anemia had a four times greater probability for persistent sarcopenia compared to the absence of anemia in both measurements, baseline and follow-up.

A recent systematic review and meta-analysis reported that anemia was associated with a higher probability of sarcopenia (OR: 1.4, CI 95%: 1.06–1.82), although these results were obtained from only two studies (11). Regarding the incidence or persistence of sarcopenia, few cohort studies have explored their association with anemia, although placing greater emphasis on continuous hemoglobin levels (Hb). A cohort study with older Chinese adults found that an increase in Hb of 1 g/dL was associated with a lower rate (8%) of sarcopenia incidence over a 4 years follow-up (26). Another study with older Australian men also reported that a 1 g/dL increase in Hb was significantly associated with a reduction in the odds of sarcopenia throughout 5 years of study (OR = 0.71, CI 95% 0.61, 0.82). In the same study, moderate anemia (OR = 3.0, $p < 0.01$) had a stronger association than mild anemia (OR = 1.73, $p < 0.01$) (15). Another study with older American adults (71 years or older) showed that anemia was associated with a higher decline in physical performance over a 4 years follow-up (16). It is important to mention that this study did not specifically analyze sarcopenia but rather a series of physical performance tests (standing balance, a timed 2.4 m walk, and a timed test of five chair rises).

Our results are consistent with those reported in these studies since anemia was significantly associated with incident and persistent sarcopenia. However, our results are not entirely comparable since we explicitly explored whether prospective changes in anemia (incidence and persistence) influenced the incidence and persistence of sarcopenia. In this sense, our study provides new, robust, and specific information about the role of the incidence and persistence of anemia concerning incident and persistent sarcopenia. Accordingly, the incidence of anemia is a significant prognostic factor for the incidence of sarcopenia, while persistent anemia accounts for a high attributable proportion of persistent sarcopenia. However, the evidence on this relationship remains controversial, and future studies with controlled designs should confirm or refute these results.

Although the specific mechanisms underlying the association between anemia and sarcopenia are not well understood, it has been proposed that low hemoglobin levels reduce oxygen delivery to all cells, impairing skeletal muscle mitochondrial respiration. Mitochondria are the primary site of ATP production and lean muscle fuel metabolism (27). Myoglobin, the protein that carries oxygen to muscle tissue, is affected by anemia, generating local hypoxia in skeletal muscle. Consequently, anemia increases the risk of muscle fatigue, and chronic fatigue impairs muscle mobilization, leading to atrophy, affecting functionality, and increasing the risk of falls and

TABLE 1 Baseline characteristics by sarcopenia groups.

	Sarcopenia[a]				*p*-value[b]
	Total *n* = 1,500	None/recovery *n* = 1,238	Incident *n* = 99	Persistent *n* = 163	
Exposure					
Anemia (%)	17.39	15.60	30.51	26.41	0.07
Covariates					
Sex (female, %)	54.48	55.75	44.70	49.47	0.44
Age (years)	61.19 (0.49)	59.77 (0.45)	69.02 (1.55)	69.86 (1.87)	<0.01
Body mass index [weight (kg) ÷ squared height (meters)]	28.69 (0.25)	28.53 (0.27)	29.51 (1.03)	29.72 (0.71)	0.27
Multimorbidity (2 or more chronic conditions) (%)	61.66	59.97	61.68	77.38	0.09
C-reactive protein (mg/L)	1.42 (0.09)	1.42 (0.10)	1.67 (0.46)	1.22 (0.22)	0.61
Physical activity (%)					
Low	45.81	44.41	67.63	46.20	
Moderate	25.85	26.27	21.84	24.24	
High	28.34	29.32	10.53	29.56	0.19
Sedentary behavior (daily sitting hours)	2.65 (0.14)	2.67 (0.15)	2.19 (0.41)	2.78 (0.33)	0.53
Alcohol consumption (%)					
Never	37.09	35.52	50.02	44.19	
Ever drinker, no longer	47.27	48.52	42.67	38.37	
Current drinker (low risk)	10.33	10.75	1.66	11.46	
Current drinker (high risk)	5.30	5.21	5.66	5.98	0.57
Tobacco use (%)					
Never	71.41	71.80	67.80	69.81	
Ever smoked, no longer	13.51	12.99	12.08	19.15	
Current smoker, not daily	2.30	2.35	4.11	0.80	
Current smoker, daily	12.79	12.86	16.01	10.24	0.82
Fruits and vegetable consumption (number of portions per day)	2.82 (0.12)	2.81 (0.13)	3.26 (0.20)	2.69 (0.30)	0.10
Food insecurity (%)					
None	78.08	78.38	86.01	70.72	
Moderate	8.08	9.02	3.66	1.92	
Severe	13.84	12.60	10.33	27.37	<0.01
Schooling (years of formal education)	6.20 (0.38)	6.51 (0.42)	4.65 (0.61)	4.26 (0.64)	<0.01
Socioeconomic status (assets index)	0.13 (0.09)	0.19 (0.10)	0.16 (0.16)	0.21 (0.25)	0.03
Dwelling area (rural, %)	22.32	24.01	18.24	9.04	0.10

[a]Cells are percentages or means (std. error).
[b]*p*-value for chi-square or ANOVA tests.

disability (28). The observed association between anemia and sarcopenia in our study might also be partially explained by chronic low-grade inflammation since this has been identified as the main contributor to anemia in one study with older adults from the southern region of Mexico (29). Chronic inflammation also contributes to the loss of muscle mass, strength, and functionality (30), and it is a common shared pathway with anemia due to chronic disease and other geriatric syndromes (31).

Anemia and sarcopenia might also share a nutritional cause as etiology, given that both conditions have been linked through malnutrition (11, 32). Previous evidence has indicated that nutritional anemia could account for one-third of all anemias in older people. Aside from iron deficiency, nutritional anemia is associated with vitamin B12 (cobalamin), which is frequently related to dietary cobalamin malabsorption and vitamin B9 (folate) deficiency (33–35). The inadequate intake of nutrients, associated with the loss of appetite during aging, is a nutritional factor leading to poor protein intake, which can also cause iron deficiency and other nutritional anemias. As for older Mexican adults, the adequacy intake of some essential micronutrients, such as iron and zinc, is low (36). However, tortillas and beans are part of the traditional Mexican diet and are consumed in large amounts. They are sources of protein, micronutrients (nonheme iron), and inhibitors of iron absorption as phytates and tannins. Despite this, previous studies have shown that although the Mexican diet has a high iron content, its bioavailability is low, which, in turn, can affect iron status (37). Poor protein quality mainly reflects the low bioavailability of iron, B12, and other critical micronutrients involved in erythropoiesis and protein synthesis needed for the anabolism of muscle fibers. However, micronutrient deficiency appears to have a low contribution to anemia in older Mexican adults (29), and its impact on the risk of sarcopenia in this population is also unknown.

The study results should be interpreted in the presence of some limitations. Hb measurement was based on a capillary dried blood sample, which might misclassify anemia diagnosis, affecting those around the cutoff value. Nonetheless, Hb data were calibrated with venous blood to minimize bias due to measurement error (38). The SMI was estimated through a formula instead of more precise methods such as DXA or BIA; this could result in low sensitivity of subjects categorized with low mass muscle. In addition, we do not have any information regarding diet and protein quality, the etiology of anemia, or the treatment for anemia, which could help explain the risk in the incidence and persistence of sarcopenia. Reverse causality could also explain the results obtained. Older adults with persistent sarcopenia have specific characteristics, such as a higher prevalence of chronic comorbidities, that increase the risk of persistent anemia, with

TABLE 2 Proportion of older adults in each sarcopenia and anemia group.

		Estimator	CI 95%	
Sarcopenia	None/recovery	85.5	81.3	88.8
	Incident	5.3	3.7	7.7
	Persistent	9.2	6.4	13.0
Anemia	None	70.7	64.7	76.0
	Recovery	12.0	8.6	16.4
	Incident	10.6	7.3	15.0
	Persistent	6.9	4.7	9.8

TABLE 3 Results of the multinomial logistic regression models.

	Incident sarcopenia				Persistent sarcopenia			
	RRR	CI 95%		*p*-value[a]	RRR	CI 95%		*p*-value[a]
Anemia (ref: none)								
Recovery	0.54	0.20	1.43	0.22	1.05	0.26	4.26	0.95
Incident	3.66	1.18	11.19	0.02	0.63	0.20	2.06	0.45
Persistent	0.75	0.18	3.20	0.70	3.59	1.14	11.27	0.03

[a]Adjusted for covariates shown in Table 1.

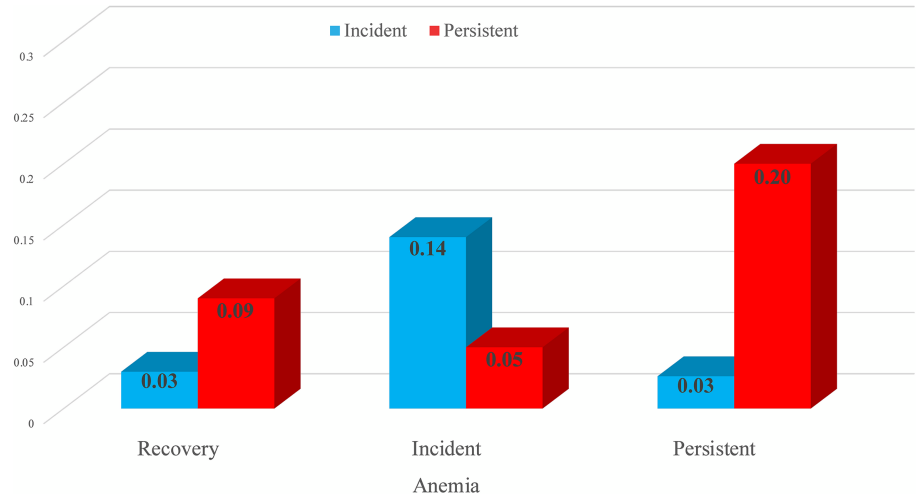

FIGURE 2
Conditional probabilities of incident and persistent sarcopenia.

low-grade inflammation as a shared common pathway where anemia by chronic disease may arise.

5 Conclusion

The results of this study have important implications for research, clinical practice, and public health policies targeting older adults. Regarding health policies, addressing the primary needs of all people to reach food security is crucial. Most anemia cases in older Mexican adults have an inflammatory component secondary to chronic disease (29); therefore, secondary prevention should be reinforced to maintain autonomy in older adults. Primary actions to promote a healthy diet rich in antioxidants, high-quality proteins, and micronutrients, as well as moderate physical activity and maintaining a healthy weight, are vital for the aging population to delay the deleterious effects of anemia and sarcopenia. For clinical practice, according to clinical guidelines in Mexico, it is recommended to assess and determine the cause of anemia and treat it whenever feasible. Individuals with micronutrient deficiencies, such as iron, B12, and folate, should be given supplements to address the deficiency. For research, further studies should explore the effect of treating anemia on the onset of broad functional outcomes in older adults. In conclusion, the current study provides evidence that anemia is an independent risk factor for incident and persistent sarcopenia. Given that both conditions are highly prevalent and modifiable, public health approaches should be focused on maintaining adequate Hb values and avoiding loss of muscle function to preserve autonomy in the older adult population.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.who.int/data/data-collection-tools/study-on-global-ageing-and-adult-health/sage-waves.

Ethics statement

The studies involving humans were approved by Research and ethics committees of the National Institute of Public Health, Cuernavaca, Mexico (CI/2020/550). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

VC-G: Investigation, Methodology, Writing – original draft, Writing – review & editing. AS-R: Investigation, Methodology, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Project administration, Software. BM-E: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing.

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Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1323450/full#supplementary-material

References

1. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Aging*. (2019) 48:16–31. doi: 10.1093/aging/afy169

2. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol*. (2003) 95:1851–60. doi: 10.1152/japplphysiol.00246.2003

3. Reijnierse EM, Trappenburg MC, Leter MJ, Blauw GJ, Sipilä S, Sillanpää E, et al. The impact of different diagnostic criteria on the prevalence of sarcopenia in healthy elderly participants and geriatric outpatients. *Gerontology*. (2015) 61:491–6. doi: 10.1159/000377699

4. Manrique-Espinoza B, Salinas-Rodríguez A, Rosas-Carrasco O, Gutiérrez-Robledo LM, Avila-Funes JA. Sarcopenia is associated with physical and mental components of health-related quality of life in older adults. *J Am Med Dir Assoc*. (2017) 18:636.e1–5. doi: 10.1016/j.jamda.2017.04.005

5. Fhon JRS, Silva ARF, Lima EFC, Santos Neto APD, Henao-Castaño ÁM, Fajardo-Ramos E, et al. Association between sarcopenia, falls, and cognitive impairment in older

people: a systematic review with meta-analysis. *Int J Environ Res Public Health*. (2023) 20:4156. doi: 10.3390/ijerph20054156

6. Beaudart C, Demonceau C, Reginster JY, Locquet M, Cesari M, Cruz Jentoft AJ, et al. Sarcopenia and health-related quality of life: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. (2023) 14:1228–43. doi: 10.1002/jcsm.13243

7. Beaudart C, Zaaria M, Pasleau F, Reginster JY, Bruyère O. Health outcomes of sarcopenia: a systematic review and meta-analysis. *PLoS One*. (2017) 12:e0169548. doi: 10.1371/journal.pone.0169548

8. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anemia, WHO vitamin and mineral nutrition information system, 1993–2005. *Public Health Nutr*. (2009) 12:444–54. doi: 10.1017/S1368980008002401

9. Salinas-Rodríguez A, De La Cruz-Góngora V, Manrique-Espinoza B. Condiciones de salud, síndromes geriátricos y estado nutricional de los adultos mayores en México. *Salud Publica Mex*. (2020) 62:777–85. doi: 10.21149/11840

10. Yuan S, Larsson SC. Epidemiology of sarcopenia: prevalence, risk factors, and consequences. *Metabolism*. (2023) 144:155533. doi: 10.1016/j.metabol.2023.155533

11. Gao Q, Hu K, Yan C, Zhao B, Mei F, Chen F, et al. Associated factors of sarcopenia in community-dwelling older adults: a systematic review and meta-analysis. *Nutrients*. (2021) 13:4291. doi: 10.3390/nu13124291

12. Tseng SH, Lee WJ, Peng LN, Lin MH, Chen LK. Associations between hemoglobin levels and sarcopenia and its components: results from the I-Lan longitudinal study. *Exp Gerontol*. (2021) 150:111379. doi: 10.1016/j.exger.2021.111379

13. Kitamura A, Seino S, Abe T, Nofuji Y, Yokoyama Y, Amano H, et al. Sarcopenia: prevalence, associated factors, and the risk of mortality and disability in Japanese older adults. *J Cachexia Sarcopenia Muscle*. (2021) 12:30–8. doi: 10.1002/jcsm.12651

14. Ko YC, Chie WC, Wu TY, Ho CY, Yu WR. A cross-sectional study about the relationship between physical activity and sarcopenia in Taiwanese older adults. *Sci Rep*. (2021) 11:11488. doi: 10.1038/s41598-021-90869-1

15. Hirani V, Naganathan V, Blyth F, Le Couteur DG, Seibel MJ, Waite LM, et al. Low hemoglobin concentrations are associated with sarcopenia, physical performance, and disability in older Australian men in cross-sectional and longitudinal analysis: the concord health and aging in men project. *J Gerontol A*. (2016) 71:1667–75. doi: 10.1093/gerona/glw055

16. Penninx BW, Guralnik JM, Onder G, Ferrucci L, Wallace RB, Pahor M. Anemia and decline in physical performance among older persons. *Am J Med*. (2003) 115:104–10. doi: 10.1016/s0002-9343(03)00263-8

17. GBD 2021 Anaemia Collaborators. Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990–2021: findings from the global burden of disease study 2021. *Lancet Haematol*. (2023) 10:e713–34. doi: 10.1016/S2352-3026(23)00160-6

18. Kowal P, Chatterji S, Naidoo N, Biritwum R, Fan W, Lopez Ridaura R, et al. Data resource profile: the World Health Organization Study on global AGEing and adult health (SAGE). *Int J Epidemiol*. (2012) 41:1639–49. doi: 10.1093/ije/dys210

19. Salinas-Rodríguez A, González-Bautista E, Rivera-Almaraz A, Manrique-Espinoza B. Longitudinal trajectories of intrinsic capacity and their association with quality of life and disability. *Maturitas*. (2022) 161:49–54. doi: 10.1016/j.maturitas.2022.02.005

20. Miu J, Negin J, Salinas-Rodriguez A, Manrique-Espinoza B, Sosa-Ortiz AL, Cumming R, et al. Factors associated with cognitive function in older adults in Mexico. *Glob Health Action*. (2016) 9:30747. doi: 10.3402/gha.v9.30747

21. Salinas-Rodríguez A, Palazuelos-González R, Rivera-Almaraz A, Manrique-Espinoza B. Longitudinal association of sarcopenia and mild cognitive impairment among older Mexican adults. *J Cachexia Sarcopenia Muscle*. (2021) 12:1848–59. doi: 10.1002/jcsm.12787

22. Howe LD, Galobardes B, Matijasevich A, Gordon D, Johnston D, Onwujekwe O, et al. Measuring socioeconomic position for epidemiological studies in low- and middle-income countries: a method of measurement in epidemiology paper. *Int J Epidemiol*. (2012) 41:871–86. doi: 10.1093/ije/dys037

23. Arokiasamy P, Uttamacharya KP, Capistrant BD, Gildner TE, Thiele E, Biritwum RB, et al. Chronic noncommunicable diseases in 6 low- and middle-income countries: findings from wave 1 of the World Health Organization's study on Global Aging and Adult Health (SAGE). *Am J Epidemiol*. (2017) 185:414–28. doi: 10.1093/aje/kww125

24. Gildner TE, Salinas-Rodríguez A, Manrique-Espinoza B, Moreno-Tamayo K, Kowal P. Does poor sleep impair cognition during aging? Longitudinal associations between changes in sleep duration and cognitive performance among older Mexican adults. *Arch Gerontol Geriatr*. (2019) 83:161–8. doi: 10.1016/j.archger.2019.04.014

25. Smith L, Jacob L, Barnett Y, Butler LT, Shin JI, López-Sánchez GF, et al. Association between food insecurity and sarcopenia among adults aged ≥65 years in low- and middle-income countries. *Nutrients*. (2021) 13:1879. doi: 10.3390/nu13061879

26. Liu Q, You J, Zhong M, Wu Z, Geng Y, Huang C. Hemoglobin level is negatively associated with sarcopenia and its components in Chinese aged 60 and above. *Front Public Health*. (2023) 11:1081843. doi: 10.3389/fpubh.2023.1081843

27. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci USA*. (2003) 100:7996–8001. doi: 10.1073/pnas.1332551100

28. Penninx BW, Pahor M, Cesari M, Corsi AM, Woodman RC, Bandinelli S, et al. Anemia is associated with disability and decreased physical performance and muscle strength in elderly individuals. *J Am Geriatr Soc*. (2004) 52:719–24. doi: 10.1111/j.1532-5415.2004.52208.x

29. De La Cruz-Góngora V, Salinas-Rodríguez A, Flores-Aldana M, Villalpando S. Etiology of Anemia in older Mexican adults: the role of hepcidin, vitamin A and vitamin D. *Nutrients*. (2021) 13:3814. doi: 10.3390/nu13113814

30. Dalle S, Rossmeislova L, Koppo K. The role of inflammation in age-related sarcopenia. *Front Physiol*. (2017) 8:1045. doi: 10.3389/fphys.2017.01045

31. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. *Cell*. (2023) 186:243–78. doi: 10.1016/j.cell.2022.11.001

32. Velázquez Alva Mdel C, Irigoyen Camacho ME, Delgadillo Velázquez J, Lazarevich I. The relationship between sarcopenia, undernutrition, physical mobility and basic activities of daily living in a group of elderly women of Mexico City. *Nutr Hosp*. (2013) 28:514–21. doi: 10.3305/nh.2013.28.2.6180

33. Andrès E, Federici L, Serraj K, Kaltenbach G. Update of nutrient-deficiency anemia in elderly patients. *Eur J Intern Med*. (2008) 19:488–93. doi: 10.1016/j.ejim.2008.01.016

34. Andrès E, Serraj K, Federici L, Vogel T, Kaltenbach G. Anemia in elderly patients: new insight into an old disorder. *Geriatr Gerontol Int*. (2013) 13:519–27. doi: 10.1111/ggi.12017

35. Yen PK. Nutritional anemia. *Geriatr Nurs*. (2000) 21:111–2. doi: 10.1067/mgn.2000.107138

36. Cruz-Góngora V, Martínez-Tapia B, Cuevas-Nasu L, Flores-Aldana M, Shamah-Levy T. Dietary intake and adequacy of energy and nutrients in Mexican older adults: results from two National Health and Nutrition Surveys. *Salud Publica Mex*. (2017) 59:285–98. doi: 10.21149/7851

37. Sánchez-Pimienta TG, López-Olmedo N, Rodríguez-Ramírez S, García-Guerra A, Rivera JA, Carriquiry AL, et al. High prevalence of inadequate calcium and iron intakes by Mexican population groups as assessed by 24-hour recalls. *J Nutr*. (2016) 146:1874S–80S. doi: 10.3945/jn.115.227074

38. Dona AC, DeLouize AM, Eick G, Thiele E, Salinas Rodríguez A, Manrique Espinoza BS, et al. Inflammation and central adiposity as mediators of depression and uncontrolled diabetes in the study on global AGEing and adult health (SAGE). *Am J Hum Biol*. (2020) 32:e23413. doi: 10.1002/ajhb.23413

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Association between obesity and anemia in an nationally representative sample of United States adults: a cross-sectional study

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Introduction: Few studies are about the relationship between anemia and obesity, and previous studies have only paid attention to BMI.

Methods and Results: We first included body fat percentage (BF%) as an assessment indicator and divided it into quartiles, grouped participants into obesity and non-obesity used data from NHANES database. After adjustment for age, gender, ethnicity, education and family income, the level of soluble transferrin receptor (sTfR), and incidence of elevated CRP or HsCRP were progressively higher with increased BF%, whereas mean cell volume (MCV), natural logarithm (Ln) serum ferritin (SF), and Ln SF/sTfR were progressively reduced. Although a higher prevalence of anemia and lower hemoglobin was observed with increased BF%, but there was no statistical difference. Women in the highest BF% group demonstrated a significantly higher risk of iron deficiency compared to those in the lowest BF% group.

Discussion: BF% should be given more attention, and women with high BF% should pay attention to iron deficiency.

KEYWORDS

anemia, obesity, body fat percentage, iron deficiency, inflammation, NHANES

Introduction

The global prevalence of obesity has been accelerated by increases in national income, coupled with a lack of physical activity and nutritionally balanced diets. Over the past 40 years, the obesity rate has risen from 3 to 11% in men and from 6 to 15% in women (1). By 2030, it is foreseen that almost half of the adult population in the United States will be obese (2).

Obesity is associated with multiple adverse outcomes, including anemia, which is a serious global public health problem. In addition to height and weight, anemia is a basic indicator that reflects the nutritional well-being of individuals. While it may appear paradoxical, obesity is also linked to nutrient deficiencies (3), most people with long-term anemia looks thinner than others. The overlooked paradox of the coexistence of obesity and anemia certainly exist. An assessment tool that has been widely used in clinical practice and research as a screening tool for obesity is the BMI. However, BMI cannot differentiate body composition or excess fat distribution. This measurement alone is insufficient to evaluate adiposity-related disease risk.

Anemia's clinical manifestations often include fatigue, pallor, shortness of breath, and an increased heart rate. Iron deficiency anemia (IDA) stands as the prevailing cause of anemia worldwide, while anemia of inflammation (AI), also known as anemia of chronic disease (ACD), is recognized as the most frequent form of anemia in hospitalized and chronically ill patients, which ranks as the second most common type of anemia globally, following IDA (4). Obesity is characterized by low-grade chronic inflammation. This results in the production of certain inflammatory cytokines, leading to elevated levels of circulating plasma inflammatory markers and inflammatory cells (5). Body mass index (BMI)-based studies have shown that obese women are associated with iron deficiency (6). Body fat percentage (BF%) is a better indicator for classifying obesity compared to BMI (7), while little attention has been paid to the relationship between BF% and anemia, iron, and inflammation.

Methods

Study design and population

National Health and Nutrition Examination Survey (NHANES) utilized a stratified multistage probability sample to represent the civilian, non-institutionalized population of the United States. The National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention conducted surveys through household interviews, followed by standardized physical examinations at Mobile Examination Centers (MECs). The NHANES protocol has been approved by the NCHS Research Ethics Review Board. NHANES data are released on a 2-year cycle. The data collection and analysis procedures for NHANES have been published (available from https://www.cdc.gov/nchs/nhanes/index.htm).

This analysis was conducted using three data cycles of NHANES: 2003–2004, 2005–2006, and 2017–2018. Institutional review board approval was not required for the current analysis. The analytical sample included adults aged 20–65 years who were not pregnant at the time of participation and had no missing data for hemoglobin, serum ferritin (SF), soluble transferrin receptor (sTfR), BMI, and Dual-Energy X-ray Absorptiometry (DEXA)-whole body measurements. Individuals who potentially had liver disease were excluded from the study, with abnormal liver enzyme levels defined as alanine aminotransferase (ALT) > 70 U/L or aspartate aminotransferase (AST) > 70 U/L (8) (Figure 1).

Laboratory methods

Serum ferritin

Serum ferritin was measured by electrochemiluminescence immunoassay. Over the years, various laboratory measurement methods have been employed for sample analysis. Due to variations in the testing methods, the NHANES team conducted cross-studies to compare the data and made necessary adjustments based on the comparative results before releasing the data to the public. For ferritin, two methods were used in 2003–2004. The National Center for Environmental Health analyzed all 2003 samples with a BioRad assay (BioRad Laboratories, Hercules, CA, United States) and all 2004 samples with a Roche/Hitachi assay (Roche Diagnostics, Basel, Switzerland). NHANES used three piecewise linear regression equations to adjust the 2003 ferritin data to be comparable to the 2004 ferritin (ng/mL) data before publishing the data. Ferritin in 2005–2006 is measured using immuno-turbidimetry method on Roche/Hitachi 912 clinical analyzer. Ferritin in 2017–2018 was measured using sandwich principle on Roche Cobas® e601. We converted 2017–2018 data from E170 to Hitachi 912, for ferritin in 2017–2018 to be accurately comparable to other years, a Deming regression analysis was performed, and the following regression was obtained for Ferritin (ng/mL):

$$\mathrm{E170}\left(2017-2018\right)=10^{**}\left(0{\cdot}989^{*}\log 10\left(\mathrm{Hitachi\ 912}\right)+0{\cdot}049\right)$$

Soluble transferrin receptor

Soluble transferrin receptor is measured using immuno-turbidimetry Roche kits on the Hitachi 912 clinical analyzer in 2003–2004 and 2005–2006, on the Cobas® c501 clinical analyzer in 2017–2018. There was no need for adjustment of the sTfR measured value. The ratio of natural logarithm (Ln) SF/sTfR reflected iron levels in the body (9). "Low" levels of Ln SF/sTfR were defined as values below the 25th percentile (< 0.818 for women, < 1.412 for men), seen as iron deficiency (ID).

Complete blood count

Hemoglobin, mean cell volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are derived from complete blood count (CBC) with five-part differential—whole blood. The methods used to derive CBC parameters are based on the Beckman Coulter (DxH 800 instrument) methodology of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. Anemia is defined as hemoglobin level less than 13 g/dL for men and less than 12 g/dL for women (10).

C-Reactive protein

National Health and Nutrition Examination Survey 2003–2004 and 2005–2006 quantified C-reactive protein (CRP) by latex-enhanced nephelometry in University of Washington, Seattle, WA, United States. Analysis of high sensitivity C-reactive protein (HsCRP) in NHANES 2017–2018 is by a two-reagent, immunoturbidimetric system, using the Roche Cobas 6000 chemistry analyzer (Cobas 6000) in University of Minnesota—Advanced Research Diagnostics Laboratory (ARDL), Minneapolis, MN, United States. Elevated CRP was defined as CRP more than 8 mg/L. Elevated HsCRP was defined as HsCRP more than 2 mg/L (11).

Anthropometry

The BMI is derived from height and weight measurements collected at the Mobile Examination Centers (MECs). Obesity was assessed using BMI and classified as non-obese (BMI < 30) and obese (BMI ≥ 30) (12). Total BF% measurements were performed using whole-body dual-energy X-ray absorptiometry (DXA) scanning (Hologic, Inc., Bedford, Massachusetts).

Assessment of potential confounding variables

Gender, race/ethnicity, age, education, marriage, and income status, which may be associated with BF% were included as potential confounders in the regression models. Race/ethnicity was categorized

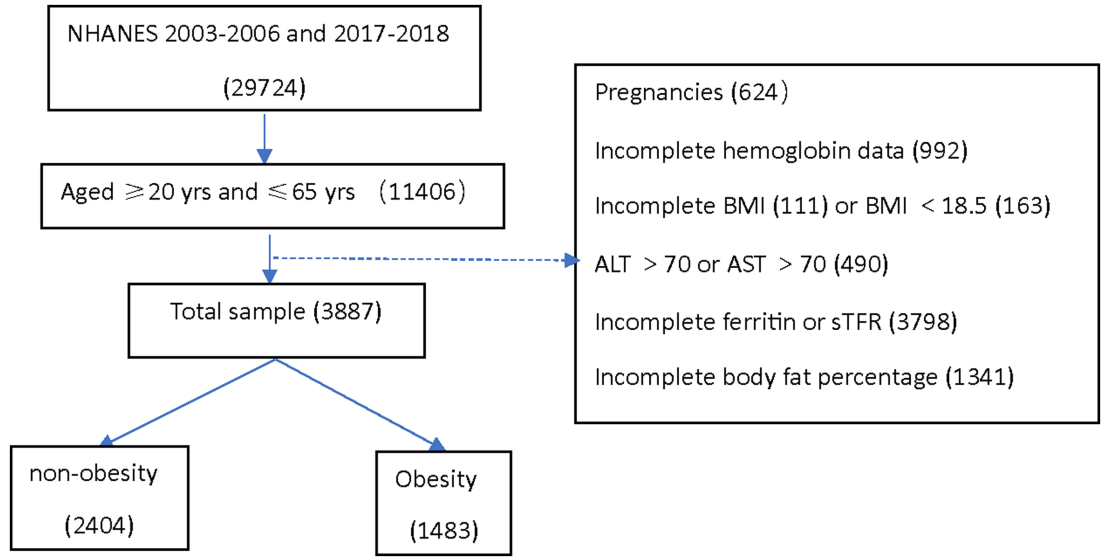

FIGURE 1
Cohort selection.

into five groups: non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other race. Education was categorized as "no college degree" and "college degree." Marriage was categorized into living with a partner or not. Income status was categorized as having an annual income of more than $20,000 or less than $20,000. Current smoking was defined as smoked at least 100 cigarettes in life and smoked within the last 30 days. Alcohol consumption was defined as having ≥3 alcohol drinks per year. Blood donations were defined as participants had donated blood in the past 12 months.

Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics software (version 28, IBM Corp., Armonk, NY, United States), which incorporated weights to account for the complex sample design. The 6-year examination weights from the NHANES data for 2003–2006 and 2017–2018 were utilized to account for non-response and oversampling in all analyses. We assessed whether there were differences in characteristics between non-obesity and obesity in the population eligible for inclusion using χ^2 or *t* tests ($p < 0.05$; two-tailed). We log-transformed SF (Ln SF) data to standardize the distribution. BF% was put into quartiles. After adjusting for the potential confounders such as age, race, education, family income, and logistic regression models compared differences in hemoglobin concentration (HB), MCV, SF, and sTfR levels between populations with different BF%. The characteristics of anemia and non-anemia in obese people were compared according to men and women separately using χ^2 or *t* tests. We performed further analysis on men and women to look for any sex-specific differences in the association between BF% and SF, and sTfR, HB, and CRP.

Results

Of the 3,887 NHANES participants analyzed in the current study, 1,483 were obesity, 2,404 were non-obesity. The mean ± SE for age was 37.64 ± 0.27 years. The proportion of men was 24.5%. Obesity was associated with higher age, a higher prevalence of non-Hispanic Black, no college education, Income poverty, elevated SF and sTfR, reduced MCV and MCHC, elevated prevalence of anemia and increased incidence of elevated CRP or HsCRP (Table 1). Blood donation in the last 12 months did not differ statistically by BMI category. No interaction between blood donation and anemia ($p = 0.92$, Pearson test).

After adjustment for age, gender, ethnicity, education and family income, the level of sTfR and percentage of elevated CRP or HsCRP were progressively higher with increased BF% category, whereas MCV, Ln SF, and Ln SF/sTfR were progressively reduced (Table 2). Although as shown in the Table 2, a higher prevalence of anemia and lower hemoglobin was observed with increased BF%, but they did not have statistically significant after adjustment for the confounders.

Further analysis targeting only obese groups showed that anemic women had lower levels of SF and higher levels of sTfR compared to non-anemic women. On the other hand, among anemic men, the percentage of individuals with elevated CRP or HsCRP levels was higher compared to non-anemic men (Table 3).

We conducted subgroup analysis according to different genders. Similar results were observed in these subgroups in the adjusted model (Table 4; Figure 2). For women, compared with participants with lowest BF%, participants with highest BF% exhibited significantly increased risk of higher Ln SF/sTfR (OR, 1.44, 95% CI, 1.04–1.99) in multivariable analysis controlled for factors like age, race, education, and family income. Results of multivariable analysis showed a graded relation between MCV and obesity, with progressively higher odds of reduced MCV. For men, it showed 4th quartile BF% has the higher risk to be lower MCV compared to 1st quartile BF%. A graded relation between elevated CRP percentage and obesity was observed in both women and men. The higher the BF% is, the more likely they have an elevated CRP (C-reactive protein) ratio. There looked like a linear relationship observed between anemia, hemoglobin, and BF%; however, this relationship did not reach statistical significance.

TABLE 1 Characteristics of participants presented by BMI category.

	Overall	Non-Obesity	Obesity	*p* value
		BMI 18.5–29.9 kg/m^2 (*n* = 2,404)	BMI ≥ 30 kg/m^2 (*n* = 1,483)	
Men, *n* (%)	943 (24.5)	593 (23.4)	350 (26.4)	0.248
Age, year, mean (SE)	37.64 ± 0.27	36.91 ± 0.41	38.91 ± 0.31	<0.001
Race, *n* (%)				<0.001
Mexican American	729 (10.2)	421 (9.1)	308 (12.2)	
Other Hispanic	281 (6.6)	173 (6.6)	108 (6.7)	
Non-Hispanic White	1,459 (62.5)	946 (64.3)	513 (59.3)	
Non-Hispanic Black	847 (11.7)	419 (9.2)	428 (16)	
Other Race—including multi-racial	571 (9)	445 (10.8)	126 (5.8)	
Married/living with a partner, *n* (%)	2,335 (62.1)	1,489 (63.1)	846 (60.4)	0.345
Ever attended college, *n* (%)	2,298 (64.8)	1,477 (67.9)	821 (59.5)	<0.001
Annual income > $20,000, *n* (%)	730 (13.4)	413 (11.8)	317 (16.1)	0.003
Current smoking, *n* (%)	717 (20.9)	442 (21.1)	275 (20.4)	0.673
Alcohol consumption, *n* (%)	841 (35.8)	511 (34.9)	330 (37.5)	0.325
Donated blood in past 12 months, *n* (%)	200 (6.3)	117 (5.9)	83 (6.8)	0.202
Ln SF	4.05 ± 0.03	3.99 ± 0.03	4.16 ± 0.04	0.005
sTfR (mg/L)	3.40 ± 0.04	3.24 ± 0.04	3.68 ± 0.07	<0.001
Hb (g/dL)	13.95 ± 0.04	13.97 ± 0.04	13.93 ± 0.07	0.601
MCV (fl)	88.77 ± 0.17	89.71 ± 0.17	87.13 ± 0.23	<0.001
MCHC (g/dL)	30.02 ± 0.06	30.39 ± 0.06	29.37 ± 0.09	<0.001
Anemia, *n* (%)	308 (5.7)	157 (4.9)	151 (7)	0.02
Elevated CRP or HsCRP, *n* (%)	1,272(31)	463 (18.3)	809 (53.3)	<0.001

Hb, Hemoglobin; MCV, Mean cell volume; Ln SF, Ln serum ferritin; sTfR, Soluble transferrin receptor; MCHC, Mean corpuscular hemoglobin concentration; CRP, C-Reactive protein; and Hs-CRP, Hypersensitive C-reactive protein.

TABLE 2 Comparison of anemia, Hb, MCV, Ln SF, Ln SF/sTfR, and elevated CRP or HsCRP of all participants by BF% quartiles.

	1st quartile BF%	2nd quartile BF%	3rd quartile BF%	4th quartile BF%	Unadjusted *p* value	Adjusted *p* value
Anemia	40 (2.9)	59 (4.3)	94 (6.9)	115 (9)	<0.001	0.633
Hb (g/dL)	14.54 ± 0.05	14.01 ± 0.06	13.66 ± 0.05	13.51 ± 0.06	<0.001	0.400
MCV (fl)	89.62 ± 0.24	89.44 ± 0.25	88.70 ± 0.23	87.12 ± 0.22	<0.001	<0.001
sTfR (mg/L)	3.00 ± 0.06	3.25 ± 0.06	3.55 ± 0.07	3.87 ± 0.07	<0.001	<0.001
Ln SF	4.47 ± 0.05	4.02 ± 0.05	3.81 ± 0.04	3.85 ± 0.33	<0.001	0.001
Ln SF/sTfR	1.67 ± 0.38	1.43 ± 0.03	1.28 ± 0.02	1.18 ± 0.02	<0.001	<0.001
Elevated CRP or HsCRP, *n* (%)	223 (22.2)	237 (22.4)	299 (29.7)	513 (52.2)	<0.001	<0.001

Hb, Hemoglobin; MCV, Mean cell volume; Ln SF, Ln serum ferritin; sTfR, Soluble transferrin receptor; CRP, C-Reactive protein; and Hs-CRP, Hypersensitive C-reactive protein.
Adjusted for: age, race, education, and family income.

Discussion

As far as we know, we are the first to use BF% instead of BMI to explore the link between obesity and iron deficiency and anemia. In the present large population-based cross-sectional representative sample of United States adults, we hypothesized that obesity may be associated with the features of IDA or AI. However, the outcome failed to demonstrate association between BF% and anemia.

Our results suggested that increasing BF% was associated with an increase in sTfR and incidence of elevated CRP or HsCRP; while on the other hand, with increasing BF%, there is an observed decrease in level of MCV, Ln ferritin and Ln ferritin/transferrin receptor. However, contrary to our hypothesis, although a decrease in HB with increasing BF%, there was no statistically significant correlation between changes in HB and the increased incidence of anemia. The prevalence of inflammatory conditions, as indicated by elevated levels CRP or HsCRP tended to increase in both women and men as BF% rose.

TABLE 3 Comparison of non-anemia and anemia in the obese population by sex.

	Women		Adjusted *p* value	Men		Adjusted *p* value
	Non-anemia	Anemia		Non-anemia	Anemia	
sTfR (mg/L)	3.58 ± 0.05	7.28 ± 0.44	<0.001*	3.01 ± 0.07	3.11 ± 0.41	0.569
Ln SF	3.97 ± 0.04	2.44 ± 0.11	<0.001*	5.07 ± 0.07	5.59 ± 0.64	0.458
Elevated CRP or HsCRP, *n* (%)	516 (51.9)	76 (49.5)	0.545	208 (57.3)	9 (100)	0.034*

*$p<0.05$. Adjusted for: age, race, education, and family income.
sTfR, Soluble transferrin receptor; Ln SF, Ln serum ferritin; CRP, C-Reactive protein; Hs-CRP, Hypersensitive C-reactive protein.

TABLE 4 Association between total BF% and anemia, iron, inflammation by BF% quartiles by different gender.

Women	1st quartile BF% (lowest–34.8%)	2nd quartile BF% (34.8–39.7%)	3rd quartile BF% (39.7–43.9%)	4th quartile BF% (43.9–highest)
Anemia, *n* (%)	54 (5.2)	69 (6.6)	71 (8.1)	94 (9)
Hb (g/dL)	13.56 ± 0.05	13.57 ± 0.05	13.55 ± 0.06	13.52 ± 0.07
MCV (fl)	90.74 ± 0.22	89.19 ± 0.27	88.11 ± 0.26	87.02 ± 0.22
MCV ≤ 80	38(3.6)	69(6.2)	75(7.7)	102(9.7)
Ln SF/sTfR	1.28 ± 0.02	1.27 ± 0.02	1.26 ± 0.03	1.14 ± 0.02
Ln SF/sTfR (<0.818)	160 (19.2)	170 (19.8)	187 (22.4)	219 (25.6)
CRP or HsCRP, *n* (%)	79 (10.3)	137 (17.9)	270 (36.2)	408 (54.7)
Men	1st quartile BF% (lowest–23.8%)	2nd quartile BF% (23.8–27.9%)	3rd quartile BF% (27.9–31.7%)	4th quartile BF% (31.7–highest)
Anemia, *n* (%)	5 (1.9)	2 (0.8)	3 (0.7)	10 (1.9)
Hb (g/dL)	15.19 ± 0.06	15.07 ± 0.06	15.27 ± 0.10	15.27 ± 0.12
MCV (fl)	90.07 ± 0.52	87.94 ± 0.31	88.01 ± 0.48	88.02 ± 0.60
MCV ≤ 80	7 (1.4)	11 (2.6)	12 (2.7)	16 (4.7)
LnSF/sTfR	1.86 ± 0.06	1.93 ± 0.04	1.90 ± 0.07	1.85 ± 0.07
LnSF/sTfR(<1.412)	57 (21.8)	54 (18.4)	56 (19.6)	68 (28.9)
CRP or HsCRP, *n* (%)	41 (16.3)	84 (32.7)	98 (44.7)	155 (61.5)

Low level of Ln SF/sTfR defined as a value below the 25th percentile, <0.818 for women, <1.412 for men.
Hb, Hemoglobin; MCV, Mean cell volume; Ln SF, Ln Serum ferritin; sTfR, Soluble transferrin receptor; CRP, C-Reactive protein; and Hs-CRP, Hypersensitive C-reactive protein.

There is no consensus on cutoffs on BF% in men and women separately, so we used the quartile method. ID was statistically significant in women compared 4th to 1st BF% group. There was a graded association between percentage of MCV ≤80 and BF% among women, which appears to be more robust. There was no statistically significant relationship between BF% and ID in men.

Lack of standards in diagnosis of ID posed challenges in accurately identifying ID (13, 14), some studies have used SF alone, as WHO has concluded that SF is a good marker of iron stores and should be used to diagnose ID in apparently healthy individuals (15). Some studies used serum iron and transferrin saturation, while few studies used sTfR. SF is not a sufficiently sensitive indicator for the diagnosis of ID in obese patients. The use of SF as the only biomarker for assessing ID may underestimate ID because obesity-associated chronic inflammation leads to elevated SF and SF may be an inflammatory marker rather than a marker of iron status in overweight and obese individuals (16). STfR is a promising candidate for the detection of ID. That is why our study also incorporated the use of sTfR, which is an inflammation-independent marker. This allows for the diagnosis of iron deficiency in patients with concurrent inflammation.

Various hypotheses have been proposed for the association between obesity and ID. One theory suggested that ID in obese subjects was due to nutritional imbalances (17). In some viewpoints, increased blood volume in obese individuals led to increased iron requirements (18). It was argued that the reason was decrease in iron-binding myoglobin in muscle due to decreased physical activity (19). While others believed that it was related to genetic predisposition (20). There were those who proposed that increased obesity was associated with decreased duodenal iron absorption (21, 22). Other physiological factors associated with chronic inflammation due to excessive obesity could also influenced the bioavailability of iron. The increased accumulation of total and visceral fat mass triggered the production of inflammatory cytokines (23). Chronic inflammation led to increased levels of feromodulin, a small peptide hormone that negatively regulated intestinal iron absorption. This hormone was inversely correlated with serum iron levels (24, 25). In addition, increased adipose tissue volume in obese individuals might directly contribute to increased ferredoxin expression (26). However, there was no difference in iron intake through diet between obese and non-obese individuals (27, 28).

Our findings were in line with some previous studies. For example, a meta-analysis reported that obese/overweight participants

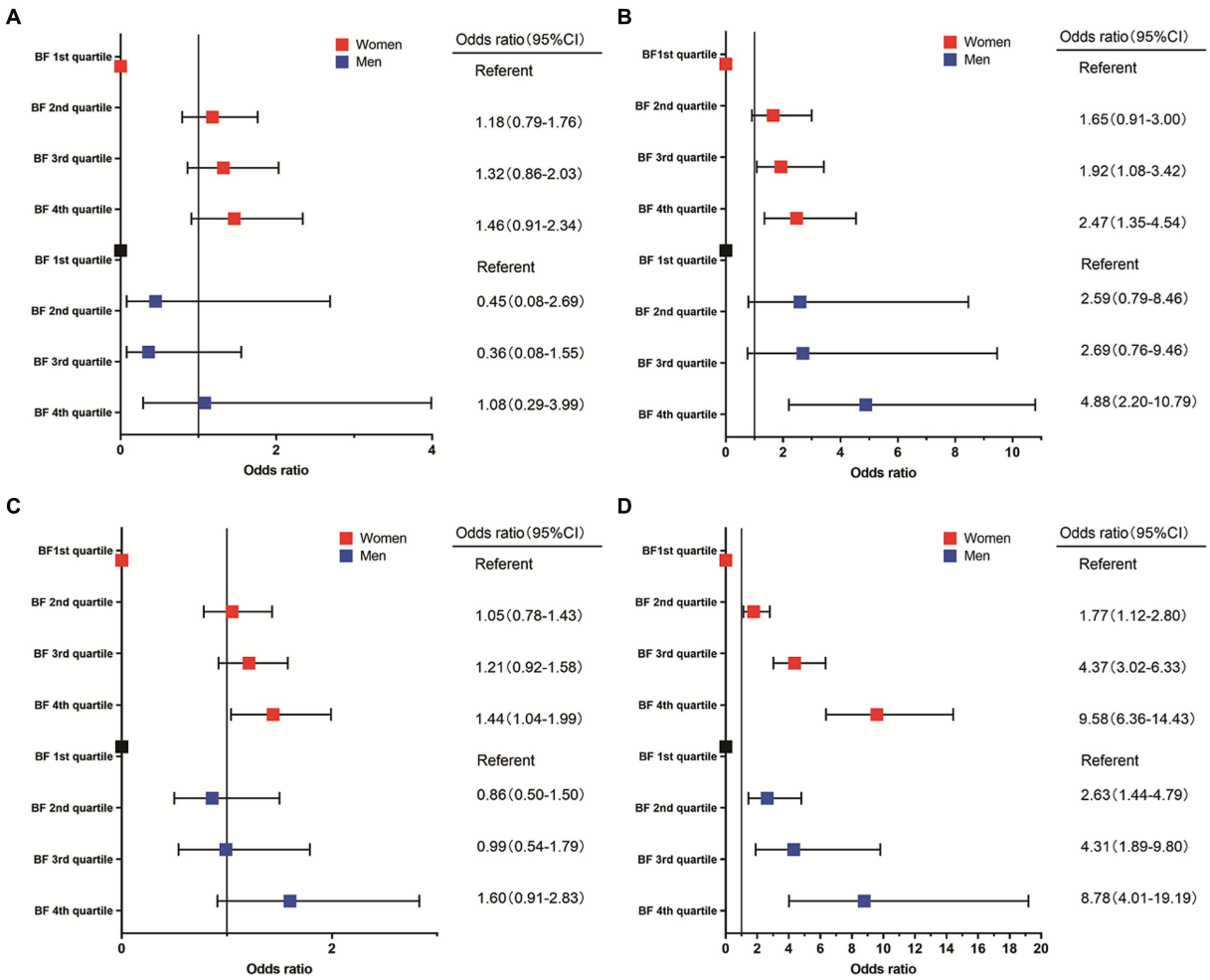

FIGURE 2
Association between incidence of anemia **(A)**, MCV ≤ 80 **(B)**, Ln SF/sTfR < the 25th percentile **(C)**, elevated CRP or HsCRP **(D)**, and body fat percentage.

were more likely to develop ID compared to normal weight participants (29). There was also another review that concluded the opposite, and although ID appeared to be a typical manifestation of severe obesity, the review concluded that most studies showed higher hemoglobin and ferritin concentrations in obese subjects compared to normal-weight adults (30). Iwasaki's study gave different results too. They found a positive correlation between SF levels and body fat index in adults (31).

According to our study, women in the highest BF% group demonstrated a significantly higher risk of ID compared to those in the lowest BF% group, which is consistent with Aguree's finding that obese women have a higher prevalence of ID (6). This also aligns with the outcomes of other researches which showed lower serum iron concentrations in overweight women, but no difference in men (32). There are also studies that yielded different results from our research findings, they claimed that no difference is in serum iron between obese and normal weight controls (33). In our study, we did not find any link between obesity and iron deficiency anemia (IDA). Some obese individuals with hidden or early-stage IDA may not show obvious signs of anemia (19). Depriving developing erythrocytes of iron supply during maturation leads to a reduction in red blood cell production (34). Consequently, this led to reduced erythrocyte synthesis, which may explain the lower MCV values observed in subjects with high BF%.

A major strength of this study is we pioneered the use of BF% instead of BMI to explore the association between obesity and iron deficiency, offering a fresh perspective on this complex relationship. Moreover, the inclusion of a nationally representative sample ensured that our results could be extended to the broader population, enhancing the external validity of our findings. The present study also has limitations. Firstly, cross-sectional design: the use of a cross-sectional design limited our ability to establish causality between obesity and iron deficiency anemia. While we can identify associations, we cannot infer the direction of causation. Secondly, limited male sample size: the insufficient number of male participants in our study hindered the precision and reliability of our extrapolations for this subgroup. Therefore, future prospective study is needed to confirm our findings.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://www.cdc.gov/nchs/nhanes/index.htm (NHANES).

Ethics statement

This research was exempt from local institutional review board review because of the de-identified data analyzed.

Author contributions

ZC: Writing – original draft, Data curation. BC: Writing – original draft, Software, Methodology. LL: Writing – original draft, Validation. XT: Writing – review & editing, Supervision. HX: Writing – review & editing, Conceptualization.

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Conflict of interest

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

Publisher's note

References

1. Jaacks LM, Vandevijvere S, Pan A, McGowan CJ, Wallace C, Imamura F, et al. The obesity transition: stages of the global epidemic. *Lancet Diabetes Endocrinol*. (2019) 7:231–40. doi: 10.1016/S2213-8587(19)30026-9

2. Ward ZJ, Bleich SN, Cradock AL, Barrett JL, Giles CM, Flax C, et al. Projected U.S. state-level prevalence of adult obesity and severe obesity. *N Engl J Med*. (2019) 381:2440–50. doi: 10.1056/NEJMsa1909301

3. Kimmons JE, Blanck HM, Tohill BC, Zhang J, Khan LK. Associations between body mass index and the prevalence of low micronutrient levels among US adults. *Medscape Gen Med*. (2006) 8:59.

4. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. (2014) 123:615–24. doi: 10.1182/blood-2013-06-508325

5. Rogero MM, Calder PC. Obesity, inflammation, toll-like receptor 4 and fatty acids. *Nutrients*. (2018) 10:432. doi: 10.3390/nu10040432

6. Aguree S, Owora A, Hawkins M, Reddy MB. Iron deficiency and Iron deficiency Anemia in women with and without obesity: NHANES 2001-2006. *Nutrients*. (2023) 15:2272. doi: 10.3390/nu15102272

7. Kilani H, Abu-Eisheh A. Optimum anthropometric criteria for ideal body composition related fitness. *Sultan Qaboos Univ Med J*. (2010) 10:74–9.

8. Fleming DJ, Jacques PF, Dallal GE, Tucker KL, Wilson PW, Wood RJ. Dietary determinants of iron stores in a free-living elderly population: the Framingham heart study. *Am J Clin Nutr*. (1998) 67:722–33. doi: 10.1093/ajcn/67.4.722

9. Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. *Am J Clin Nutr*. (2017) 106:1606S–14S. doi: 10.3945/ajcn.117.155887

10. WHO (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 3.

11. Puri R, Nissen SE, Arsenault BJ, St John J, Riesmeyer JS, Ruotolo G, et al. Effect of C-reactive protein on lipoprotein(a)-associated cardiovascular risk in optimally treated patients with high-risk vascular disease: a prespecified secondary analysis of the ACCELERATE trial. *JAMA Cardiol*. (2020) 5:1136–43. doi: 10.1001/jamacardio.2020.2413

12. Garvey WT, Garber AJ, Mechanick JI, Bray GA, Dagogo-Jack S, Einhorn D, et al. American Association of Clinical Endocrinologists and American College of endocrinology position statement on the 2014 advanced framework for a new diagnosis of obesity as a chronic disease. *Endocr Pract*. (2014) 20:977–89. doi: 10.4158/EP14280.PS

13. Cook JD. Defining optimal body iron. *Proc Nutr Soc*. (1999) 58:489–95. doi: 10.1017/S0029665199000634

14. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. *Blood*. (2003) 101:3359–63. doi: 10.1182/blood-2002-10-3071

15. World Health Organization (2020). WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva.

16. Khan A, Khan WM, Ayub M, Humayun M, Haroon M. Ferritin is a marker of inflammation rather than Iron deficiency in overweight and obese people. *J Obes*. (2016) 2016:1937320.

17. Pinhas-Hamiel O, Newfield RS, Koren I, Agmon A, Lilos P, Phillip M. Greater prevalence of iron deficiency in overweight and obese children and adolescents. *Int J Obes Relat Metab Disord*. (2003) 27:416–8. doi: 10.1038/sj.ijo.0802224

18. Failla ML, Kennedy ML, Chen ML. Iron metabolism in genetically obese (Ob/Ob) mice. *J Nutr*. (1988) 118:46–51. doi: 10.1093/jn/118.1.46

19. SELTZER CC, MAYER J. Serum Iron and Iron-binding capacity in adolescents. *Am J Clin Nutr*. (1963) 13:354–61. doi: 10.1093/ajcn/13.6.354

20. Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M. Overweight children and adolescents: a risk group for iron deficiency. *Pediatrics*. (2004) 114:104–8. doi: 10.1542/peds.114.1.104

21. Thankachan P, Walczyk T, Muthayya S, Kurpad AV, Hurrell RF. Iron absorption in young Indian women: the interaction of iron status with the influence of tea and ascorbic acid. *Am J Clin Nutr*. (2008) 87:881–6. doi: 10.1093/ajcn/87.4.881

22. Mujica-Coopman MF, Brito A, López de Romaña D, Pizarro F, Olivares M. Body mass index, iron absorption and iron status in childbearing age women. *J Trace Elem Med Biol*. (2015) 30:215–9. doi: 10.1016/j.jtemb.2014.03.008

23. Subramanian V, Ferrante AW Jr. Obesity, inflammation, and macrophages. *Nestle Nutr Workshop Ser Pediatr Progr*. (2009) 63:151–9. doi: 10.1159/000209979

24. Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Guzman G, Holterman AXL, et al. Elevated systemic hepcidin and iron depletion in obese premenopausal females. *Obesity (Silver Spring)*. (2010) 18:1449–56. doi: 10.1038/oby.2009.319

25. Stoffel NU, el-Mallah C, Herter-Aeberli I, Bissani N, Wehbe N, Obeid O, et al. The effect of central obesity on inflammation, hepcidin, and iron metabolism in young women. *Int J Obes*. (2020) 44:1291–300. doi: 10.1038/s41366-020-0522-x

26. Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh B, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology*. (2006) 131:788–96. doi: 10.1053/j.gastro.2006.07.007

27. Menzie CM, Yanoff LB, Denkinger BI, McHugh T, Sebring NG, Calis KA, et al. Obesity-related hypoferremia is not explained by differences in reported intake of heme and nonheme, iron or intake of dietary factors that can affect iron absorption. *J Am Diet Assoc*. (2008) 108:145–8. doi: 10.1016/j.jada.2007.10.034

28. Cepeda-Lopez AC, Osendarp SJM, Melse-Boonstra A, Aeberli I, Gonzalez-Salazar F, Feskens E, et al. Sharply higher rates of iron deficiency in obese Mexican women and children are predicted by obesity-related inflammation rather than by differences in dietary iron intake. *Am J Clin Nutr*. (2011) 93:975–83. doi: 10.3945/ajcn.110.005439

29. Zhao L, Zhang X, Shen Y, Fang X, Wang Y, Wang F. Obesity and iron deficiency: a quantitative meta-analysis. *Obes Rev*. (2015) 16:1081–93. doi: 10.1111/obr.12323

30. Cheng HL, Bryant C, Cook R, O'Connor H, Rooney K, Steinbeck K. The relationship between obesity and hypoferraemia in adults: a systematic review. *Obes Rev*. (2012) 13:150–61. doi: 10.1111/j.1467-789X.2011.00938.x

31. Iwasaki T, Nakajima A, Yoneda M, Yamada Y, Mukasa K, Fujita K, et al. Serum ferritin is associated with visceral fat area and subcutaneous fat area. *Diabetes Care*. (2005) 28:2486–91. doi: 10.2337/diacare.28.10.2486

32. Micozzi MS, Albanes D, Stevens RG. Relation of body size and composition to clinical biochemical and hematologic indices in US men and women. *Am J Clin Nutr*. (1989) 50:1276–81. doi: 10.1093/ajcn/50.6.1276

33. Ozata M, Mergen M, Oktenli C, Aydin A, Yavuz Sanisoglu S, Bolu E, et al. Increased oxidative stress and hypozincemia in male obesity. *Clin Biochem*. (2002) 35:627–31. doi: 10.1016/S0009-9120(02)00363-6

34. Hastka J, Lasserre JJ, Schwarzbeck A, Reiter A, Hehlmann R. Laboratory tests of iron status: correlation or common sense? *Clin Chem*. (1996) 42:718–24. doi: 10.1093/clinchem/42.5.718

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Comparison of venous and pooled capillary hemoglobin levels for the detection of anemia among adolescent girls

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Introduction: Blood source is a known preanalytical factor affecting hemoglobin (Hb) concentrations, and there is evidence that capillary and venous blood may yield disparate Hb levels and anemia prevalence. However, data from adolescents are scarce.

Objective: To compare Hb and anemia prevalence measured by venous and individual pooled capillary blood among a sample of girls aged 10–19 years from 232 schools in four regions of Ghana in 2022.

Methods: Among girls who had venous blood draws, a random subsample was selected for capillary blood. Hb was measured using HemoCue® Hb-301. We used Lin's concordance correlation coefficient (CCC) to quantify the strength of the bivariate relationship between venous and capillary Hb and a paired t-test for difference in means. We used McNemar's test for discordance in anemia cases by blood source and weighted Kappa to quantify agreement by anemia severity. A multivariate generalized estimating equation was used to quantify adjusted population anemia prevalence and assess the association between blood source and predicted anemia risk.

Results: We found strong concordance between Hb measures (CCC = 0.86). The difference between mean venous Hb (12.8 g/dL, ± 1.1) and capillary Hb (12.9 g/dL, ± 1.2) was not significant ($p = 0.26$). Crude anemia prevalence by venous and capillary blood was 20.6% and 19.5%, respectively. Adjusted population anemia prevalence was 23.5% for venous blood and 22.5% for capillary ($p = 0.45$). Blood source was not associated with predicted anemia risk (risk ratio: 0.99, 95% CI: 0.96, 1.02). Discordance in anemia cases by blood source was not significant (McNemar $p = 0.46$). Weighted Kappa demonstrated moderate agreement by severity ($\kappa = 0.67$). Among those with anemia by either blood source ($n = 111$), 59% were identified by both sources.

Conclusion: In Ghanaian adolescent girls, there was no difference in mean Hb, anemia prevalence, or predicted anemia risk by blood source. However, only 59% of girls with anemia by either blood source were identified as having anemia by both sources. These findings suggest that pooled capillary blood may

be useful for estimating Hb and anemia at the population level, but that caution is needed when interpreting individual-level data.

KEYWORDS

hemoglobin, anemia, blood source, capillary, venous, adolescents

Introduction

Anemia is a common blood condition and a global health problem that affects approximately 40% of children aged 6–59 months, 30% of nonpregnant women aged 15–49 years, and 36% of pregnant women aged 15–49 years (1). Iron deficiency is a key cause of anemia (2, 3), and evidence has shown that 25% of anemia cases in preschool children and 37% in women of reproductive age are attributable to iron deficiency (4). The World Health Organization (WHO) has established population prevalence ranges to indicate the magnitude of iron deficiency and anemia as public health problems, with prevalence of 5–19.9% considered mild, 20–39.9% considered moderate, and 40% and above considered high (3, 5). These ranges exist alongside recommendations for iron supplementation in school-age children and menstruating women in settings where the prevalence of anemia is 20% or higher (6, 7). The consequences of iron deficiency and anemia include adverse pregnancy outcomes (i.e., low birth weight, prematurity, and increased risk of maternal illness and mortality), compromised growth, suppressed immune function, impaired cognitive development, and loss of individual and national productivity (8–11). The prevention and detection of anemia in adolescent girls is of particular importance due to the increased need for micronutrients during this developmental life stage, with estimated average requirements for iron increasing by nearly 40% between the ages of 13 and 14 years (11, 12).

Anemia is diagnosed by comparing hemoglobin (Hb) levels, which can be measured in venous or capillary blood, to globally accepted thresholds (3, 13, 14). Depending on the context, either or both blood sources may be used to estimate population-level anemia prevalence and/or to screen for anemia at the individual level. Venous blood assessed by automated hematology analyzer has been established as the gold standard for anemia diagnosis (14, 15). However, due to the considerable cost and logistic demand of collecting venous blood (i.e., strict cold chain requirements for transportation and storage), governments and their partners must weigh the advantages of using the reference method against the significant inputs needed to successfully conduct national-scale health surveys. Collecting capillary blood via finger prick requires less training, uses only a small volume of blood, and does not require cold storage if analyzed immediately using a point-of-care hemoglobinometer (16). In real-world settings of Hb measurement, the single drop method is more commonly used than pooled capillary blood as it is relatively quicker to implement and requires fewer materials (e.g., microtubes, pipettes, and parafilm).

Blood source is a known preanalytical factor affecting Hb levels and anemia prevalence, alongside postural effect (i.e., sitting vs. standing), and environmental factors such as temperature and humidity (17). In a 2019 review, Whitehead et al. identified 12 studies which found higher mean Hb in capillary blood compared to venous blood, and three studies which found lower Hb levels in capillary blood compared to venous (17). In this same review, the authors identified four studies which compared single drop capillary blood to pooled capillary blood, finding distinct differences. Such inconsistencies in Hb levels by blood source translate into discrepancies in anemia diagnosis, which in turn influence clinical decisions and the interpretation of the anemia burden, intervention response, and program decision-making (14). A recent review of survey data from multiple countries found that anemia prevalence estimates were consistently lower in surveys using venous blood compared to capillary (18).

A limited number of studies have compared pooled capillary blood to venous blood for the measurement of Hb and detection of anemia. A recent study in Mexican children and adults found that venous and pooled capillary blood performed comparably in the determination of Hb levels (19), and a study among Indian women found that Hb estimates and anemia prevalence were not significantly different using pooled capillary blood compared to venous blood (20). Similarly, two earlier studies found that pooled capillary blood, compared to single drop, improved the precision of Hb measurement compared to estimates obtained using venous blood (21, 22). Although multiple studies have compared Hb level and/or anemia prevalence by blood source among young children and adults (14, 18–25), our literature review yielded no publications that have made such comparisons in adolescents aged 10–19 years.

Our objective is to compare Hb levels and anemia prevalence among adolescent girls in Ghana as detected by venous and individual pooled capillary blood and to ascertain if blood source is associated with adjusted population anemia risk. Such information could guide decision-making around preferred methods for estimating Hb for screening and clinical purposes, and for monitoring the anemia situation in-country to inform timely public health interventions.

Methods

Data collection and point-of-care procedures

We collected data in 2022 from 4,833 adolescent girls aged 10–19 years in Ghana participating in the government-led Girls' Iron-Folate Tablet Supplementation (GIFTS) program (26) (Figure 1). We used a multistage complex survey design in which schools (clusters) were selected by probability proportional to size sampling, from four ethnically and geographically diverse regions spanning three ecologic zones (strata), to achieve a nationally representative sample of school-going adolescents. Venous blood draws were obtained by trained phlebotomists from 4,819 girls. A random subsample of 441 girls was selected for additional individual pooled

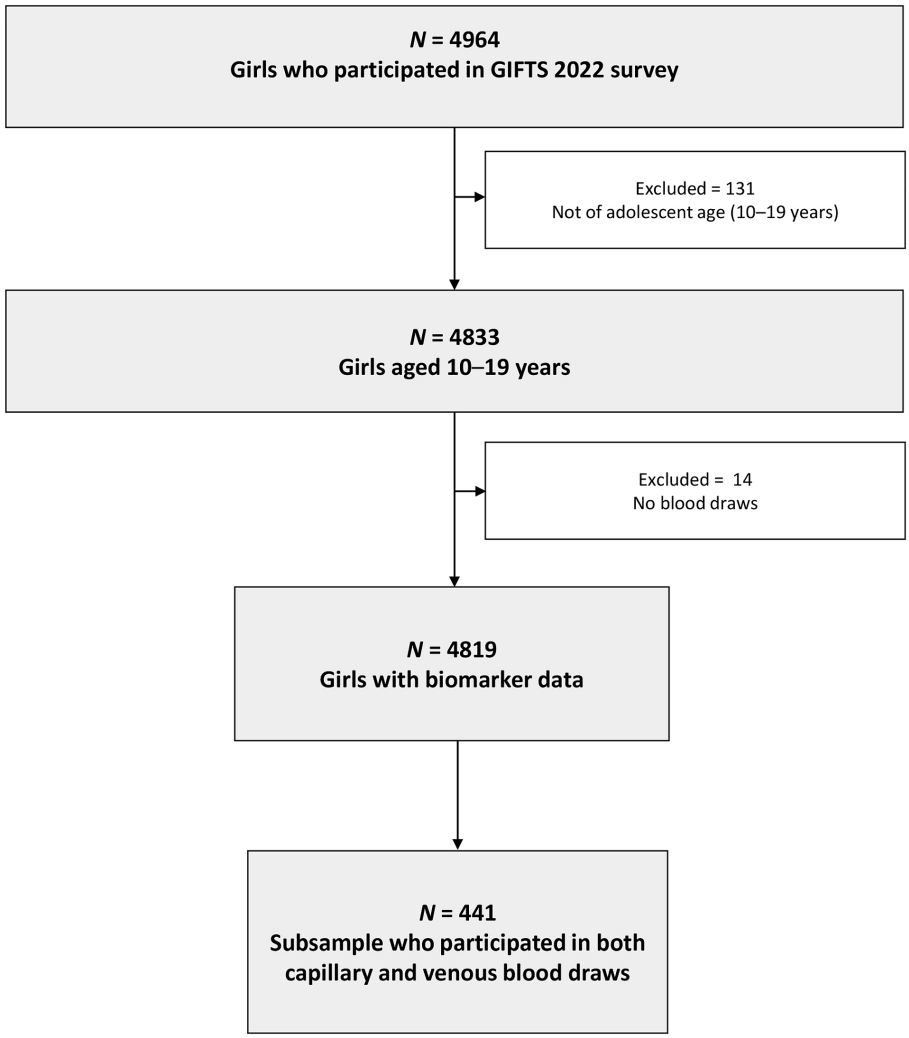

FIGURE 1
Participant flow chart of school-going adolescent girls from a nationally representative survey in Ghana, 2022. GIFTS, Girls' Iron-Folate Tablet Supplementation Program.

capillary blood draws. The HemoCue® Hb-301 System was used by phlebotomists to determine Hb status using venous blood at the point of care as well as pooled capillary blood among those in the subsample. Anemia status and severity were defined using the WHO Hb concentrations for the diagnosis of anemia (3). All girls who tested positive for malaria and/or had anemia were given a referral to seek medical care at a health facility per Ghana Ministry of Health protocol.

Statistical analyses

Individual-level data were analyzed and pooled to estimate population mean Hb and anemia prevalence from both venous and pooled capillary blood. We used a Bland–Altman plot to evaluate agreement between continuous venous and capillary Hb, Lin's concordance correlation coefficient (CCC) to quantify the strength of the bivariate relationship, and a paired t-test for difference in population mean Hb. In cross-tabulation analysis, we calculated agreement statistics (i.e., sensitivity, specificity, and accuracy) to determine the appropriateness of capillary blood for diagnosing anemia compared to venous blood as the reference method. McNemar's test was conducted to assess discordance in anemia cases identified by each blood source, and weighted Kappa was used to quantify agreement between the two at varying levels of anemia severity. We used a multivariate generalized estimating equation (GEE) with identity link function to quantify adjusted population anemia prevalence and assess if blood source is associated with the predicted risk of anemia (27). This model accounted for two blood measurements from each adolescent girl (1 venous, 1 pooled capillary) and multiple students from each school as random effects, as well as potential confounders (i.e., child age, school level, and region) as fixed effects. Covariance decomposition was used to partition intra-cluster correlation coefficients (ICC) and determine the variance in anemia prevalence due to intra-individual differences vs. phlebotomist technique (intra-school differences). All analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC) and R Studio version 4.3.1. Statistical significance was set at a two-sided alpha level of 0.05.

TABLE 1 Basic characteristics of school-going adolescent girls from a nationally representative survey in Ghana, 2022.

	Subsample with capillary blood draw (n = 441)		Sample without capillary blood draw (n = 4,378)		Overall sample (N = 4,819)		p-value[a]
	n	Mean ± SD/% (95% CI)	n	Mean ± SD/% (95% CI)	n	Mean ± SD/% (95% CI)	
Age, years	441	16.4 ± 1.7	4,378	16.4 ± 1.8	4,819	16.4 ± 1.8	0.91
Post-menarche	408	92.5 (90.1, 95.0)	4,043	92.3 (91.6, 93.1)	4,451	92.4 (91.6, 93.1)	0.90
School level							
Junior high school	222	50.3 (45.7, 55.0)	2,152	49.2 (47.7, 50.6)	2,374	49.2 (47.9, 50.7)	0.64
Senior high school	219	49.7 (45.0, 54.3)	2,226	50.8 (49.4, 52.3)	2,445	50.7 (49.3, 52.1)	0.64
School location							
Rural	128	29.0 (24.8, 33.3)	1,230	28.1 (26.8, 29.4)	1,358	28.2 (26.9, 29.5)	0.68
Peri-urban	37	8.4 (5.8, 11.0)	366	8.4 (7.5, 9.2)	403	8.4 (7.6, 9.1)	0.98
Urban	276	62.6 (58.1, 67.1)	2,782	63.5 (62.1, 65.0)	3,058	63.5 (62.1, 64.8)	0.69
Heard of anemia	284	64.4 (59.9, 68.9)	2,643	60.4 (58.9, 61.8)	2,927	60.7 (59.4, 62.1)	0.10
Ever had anemia (self-report)[b]	10	3.5 (1.4, 5.7)	142	5.4 (4.5, 6.2)	152	5.2 (4.4, 6.0)	0.26
Venous Hb (g/dL)	441	12.8 ± 1.1	4,378	12.6 ± 1.2	4,819	12.7 ± 1.2	0.03
Venous anemia	91	20.6 (16.8, 24.4)	984	22.5 (21.2, 23.7)	1,075	22.3 (21.1, 23.5)	0.38

Estimates are unweighted to ensure comparability between the overall sample and subsample. SD, standard deviation; CI, confidence interval. [a]p-values represent a comparison of the subsample with capillary blood draws (n = 441) to those without capillary blood draws (n = 4,378). [b]Among those who had heard of anemia.

Results

Basic characteristics

Among the subsample of 441 girls, the mean age was 16.4 (± 1.7) years and most girls (92.5%) had reached menarche (Table 1). The majority of girls attended school in urban areas (62.6%). Awareness of anemia was moderate (64.4%), and 10 girls (3.5%) reported that they had ever had anemia. A comparison of participant characteristics between the random subsample with capillary blood draw (n = 441) and those without (n = 4,378) suggested no selection bias (Table 1). The only significant difference observed between the two samples was for venous Hb, and the magnitude of difference was small (0.2 g/dL).

Hemoglobin

A density distribution plot indicated a complete overlap of Hb distributions with comparable means; venous Hb was 12.8 g/dL (± 1.1) while capillary Hb was 12.9 g/dL (± 1.2) (Figure 2; Table 2). No statistically significant difference in mean venous and pooled capillary Hb was observed (paired t-test, p = 0.26). From Bland–Altman analysis, the population mean difference in Hb was very low at −0.09 g/dL with 95% limits of agreement of −1.32 and + 1.15 g/dL (Figure 2). Further, CCC analysis demonstrated strong concordance between the two Hb measures (CCC = 0.86, precision = 0.86, accuracy = 0.99).

Anemia

The crude prevalence of anemia by venous and pooled capillary blood was 20.6% (91 of 441 girls) and 19.5% (86 of 441 girls), respectively, and the majority of cases were mild (Table 2). The sensitivity and specificity of capillary blood for diagnosing anemia compared to venous blood were 0.73 and 0.94, respectively, while accuracy was 0.90. The discordance in anemia cases identified by the two blood sources was not significant (McNemar's test, p = 0.46). A total of 111 anemia cases were identified by either blood source, and among these cases, 66 (59%) were identified by both sources (Figure 3). By anemia severity—mild (n = 93), moderate (n = 32), and severe (n = 2)—38, 47, and 0% of cases were identified by both blood sources, respectively (Figure 3). Weighted Kappa demonstrated moderate agreement by severity (κ = 0.67). Because only two cases were identified as severe anemia (one by each blood source), we calculated a second weighted Kappa after combing moderate and severe cases and found that the outcome was not meaningfully different (κ = 0.68) (28).

Our GEE model to assess the association between blood source and risk of anemia, controlling for child age, school level, and geographic region as fixed effects, yielded an adjusted anemia prevalence of 23.5% for venous blood and 22.5% for capillary, a difference which was not significant (p = 0.45) (Figure 2). Blood source was not associated with the predicted risk of anemia (risk ratio: 0.99, 95% confidence interval: 0.96, 1.02). Covariance analysis indicated that intra-individual differences accounted for the majority of variance in anemia prevalence (ICC: 0.67), while school effect or phlebotomist technique accounted for minimal variance (ICC: 0.02). The remaining 31% of variance resulted from fixed effect variables, i.e., child age, school level, and geographic region of survey.

Discussion

In our sample of Ghanaian adolescent girls, there was no difference in mean Hb or anemia prevalence as estimated by venous and pooled

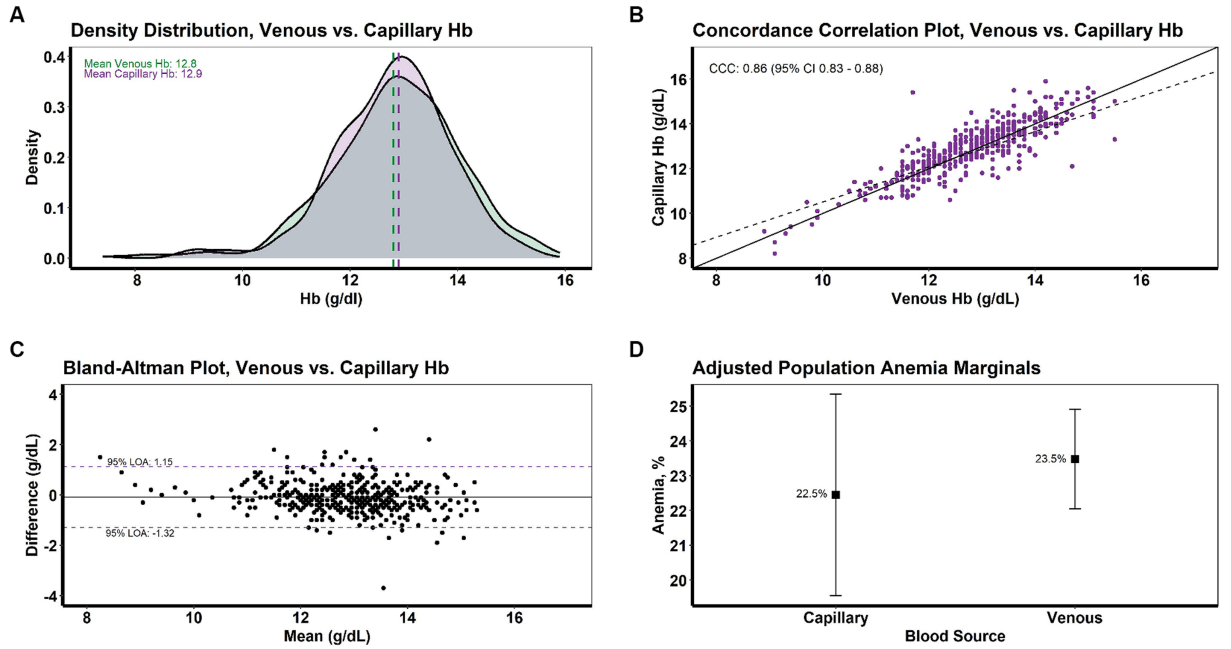

FIGURE 2
Intra-individual concordance of venous and pooled capillary hemoglobin levels and anemia among school-going adolescent girls from a nationally representative survey in Ghana, 2022 (*N* = 441). Comparisons shown include **(A)** density distribution for Hb, **(B)** concordance correlation plot for Hb, **(C)** Bland-Altman plot for Hb, and **(D)** adjusted population anemia marginals. Hb, hemoglobin; CCC, concordance correlation coefficient; LOA, limit of agreement.

TABLE 2 **Comparison of hemoglobin levels and anemia prevalence by blood source among school-going adolescent girls from a nationally representative survey in Ghana, 2022 (*N* = 441).**

	Venous		Capillary		Measure of agreement/ Difference
	n	Mean ± SD/ Proportion (95% CI)	*n*	Mean ± SD/ Proportion (95% CI)	
Mean Hb (g/dL) ± SD	441	12.8 ± 1.1	441	12.9 ± 1.2	CCC = 0.86, precision = 0.86, accuracy = 0.99[a] *T*-test, $p = 0.26$
Anemia	91	20.6 (16.8, 24.2)	86	19.5 (15.8, 23.2)	McNemar, $p = 0.46$ Weighted Kappa, $\kappa = 0.67$
Mild	67	15.2 (11.8, 18.6)	61	13.8 (10.6, 17.1)	
Moderate	23	5.2 (3.1, 7.3)	24	5.4 (3.3, 7.6)	
Severe	1	0.2 (0.0, 0.7)	1	0.2 (0.0, 0.7)	
Anemia[b]	91	20.6 (16.8, 24.2)	86	19.5 (15.8, 23.2)	Sensitivity: 0.73 Specificity: 0.94 Accuracy: 0.90

SD, standard deviation; CI, confidence interval; CCC, concordance correlation coefficient. [a]The CCC contains both precision and accuracy measures, where precision represents the Pearson correlation coefficient and accuracy represents a bias correction factor which measures how far the best-fit line deviates from the line of origin. [b]Agreement statistics were calculated in cross-tabulation analysis using venous blood as the reference method.

capillary blood, suggesting that blood source is not a factor in determining anemia status in this population. These findings are consistent with recent studies in Mexico and India which also found that pooled capillary blood and venous blood performed similarly for determining Hb and detecting anemia (19, 20). In linear analysis, we found that blood source was not associated with anemia risk, with adjusted population anemia prevalence differing by a single percentage point. However, despite substantial agreement between the two blood sources (e.g., sensitivity of 0.73 and accuracy of 0.90 with venous blood as the reference method), we found that they do not detect the same girls as having anemia; only 59% (66 of 111) of anemia cases identified by either blood source were identified by both sources (Figure 3). This finding may have practical implications for researchers and public health workers seeking the most appropriate method for screening and diagnosing iron deficiency and anemia. While pooled capillary blood may be appropriate for population-level analyses, the precision offered by venous blood measures may be preferrable for measuring Hb and diagnosing anemia at the individual level. Decision-makers must weigh the operational feasibility of implementing gold standard procedures compared to those that are simpler and less expensive to execute.

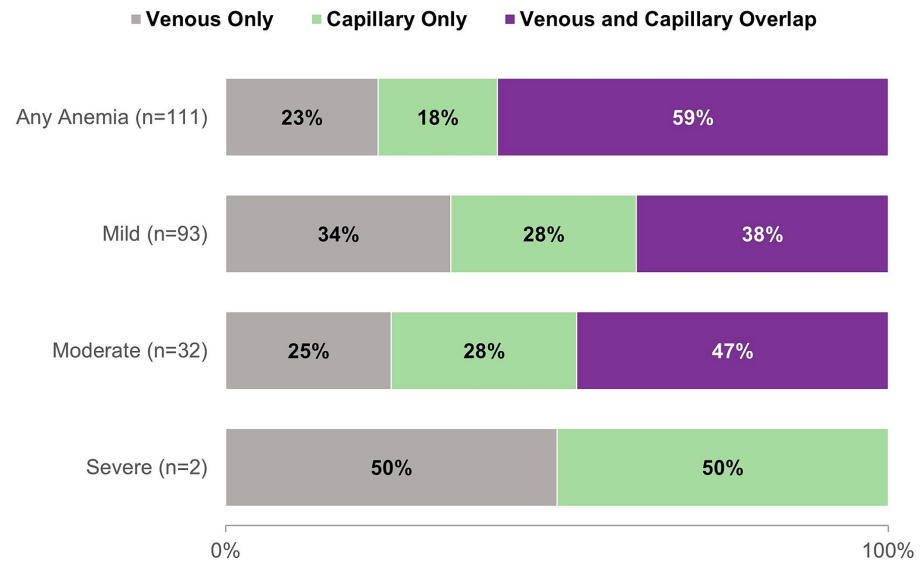

FIGURE 3
Proportion of anemia cases diagnosed by venous and pooled capillary Hb for any anemia and by severity among school-going adolescent girls from a nationally representative survey in Ghana, 2022 ($N = 111$).

Our analysis of anemia prevalence by blood source and severity showed only moderate agreement ($\kappa = 0.67$), with less than half of cases identified by either blood source being identified by both sources for all levels of severity. This finding is likely driven by the difference in mild vs. moderate anemia diagnosis by blood source, as the two categories are distinguished by a difference in Hb of only 0.1 g/dL (3).

Historically, a single drop of capillary blood has been used to assess anemia in the Demographic and Health Survey (DHS). The 2014 and 2022 DHS in Ghana found an anemia prevalence of 48% and 44% among girls aged 15–19, respectively (29, 30). Yet, the Ghana National Micronutrient Survey conducted in 2017 using venous blood from nonpregnant women 15–49 years revealed an anemia prevalence of 26% among adolescent girls aged 15–19 (31), substantially lower than the DHS findings in the same age group. Such discrepancies create doubt regarding the use of capillary blood for population surveys. The findings from our study have suggested that, with quality training on technique, pooled capillary blood may be a viable alternative to single drop in Ghana and could be relevant to other resource-constrained settings where it is not feasible to incorporate venous blood draws into clinical settings, annual surveys, and surveillance systems. We found that only 2% of variance in intra-individual anemia prevalence was attributable to phlebotomist technique, an encouraging result suggesting strong capacity within existing public health service platforms in Ghana to collect high-quality biological data.

Strengths and limitations

The strengths of our study include a large nationally representative sample of school-going Ghanaian adolescent girls from whom multiple biological samples were collected, enabling adjustment for intra-individual variation while isolating the role of blood source on population Hb levels and anemia prevalence. Further, our comparison of the random subsample of girls with capillary blood draw to those without suggested no selection bias, and thus our findings may be generalizable to the broader population of school-going adolescent girls in Ghana. However, as our data are from a specific population in a single country, findings should be interpreted cautiously and might not be applicable to other population groups, even within Ghana.

Conclusion

Among Ghanaian adolescent girls, there was no difference in mean Hb, anemia prevalence, or predicted anemia risk by blood sample collection method. However, there was some discrepancy in anemia cases identified by venous and pooled capillary blood at the individual level, for any anemia and by severity. These findings suggest that, while pooled capillary blood may be useful for estimating Hb and anemia at the population level among Ghanaian adolescent girls, the same might not be true for individual-level analyses. Future efforts in this context should involve careful consideration of goals and objectives to inform the choice of blood sample collection method.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethical Review Committee of the Ghana Health Service. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

MiJ: Resources, Supervision, Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. EA: Resources, Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. MA: Supervision, Resources, Writing – review & editing, Project administration, Investigation, Conceptualization. VQ: Writing – review & editing, Supervision, Project administration, Investigation, Resources, Conceptualization. RS: Resources, Writing – review & editing, Project administration. PO-A: Resources, Writing – review & editing, Project administration, Investigation. JA: Resources, Writing – review & editing, Project administration, Investigation. MD: Writing – review & editing, Project administration, Investigation. LG: Writing – review & editing, Project administration, Conceptualization. CM: Writing – review & editing, Investigation. MaJ: Resources, Project administration, Writing – review & editing, Supervision, Conceptualization. OA: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Conflict of interest

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

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References

1. Stevens GA, Paciorek CJ, Flores-Urrutia MC, Borghi E, Namaste S, Wirth JP, et al. National, regional, and global estimates of anaemia by severity in women and children for 2000-19: a pooled analysis of population-representative data. *Lancet Glob Health*. (2022) 10:e627–39. doi: 10.1016/S2214-109X(22)00084-5

2. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. (2014) 123:615–24. doi: 10.1182/blood-2013-06-508325

3. World Health Organization. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Available at: https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?ua=1

4. Petry N, Olofin I, Hurrell RF, Boy E, Wirth JP, Moursi M, et al. The proportion of Anemia associated with Iron deficiency in low, medium, and high human development index countries: a systematic analysis of National Surveys. *Nutrients*. (2016) 8:693. doi: 10.3390/nu8110693

5. World Health Organization. *WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations*. Geneva: World Health Organization (2020).

6. World Health Organization. *WHO Guideline: Intermittent iron supplementation in preschool and school-age children*. Geneva: World Health Organization (2011).

7. World Health Organization. *WHO Guideline: Intermittent iron and folic acid supplementation in menstruating women*. Geneva: World Health Organization (2011).

8. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet*. (2013) 382:427–51. doi: 10.1016/S0140-6736(13)60937-X

9. Georgieff MK. Iron deficiency in pregnancy. *Am J Obstet Gynecol*. (2020) 223:516–24. doi: 10.1016/j.ajog.2020.03.006

10. Darnton-Hill I, Webb P, Harvey PW, Hunt JM, Dalmiya N, Chopra M, et al. Micronutrient deficiencies and gender: social and economic costs. *Am J Clin Nutr*. (2005) 81:1198S–205S. doi: 10.1093/ajcn/81.5.1198

11. Norris SA, Frongillo EA, Black MM, Dong Y, Fall C, Lampl M, et al. Nutrition in adolescent growth and development. *Lancet*. (2022) 399:172–84. doi: 10.1016/S0140-6736(21)01590-7

12. Institute of Medicine. *Dietary reference intakes: The essential guide to nutrient requirements*. Washington, DC: The National Academies Press (2006).

13. Cascio MJ, DeLoughery TG. Anemia: evaluation and diagnostic tests. *Med Clin North Am*. (2017) 101:263–84. doi: 10.1016/j.mcna.2016.09.003

14. Neufeld LM, Larson LM, Kurpad A, Mburu S, Martorell R, Brown KH. Hemoglobin concentration and anemia diagnosis in venous and capillary blood: biological basis and policy implications. *Ann N Y Acad Sci*. (2019) 1450:172–89. doi: 10.1111/nyas.14139

15. Fothergill A, Crider KS, Johnson CB, Raj MP, Guetterman HM, Bose B, et al. Comparison of Anemia screening methods using paired venous samples in women of reproductive age in southern India. *J Nutr*. (2023) 152:2978–92. doi: 10.1093/jn/nxac218

16. Bond MM, Richards-Kortum RR. Drop-to-drop variation in the cellular components of fingerprick blood: implications for point-of-care diagnostic development. *Am J Clin Pathol*. (2015) 144:885–94. doi: 10.1309/AJCP1L7DKMPCHPEH

17. Whitehead RD Jr, Mei Z, Mapango C, Jefferds MED. Methods and analyzers for hemoglobin measurement in clinical laboratories and field settings. *Ann N Y Acad Sci*. (2019) 1450:147–71. doi: 10.1111/nyas.14124

18. Hruschka DJ, Williams AM, Mei Z, Leidman E, Suchdev PS, Young MF, et al. Comparing hemoglobin distributions between population-based surveys matched by country and time. *BMC Public Health*. (2020) 20:422. doi: 10.1186/s12889-020-08537-4

19. De la Cruz-Gongora V, Mendez-Gomez-Humaran I, Gaona-Pineda EB, Shamah-Levy T, Dary O. Drops of capillary blood are not appropriate for hemoglobin measurement with point-of-care devices: a comparative study using drop capillary, pooled capillary, and venous blood samples. *Nutrients*. (2022) 14:346. doi: 10.3390/nu14245346

20. Dasi T, Palika R, Pullakhandam R, Augustine LF, Boiroju NK, Prasannanavar DJ, et al. Point-of-care Hb measurement in pooled capillary blood by a portable autoanalyser: comparison with venous blood Hb measured by reference methods in cross-sectional and longitudinal studies. *Br J Nutr*. (2022) 128:1108–17. doi: 10.1017/S0007114521004347

21. Conway AM, Hinchliffe RF, Earland J, Anderson LM. Measurement of haemoglobin using single drops of skin puncture blood: is precision acceptable? *J Clin Pathol*. (1998) 51:248–50. doi: 10.1136/jcp.51.3.248

22. Mills AF, Meadows N. Screening for anaemia: evaluation of a haemoglobinometer. *Arch Dis Child*. (1989) 64:1468–71. doi: 10.1136/adc.64.10.1468

23. Neufeld L, Garcia-Guerra A, Sanchez-Francia D, Newton-Sanchez O, Ramirez-Villalobos MD, Rivera-Dommarco J. Hemoglobin measured by Hemocue and a reference method in venous and capillary blood: a validation study. *Salud Publica Mex*. (2002) 44:219–27. doi: 10.1590/S0036-36342002000300005

24. Patel AJ, Wesley R, Leitman SF, Bryant BJ. Capillary versus venous haemoglobin determination in the assessment of healthy blood donors. *Vox Sang*. (2013) 104:317–23. doi: 10.1111/vox.12006

25. Boghani S, Mei Z, Perry GS, Brittenham GM, Cogswell ME. Accuracy of capillary hemoglobin measurements for the detection of Anemia among U.S. low-income toddlers and pregnant women. *Nutrients*. (2017) 9:253. doi: 10.3390/nu9030253

26. Gosdin L, Sharma AJ, Tripp K, Amoaful EF, Mahama AB, Selenje L, et al. A school-based weekly Iron and folic acid supplementation program effectively reduces Anemia in a prospective cohort of Ghanaian adolescent girls. *J Nutr*. (2021) 151:1646–55. doi: 10.1093/jn/nxab024

27. Liang KL, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika*. (1986) 73:13–22. doi: 10.1093/biomet/73.1.13

28. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. (2012) 22:276–82. doi: 10.11613/BM.2012.031

29. Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF International. *Ghana demographic and health survey 2014*. Rockville, MD: GSS, GHS, and ICF International (2015).

30. Ghana Statistical Service (GSS) and ICF. *Ghana demographic and health survey 2022: Key indicators report*. Accra, Ghana: GSS and ICF (2023).

31. Wegmuller R, Bentil H, Wirth JP, Petry N, Tanumihardjo SA, Allen L, et al. Anemia, micronutrient deficiencies, malaria, hemoglobinopathies and malnutrition in young children and non-pregnant women in Ghana: findings from a national survey. *PLoS One*. (2020) 15:e0228258. doi: 10.1371/journal.pone.0228258

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The increased tendency for anemia in traditional Chinese medicine deficient body constitution is associated with the gut microbiome

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Background: Constitution is a valuable part of traditional Chinese medicine theory; it is defined as the internal foundation for the occurrence, development, transformation and outcome of diseases, and has its characteristic gut microbiota. Previous study showed that deficiency constitution was related to lower Hb counts. However, no research has examined how alterations in the gut microbiome induced by deficiency constitution may increase the tendency for anemia.

Methods: We used a multiomics strategy to identify and quantify taxonomies and compounds found under deficient constitution individuals and further explore the possible pathological factors that affect red blood cell indices.

Results: ① People with deficient constitution showed lower hemoglobin (Hb), more Firmicutes, less Bacteroidetes, and higher α diversity. ② We identified *Escherichia coli*, *Clostridium bolteae*, *Ruminococcus gnavus*, *Streptococcus parasanguinis* and *Flavonifractor plautii* as potential biomarkers of deficient constitution. ③ *Slackia piriformis*, *Clostridium_sp_L2_50* and *Bacteroides plebeius* were enriched in balanced-constitution individuals, and *Parabacteroides goldsteinii* was the key bacterial marker of balanced constitution. ④ *Flavonifractor plautii* may be a protective factor against the tendency for anemia among deficient individuals. ⑤ *Ruminococcus gnavus* may be the shared microbe base of deficiency constitution-related the tendency for anemia. ⑥ The microorganism abundance of the anaerobic phenotype was lower in deficient constitution group. ⑦ Alterations in the microbiome of deficient-constitution individuals were associated with worse health status and a greater risk of anemia, involving intestinal barrier function, metabolism and immune responses, regulated by short-chain fatty acids and bile acid production.

Conclusion: The composition of the gut microbiome was altered in people with deficient constitution, which may explain their poor health status and tendency toward anemia.

KEYWORDS

traditional Chinese medicine constitution, deficient constitution, anemia, gut microbiome, serum metabolomics

1 Introduction

Constitution is an important aspect of traditional Chinese medicine theory. Constitution is defined as the internal foundation for the emergence, development, transformation and outcome of diseases. The discipline of traditional Chinese medicine constitution divides people into nine subgroups: balanced, relatively healthy constitution and eight unbalanced constitutions (qi-deficient, yang-deficient, yin-deficient, phlegm-dampness, dampness-heat, blood stasis, qi-stagnation, and inherited special constitution). People in each unbalanced constitution subgroup are susceptible to certain diseases, a classification that helps us choose specific intervention and disease prevention strategies (1).

According to the traditional Chinese medicine theory, people with a balanced constitution are full of energy, fit, and not predisposed to becoming ill. People in the excessive constitution are likely stucked by some harmful substances (phlegm-dampness, dampness-heat, blood stasis or qi) disturbing physical functions, while people in the deficient constitution are likely lack of some important substances (yang-qi or yin-liquid) maintaining physical functions. A literature review of 1,639 clinical studies showed that none of the research found a relation between the balanced constitution and any disease, while 333 kinds of diseases are closely associated with eight unbalanced constitutions. People in the yang-deficient constitution, yin-deficient constitution and qi-deficient constitution groups, are likely to experience diabetes, stroke and hypertension (especially females) (2). Previous study showed that qi-deficient constitution is accompanied by immune dysfunction leading to inability to resist pathogenic microorganisms (3); yang-deficient constitution exhibit deregulation of various genes involved in energy, lipid, and glucose metabolism (4); yin-deficient constitution related to certain differentially expressed genes associated with the hypothalamus-pituitary–adrenal axis, hypothalamus-pituitary-thyroid axis, cyclic nucleoside system, and immune function (5).

Overall, people in the these three groups all present deficiency statuses of physical and functional, related immunocompromised and hypometabolism (6, 7). One study showed that creatinine (CRE), thyroid-stimulating hormone (TSH), red blood cells (RBCs) and hemoglobin (Hb) levels were lower in people with yang-deficient constitution than in those without (8). Another study of 60 female volunteers aged 35–49, showed that the values of triglyceride (TG), Hb, platelet distribution width (PDW), mean platelet volume (MPV), red cell distribution width standard deviation (RDW-SD) and platelet/larger cell ratio were lower in the yin deficiency group, while the values of white blood cell (WBC) count and RDW coefficient of variation (RDW-CV) were higher in the balanced group (9). Coincidentally, we also found lower Hb counts in individuals with a deficient constitution based on the data from regular physical examinations among hospital staff, and most of these individuals were female.

The World Health Organization defines anemia as a condition in which the number of RBCs or the Hb concentration within them is lower than normal. In adults, anemia is defined as Hb levels of <12.0 g/dL in women and <13.0 g/dL in men (10). Anemia can be categorized into three types: decreased production, increased destruction and loss of red blood cells through bleeding. All types of anemia result in impaired oxygen delivery to tissues, causing symptoms like weakness and fatigue. The causes of anemia are often multifactorial. Nutritional deficiencies are the most common risk factors for anemia in developing countries. These can result from insufficient dietary intake, increased nutrient losses (e.g., blood loss from parasites, childbirth hemorrhage, or heavy menstrual bleeding), damaged absorption (e.g., lack of intrinsic factor for vitamin B12 absorption, high phytate intake, or *Helicobacter pylori* infection affecting iron absorption), or altered nutrient metabolism (e.g., vitamin A or riboflavin deficiency impaired iron mobilization) (11, 12).

The results of RBC indices are used to diagnose different types of anemia, and each type has a different effect on the size, shape, and/or quality of red blood cells (13). Specifically, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were earliest purposed by Wintrobe in 1929 to define the size and hemoglobin content of red blood cells. If people with larger MCV and lower MCH than normal, it may indicates iron deficiency anemia. If people with smaller MCV and higher MCH than normal, it may indicates vitamin B deficiency related anemia. If people with larger MCV and lower MCHC than normal, it may indicates Thalassemia. If people with higher MCHC than normal, it may indicates hemolytic anemia or hereditary spherocytosis (14). Red cell distribution width (RDW) can be used to quantify the variation in the size of red cells. If people with increased RDW, it may indicates nutritional anemia and sideroblastic anemia. Hematocrit (HCT) is the proportion of blood volume occupied by packed RBC. For many practical approaches, a decrease in HCT is considered equivalent to a decreased Hb concentration (15). These aforementioned values categorized as red blood cell indices are all related to different etiology of anemia, so we assumed people with or without anemia but with one of abnormal red blood cell indices (including Hb, RBC, MCV, MCH, MCHC, RDW and HCT) may have the tendency for anemia in this study.

Over recent years, research on the traditional Chinese medicine and its regulation of intestinal microbiome to improve the symptoms of disease is very extensive (16–19). Different constitution subgroups in Chinese medicine have not only specific clinical characteristics but also characteristic gut microbiota and pathological bases (1, 20–23). A previous report indicated that the α diversity of balanced constitution individuals was considerably higher than that of qi-deficient constitution individuals and the contrast of the β diversity

Abbreviations: CCMQ, Constitution in Chinese Medicine Questionnaires; BCNG, Balanced Constitution Normal Group; BCAG, Balanced Constitution Abnormal Group; DCNG, Deficiency Constitution Normal Group; DCAG, Deficiency Constitution Abnormal Group; BCG, Balanced Constitution Group; DCG, Deficiency Constitution Group; NG, Normal Group; AG, Abnormal Group.

was notably detected between them, having 122 and 4 bacterial taxa that were significantly overactive, respectively. The operational potential of qi-deficient constitution bacterial taxa was decreased in butanoate metabolism and fatty acid metabolism (23). Another randomized clinical trial demonstrated that yang-deficient constitution samples exhibited significantly lower species and showed an increased abundance of Bacteroidetes and Bacteroides, as well as increased levels of gut microbial-derived urinary metabolites, compared to balanced-constitution samples (21).

The intestinal environment is complicated and dynamic, and it significantly influences the host's constitution. As the lumen of the colon lacks oxygen, anaerobic microbes, such as Bacteroides, Clostridium, and Ruminococcus, are its main occupants, but the numbers of oxygen-tolerant facultative anaerobes and anaerobes increase during dysbiosis (24, 25). Aplastic anemia (26), gestational anemia (27, 28), iron deficiency anemia (29) and cancer-related anemia (30) have been associated with changes in the gut microbiome. On the basis of these conclusions, we hypothesized that changes in the intestinal anaerobic environment may be related to deficient constitution-related increase tendency for anemia.

Therefore, we hypothesized that women with deficient constitution have different microbial compositions and structures from those with a balanced constitution, which predisposes them to specific diseases, such as anemia, associated with intestinal anaerobic environmental disorders.

2 Materials and methods

2.1 Subjects

The study was conducted at Guangdong Provincial Hospital of Chinese Medicine, Fangcun Branch, Guangzhou, China, among internal clinical staff. This study conformed to the Declaration of Helsinki and the protocol was officially agreed upon the Ethics Committee of the Guangdong Provincial Hospital of Chinese Medicine (Ethical review number: B2017-199-01) and was conducted according to approved guidelines and regulations. All subjects offered verbal informed consent and were able to quit at any time. Data privacy was protected throughout this study.

2.2 Design and study population

The hospital staff members underwent a health assessment at the hospital, involving medical history, physical examination, blood hematology, biochemistry analyses and traditional Chinese medicine constitution assessment. The initial details included sex and age. Systolic blood pressure (SBP), diastolic blood pressure (DBP), height, weight, waist circumference and hip circumference were also collected. We used the Constitution in Chinese Medicine Questionnaires (CCMQ) to identify traditional Chinese medicine constitution (31). People with clinical conditions of yang deficiency, yin deficiency and qi deficiency according to the CCMQ responses were categorized as the deficient constitution group (DCG), while people without any clinical conditions of basis constitutions were cataloged as the balanced constitution group (BCG). Fifty-two clinical features were assessed in this study. According to the results Hb, RBC, HCT, MCV, MCH, MCHC and RDW, people were also divided into a normal group (NG) and an abnormal group (AG) if one of the indices was out of the normal range. We assumed people with one of abnormal red blood cell indices (including Hb, RBC, MCV, MCH, MCHC, RDW and HCT) may have the tendency for anemia in this study. As for sample size, a convenience sample was chosen because it was beyond the scope of the study to include a statistically powered sample size due to the limitation of resources.

2.2.1 Inclusion criteria of participants

① Age ≥ 18 years and ≤ 60 years; ② Female; ③ Underwent a health assessment in the hospital, including medical history, physical examination, blood hematology, biochemistry analyses and traditional Chinese medicine constitution assessment; ④ Categorized as the deficient constitution (including yang deficiency, yin deficiency and qi deficiency) or balanced constitution according to the CCMQ; ⑤ The informed consent shall be agreed by the participants.

2.2.2 Exclusion criteria of participants

① Age < 18 years or > 60 years; ② Male; ③ During menstruation, pregnancy, child birth and/or baby nursing period; ④ Accompanied by hematological malignancies or other diseases that could significantly affect survival; ⑤ Categorized as the excesssive constitution (including phlegm-dampness, dampness-heat, blood-stasis, and qi-depression) or special-diathesis constitution according to the CCMQ; ⑥ Used antibiotics in last 6 months.

Finally, 129 participants, including 23 individuals in the deficient constitution associated with the DCAG, 56 individuals in the DCNG, 15 individuals in the BCAG and 35 individuals in the BCNG, were selected. A total of 127 fecal and serum samples were collected.

2.3 Traditional Chinese medicine constitution assessment

Considering its validity and reliability, the CCMQ was used to assess traditional Chinese medicine constitution, which comprises 9 subscales with a total of 60 questions, of each scored from 1 (none) to 5 (always), evaluating the constitution type by adding the item scores based on Wang Qi's nine-point method of constitution classification. This method divides human constitution into nine types, among which balanced constitution is the healthiest type, while the other eight types are pathological types hinting at unsatisfactory status (31). People with qi-deficient, yang-deficient, and yin-deficient constitutions are deficient, while those with phlegm-dampness, dampness-heat, blood-stasis, and qi-depression constitutions are excesssive.

2.4 Blood biochemical analysis

This study assessed 52 biomarkers as the clinical features: albumin (ALB), globulin (GLB), albumin/globulin (ALB/GLB), alanine transaminase (ALT), aspartate transaminase (AST), aspartate transaminase/alanine transaminase (AST/ALT), serum gamma-glutamyl transferase (GGT), alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), creatinine (Cre), urine acid (UA), fasting blood glucose (Glu), serum total cholesterol (TC), TG,

high-density lipoprotein cholesterol (HDLC), nonhigh-density lipoprotein cholesterol (non HDLC), low-density lipoprotein cholesterol (LDLC), serum total bile acid (TBA), TSH, C-reactive protein (CRP), anti-cyclic peptide containing citrulline (anti-CCP), Epstein–Barr virus nuclear antigen immunoglobulin A (NA IgA), Epstein–Barr virus viral capsid antigen immunoglobulin A (VCA IgA), procalcitonin (PCT), functional thyroid 3 (FT3), free triiodothyronine (FT3), free thyroxine (FT4), total protein (TP), urea, estimated glomerular filtration rate (eGFR), interleukin-27 (IL27), RBC, WBC count, Hb, HCT, MCV, MCH, MCHC, platelet (PLT), neutrophil (NEUT), percentage of neutrophils (NEUT%), lymphocyte (LYM), percentage of lymphocyte (LYM%), monocyte (MONO), percentage of monocyte (MONO%), eosinophil (EOSIN), percentage of eosinophil (EOSIN%), basophil (BASO), percentage of basophil (BASO%), Meam platelet volume (MPV), RDW and platelet distribution width (PDW).

2.5 Fecal sample collection, sequencing and microbial data analysis

Stool samples of at least 2 g were saved, placed in arid tubs and stored at −80°C before gDNA extraction. After isolation, the DNA was sonicated for fragmentation, resulting a size of 350 bp. The library was prepared by using the TruSeq DNA HT Sample Prep Kit. Suitable libraries were screened and sequenced by a paired-end strategy with the Illumina HiSeq 2000 platform. After filtering out low-quality and host derived sequences, metagenomics bioinformatics pipeline was performed according to the bioBakery3 (32). After preprocessing, there were around 6 G of clean data for each sample. We used MetaPhlan2 to obtain the taxonomy of the metagenomes and applied HUMAnN2 to characterize the microbial gene and biochemical pathways (33, 34). The bacterial α diversity was evaluated by the Shannon index, observed species, and simpson index. Differences in microbial characteristics were examined by the Wilcoxon rank-sum test and linear discriminant analysis effect size (LEfSe) (35).

2.6 Serum sample collection, sequencing and metabolic data analysis

Blood and stool samples were taken from each volunteer. The 100 μL serum of every sample was positioned in a 96-well plate and mixed by 300 μL of precooled extract solution (methanol: acetonitrile, 2:1, v/v). After brief vortex, the samples were incubated for 2 h at −20°C, centrifuged at 4000 rpm for 20 min, freeze-dried, redissolved in 150 μL of 50% methanol, and then centrifuged at 4000 rpm for 30 min. One hundred microliters of sample was drawn out aim for metabolite extraction. A quality control sample was made by combining equal amounts of supernatants from all samples. LC–MS/MS analysis was conducted using the UHPLC system (Vanquish, Thermo Fisher Scientific) coupled to a Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). After converting the raw data to mzXML format, peak detection, extraction, alignment and integration were performed sequentially using ProteoWizard. PCA and OPLS-DA models were used to discriminate the classifications for different groups in score plots. Then, based on OPL-DA loading plots, we selected ions with VIP > 1.5 to identify significant metabolites in each group. Next, univariate analysis of t test and fold-change were used for those notable distinctive ions with VIP > 1.5 and fold-changes > 1.5 (or < 0.67). They were recognized and interpreted according to studies of their exact masses in metabolomic-associated databases: METLIN,[1] HMDB[2] and KEGG.[3] Metabolomics bioinformation analysis was performed by MetaboAnalyst software.[4]

2.7 Statistical analysis

All data were double recorded to guarantee correct entry. The fundamental characteristics of the objects were reported by counts and abundances. Statistical analysis was performed using the R statistical package (v.4.0.4). The vegan package was used for microbial community ecology analysis, and the distribution-free test, Kruskal–Wallis test, Wilcoxon test, and Spearman correlation test were uesd for relative abundance analysis. Factor analysis and one-way analysis of variance were used for normally distributed data. MaAsLin 2(Microbiome Multivariable Association with Linear Models) was conducted to explore the association between metobolites as an independent variable and the count of clinical biomarkers (including deficiency constitution group, age, BMI, MCHC, MCH, MCV, RDW, HCT, RBC, Hb counts) as the dependent variable or covariates. $P < 0.05$ was defined as statistically significant.

2.8 Integrative multi-omic analysis

Procrustes analysis was performed to examine the congruence of the two-dimensional shapes generated by PCAs between metabolomics and microbiome by R vegan package.

Two-way Orthogonal Partial Least Squares analysis (O2PLS) performed by R OmicsPLS package was used to reveal the relationship between microbiota at species level and metabolites, in which, microbiota data were mapped to metabolites on low dimensional hyper planes. Microbiota with larger VIP value (>1), are the most related to explain metabolites. The correlation matrix display the pair-wise correlation between all variables.

MetOrigin was uesd to trace the origins of bacteria and identify their certain metabolic reactions involved for understanding the complicated interaction between microbiome and metabolites. It was performed on its authorized website.[5]

Stepwise mediation analysis performed by R mediate package was conducted for multi-omic modules along the microbiome-metabolite-host axis to assess the relationship of the constitution related-microbiome with the tendency for anemia, with Hb, RBC, HCT, MCV, MCH, MCHC and RDW being a continuous dependent variable, and the tendency for anemia being a discrete dependent variable.

1 www.metlin.scripps.edu
2 www.hmdb.ca
3 www.genome.jp/kegg
4 http://www.metaboanalyst.ca/
5 http://metorigin.met-bioinformatics.cn

3 Results

3.1 Summary of clinical characteristics

A total of 129 female hospital staff members were included in our study, including 35 people in the balanced constitution mormal group (BCNG), 15 people in the balanced constitution abnormal group (BCAG), 56 people in the deficient constitution normal group (DCNG) and 23 people in the deficient constitution abnormal group (DCAG). Compared to the broad balanced constitution group (BCG), the broad deficient constitution group (DCG) had lower Hb, mean corpuscular hemoglobin concentration (MCHC), systolic blood pressure (SBP), and total bile acids (TBA) values, tending toward anemia characteristics. There was no significant difference in other indicators between the two groups including age (Table 1).

3.2 Differences in community composition of the gut microbiota

To distinguish the relative abundances of gut microbiota between groups, we examined the taxonomic composition in gut microbiota of different group samples at the phylum level (Figure 1A), and further explored the differential taxonomic composition of the DCG and BCG at the species level (Figure 1B). Bacteroides and Firmicutes were the two dominant categories, but there was more Firmicutes and fewer Bacteroidetes in the DCG than in the BCG.

The Venn diagram showed that 50 species were shared between the DCNG, DCAG, BCNG and BCAG, 2 species were unique to the DCNG, 1 species was unique to the DCAG, 2 species were unique to the BCNG, and 7 species were unique to the BCAG (Figure 1C).

Then, we analyzed the α diversity of all groups based upon pairwise comparisons by the Wilcoxon rank-sum test (Figure 1D). Generally, no significant differences were found in the α diversity based on the Shannon index of the microbiota ($p > 0.05$). We also analyzed their β diversity. Based on the PCoA results, a contrast in the gut microbiota was detected between the DCG and BCG samples (Figure 1E).

3.3 Differences in biological markers of the gut microbiota

To identify characteristic gut microbiota in the DCG and BCG, we analyzed bacterial markers by LEfSe analysis to distinguish people between them on the grounds of the relative abundance of each taxon (Figure 2A). The cladogram displays the phylogenetic distribution between the DCG and the BCG from the phylum to the genus-level characteristic organisms. It highlights microbial subtrees that were differentially abundant by LEfSe, and their effect sizes as assessed by linear discriminant analysis (Figure 2B). As shown in the figure, there was a greater abundance of the *Slackia piriformis, Clostridium*_sp_L2_50, and *Bacteroides plebeius* species in the BCG. There was a greater abundance of *Flavonifractor plautii* (family Clotridiaceae), *Streptococcus parasanguinis* (family Streptococcaceae), ordor Lactobacillales, class Bacilli, as well as Gammaproteobacteria, Veillonella and Coprobacillus, and *Clostridium bolteae*, in the DCG. Overall, significant differences were found in both species abundance and composition between the two groups of samples at the class, order, family, genus, species and strain levels.

Based on specificity occupancy (SPEC-OCCU) analysis, *Escherichia coli*, *Clostridium bolteae* and *Ruminococcus gnavus* were distinguished as dominant bacterial markers of the DCG, while *Parabacteroides goldsteinii* was considered the dominant bacterial marker in the BCG (Figure 2C).

The same method was used for LEfSe analysis to distinguish between people with tendency for anemia and relatively healthy people. *Sutterella wadsworthensis* had a notably higher abundance in the AG, while *Megasphaera unclassified* and *Clostridium asparagiforme* were higher in the NG (Supplementary Figure S1A). Taken together, the data showed that significant differences were present in both species abundance and composition between the AG and the NG at the species level, but they were incompatible with the characteristics of gut microbiota in the DCG and the BCG. This suggests that differential gut microbiota in traditional Chinese medicine constitution may not be directly associated with the risk of anemia, and not all the people with deficient constitution progress to anemia.

To reveal the role of the microbiota in the pathological process of anemia, we measured bacterial markers in the four groups (DCNG vs. DCAG vs. BCNG vs. BCAG). At the species level, the abundances of *Streptococcus australis* and *Flavonifractor plautii* were markedly higher in the BCAG, while the abundance of *Clostridium leptum* was notably higher in the DCNG than in the other groups (Supplementary Figure S1B). To explore how constitution factors influence people who are prone to anemia, we quantified the differentially expressed microbiota between DCs and BCs in normal or abnormal situations. For comparisons between the DCNG and the BCNG, the differential analysis at the species level displayed markedly higher abundance of *Bacteroides thetaiotaomicron*, *Streptococcus parasanguinis*, *Clostridium bolteae*, *Streptococcus australis*, *Flavonifractor plautii*, *Streptococcus salivarius* and *Lachnospiraceae* bacterium_7_1_58FFA in the DCNG, while there was a higher abundance of *Peptostreptococcaceae* noname unclassified and *Anaerotruncus* unclassified in the BCNG (Supplementary Figure S1C). Notably, *Flavonifractor plautii, Streptococcus parasanguinis* and *Clostridium bolteae* matched the results of differences in gut microbiota between the DCG and the BCG. To compare the results between the DCAG and BCAG, the differential analysis at the species level displayed markedly higher abundances of *Bacteroides vulgatus*, *Ruminococcus gnavus*, *Parabacteroides* unclassified and *Coprobacillus* unclassified in the DCAG, while the abundances of *Akkermansia muciniphila*, *Eubacterium eligers*, *Alistipes finegens*, *Bacteroides nordii*, *Parabacteroides goldsteinii*, *Clostridium leptum*, *Subdobigranulum* unclassified and *Bacteroides plebeius* were notably higher in the BCAG (Supplementary Figure S1D). Notably, *Bacteroides plebeius* and *Parabacteroides goldsteinii* were characteristic gut species distinguishing the DCG from the BCG.

To distinguish the association between clinical characteristics and the microbial society structure, we inspected the contributions of clinical characteristics to the variety of microbial societies and differences in the relevant abundances of microbial species on the basis of the correlation coefficient and multiple regression model

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TABLE 1 Clinical Characteristics of the DCG and BCG.

Characteristics			
Characteristic	Balance, $N = 50$[a]	Deficiency, $N = 79$[a]	p-value[b]
Red blood cell indices (counts)			>0.99
Abnormal	15 (30%)	23 (29%)	
Normal	35 (70%)	56 (71%)	
Groups (counts)			<0.001
BalanceAbnormal	15 (30%)	0 (0%)	
BalanceNormal	35 (70%)	0 (0%)	
DeficiencyAbnormal	0 (0%)	23 (29%)	
DeficiencyNormal	0 (0%)	56 (71%)	
Age (yrs)	34 (6)	35 (6)	0.32
BMI (Body mass index, kg/m^2)	21.62 (3.34)	21.01 (2.57)	0.27
SBP (Systolic blood pressure, mmHg)	113 (8)	109 (12)	0.027
DBP (Diastolic blood pressure, mmHg)	69 (6)	68 (8)	0.43
GLU (Blood glucose, mmol/L)	5.20 (0.76)	5.15 (0.55)	0.68
LDLC (Low density lipoprotein, mmol/L)	2.85 (0.68)	2.77 (0.62)	0.46
TC (Total cholesterol, mmol/L)	4.75 (0.77)	4.65 (0.71)	0.46
TG (Triglyceride, mmol/L)	0.91 (0.37)	0.88 (0.37)	0.67
HDLC (High density lipoprotein cholesterol, mmol/L)	1.53 (0.25)	1.50 (0.32)	0.5
CRP (C-reactive protein, mg/L)	1.08 (2.00)	1.03 (2.35)	0.88
NEUT (Neutrophil, 10^9/L)	3.50 (1.37)	3.32 (1.23)	0.46
MONO (Monocyte, 10^9/L)	0.38 (0.11)	0.37 (0.11)	0.92
EOSIN (Eosinophil. 10^9/L)	0.12 (0.10)	0.11 (0.08)	0.47
MCHC (Mean corpuscular hemoglobin concentration, g/L)	330 (9)	326 (12)	0.02
MCH (Mean corpuscular hemoglobin, pg)	29.1 (2.4)	28.9 (3.2)	0.67
MCV (Mean corpuscular volume, fL)	88 (6)	89 (8)	0.75
MPV (Meam platelet volume, fL)	10.03 (0.95)	10.32 (0.87)	0.091
LYM (Iymphocyte, 10^9/L)	1.98 (0.52)	1.82 (0.48)	0.086
WBC (White blood cell, 10^9/L)	6.01 (1.62)	5.66 (1.53)	0.22
RDW (Red blood cell distribution width, %)	12.47 (1.04)	12.63 (1.38)	0.45
HCT (Hematocrit, %)	40.11 (2.64)	39.25 (2.36)	0.065
RBC (Red blood cell, 10^{12}/L)	4.57 (0.47)	4.47 (0.49)	0.23
PDW (Platelet distribution width, fL)	11.44 (2.08)	11.78 (2.34)	0.39
PCT (Procalcitonin, ng/mL)	0.27 (0.04)	0.26 (0.05)	0.51
PLT (Platelet, 10^9/L)	269 (50)	254 (57)	0.12
Hb (Hemoglobin, g/L)	132 (10)	128 (9)	0.01
TBA (μmol/L)	6.20 (7.15)	3.65 (2.27)	0.018
TP (Total protein, g/L)	74.8 (4.0)	74.7 (3.6)	0.82
IL27 (Interleukin-27, pg./mL)	697 (1,281)	566 (1,276)	0.57

[a]*n* (%); Mean (SD). [b]Fisher's exact test; Welch Two Sample *t*-test.

(Figure 3), and the major predictors were identified. Statistical significance was found for microbial species of *Paraprevotella* unclassified ($p < 0.05$), and stronger statistical significance was found for *Sutterella wadsworthensis*, *Megasphaera* unclassified and *Bacteriudes plebeius* ($p < 0.01$), while extreme statistical significance was found for *Clostridium*_sp_L2_50, *Slackia piriformis*, *Clostridium butyricum*, *Neisseria meningitidis* and *Eubacterium brachy* ($p < 0.001$). Anemia-related indices, including MCV, MCH, Hb, and RBC, represented the most variable importance to the bacterial species mentioned above. Immunity

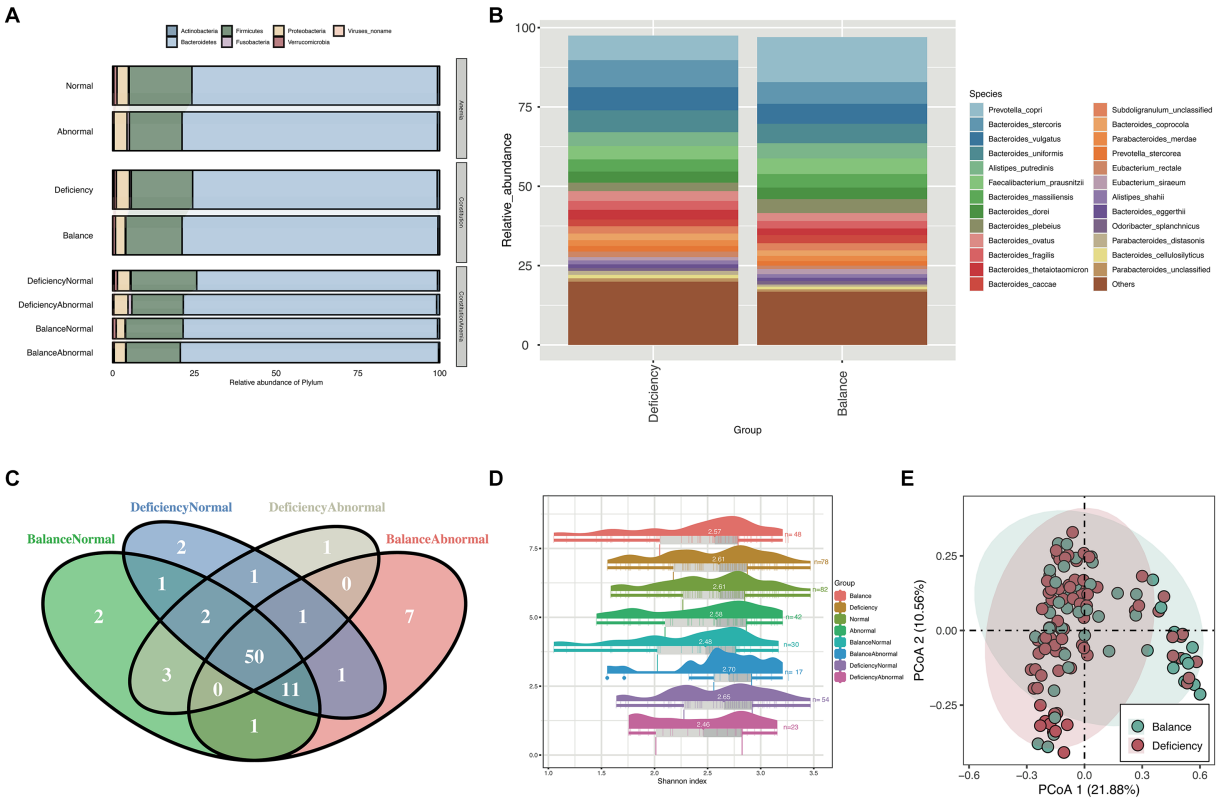

FIGURE 1
Taxonomy composition and diversity analysis. **(A)** Stacked bar chart for the relative abundance of phylum between different groups and **(B)** stacked bar chart for the relative abundance of species between the BCG and the DCG. Different colors represent different bacteria. **(C)** Venn diagram of species counts (median value) in the DCNG, DCAG, BCNG and BCAG. **(D)** Raincloud plots of Shannon indices for the α-diversity analysis in the BCG, DCG, normal blood indices group (NG), abnormal blood indices group (AG), DCNG, DCAG, BCNG and BCAG. **(E)** PCoA analysis for β diversity analysis in DCG and BCG based on Bray–Curtis distance.

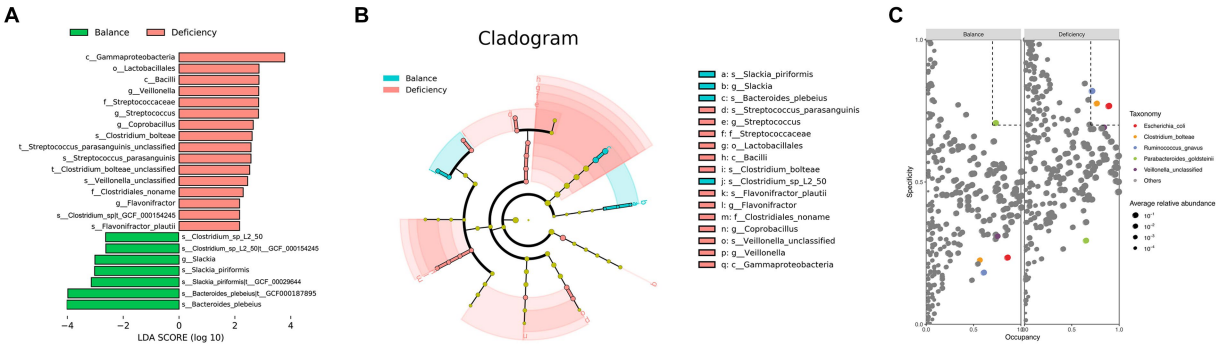

FIGURE 2
Taxonomy differences and species dominance analysis. **(A)** Bar chart of LDA logarithmic scores of biological markers spotted by LEfSe ($p < 0.05$ and LDA cutoff >2.0) for all levels of features differentially abundant between DGC and BCG. **(B)** Cladogram representing the differences by LEfSe between the DCG and the BCG. Colors distinguish between BCG (blue) and DCG (red), and the size of the dot relates to its relative and logarithmically scaled abundances. **(C)** SPEC-OCCU analysis plot to identify the specialist species between the DCG and the BCG (occupancy>70%, specificity>70%).

indicators, including WBC, LYM, and TG, also contributed to the explanatory degree of the variance change in gut microbiota. The heatmap reflects the correlation between primary clinical characteristics and different characteristic species in the comparison of different groups (BCG vs. DCG; AG vs. NG; DCNG vs. DCAG vs. BCNG vs. BCAG).

For the communication of bacteria, we performed network analysis of the bacteria of the DCG and the BCG. Although the numbers of the keystone species in the DCG was more than that in the BCG, there were stronger networks among characteristic bacterial communities in the BCG (Figure 4A), and more independent communities in the DCG (Figure 4B).

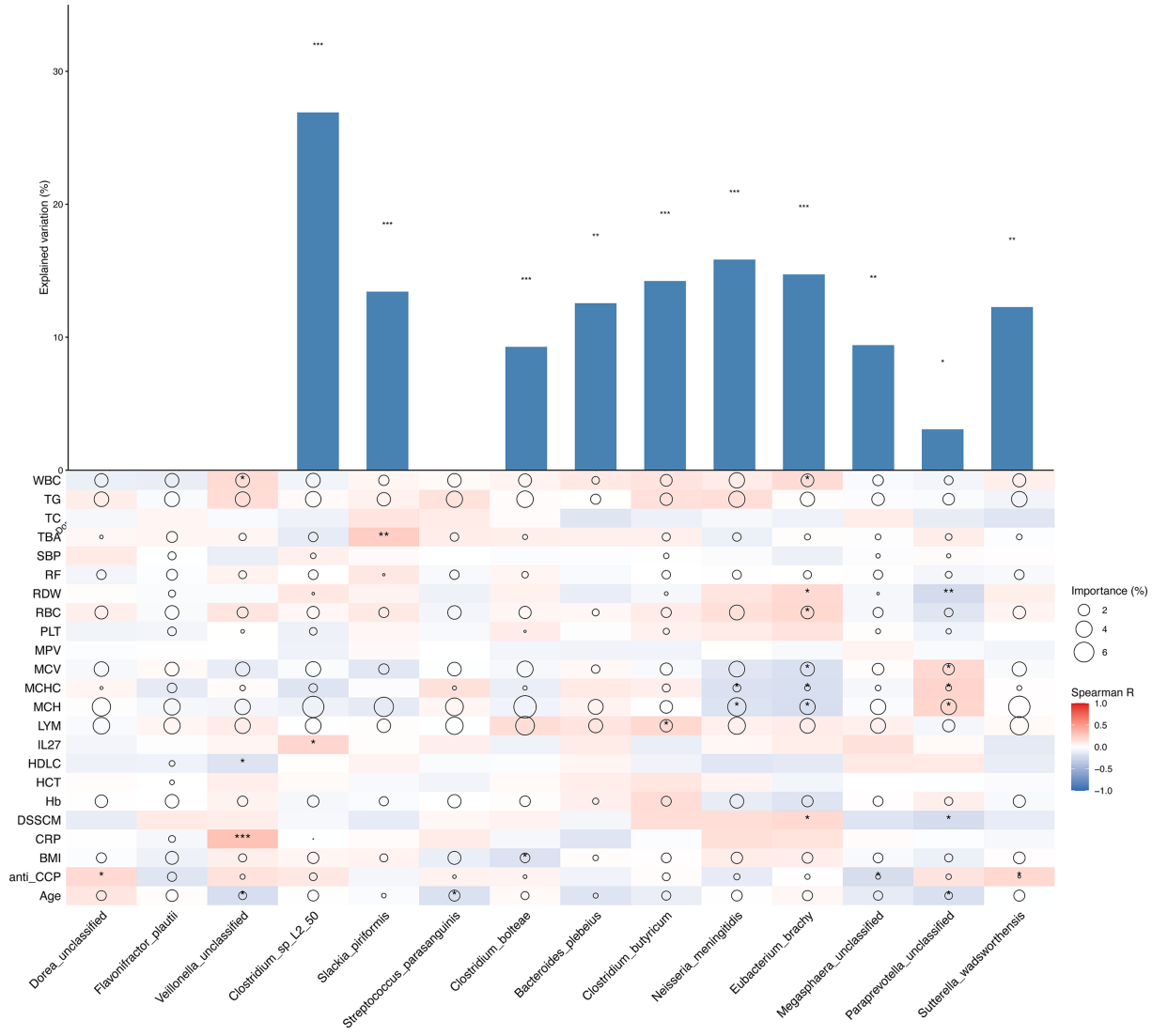

FIGURE 3
Contribution of phenotype to species relative abundance differences on the basis of correlation analysis and a random forest model between different groups (BCG vs. DCG; AG vs. NG; DCNG vs. DCAG vs. BCNG vs. BCAG). The size of each circle denotes the variable importance [calculated by means of multiple regression modeling and variance decomposition analysis (Maaslin2)]. Colors denote the strength of the Spearman correlation coefficients.

3.4 Differences in metagenomic functional pathways

To examine the possible operation of the intestinal microbial society and their relationship in deficient constitution-related the tendency for anemia, we analyzed the relevant abundance of 339 metabolic pathways based on the UniRef90 database (Figure 5A). Of note, 252 (74%) of the pathways were related to at least one comparison in groups.

Compared to the BCG, seven pathways were extremely significantly upregulated in the DCG ($p < 0.005$). They were PWY_5484 (glycolysis II, from fructose 6-phosphate), GLYCOLOLYSIS, PWY66_400 (glycolysis VI, metazoan), PWY_409 (superpathway of purine nucleotide salvage), ANAEROFRUCAT_PWY (homolactic fermentation), BIOTIN_BIOSYNTHESIS_PWY, and PWY_5695 (urate biosynthesis/inosine 5′-phosphate degradation). Most of these pathways were associated with energy metabolism, especially the glycolytic cycle. Comparisons of DCG vs. BCG and AG vs. NG, also showed a difference in ANAEROFRUCAT_PWY (homolactic fermentation), a process of transformation from sugars to cellular energy and the metabolic byproduct lactate. Based on the threshold of $p < 0.05$, more shared pathways were observed in the comparisons of DCG vs. BCG, AG vs. NG and BCAG vs. BCNG vs. DCNG vs. DCAG. In each pair, the activity of 10 pathways was downregulated in the former group (including PEPTIDOGLYCANSYN-PWY, PWY-2942, PWY-5097, PWY-6386, PWY-6387, PWY-7219, PWY-7221, PWY-6122, PWY-6277 and PWY-6121, which are mainly related to nucleotide biosynthesis and peptidoglycan biosynthesis), while 8 were upregulated (including NAEROFRUCAT-PWY, NONOXIPENT-PWY, TRPSYN-PWY, PWY4FS-8, PWY4FS-7, PWY0-1296, COA-PWY and GLCMANNANAUT-PWY, which are mainly related to survival of *Escherichia coli* and multiroute energy metabolism). These results

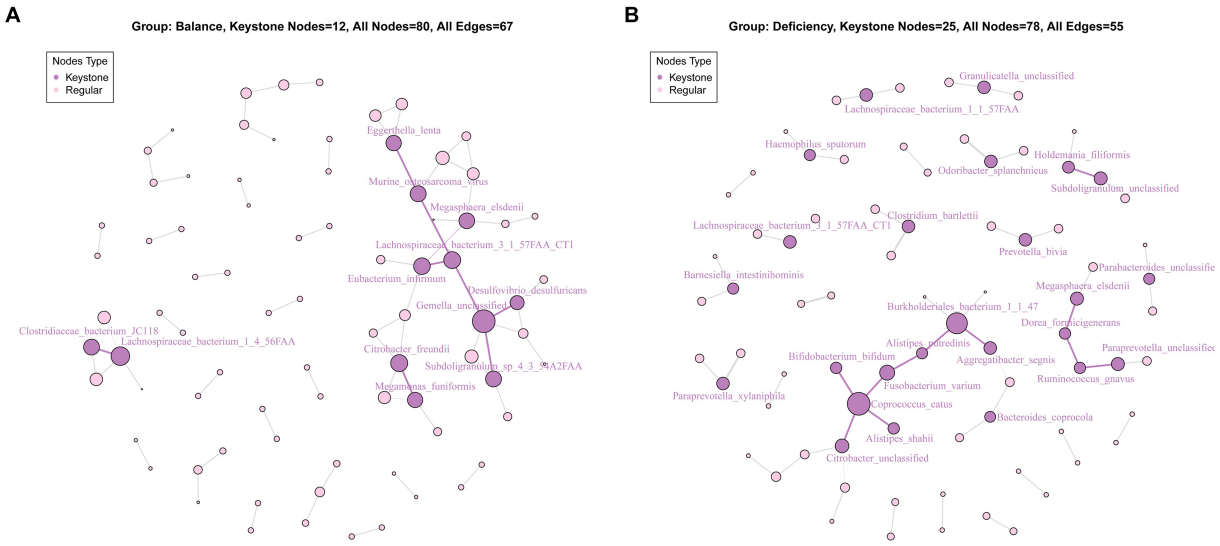

FIGURE 4
Key species analysis based on community interaction networks in the BCG **(A)** and DCG **(B)**. The lines between species indicate correlations: red bold edge means negative correlation; black weak edge means positive correlation. The size of a node means the degree of importance in the network; the dark purple nodes show the keystone species.

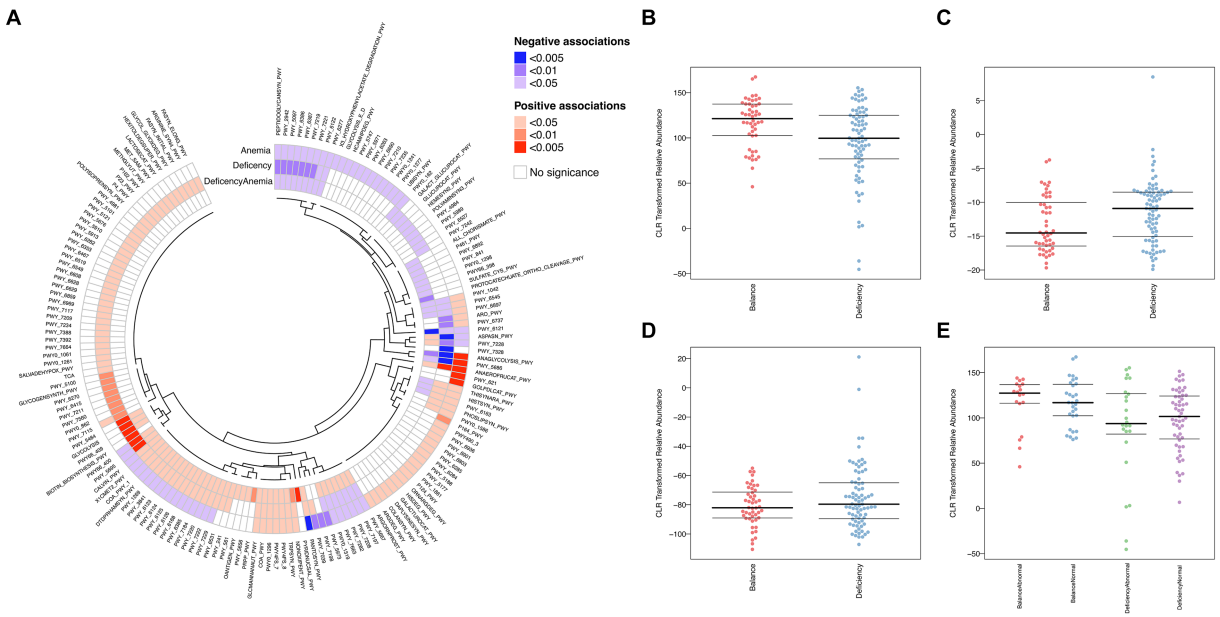

FIGURE 5
Differentially functional microbiota in different groups of samples. **(A)** Heatmap and circular dendrogram of the microbial, describing the notable enrichment (red) or notable underrepresentation (blue) in each comparison (deficiency: DCG vs. BCG; anemia: NG vs. AG; deficiency anemia: BCNG vs. DCAG). **(B–E)** BugBase analysis for organism-level microbiome phenotypes, including anaerobic **(B)**, aerobic **(C)**, and facultative-anaerobic **(D)**, between the DCG and the BCG samples and **(E)** anaerobic features among the DCNG, DCAG, BCNG and BCAG.

indicated the pathological basis of deficient constitution-related increases the tendency for anemia and emphasized *Escherichia coli* as the dominant bacterial marker in the DCG.

We then applied BugBase to predict organism-level microbiome phenotypes between the DCG and the BCG. The results showed that the abundance of microorganism with an anaerobic phenotype was higher in the BCG than the DCG, while the abundance of microorganism with aerobic and facultative-anaerobic phenotypes was lower in the BCG (Figures 5B–D). More specifically, the anaerobic phenotype was most abundant in the BCAG, followed by BCNG, DCNG and DCAG in descending ordor (Figure 5E).

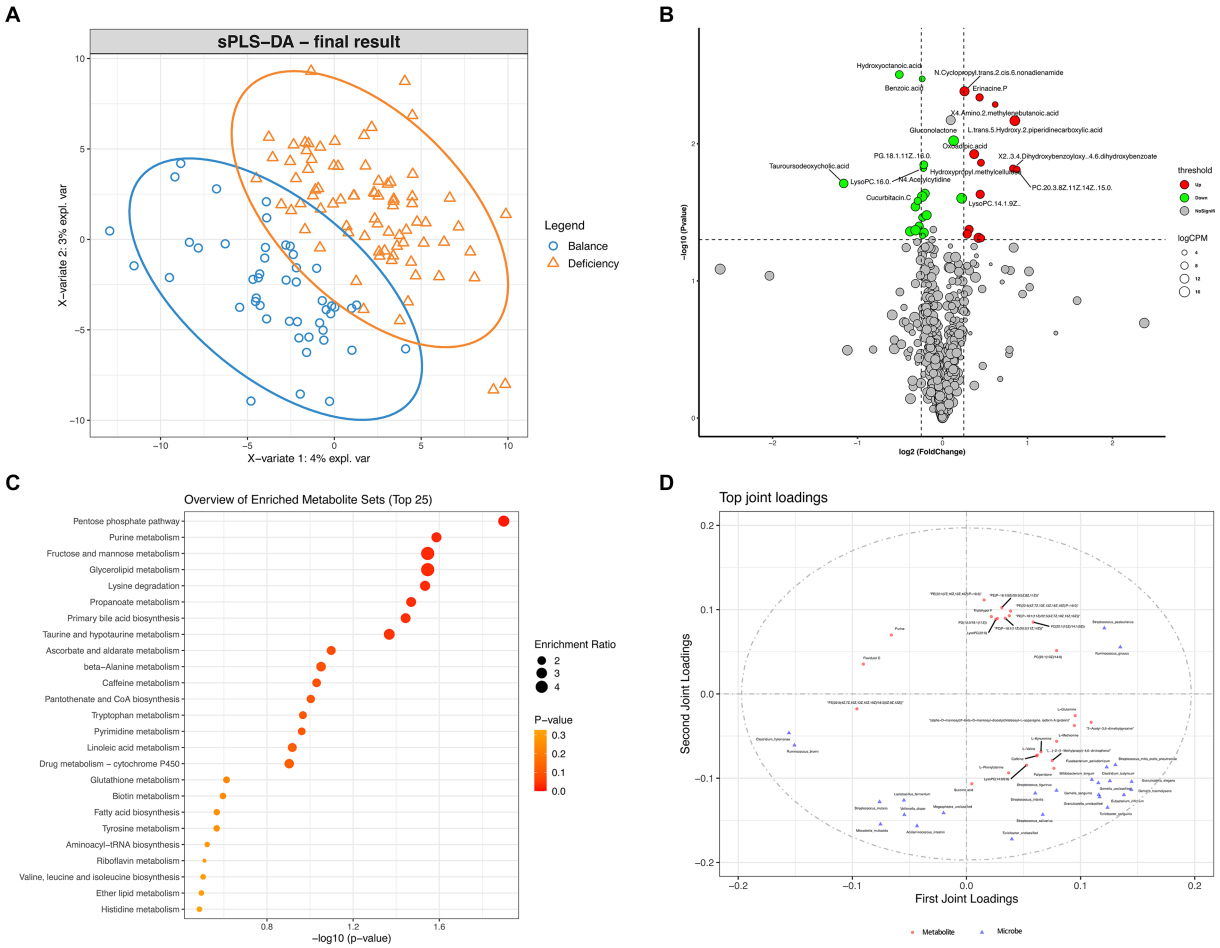

FIGURE 6
Differential analysis of metabolome features. **(A)** PLS-DA analysis of metabolites between DCGs and BCGs. **(B)** Volcano plot of differential metabolites between the DCG and the BCG. **(C)** Scatter plot for functional enrichment analysis of differential metabolites between the DCG and the BCG. **(D)** O2PLS analysis of microbiota metabolite relationships.

3.5 Identification of differentially abundant metabolites

Differentially aboundant metabolites in the serum of DCG and BCG individuals were recognized by metabolomic profiling. Score plots of the PLS-DA models showed discrimination between them in the serous metabolic profile (Figure 6A). Differential metabolites are displayed in the volcano plot (Figure 6B), and we noticed 13 metabolites that were significantly upregulated (including oxoadipic acid, etc.) and 9 metabolites that were significantly downregulated (including cucurbitacin C, decanoylcarnitine, tauroursodeoxycholic acid, glycerol triundecanoate, etc.) in the serum of DCG individuals ($p < 0.05$). The same method was applied to discover differential metabolites in the serum of AG individuals, and 14 metabolites were upregulated (inclusive of 2-hydroxybutyric acid, L-methionine, etc.), while 33 metabolites were notably downregulated (including cucurbitacin C and cholic acid, etc.; $p < 0.05$; Supplementary Figure S2).

To explore the potential correlations between the changes in clinical index and metabolomic features, we used spearman's correlation coefficients to measure the linear relationship between clinical index and metabolomic features. There are some significant correlation was displayed between them (Supplementary Figure S3; $p < 0.05$). Considering that these metabolites may be related to confounding factors of aforementioned clinical features, we made linear models with covariate adjustment analysis of bacteria in the DCG (Supplementary Figure S4). The resulting plot compares the p values for each metabolite both before (x-axis) and after (y-axis) covariate adjustment. It shows that L-gluconolactone and pentaneitrile were significant only after adjusting for significantly differential clinical features, including age, BMI, MCHC, MCH, MCV, RDW, HCT, RBC and Hb, while gluconolactone and hydroxyoctanoic acid were significant before and after adjustment.

We further used Maaslin2 to find multivariate associations between clinical data and metabolomic features of the bacterium in the DCG, taking deficiency constitution, age, BMI, MCHC, MCH, MCV, RDW, HCT, RBC and Hb into account. Galacturonic acid (Supplementary Figure S5A; FDR < 0.001) and ajocysteine (Supplementary Figure S5B; FDR < 0.001) had a negative linear correlation with Hb, while linoelaidic acid had a positive linear correlation with RBCs (Supplementary Figure S5C; FDR < 0.001).

Then, a scatter plot for functional enrichment analysis of differential metabolites between the DCG and the BCG was used to

explore the biological pathways of the DCG (Figure 6C; $p < 0.05$). A total of 65 pathways were significantly enriched, 25 of which were enriched in protein metabolism (including "Lysine degradation," "beta-Alanine metabolism," "Tryptophan metabolism," "Biotin metabolism," "Tyrosine metabolism," "Aminoacyl-tRNA biosynthesis," "Valine, leucine and isoleucine biosynthesis," and "Histidine metabolism") and glycolipid metabolism (including "Fructose and mannose metabolism," "Glycerolipid metabolism," "Fatty acid biosynthesis," "Linoleic Acid," "Biotin metabolism," "Ether lipid metabolism," "Primary bile acid biosynthesis," and "Taurine and hypotaurine metabolism"). The subsequent reaction converts the products to CoA and can be shunted to the TCA cycle (including "pantothenate and CoA biosynthesis" and "pyrimidine metabolism"). Notably, taking the enrichment ratio and *p* value into consideration, the most significant biological pathway was related to the "pentose phosphate pathway," as the first-line defense against oxidative stress and is critical to cell proliferation, survival, and senescence. "glutathione metabolism" and "ascorbate and aldarate metabolism" were also connected with oxidant stress. The results indicated that complex energy metabolism and oxidative stress alterations are involved in deficient constitution.

To recognize the associations between different members of the intestinal microbiome and metabolites, we assembled a network of cooccurring bacteria and metabolites and interrogated it for modules via Procrustes analysis and O2PLS analysis. Procrustes analysis showed a significant correlation between the microbial community and metabolites in different samples (Supplementary Figure S6; M2 = 0.217, $p < 0.001$). O2PLS analysis revealed the significant and biologically relevant coordination between bateria and metabolites (Figure 6D). Several kinds of phosphatidylcholine (PC, the basic components of the lipid bilayer of cells involved in metabolism and signaling) were significantly correlated with *Ruminococcus gnavus* (the dominant bacterial marker of DCG).

To characterize this intricate interaction between the microbiome and metabolites, we used MetOrigin to distinguish which bacteria contribute and how to specific metabolic reactions by determining where metabolites come from. Taking characteristic metabolites of deficiency constitution and those correlated with Hb and RBC into account, 3 metabolites were from microbiota, 7 metabolites were from both host and bacteria (cometabolism), and others were related to drugs, food or something else (Supplementary Figure S7A). Based on the metabolites involved, 13 pathways were enriched, among which "C5-branched dibasic acid metabolism" (derived solely from microbiota) was most significant and was found to be distributed in the class Gammaproteobacteria (the greatest abundance of differential microbiota in DCG) (Supplementary Figures S7B,C). *Escherichia coli*, as the dominant bacterial marker of DCG based on SPEC-OCCU analysis, was from the class Gammaproteobacteria. Moreover, the class Gammaproteobacteria and class Bacilli (the second most abundant of differential microbe in the DCG) also contributed to the pathways "pentose and glucuronate interconversions" and "ascorbate and aldarate metabolism" (Supplementary Figures S7D,E), while the order Lactobacillales (the third most abundant differential microbe in the DCG) contributed to the pathways "Amino sugar and nucleotide sugar metabolism" (Supplementary Figure S7F). These pathways were all related to galacturonic acid, which had a negative linear correlation with Hb.

To explore the relationship among metabolites, microbial community and tendency for anemia, we used mediation analysis to investigate metabolites as the mediator of the relation between microbial community and tendency for anemia based on their abundances and counts. We discovered L-Glutamine and D-Glutamine mediated the *Bacteroides plebeius*-tendency for anemia association, while alpha−Santalyl phenylacetate mediated the *Flavonifractor plautii*-tendency for anemia association, both in a negative way (Supplementary Figure S8A; $p < 0.05$). As for the tendency for anemia, we also found alpha−Santalyl phenylacetate mediated the *Bacteroides plebeius*-MCHC association in a negative way (Supplementary Figure S8B; $p < 0.05$).

4 Discussion

In this study, we described the clinical characteristics of individuals with deficient constitution, and our microbiome study identified the distinctive compositional and functional patterns of their intestinal microenvironments. Based on the negative correlation between deficient constitution and Hb concentration, we suggest that people with deficient constitution are at risk of anemia. Therefore, we divided people into four groups according to traditional Chinese medicine constitution (deficient constitution versus balanced constitution) and the tendency for anemia (based on laboratory-measured Hb counts, RBC, HCT, MCV, MCH, MCHC and RDW), including the BCNG, BCAG, DCNG and DCAG, and we explored conceivable pathological processes of deficient constitution-related increases the tendency for anemia.

Previous research on the gut microbiota of the qi-deficient constitution, yang-deficient constitution and yin-deficient constitution showed a lower Shannon index of α diversity and differential microbiota profiles compared to the balanced constitution. These studies were mainly carried out in the northern part of China, such as Shandong Province and Beijing (3, 36). There were two studies in the southern part of China: one study screened 461 healthy balanced-constitution individuals and 166 qi-deficient individuals from 5,987 college students (23), and another enrolled 461 balanced-constitution and 76 yang-deficient constitution undergraduates aged 18 to 22 (21). In contrast with previous findings, the Shannon index of α diversity of the microbiota from the DCG was higher than that of the microbiota from the BCG at all levels in this study. Humans are omnivorous primates, and their gut microbiota varies widely across geography (37, 38) and age (39, 40). Our study enrolled female hospital staff members (age from 24 to 54 years old, mean = 34.40) in Guangzhou, southern China. We assumed that differences in age, geographical location, lifestyle and long-term diet may have contributed to this unexpected result. The Shannon index reflects the richness and evenness of species, so we concluded that there were more heterogeneous biomes in the DCG than in the BCG.

The species had roughly the same counts between the DCG and the BCG, but there were increased Firmicutes and decreased Bacteroidetes in DCG. In latest reports, a higher Firmicutes-to-Bacteroidetes ratio was spotted in both overweight rats and humans, involving energy metabolism dysfunction (41–43). The bacterial taxon information of participants was identical to that of healthy Asian populations published in earlier studies (44), but the microbiota were unalike between DCG and BCG.

Through LEfSe analysis and SPEC-OCCU analysis, we focused on *Flavonifractor plautii*, *Streptococcus parasanguinis*, *Clostridium bolteae*, *Escherichia coli*, and *Ruminococcus gnavus* as markers specifically enriched in the DCG at the species level. In line with our findings, as a key bacterial marker of DCG, *Escherichia coli*, with higher occupancy and specificity, is a most facultatively anaerobic and opportunistic pathogen. For *Clostridium bolteae,* its abundance was mostly attributed to MCH, LYM, MCV, TG, WBC counts and RBC counts in this study, indicating that *Clostridium bolteae* might be connected with anemia, immunity and lipid metabolism. It is usually considered an opportunistic pathogen (45) that is associated with some kinds of rare autoimmune diseases, such as immunoglobulin G4-related disease, systemic sclerosis and neuromyelitis optica spectrum disorders (46, 47). A previous study demonstrated that *Flavonifractor plautii* treatment calmed antigen-stimulated Th2 immune responses, consequently restraining interleukin (IL)-4 and ovalbumin-specific IgE production in ovalbumin-sensitized mice (48). Another study showed that Gd-IgA1-associated α-galactosidase and α-N-acetylgalactosaminidase from *Flavonifractor plautii* were conspicuous in IgA nephropathy patients. Thus, *Flavonifractor plautii* has an impact on the host immune system. On the other hand, *Flavonifractor plautii*, found to be depleted in the microbiota of obese subject, has been investigated as a putative biomarker of healthy status, related to butyrate and propionate (48–50). Comparison of the four groups showed that *Flavonifractor plautii* was not only the characteristic bacteria enriched in the DCG but also a significant species in the DCNG, and was negative associated with the tendency for anemia mediated by alpha−Santalyl phenylacetate, which indicates that it may be a beneficial factor that helps some deficient-constitution individuals avoid anemia. People with a deficient constitution are characterized by hypoimmunity and low energy (6). These individuals also had increased activity in pathways associated with energy metabolism, especially the glycolytic cycle, based on the UniRef90 database of microbiota in the DCG. While the host activates immune signaling to fight infection, intestinal epithelial cells react to aerobic glycolysis by quickly transferring bioenergetic molecules, which causes oxygenation of the epithelium, an instant increase in mucosal-associated commensal Enterobacteriaceae and a decrease in obligate anaerobes (51).

In the case of gut microbial taxa in balanced-constitution people, *Bacteroides plebeius*, *Slackia piriformis*, *Clostridium*_sp_L2_50 and *Veillonella* unclassified were found notably based on LEfSe analysis. The abundance of *Bacteroides plebeius* was mostly attributed to MCH, LYM, TG and WBC counts. It was positively associated with Hb and RBC counts, negative associated with the tendency for anemia mediated by L-Glutamine and D-Glutamine, negative associated with MCHC mediated by alpha−Santalyl phenylacetate in this study, suggesting that *Bacteroides plebeius* might help avoid anemia and might be related to immunity. *Bacteroides plebeius* is related to the dietary components of seaweed and coffee phenolic compounds (52, 53). Other research has suggested that intestinal microbes support the human body in energy from dietary polysaccharides via carbohydrate active enzymes, which are lacking in the human genome, possibly from seaweed-associated marine bacteria (54). One report has shown that, compared to Crohn's disease patients with recurrence after surgery, remitted patients had higher proportions of *Bacteroides plebeius*, which is a relative of butyrate-producing bacteria (55). Although *Slackia piriformis* and *Clostridium*_sp_L2_50 were rarely reported previously, we found a positive association between *Slackia piriformis* and TBA ($p < 0.01$) and a positive association between *Clostridium*_sp_L2_50 and IL27 ($p < 0.05$), implying that these bacteria are probably probiotic. Based on SPEC-OCCU analysis, *Parabacteroides goldsteinii*, as a key bacterial marker of BCG, is a SCFA producer and possesses gut barrier-maintaining functions and potently protects against pathogenic bacterial lipopolysaccharide-induced inflammation (56). The puzzle appeared when *Clostridium leptum* was increased significantly in the BCAG in the comparison among the four groups, which is acknowledged as a probiotic. *Clostridium leptum* is a member of the Ruminococcaceae family acknowledged to be capable of 7α-dehydroxylation and producing secondary BAs that mainly have anti-inflammatory activities (57, 58). The SCFAs generated by this community modulate the expression of Foxp3, an important gene governing the development of regulatory T cells (59). This may indicate that although people were out of the normal blood index range and tended to have anemia, they may still take advantage of their balanced constitution.

We then went a step further to reveal that people of different constitutions tend to have anemia or avoid anemia under the influence of their particular intestinal microbiota. Distinguished from people of balanced constitution, the differential microbiota of people with deficient constitution showed less auspicious results, although they were all within the normal blood index ranges at the time. In addition to *Clostridium bolteae*, *Streptococcus parasanguinis* and *Flavonifractor plautii*, distinguished as characteristic microbiota in the DCG as stated, *Bacteroides thetaiotaomicron*, *Streptococcus australis*, *Streptococcus salivarius* and *Lachnospiraceae* bacterium_7_1_58FFA showed markedly higher abundance in the DCNG. *Streptococcus australis*, *Streptococcus parasanguinis* and *Streptococcus salivarius*, as oral bacteria, are usually found along with each other, and they are related to precancerous lesions of gastric cancer, Takayasu arteritis, COPD and COVID-19-related mortality (60–63). On the other hand, *Bacteroides thetaiotaomicron* has a strong ability to degrade dietary polysaccharides, produce host-absorbable short-chain and organic acids as a resource of energy, transform cholesterol to cholesterol sulfate, and ameliorate inflammation by producing bacterial extracellular vesicles (64–66).

However, when we focused on people out of the normal red blood cell index ranges, the differential microbiota of people with a balanced constitution showed good indications compared to people with a deficient constitution. *Akkermansia muciniphila, Eubacterium eligers, Alistipes finegens, Bacteroides nordii, Bacteroides plebeius, Parabacteroides goldsteinii* and *Clostridium leptum* were enriched in the BCAG, most of which are regarded as probiotics. These results demonstrate that people with tendency for anemia but a balanced constitution may be in a more beneficial situation than those with a deficient constitution. *Akkermansia muciniphila*, *Eubacterium eligers*, and *Parabacteroides goldsteinii* are all SCFA producers (59, 67). *Akkermansia muciniphila* can maintain gut barrier function, reorganize disturbed microorganisms, enhance SCFA secretion, and alleviate APAP-induced oxidative stress and the inflammatory response (68, 69). *Parabacteroides goldsteinii* also possesses gut barrier-maintaining functions and potently protects against pathogenic bacterial lipopolysaccharide-induced inflammation (56). For the characteristic gut microbiota in the DCAG, *Bacteroides vulgatus* and *Ruminococcus gnavus* were considered hominoxious and reported to be related to inflammatory bowel disease, metabolic syndrome, postacute COVID-19 syndrome, spondyloarthritis and type 2 diabetes mellitus and other diseases (70–75). Moreover, *Ruminococcus*

gnavus was a key bacterial marker in the DCG, with higher occupancy and specificity in deficient-over balanced-constitution samples in this study. We consider *Ruminococcus gnavus* to be a shared microbial basis of deficient constitution and tendency for anemia.

According to previous research, anemia is related to alterations in the gut microbiome (26–30). In our study, aerobic and facultative-anaerobic bacteria were more abundant in the DCG, while its anaerobic bacteria were less abundant. As we known, low availability of oxygen characterizes the intestinal lumen. The consumption of oxygen by mammalian intestinal epithelial cells (IECs) leads to intestinal hypoxia, which is maintained by SCFAs produced by some specific microbiota (especially by anaerobic bacteria). This finding provides evidence that the change of intestinal anaerobic environment may be associated with deficient constitution.

Metabonomics provides some clues for identifying differences between deficient constitution and balanced constitution individuals. In brief, samples with a deficient constitution were more likely to have energy metabolism disorders, including amino acid metabolism and glycolipid metabolism, associated with low-energy and immunologic derangement. 2-Oxoadipate (upregulated metabolite) can be converted to acetyl-CoA in mitochondria (76), and abnormal quantities of 2-aminoadipic acid are found in patients with 2-oxoadipic aciduria (77). Cucurbitacin C (downregulated metabolite), with antioxidant ability shows growth inhibition capabilities against tumor cells by activating cellular immunity (78). Cucurbitacin D promotes fetal hemoglobin synthesis by activating the p38 pathway and stabilizing γ-globin mRNA (79). Decanoylcarnitine (downregulated metabolite), an acylcarnitines, can produce energy by transporting and breaking down organic acids and fatty acids (80). Tauroursodeoxycholic acid (downregulated metabolite), a type of bile acid, is an essential component for cholesterol homeostasis, by regulating the secretion of bile and lipids for the digestion of dietary fats and vitamins (81). A previous study in mice reported that the LPS-induced inflammatory damage group had lower levels of glycerol triundecanoate (downregulated metabolite) than the control group (82).

After adjusting fo age, BMI, MCHC, MCH, MCV, RDW, HCT, RBC count and Hb level by covariate adjustment analysis, L-gluconolactone, pentanenitrile, gluconolactone and hydroxyoctanoic acid were significantly more abundant in the DCG. Gluconolactone is generated by enzymatic oxidation of D-glucose by the enzyme glucose oxidase, with antioxidant free radical scavenging activities. Additionally, the most significant biological pathway in the DCG was related to the pentose phosphate pathway, which is the first-line defense response to oxidative stress. In addition to being absorbed from the diet, gluconolactone is one of the metabolites of the gut microbiota, affecting biological and pathological processes once intestinal homeostasis changes (83). Gluconolactone in feces increased after a half-marathon race and after a high-fat diet (84, 85), indicating that gluconolactone may play an important role in the gut microbiota and human health. L-Gulonolactone and pentanenitrile come mostly from a few particular food and have uncertain roles in humans.

Lower Hb counts was observed in the DCG, and galacturonic acid displayed a negative linear relationship with Hb in this study. Hb is formed by α- and β-globin subunits attached to heme prosthetic groups, which can transport oxygen and carbon dioxide. Hb molecules limit possible pathologies caused by associated iron and free oxygen, reactive molecules capable of inflicting damage through the production of reactive oxygen species (86). It is believed that modification of HbA at its amino terminus with galacturonic acid influences the O_2 affinity of the molecule (87). The top 3 characteristic bacterial constituents of the deficient constitution, the Gammaproteobacteria class, Bacilli class and Lactobacillales order, contributed to the enrichment of metabolic pathways of galacturonic acid. Pectin oligosaccharides, emerging prebiotics consisting of both galacturonic acids and neutral sugars, have an effect on the microbiome, and the latter also impact the cholesterol-reducing effects of pectin oligosaccharides via specific bacteria and their SCFA metabolites (88). Therefore, we assumed that the microbiome of the DCG may also impact the Hb-regulating effects of galacturonic acids, but the specific mechanisms need further study.

Metabonomics analysis was applied to distinguish between the AG and NG. Generally, individuals with a tendency for anemia were in the DCG and mainly had deficient energy metabolism and immunologic derangement. Recently, 2-hydroxybutyrate (upregulated metabolite) in the plasma has been noted as a favorable indicator for type 2 diabetes in the early-stage and is commonly found in the urine of lactic acidosis and ketoacidosis patients, which have high concentrations of it related to energy-deficient metabolism (e.g., birth asphyxia) (89–91). L-Methionine (upregulated metabolite) is a necessary amino acid, but persistently excessive methionine is related to homocystinuria-megaloblastic anemia (92). Cholic acid (downregulated metabolite), as primary bile acid, maintains cholesterol homeostasis by regulating all vital enzymes (93).

The human gut microbiome is fed by dietary nutrition to generate bile acids, SCFAs and other bioactive compounds, which are necessary for maintaining host physiology involving in protein metabolism, glucose metabolism and lipid metabolism (94). Herein, we used multiple technologies including metagenomics and metabolomics, to identify, categorize, and quantify compounds of deficient individuals, and found that decreased bacteria relevant to SCFA production and bile acid secretion, which influence energy metabolism and inflammation, are prominent features of deficient individuals. SCFA production is one of the well-known metabolic attributes of human gut bacteria and is connected with health situations (95). They can boost mitochondrial β-oxidation, deplete oxygen and consequently reduce the degradation of hypoxia induction factor (HIF), affecting host ATP production and immune responses (96–98). HIF-1 can influence the barrier function of the epithelium by regulating the expression of several relevant genes (96). HIF-2α is a master transcription factor of intestinal iron absorption and is related to intestinal iron absorption in the host. It has been suggested that activation of HIF-2α by microbiome-based therapeutics would lead to increased intestinal iron absorption and alleviate anemic disorders (99). In addition, because of lower Hb counts in people with deficient constitution, cells exposed to prolonged hypoxia may activate HIF (100), and metabolic pathways of the defense response to oxidative stress were observed in this study.

This study may have some potential limitations. We used a convenience sample due to the limitation of resources, and it may cause sampling bias because of geographic specificity, diet and other confounding factors. This study is a cross-sectional study, the causal inference and the identified associations need further studies.

To our knowledge, this study comprehensively investigated a relatively unexplored area—the relationship between alterations in the gut microbiome due to deficiency constitution and the tendency toward anemia. In the future research, larger sample sizes and longitudinal cohort studies will be performed to reveal the more

specific associations between them and offer potential interventions to reduce the risk of anemia in individuals with deficiency constitution.

5 Conclusion

Our study suggests that the composition of the gut microbiome differs between deficient-constitution and balanced-constitution subjects. Alterations in the microbiome of people with deficient constitution were associated with worse health status and a greater risk of anemia, involving intestinal barrier function, metabolism and immune responses, which were regulated by SCFAs and bile acid production. These findings may offer a new perspective of traditional Chinese medicine constitution regarding the etiology of anemia with implications for developing new strategies for prevention of anemia.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, CNP0003844.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Guangdong Provincial Hospital of Chinese Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the subjects in this study were internal clinical staff from Guangdong Provincial Hospital of Chinese Medicine, Fangcun Branch, Guangzhou, China. All subjects offered verbal informed consent. The data were collected from their health assessment with no intervention and were transformed to anonymity. The requirement for informed consent was therefore waived.

Author contributions

YLia: Investigation, Visualization, Writing – original draft, Writing – review & editing. YC: Formal analysis, Methodology, Software, Writing – review & editing. YLin: Methodology, Project administration, Resources, Supervision, Writing – review & editing. WH: Project administration, Supervision, Writing – review & editing. QQ: Data curation, Formal analysis, Methodology, Writing – review & editing. CS: Project administration, Supervision, Writing – review & editing. JY: Project administration, Supervision, Writing – review & editing. NX: Data curation, Writing – review & editing. XC: Project administration, Supervision, Writing – review & editing. FX: Project administration, Supervision, Writing – review & editing. XS: Data curation, Formal analysis, Writing – review & editing. YD: Data curation, Formal analysis, Writing – review & editing. YLiu: Data curation, Formal analysis, Writing – review & editing. FT: Project administration, Supervision, Writing – review & editing. CH: Project administration, Writing – review & editing. JL: Data curation, Formal analysis, Writing – review & editing. QD: Data curation, Writing – review & editing. XZ: Project administration, Supervision, Writing – review & editing. HG: Project administration, Supervision, Writing – review & editing. YongL: Project administration, Writing – review & editing. XF: Data curation, Formal analysis, Project administration, Resources, Supervision, Writing – review & editing. XJ: Data curation, Formal analysis, Software, Writing – review & editing. LHa: Formal analysis, Methodology, Resources, Software, Writing – review & editing. ZY: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. LHu: Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing.

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Conflict of interest

LHa and XJ is employed by Kangmeihuada GeneTech Co., Ltd.

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1359644/full#supplementary-material

References

1. Wang Q. Individualized medicine, health medicine, and constitutional theory in Chinese medicine. *Front Med*. (2012) 6:1–7. doi: 10.1007/s11684-012-0173-y

2. Liang X, Wang Q, Jiang Z, Li Z, Zhang M, Yang P, et al. Clinical research linking traditional Chinese medicine constitution types with diseases: a literature review of 1639 observational studies. *J Tradit Chin Med*. (2020) 40:690–702. doi: 10.19852/j.cnki.jtcm.2020.04.019

3. Zhen J, Zhao P, Huang G, Li Y, Zhao L, Zhang Y, et al. Fecal-associated microbiome differences between traditional Chinese medicine qi-deficiency and balanced constitutions. *J Tradit Chin Med Sci*. (2020) 7:104–13. doi: 10.1016/j.jtcms.2020.03.002

4. Qi Y, Yangshuai L. NMR-based Metabonomics studies on serum and urine of Yang-deficiency constitution. *Chem J Chin Univ Chin*. (2011) 32:2521.

5. Wang Q, Ren XJ, Yao SL, Wu HD. Clinical observation on the endocrinal and immune functions in subjects with yin-deficiency constitution. *Chin J Integr Med*. (2010) 16:28–32. doi: 10.1007/s11655-010-0028-9

6. Chiang HC, Yang ST, Lee KC, Huang PY, Hsu M, Chang HH. From theory to clinic: key components of qi deficiency in traditional Chinese medicine. *Altern Ther Health Med*. (2012) 18:28–36.

7. Wang J, Li Y, Ni C, Zhang H, Li L, Wang Q. Cognition research and constitutional classification in Chinese medicine. *Am J Chin Med*. (2011) 39:651–60. doi: 10.1142/S0192415X11009093

8. Chen HJ, Lin YJ, Wu PC, Hsu WH, Hu WC, Wu TN, et al. Study on Yang-Xu using body constitution questionnaire and blood variables in healthy volunteers. *Evid Based Complement Alternat Med*. (2016) 2016:1–7. doi: 10.1155/2016/9437382

9. Qi L, Bin L, Jinfeng L, Chunan R, Yan T, Ruoxi Y. Analysis of blood biochemical indexes and intestinal microflora diversity in women with constitution of Yin deficiency and constitution of Yin-yang harmony. *Acta Chin. Med.* (2020) 35:1514–9.

10. World Health Organization. *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity* (2011). Available at: https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.1.

11. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. *Ann N Y Acad Sci*. (2019) 1450:15–31. doi: 10.1111/nyas.14092

12. Benson CS, Shah A, Stanworth SJ, Frise CJ, Spiby H, Lax SJ, et al. The effect of iron deficiency and anaemia on women's health. *Anaesthesia*. (2021) 76:84–95. doi: 10.1111/anae.15405

13. Clark VL, Kruse JA. Clinical methods: the history, physical, and laboratory examinations. *JAMA*. (1990) 264:2808–9. doi: 10.1001/jama.1990.03450210108045

14. National Library of Medicine. *Red blood cell (RBC) indices*. (2021). Available at: https://medlineplus.gov/lab-tests/red-blood-cell-rbc-indices/. (Accessed October 5, 2021).

15. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol*. (2016) 38:123–32. doi: 10.1111/ijlh.12500

16. Wang K, Ma J, Li Y, Han Q, Yin Z, Zhou M, et al. Effects of essential oil extracted from Artemisia argyi leaf on lipid metabolism and gut microbiota in high-fat diet-fed mice. *Front Nutr*. (2022) 9:1024722. doi: 10.3389/fnut.2022.1024722

17. Duan J, Sun J, Jiang T, Ma X, Li X, Wang Y, et al. Podophyllotoxin-mediated neurotoxicity via the microbiota-gut-brain axis in SD rats based on the toxicological evidence chain (TEC) concept. *Sci Total Environ*. (2024) 907:168106. doi: 10.1016/j.scitotenv.2023.168106

18. Su M, Hu R, Tang T, Tang W, Huang C. Review of the correlation between Chinese medicine and intestinal microbiota on the efficacy of diabetes mellitus. *Front Endocrinol (Lausanne)*. (2022) 13:1085092. doi: 10.3389/fendo.2022.1085092

19. Ming T, Lei J, Peng Y, Wang M, Liang Y, Tang S, et al. Curcumin suppresses colorectal cancer by induction of ferroptosis via regulation of p53 and solute carrier family 7 member 11/glutathione/glutathione peroxidase 4 signaling axis. *Phytother Res*. (2024) 2024:8258. doi: 10.1002/ptr.8258

20. Zhao H, Zong Y, Li W, Wang Y, Zhao W, Meng X, et al. Damp-heat constitution influences gut microbiota and urine metabolism of Chinese infants. *Heliyon*. (2023) 9:e12424. doi: 10.1016/j.heliyon.2022.e12424

21. Jing Y, Han S, Chen J, Lai Y, Cheng J, Li F, et al. Gut microbiota and urine Metabonomics alterations in constitution after Chinese medicine and lifestyle intervention. *Am J Chin Med*. (2021) 49:1165–93. doi: 10.1142/S0192415X21500567

22. Chen J, Cheng J, Li F, Deng Y, Li Y, Li H, et al. Gut microbiome and metabolome alterations in traditional Chinese medicine damp-heat constitution following treatment with a Chinese patent medicine and lifestyle intervention. *Phytomedicine*. (2024) 131:155787. doi: 10.1016/j.phymed.2024.155787

23. Ma K, Chen J, Kuang L, Bi J, Cheng J, Li F, et al. Qi-deficiency related increases in disease susceptibility are potentially mediated by the intestinal microbiota. *Evid Based Complement Alternat Med*. (2018) 2018:1–10. doi: 10.1155/2018/1304397

24. Wakita Y, Saiki A, Kaneda H, Segawa S, Tsuchiya Y, Kameya H, et al. Analysis of free radical production capacity in mouse faeces and its possible application in evaluating the intestinal environment: a pilot study. *Sci Rep*. (2019) 9:19533. doi: 10.1038/s41598-019-56004-x

25. Larsen PE, Dai Y. Metabolome of human gut microbiome is predictive of host dysbiosis. *Gigascience*. (2015) 4:42. doi: 10.1186/s13742-015-0084-3

26. Shao Y, Qi W, Zhang X, Ran N, Liu C, Fu R, et al. Plasma Metabolomic and intestinal microbial analyses of patients with severe aplastic Anemia. *Front Cell Dev Biol*. (2021) 9:669887. doi: 10.3389/fcell.2021.669887

27. Long Y, Liang F, Guo R, Zhu C, Zhao X, Wang X, et al. Gut Microbiota Signatures in Gestational Anemia. *Front Cell Infect Microbiol*. (2021) 11:549678. doi: 10.3389/fcimb.2021.549678

28. Wei H, Deng S, Qin Y, Yang X, Chen T, Wang X, et al. Insight into the potential value of gut microbial signatures for prediction of gestational Anemia. *Front Cell Infect Microbiol*. (2021) 11:734561. doi: 10.3389/fcimb.2021.734561

29. Muleviciene A, D'Amico F, Turroni S, Candela M, Jankauskiene A. Iron deficiency anemia-related gut microbiota dysbiosis in infants and young children: a pilot study. *Acta Microbiol Immunol Hung*. (2018) 65:551–64. doi: 10.1556/030.65.2018.045

30. Shi XQ, Zhu ZH, Yue SJ, Tang YP, Chen YY, Pu ZJ, et al. Studies on blood enrichment and anti-tumor effects of combined Danggui Buxue decoction, Fe and rhEPO based on colon cancer-related anemia model and gut microbiota modulation. *Chin J Nat Med*. (2021) 19:422–31. doi: 10.1016/S1875-5364(21)60041-9

31. Bai MH, Wong W, Hou SJ, Zheng YF, Li QR, Li ZQ, et al. Development and evaluation of short form of constitution in Chinese medicine questionnaire: a national epidemiological survey data of 21 948 case. *J Tradit Chin Med*. (2022) 42:122–31. doi: 10.19852/j.cnki.jtcm.20211228.001

32. Beghini F, McIver LJ, Blanco-Miguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *eLife*. (2021) 10:10. doi: 10.7554/eLife.65088

33. Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat Methods*. (2015) 12:902–3. doi: 10.1038/nmeth.3589

34. Franzosa EA, McIver LJ, Rahnavard G, Thompson LR, Schirmer M, Weingart G, et al. Species-level functional profiling of metagenomes and metatranscriptomes. *Nat Methods*. (2018) 15:962–8. doi: 10.1038/s41592-018-0176-y

35. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol*. (2011) 12:R60. doi: 10.1186/gb-2011-12-6-r60

36. Changyun Z, Cai J, Ning X, Xin Z, Huijuan Y, Yang L, et al. Study on characteristics of intestinal Flora in Yang deficiency constitution through rDNA gene sequencing analysis. Shandong. *J Tradit Chin Med*. (2020) 39:697–703+10.

37. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, et al. Evolution of mammals and their gut microbes. *Science*. (2008) 320:1647–51. doi: 10.1126/science.1155725

38. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A*. (2010) 107:14691–6. doi: 10.1073/pnas.1005963107

39. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature*. (2012) 486:222–7. doi: 10.1038/nature11053

40. Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci U S A*. (2011) 108:4586–91. doi: 10.1073/pnas.1000097107

41. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. (2006) 444:1027–31. doi: 10.1038/nature05414

42. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. (2006) 444:1022–3. doi: 10.1038/4441022a

43. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature*. (2009) 457:480–4. doi: 10.1038/nature07540

44. Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, et al. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J*. (2015) 9:1979–90. doi: 10.1038/ismej.2015.11

45. Dehoux P, Marvaud JC, Abouelleil A, Earl AM, Lambert T, Dauga C. Comparative genomics of *Clostridium bolteae* and *Clostridium clostridioforme* reveals species-specific genomic properties and numerous putative antibiotic resistance determinants. *BMC Genomics*. (2016) 17:819. doi: 10.1186/s12864-016-3152-x

46. Plichta DR, Somani J, Pichaud M, Wallace ZS, Fernandes AD, Perugino CA, et al. Congruent microbiome signatures in fibrosis-prone autoimmune diseases: IgG4-related disease and systemic sclerosis. *Genome Med*. (2021) 13:35. doi: 10.1186/s13073-021-00853-7

47. Pandit L, Cox LM, Malli C, D'Cunha A, Rooney T, Lokhande H, et al. *Clostridium bolteae* is elevated in *Neuromyelitis optica* spectrum disorder in India and shares

sequence similarity with AQP4. *Neurol Neuroimmunol Neuroinflamm*. (2021) 8:907. doi: 10.1212/NXI.0000000000000907

48. Mikami A, Ogita T, Namai F, Shigemori S, Sato T, Shimosato T. Oral Administration of *Flavonifractor plautii*, a Bacteria increased with green tea consumption, promotes recovery from acute colitis in mice via suppression of IL-17. *Front Nutr*. (2020) 7:610946. doi: 10.3389/fnut.2020.610946

49. Borgo F, Garbossa S, Riva A, Severgnini M, Luigiano C, Benetti A, et al. Body mass index and sex affect diverse microbial niches within the gut. *Front Microbiol*. (2018) 9:213. doi: 10.3389/fmicb.2018.00213

50. Engels C, Ruscheweyh HJ, Beerenwinkel N, Lacroix C, Schwab C. The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation. *Front Microbiol*. (2016) 7:713. doi: 10.3389/fmicb.2016.00713

51. Mullineaux-Sanders C, Sanchez-Garrido J, Hopkins EGD, Shenoy AR, Barry R, Frankel G. *Citrobacter rodentium*-host-microbiota interactions: immunity, bioenergetics and metabolism. *Nat Rev Microbiol*. (2019) 17:701–15. doi: 10.1038/s41579-019-0252-z

52. Calderón-Pérez L, Llauradó E, Companys J, Pla-Pagà L, Pedret A, Rubió L, et al. Interplay between dietary phenolic compound intake and the human gut microbiome in hypertension: a cross-sectional study. *Food Chem*. (2021) 344:128567. doi: 10.1016/j.foodchem.2020.128567

53. Peters BA, Yi SS, Beasley JM, Cobbs EN, Choi HS, Beggs DB, et al. US nativity and dietary acculturation impact the gut microbiome in a diverse US population. *ISME J*. (2020) 14:1639–50. doi: 10.1038/s41396-020-0630-6

54. Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature*. (2010) 464:908–12. doi: 10.1038/nature08937

55. Mondot S, Lepage P, Seksik P, Allez M, Tréton X, Bouhnik Y, et al. Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. *Gut*. (2016) 65:954–62. doi: 10.1136/gutjnl-2015-309184

56. Lai CH, Lin TL, Huang MZ, Li SW, Wu HY, Chiu YF, et al. Gut commensal *Parabacteroides goldsteinii* MTS01 alters gut microbiota composition and reduces cholesterol to mitigate *Helicobacter pylori*-induced pathogenesis. *Front Immunol*. (2022) 13:916848. doi: 10.3389/fimmu.2022.916848

57. Sinha SR, Haileselassie Y, Nguyen LP, Tropini C, Wang M, Becker LS, et al. Dysbiosis-induced secondary bile acid deficiency promotes intestinal inflammation. *Cell Host Microbe*. (2020) 27:659–70.e5. doi: 10.1016/j.chom.2020.01.021

58. Liu S, Zhao W, Lan P, Mou X. The microbiome in inflammatory bowel diseases: from pathogenesis to therapy. *Protein Cell*. (2021) 12:331–45. doi: 10.1007/s13238-020-00745-3

59. Laila AH. Novel prebiotics and next-generation probiotics: opportunities and challenges In: AH Laila, editor. Functional foods and nutraceuticals in metabolic and non-communicable diseases. Cambridge, MA: Academic Press (2022). 431–57.

60. Ren L, Wang Y, Zhong J, Li X, Xiao Y, Li J, et al. Dynamics of the upper respiratory tract microbiota and its association with mortality in COVID-19. *Am J Respir Crit Care Med*. (2021) 204:1379–90. doi: 10.1164/rccm.202103-0814OC

61. Wu F, Yang L, Hao Y, Zhou B, Hu J, Yang Y, et al. Oral and gastric microbiome in relation to gastric intestinal metaplasia. *Int J Cancer*. (2022) 150:928–40. doi: 10.1002/ijc.33848

62. Fan L, Chen J, Pan L, Xin X, Geng B, Yang L, et al. Alterations of gut microbiome, metabolome and lipidome in Takayasu arteritis. Arthritis. *Rheumatol*. (2022) 75:266–78. doi: 10.1002/art.42331

63. Bowerman KL, Rehman SF, Vaughan A, Lachner N, Budden KF, Kim RY, et al. Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. *Nat Commun*. (2020) 11:5886. doi: 10.1038/s41467-020-19701-0

64. Porter NT, Luis AS, Martens EC. Bacteroides thetaiotaomicron. *Trends Microbiol*. (2018) 26:966–7. doi: 10.1016/j.tim.2018.08.005

65. Gul L, Modos D, Fonseca S, Madgwick M, Thomas JP, Sudhakar P, et al. Extracellular vesicles produced by the human commensal gut bacterium *Bacteroides thetaiotaomicron* affect host immune pathways in a cell-type specific manner that are altered in inflammatory bowel disease. *J Extracell Vesicles*. (2022) 11:e12189. doi: 10.1002/jev2.12189

66. Le HH, Lee MT, Besler KR, Comrie JMC, Johnson EL. Characterization of interactions of dietary cholesterol with the murine and human gut microbiome. *Nat Microbiol*. (2022) 7:1390–403. doi: 10.1038/s41564-022-01195-9

67. Lu H, Xu X, Fu D, Gu Y, Fan R, Yi H, et al. Butyrate-producing *Eubacterium rectale* suppresses lymphomagenesis by alleviating the TNF-induced TLR4/MyD88/NF-κB axis. *Cell Host Microbe*. (2022) 30:1139–50.e7. doi: 10.1016/j.chom.2022.07.003

68. Xia J, Lv L, Liu B, Wang S, Zhang S, Wu Z, et al. *Akkermansia muciniphila* ameliorates acetaminophen-induced liver injury by regulating gut microbial composition and metabolism. *Microbiol Spectr*. (2022) 10:e0159621. doi: 10.1128/spectrum.01596-21

69. Ghaffari S, Abbasi A, Somi MH, Moaddab SY, Nikniaz L, Kafil HS, et al. *Akkermansia muciniphila*: from its critical role in human health to strategies for promoting its abundance in human gut microbiome. *Crit Rev Food Sci Nutr*. (2022) 63:7357–77. doi: 10.1080/10408398.2022.2045894

70. Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, et al. A novel *Ruminococcus gnavus* clade enriched in inflammatory bowel disease patients. *Genome Med*. (2017) 9:103. doi: 10.1186/s13073-017-0490-5

71. Grahnemo L, Nethander M, Coward E, Gabrielsen ME, Sree S, Billod JM, et al. Cross-sectional associations between the gut microbe *Ruminococcus gnavus* and features of the metabolic syndrome. *Lancet Diabetes Endocrinol*. (2022) 10:481–3. doi: 10.1016/S2213-8587(22)00113-9

72. Liu Q, Mak JWY, Su Q, Yeoh YK, Lui GC, Ng SSS, et al. Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. *Gut*. (2022) 71:544–52. doi: 10.1136/gutjnl-2021-325989

73. Ruuskanen MO, Erawijantari PP, Havulinna AS, Liu Y, Méric G, Tuomilehto J, et al. Gut microbiome composition is predictive of incident type 2 diabetes in a population cohort of 5,572 Finnish adults. *Diabetes Care*. (2022) 45:811–8. doi: 10.2337/dc21-2358

74. Breban M, Tap J, Leboime A, Said-Nahal R, Langella P, Chiocchia G, et al. Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. *Ann Rheum Dis*. (2017) 76:1614–22. doi: 10.1136/annrheumdis-2016-211064

75. Hegazy AN, West NR, Stubbington MJT, Wendt E, Suijker KIM, Datsi A, et al. Circulating and tissue-resident CD4(+) T cells with reactivity to intestinal microbiota are abundant in healthy individuals and function is altered during inflammation. *Gastroenterology*. (2017) 153:1320–37.e16. doi: 10.1053/j.gastro.2017.07.047

76. Fiermonte G, Dolce V, Palmieri L, Ventura M, Runswick MJ, Palmieri F, et al. Identification of the human mitochondrial oxodicarboxylate carrier. Bacterial expression, reconstitution, functional characterization, tissue distribution, and chromosomal location. *J Biol Chem*. (2001) 276:8225–30. doi: 10.1074/jbc.M009607200

77. Schulze-Bergkamen A, Okun JG, Spiekerkötter U, Lindner M, Haas D, Kohlmüller D, et al. Quantitative acylcarnitine profiling in peripheral blood mononuclear cells using in vitro loading with palmitic and 2-oxoadipic acids: biochemical confirmation of fatty acid oxidation and organic acid disorders. *Pediatr Res*. (2005) 58:873–80. doi: 10.1203/01.PDR.0000181378.98593.3E

78. Wu D, Wang Z, Lin M, Shang Y, Wang F, Zhou J, et al. In vitro and in vivo antitumor activity of Cucurbitacin C, a novel natural product from cucumber. *Front Pharmacol*. (2019) 10:1287. doi: 10.3389/fphar.2019.01287

79. Liu K, Xing H, Zhang S, Liu S, Fung M. Cucurbitacin D induces fetal hemoglobin synthesis in K562 cells and human hematopoietic progenitors through activation of p38 pathway and stabilization of the γ-globin mRNA. *Blood Cells Mol Dis*. (2010) 45:269–75. doi: 10.1016/j.bcmd.2010.09.004

80. Fritz IB. Action of carnitine on long chain fatty acid oxidation by liver. *Am J Phys*. (1959) 197:297–304. doi: 10.1152/ajplegacy.1959.197.2.297

81. Claudel T, Staels B, Kuipers F. The Farnesoid X receptor: a molecular link between bile acid and lipid and glucose metabolism. *Arterioscler Thromb Vasc Biol*. (2005) 25:2020–30. doi: 10.1161/01.ATV.0000178994.21828.a7

82. Ding S, Jiang H, Fang J, Liu G. Regulatory effect of resveratrol on inflammation induced by lipopolysaccharides via reprograming intestinal microbes and ameliorating serum metabolism profiles. *Front Immunol*. (2021) 12:777159. doi: 10.3389/fimmu.2021.777159

83. Qin X, Liu B, Gao F, Hu Y, Chen Z, Xu J, et al. Gluconolactone alleviates myocardial ischemia/reperfusion injury and arrhythmias via activating PKCε/extracellular signal-regulated kinase signaling. *Front Physiol*. (2022) 13:856699. doi: 10.3389/fphys.2022.856699

84. Zhao X, Zhang Z, Hu B, Huang W, Yuan C, Zou L. Response of gut microbiota to metabolite changes induced by endurance exercise. *Front Microbiol*. (2018) 9:765. doi: 10.3389/fmicb.2018.00765

85. Zheng X, Huang F, Zhao A, Lei S, Zhang Y, Xie G, et al. Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice. *BMC Biol*. (2017) 15:120. doi: 10.1186/s12915-017-0462-7

86. Thom CS, Dickson CF, Gell DA, Weiss MJ. Hemoglobin variants: biochemical properties and clinical correlates. *Cold Spring Harb Perspect Med*. (2013) 3:a011858. doi: 10.1101/cshperspect.a011858

87. Acharya AS, Bobelis DJ, White SP. Electrostatic modification at the amino termini of hemoglobin a. *J Biol Chem*. (1994) 269:2796–804. doi: 10.1016/S0021-9258(17)42013-8

88. Hu H, Zhang S, Liu F, Zhang P, Muhammad Z, Pan S. Role of the gut microbiota and their metabolites in modulating the cholesterol-lowering effects of Citrus pectin oligosaccharides in C57BL/6 mice. *J Agric Food Chem*. (2019) 67:11922–30. doi: 10.1021/acs.jafc.9b03731

89. Gall WE, Beebe K, Lawton KA, Adam KP, Mitchell MW, Nakhle PJ, et al. Alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PLoS One*. (2010) 5:e10883. doi: 10.1371/journal.pone.0010883

90. Landaas S, Pettersen JE. Clinical conditions associated with urinary excretion of 2-hydroxybutyric acid. *Scand J Clin Lab Invest*. (1975) 35:259–66. doi: 10.3109/00365517509095738

91. Li X, Xu Z, Lu X, Yang X, Yin P, Kong H, et al. Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: biomarker discovery for diabetes mellitus. *Anal Chim Acta*. (2009) 633:257–62. doi: 10.1016/j.aca.2008.11.058

92. van de Poll MC, Dejong CH, Soeters PB. Adequate range for sulfur-containing amino acids and biomarkers for their excess: lessons from enteral and parenteral nutrition. *J Nutr*. (2006) 136:1694s–700s. doi: 10.1093/jn/136.6.1694S

93. St-Pierre MV, Kullak-Ublick GA, Hagenbuch B, Meier PJ. Transport of bile acids in hepatic and non-hepatic tissues. *J Exp Biol*. (2001) 204:1673–86. doi: 10.1242/jeb.204.10.1673

94. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med*. (2016) 22:1079–89. doi: 10.1038/nm.4185

95. Asim KD. Bioactive lipids and their impacts on epigenetics. Amsterdam, Netherlands: Elsevier 21–30. (2021).

96. Pral LP, Fachi JL, Corrêa RO, Colonna M, Vinolo MAR. Hypoxia and HIF-1 as key regulators of gut microbiota and host interactions. *Trends Immunol*. (2021) 42:604–21. doi: 10.1016/j.it.2021.05.004

97. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe*. (2015) 17:662–71. doi: 10.1016/j.chom.2015.03.005

98. Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, et al. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat Commun*. (2020) 11:4457. doi: 10.1038/s41467-020-18262-6

99. Das NK, Schwartz AJ, Barthel G, Inohara N, Liu Q, Sankar A, et al. Microbial metabolite signaling is required for systemic Iron homeostasis. *Cell Metab*. (2020) 31:115–30.e6. doi: 10.1016/j.cmet.2019.10.005

100. Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat Rev Mol Cell Biol*. (2020) 21:268–83. doi: 10.1038/s41580-020-0227-y

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Global burden of anemia and cause among children under five years 1990–2019: findings from the global burden of disease study 2019

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Background: Anemia represents a significant global health issue affecting numerous children and women, characterized by diminished hemoglobin levels that may impede cognitive and developmental progress. Although commonly attributed to iron deficiency, the etiology of anemia in this demographic is multifaceted, encompassing nutritional, genetic, and infectious contributors. Nonetheless, there is a lack of high-quality data on anemia prevalence and causes analysis among children under 5 years. The aim of this study was to provide a comprehensive global assessment of the burden of anemia and its causes among children under 5 years, using data from the Global Burden of Disease Study 2019.

Methods: This investigation utilized data from the Global Burden of Disease Study (GBD) 2019 to assess the prevalence and years lived with disability (YLD) attributable to anemia in children under five from 1990 to 2019. Analyses were conducted to delineate age-specific YLD, prevalence rates, and etiological factors, with stratification by gender and Socio-Demographic Index (SDI).

Results: In 2019, anemia imposed a substantial global burden on children under five, with a reported YLD of 1,252.88 (95% UI: 831.62–1,831.34) per 100,000 population. The prevalence of moderate anemia was highest at 985.46 (95% UI: 646.24–1,450.49) per 100,000, surpassing both severe anemia at 197.82 (95% UI: 132.53–277.80) per 100,000 and mild anemia at 69.59 (95% UI: 24.62–152.53) per 100,000. Globally, the total prevalence was 39,517.75 (95% UI: 38,784.81 - 40,239.62) cases per 100,000 population. Notably, disparities were evident between genders, with males demonstrating higher YLD and prevalence rates than females. Iron deficiency emerged as the leading cause globally, with significant contributions from hemoglobinopathies and other nutritional deficiencies. Regions with a low Socio-Demographic Index, particularly sub-Saharan Africa and South Asia, exhibited the most pronounced burdens. Despite a declining trend over three decades, persistent regional and gender-based disparities highlight the necessity for continuous and focused public health interventions.

Conclusion: The burden of anemia among children under five continues to be considerable, marked by stark regional and socioeconomic disparities. These findings underscore the urgent need for advanced nutritional and healthcare strategies tailored to alleviate anemia in this vulnerable population, with a

particular emphasis on regions exhibiting low SDIs. The sustained prevalence of high anemia rates in these areas underscores the imperative for persistent, localized intervention efforts.

KEYWORDS

anemia, iron deficiency, children, etiology, disease burden

Introduction

Anemia represents a significant global health challenge, particularly among the most vulnerable populations, including children under the age of five (1). A reduction in either the quantity or functionality of red blood cells and hemoglobin characterizes anemia. This condition impairs the blood's capacity to transport oxygen efficiently. As a result, individuals with anemia often experience symptoms such as fatigue, weakness, and shortness of breath (2, 3). The condition's etiology is multifaceted, encompassing nutritional deficiencies, genetic disorders, infectious diseases, and more (4). This complexity necessitates a comprehensive understanding of anemia's various causes and their specific impacts on different demographic groups, especially young children (5–7). Their heightened vulnerability to anemia's adverse effects underscores the importance of early detection, prevention, and intervention strategies to safeguard their health and development.

The epidemiological landscape of anemia is diverse and influenced by an interplay of genetic, environmental, and socio-economic factors. Globally, anemia affects approximately one-fourth of the population, with pronounced disparities observed across regions, ages, and genders (8–10). The prevalence is notably higher in developing countries, where factors such as malnutrition, inadequate healthcare infrastructure, and a higher burden of infectious diseases like malaria and HIV/AIDS exacerbate the risk of anemia (11, 12). Children under five in these regions are particularly affected, facing increased morbidity and mortality risks (8). Anemia's impact extends beyond immediate health concerns, contributing to long-term developmental delays, impaired cognitive function (11, 13), and diminished educational performance (14, 15), thereby perpetuating cycles of poverty and disadvantage.

Despite significant advances in understanding anemia's global burden (7), a gap remains in current, comprehensive data that delineates the complex causes and consequences of anemia in children under 5 years. The GBD data, while extensive, lacks focus on this specific age group, and detailed findings are yet to be made public. This study leverages the most recent 2019 GBD data to examine the point prevalence, YLDs, and attributable causes of anemia in children under 5 years globally from 1990 to 2019. Through this analysis, we aim to enhance the global understanding of anemia's impact on younger populations, guiding future research and informing prevention, diagnosis, and management strategies to mitigate the health burden of anemia among children and adolescents.

Methods

Overview of the global burden of disease study 2019

The GBD Study 2019 offers a comprehensive analysis of health challenges worldwide, covering 369 diseases and injuries along with 87 risk factors from 1990 to 2019, across 204 countries and territories (16). Utilizing robust methodologies, previously detailed in extensive publications, this study highlights trends in epidemiological patterns. The GBD 2019's extensive dataset, accessible via the Global Health Data Exchange (GHDX) and the interactive GBD Compare platform, serves as an invaluable resource for delineating the evolving landscape of global health challenges, including both fatal and non-fatal health outcomes.

Definitions and classification of anemia

Anemia is defined in this study by age-adjusted reduced hemoglobin levels, following specific criteria for classification. This analysis, spanning the years 1990 to 2019 across 204 nations, calculates the distribution of hemoglobin concentrations, anemia prevalence, and YLDs due to anemia across 37 identified etiologies, covering all genders and 25 age categories. The gradation of anemia into mild, moderate, and severe categories is based on hemoglobin concentration thresholds that vary by age, gender, and pregnancy status, in alignment with World Health Organization standards and adapted for specific demographic nuances.

Methodological approach to assessing anemia prevalence

The assessment of anemia prevalence in children under five utilized demographic health surveys, scholarly articles, and governmental reports. Data were aligned with specific geographic, age, gender, and pregnancy demographics, following GBD study protocols, including adjustments for elevation based on the WHO formula. The method did not alter hemoglobin sample collection or analysis techniques. Spatiotemporal Gaussian process regression and linear mixed-effects models estimated hemoglobin levels and anemia prevalence, applying inverse weighting for model accuracy. Discrepancies were smoothed across various dimensions, enhancing the precision of uncertainty quantification in the final results.

Assessment of YLDs

The YLDs calculation for anemia in children under five was conducted by correlating the number of anemia cases at each severity level with severity-specific disability weights. These weights represent the degree of health impairment on a scale from 0 (no impairment) to 1 (death), allowing for a nuanced understanding of anemia's impact on early childhood development.

Causal attribution of anemia

Our analysis identified several causes of anemia in children under five, employing a mutually exclusive and collectively exhaustive approach to assign each anemia case to one of the 37 defined causes. This involved utilizing distributions of hemoglobin concentration, overall anemia prevalence by severity, disease prevalence or incidence, and cause-specific hemoglobin shifts derived from extensive research. This detailed methodology facilitates a comprehensive understanding of anemia's etiology in young children, which is essential for targeted health interventions.

Analysis of anemia trends in relation to socio-economic progress

We explored the relationship between anemia prevalence in children under five and socio-economic development, analyzing how anemia's prevalence and impact on YLDs correlate with the SDI across different regions. Employing meta-regression models, we investigated the potentially non-linear relationships between anemia burden and SDI, identifying countries where anemia prevalence significantly deviates from expectations based on socio-economic status. This multifaceted analysis elucidates the influence of socio-economic factors on anemia prevalence and disability in young children, informing health policy and resource distribution.

Results

Global level

Anemia's impact on the global population of children under five in 2019 was profound, with the total YLDs amounting to 1,252.88 (95% UI: 831.62–1,831.34) per 100,000 population. A nuanced breakdown by severity reveals the differential impact of the condition: mild anemia contributed to a YLDs rate of 69.59 (95% UI: 24.62–152.53) per 100,000 population, moderate anemia presented a considerably higher YLDs rate of 985.46 (95% UI: 646.24–1,450.49) per 100,000 population, and severe anemia resulted in a YLDs rate of 197.82 (95% UI: 132.53–277.80) per 100,000 population (Supplementary Table S1).

Furthermore, the prevalence of anemia in 2019 among this demographic elucidates the extent of its reach. The overall prevalence was reported at a rate of 39,517.75 (95% UI: 38,784.81 - 40,239.62) per 100,000 population. Disaggregating this data by severity, mild anemia exhibited a prevalence rate of 18,880.01 (95% UI: 18,527.70 - 19,193.64) per 100,000, moderate anemia impacted 19,281.86 (95% UI: 18,822.14 - 19,795.88) per 100,000, and severe anemia was less common but still significant, with a prevalence rate of 1,355.87 (95% UI: 1,272.73 - 1,444.12) per 100,000 (Supplementary Table S1).

Regional level

The analysis reveals stark regional disparities in childhood anemia, correlated with the SDI. Low SDI regions face the most severe burden, with a YLDs rate of 2310.58 per 100,000 population (95%UI: 1,522.92–3,346.29). This rate decreases in low-middle SDI regions to 1,487.04 (95% UI: 975.95–2,185.34), and further to 1,252.88 (95% UI: 831.62–1,831.34) globally. Higher SDI regions experience significantly lower rates, with middle, high-middle, and high SDI regions reporting YLDs of 725.25 (95% UI: 475.04–1,072.18), 448.08 (95% UI: 286.17–679.03), and 159.53 (95% UI: 97.23–251.78) respectively. In terms of prevalence, 67.43% of children under five in low SDI regions suffer from anemia, contrasting with only 14.40% in high SDI areas. Western and Central Sub-Saharan Africa record the highest rates, emphasizing extreme regional disparities in the impact of anemia.

National level

Next, we elucidate the national disparities in the burden and prevalence of anemia among children under the age of 5 years in 2019. Notably, Yemen emerged as the country with the highest YLDs rate for anemia among children under five, registering 4,356.99 YLDs per 100,000 (95%UI: 2,894.25 - 6,249.51). This was closely followed by Burkina Faso with a rate of 4,060.77 YLDs per 100,000 (95% UI: 2,743.77 - 5,856.50), and Bhutan, which recorded a rate of 3,543.98 YLDs per 100,000 (95% UI: 2,266.97 - 5,124.99). Other countries with significant YLDs rates included Mali (3,458.41 YLDs, 95% UI: 2,288.55 - 4,958.01), Sierra Leone (3,085.13 YLDs, 95% UI: 2,019.33 - 4,577.64), and the Central African Republic (2,682.17 YLDs, 95% UI: 1,644.53 - 4,000.34), underscoring the acute burden of anemia in these nations (Figure 1A).

The prevalence of anemia among children under five further illustrates the severe impact of this condition. Bhutan reported the highest prevalence at 96.15% (95% UI: 89.49–100%), closely followed by Yemen with a prevalence of 92.61% (95% UI: 87.99–97.10%), and Burkina Faso at 88.95% (95% UI: 84.75–92.69%). Mali (83.86, 95% UI: 78.97–88.13%), Sierra Leone (81.71, 95% UI: 77.08–86.08%), and the Central African Republic (80.82, 95% UI: 72.86–87.86%) also exhibited high prevalence percentages, indicating a widespread impact across these regions (Figure 1B).

Temporal trends and gender disparities

Globally, YLDs attributable to anemia have generally decreased over the past three decades for both genders across all age groups, with males consistently showing higher rates than females. For neonates (0–6 days), male YLDs decreased from 576.08 (95% UI: 369.47 to 859.27) to 541.04 (95% UI: 346.39 to 816.84), while female YLDs went from 336.29 (95% UI: 207.35 to 538.78) to 323.95 (95% UI: 197.22 to 517.40). In the 7–27 days age group, male YLDs saw a temporary rise from 2,557.11 (95% UI: 1,667.66 to 3,760.10) to 2,648.77 (95% UI: 1,745.83 to 3,843.95), contrasting with a decrease in females from 2,426.25 to 2,288.63. Both genders exhibited a downward trend in the 28–364 days category, with male YLDs reducing from 1,645.17 to 1,511.28, and female YLDs from 1,600.82 to 1,413.55. Children aged 1–4 years also saw declines, with male YLDs dropping from 1,556.87 to 1,225.95, and females from 1,455.92 (95% UI: 957.66 to 2,104.46) to 1,153.24 (95% UI: 755.59 to 1,688.78).

Prevalence trends varied by age. Newborns (0–6 days) saw an increase in anemia rates, with male rates rising from 27,938.12 per 100,000 to 29,744.13 per 100,000, and female rates from 23,356.06 per

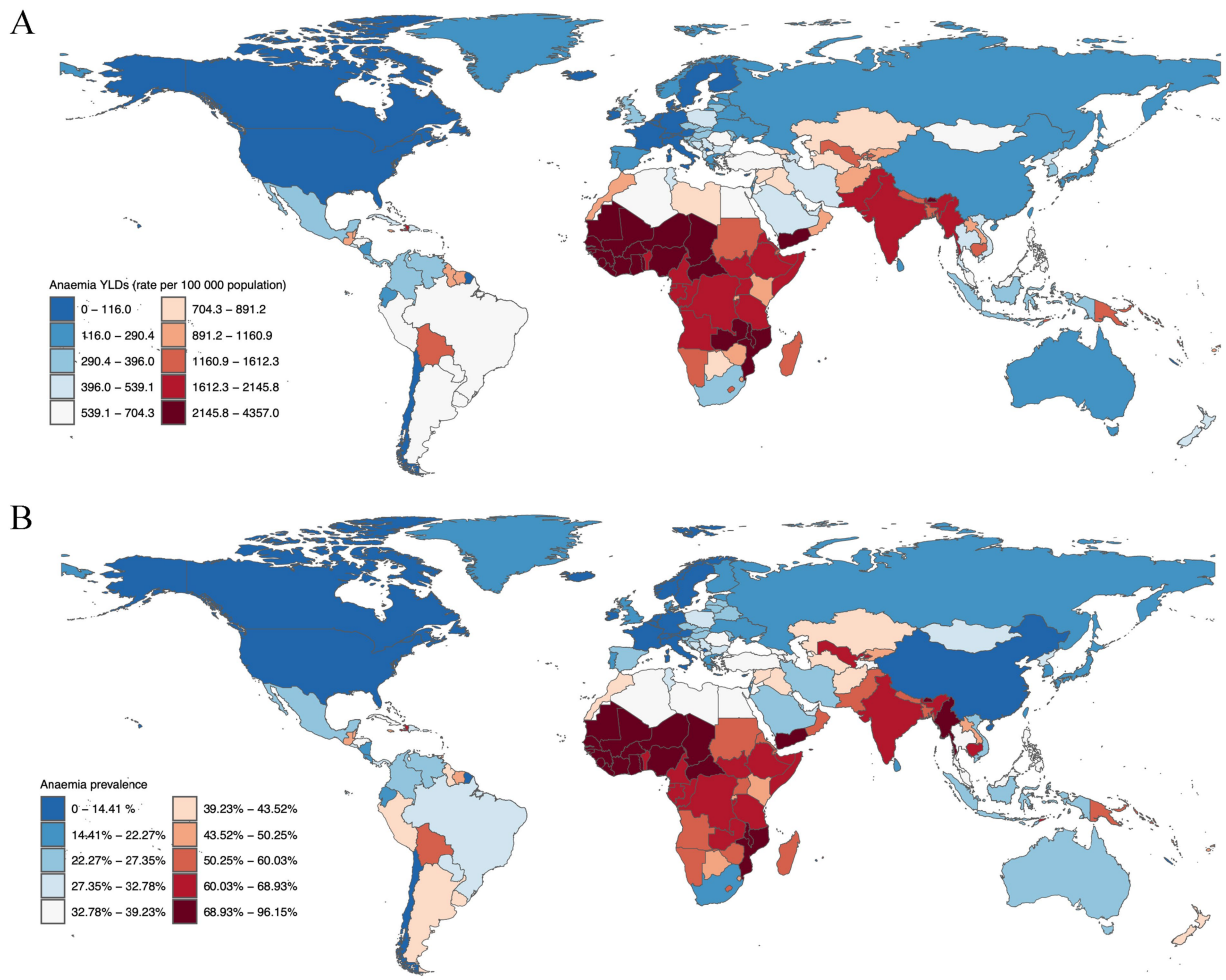

FIGURE 1
Anaemia burden in children under five, 2019. **(A)** Anaemia YLDs (rate per 100,000 population) for children under 5 years old, separated by gender, 2019. **(B)** Anaemia prevalence for children under 5 years old, separated by gender, 2019.

100,000 to 24,406.13 per 100,000. Infants aged 7–27 days experienced slight increases in prevalence, while those in the 28–364 days group saw minor declines. The most notable decrease occurred in children aged 1–4 years, with male prevalence dropping from 44,744.73 per 100,000 to 38,777.18 per 100,000, and female rates from 41,780.57 per 100,000 to 36,499.94 per 100,000 (Figure 2).

Causal attribution of anemia

Our findings reveal that dietary iron deficiency emerges as the leading global cause of anemia-related YLDs, followed in significance by hemoglobinopathies and hemolytic anemias. Other notable causes include neglected tropical diseases, malaria, vitamin A deficiency, and unspecified infectious diseases. The highest incidence rates were observed in tropical Latin America, South Asia, and both western and eastern sub-Saharan Africa, indicating geographical variations in anemia etiology.

Significant disparities in the predominant causes of anemia were noted globally and within specific regions (Figure 3A). While dietary iron deficiency was identified as the primary cause of anemia-related YLDs on a global scale and across most regions, the African regions exhibited a higher relative burden from malaria, neglected tropical diseases, and hemoglobinopathies/hemolytic disorders.

A detailed heat map analysis of the prevalence rates attributable to 15 causes of anemia among children under five further corroborates the variability in anemia etiology (Figure 3B). Consistent with the YLDs data, dietary iron deficiency ranked as the foremost contributor to the child anemia burden globally, succeeded by hemoglobinopathies and hemolytic anemias. Other significant causes encompassed neglected tropical diseases, malaria, vitamin A deficiency, and unspecified infectious diseases, with the highest prevalence rates recorded in tropical Latin America, South Asia, and sub-Saharan Africa.

The burden of age-specific leading causes of anemia

In neonates, hemoglobinopathies are the primary contributors to anemia-related YLDs, accounting for 25% in males and 30% in females, followed by dietary iron deficiency which impacts 30% of

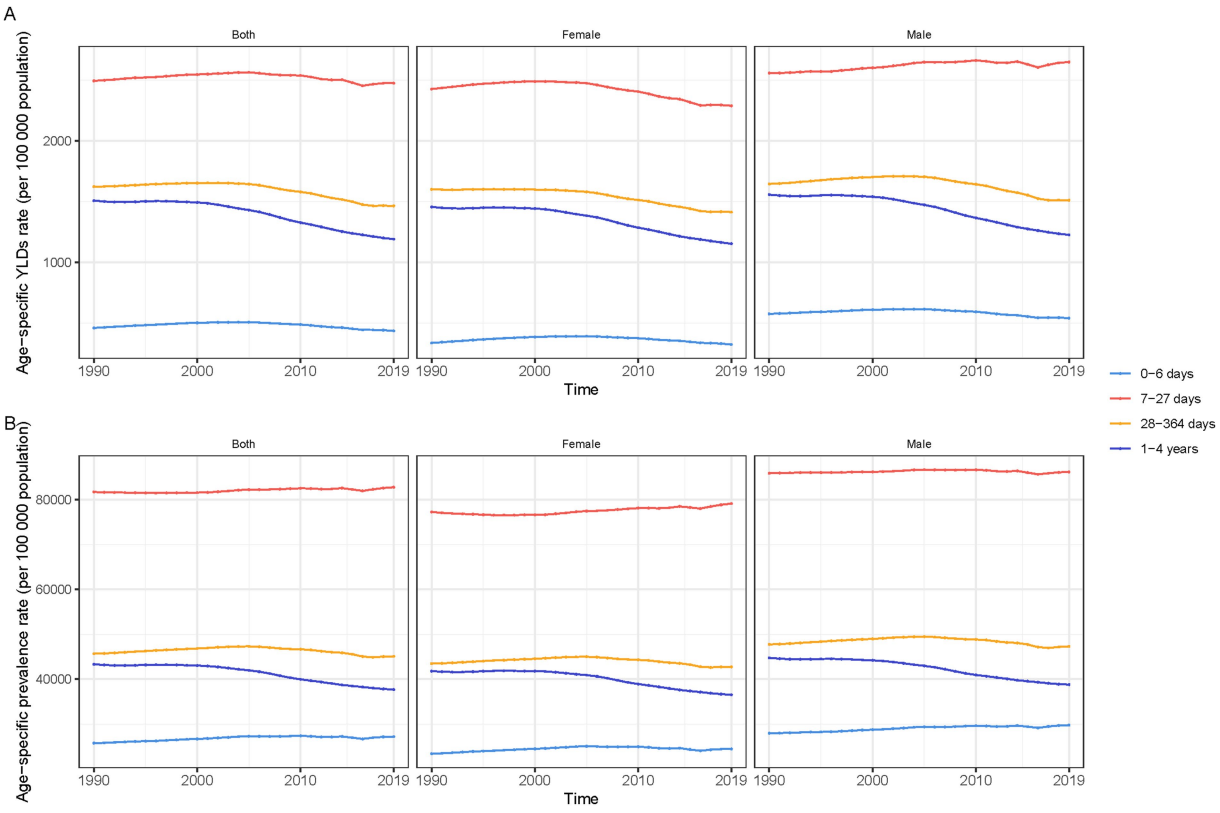

FIGURE 2
Trends in anaemia burden among children under five by age group and gender, 1990–2019, globally. **(A)** Anaemia YLDs (rate per 100,000 population). **(B)** Anaemia prevalence.

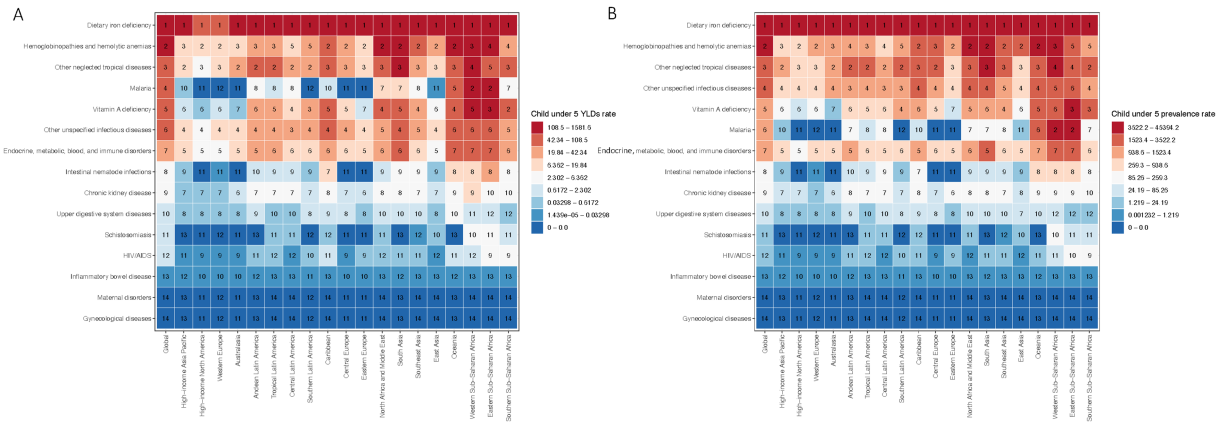

FIGURE 3
Global and regional rankings of anemia-related burden in children under five. **(A)** Global and regional rankings of anemia-related YLDs by cause in children under five, 2019. **(B)** Global and regional rankings of anemia with prevalence by cause in children under five, 2019.

males and 36% of females. Vitamin A deficiency affects 13% of males and 15% of females, with malaria contributing to around 9%. During the early infancy stage (7–27 days), dietary iron deficiency dominates, representing 35% of male and 37% of female anemia YLDs, with hemoglobinopathies causing around 12%. Vitamin A deficiency and malaria also contribute, albeit less significantly. For infants aged 28 to 364 days, dietary iron deficiency remains the largest factor, influencing 49% of male and 50% of female YLDs, while hemoglobinopathies affect about 9%. Children aged 1 to 4 years see dietary iron deficiency causing 47% of male and 48% of female anemia YLDs, with hemoglobinopathies contributing to around 6% (Figure 4A).

Prevalence patterns echo these findings, with dietary iron deficiency being most prevalent in neonates, affecting 28% of males and 32% of females, and similarly high figures persist across all age

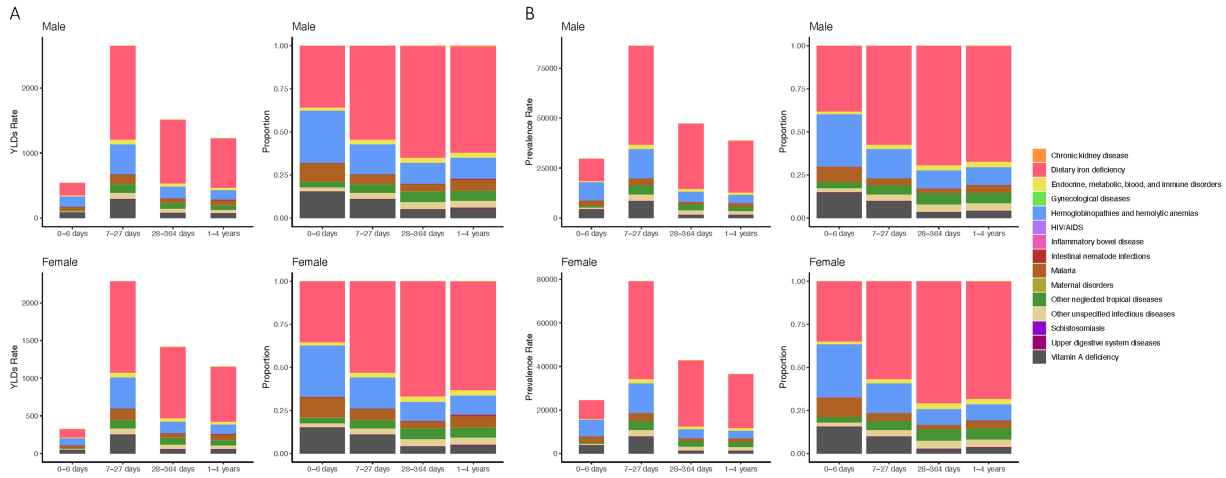

FIGURE 4
Anemia etiological contributors, prevalence, and YLDs rates by age and gender. **(A)** YLDs rate and proportional distribution by cause in male and female children under five across four age categories. **(B)** Prevalence rate and proportional distribution by cause in male and female children under five across four age categories.

groups. Hemoglobinopathies and vitamin A deficiency remain consistent causes across different ages, while malaria's impact is relatively lower but persistent (Figure 4B). This comprehensive assessment highlights the varied etiological landscape of anemia, dominated by nutritional deficiencies and genetic disorders across different developmental stages.

Socio-economic development and its impact on childhood anemia

Our study reveals a strong inverse relationship between socio-economic development, measured by the SDI, and childhood anemia rates. Regions with lower SDI values, particularly below 0.5 in sub-Saharan Africa and South Asia, experience high anemia YLDs over 2000 per 100,000 population (Figure 5A). In contrast, more developed regions with SDI values above 0.7, such as North America, Europe, and high-income areas in Asia and Latin America, show significantly lower YLDs under 1000 per 100,000 (Figure 5B). The decline in anemia rates correlates with socio-economic improvements, especially noted in East and Southeast Asia from 1990 to 2019. However, Oceania, with a moderate SDI of 0.6, contradicts this trend, suggesting other factors may influence anemia rates. A similar pattern is observed in anemia prevalence, with high rates in low SDI regions and marked reductions in areas with higher SDIs (Figures 5C,D). Overall, the global data confirms that socio-economic advancement plays a crucial role in reducing the impact of childhood anemia.

Discussion

This study, leveraging data from the Global Burden of Disease Study 2019, furnishes a comprehensive global overview of anemia in children under 5 years, a paramount public health issue. Despite a global decline in anemia disability rates among adults from 1990 to 2019, the burden of anemia in children under five remains pronounced, signifying the persistent public health challenge anemia poses to this vulnerable demographic.

In 2019, the age-standardized YLDs attributable to anemia in children under five was calculated at 1,252.88 per 100,000. Notably, moderate anemia emerged as the most prevalent form, with a rate of 985.46 per 100,000. Nevertheless, the persistently high YLDs rates among children under five underscore the significant adverse effects of anemia on early developmental stages, potentially resulting in long-term cognitive and physical development impairments, thereby affecting future health and productivity. The observed global reduction in anemia disability rates reflects the impact of effective public health interventions and advancements in nutritional standards (7). This includes fortifying foods with essential nutrients, ensuring access to quality healthcare, and educating communities about the importance of nutrition and regular health check-ups (17).

The disparity in anemia burden between regions with low and high SDI scores in 2019 is particularly striking, with a higher prevalence of anemia reported in low SDI areas (67.43%) compared to high SDI areas (14.40%). The significant contribution of countries like Yemen, the Central African Republic, and Bhutan to the YLDs and prevalence of anemia in children under five underscores the regional disparities in the burden of anemia. Similarly, high YLDs rates in Sierra Leone, Mali, and Burkina Faso point to regional health challenges and resource constraints. These disparities highlight a correlation between socio-economic development, as measured by the SDI, and anemia rates in children, illustrating how economic and social factors profoundly influence public health. Improved living standards, better healthcare access, and enhanced nutrition, often byproducts of higher SDI, contribute to lower anemia rates. Regions with high SDI such as North America, Australasia, Europe, parts of Latin America, and the Asia-Pacific demonstrate the benefits of socio-economic development, exhibiting lower rates of anemia-related YLDs. These areas generally benefit from access to diverse, nutritious foods, comprehensive healthcare systems, and effective public health policies, all of which help to mitigate the burden of anemia (7, 17). These facts indicate the significant impact of socio-economic factors

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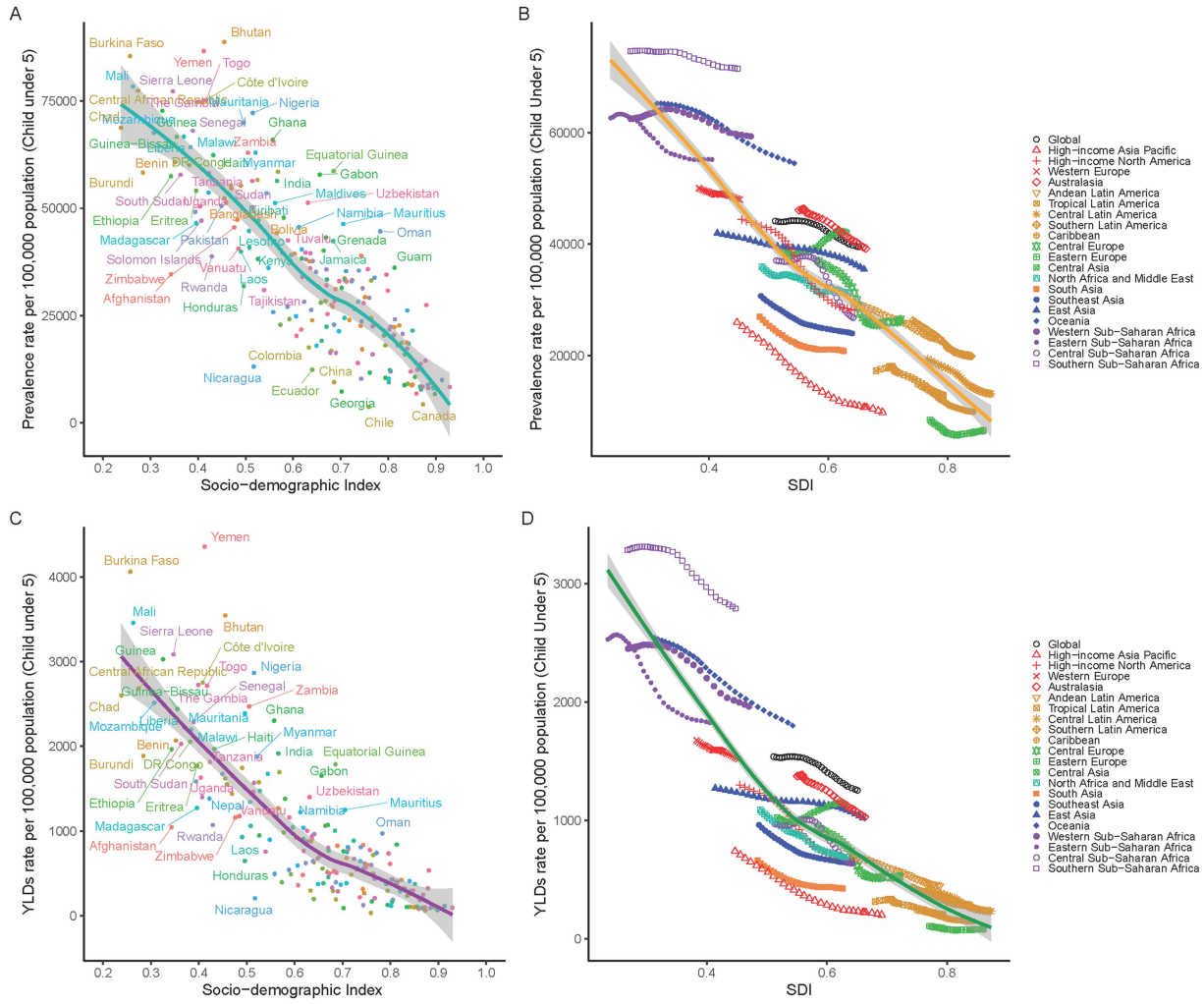

FIGURE 5
Correlation between SDI and Anemia Burden in children under5 by national and 21 GBD regions. **(A)** Anemia prevalence in children under five at the national level in relation to SDI in 2019. **(B)** Trends in anemia prevalence in children under five across 21 GBD regions, correlated with SDI from 1990 to 2019. **(C)** YLDs rates for anemia in children under five at the national level in relation to SDI in 2019. **(D)** Trends in YLD rates for anemia in children under five across 21 GBD regions, correlated with SDI from 1990 to 2019.

on health and suggests that interventions need to be tailored to the specific needs and constraints of each region. This correlation emphasizes the importance of socio-economic investments in improving living standards, healthcare access, and nutrition to alleviate the global burden of anemia.

Typically, anemia is more common in females, especially during reproductive years, due to menstrual blood loss and the increased iron demands of pregnancy (18–21). Our results reveal a higher anemia disability rate in male children under 5 years. Biological factors such as differences in iron metabolism or genetic predispositions might explain the higher anemia rates in males (22). Males and females have different patterns of iron metabolism, which may contribute to the gender disparity in anemia rates. There could be developmental differences between male and female children in this age group that affect their vulnerability to anemia. For instance, rapid growth phases, differences in gut microbiota, or varying rates of nutrient absorption might influence anemia risk (23). This unexpected trend in early childhood suggests potential biological differences in iron metabolism or genetic predispositions contributing to the increased vulnerability of male children to anemia. These findings suggest the need for gender-sensitive approaches in public health policies addressing childhood anemia. This includes tailored nutritional programs, equitable healthcare access, and awareness campaigns that cater to the specific needs of both male and female children.

For neonates aged 0–6 days, hemoglobinopathies, rather than iron deficiency anemia, are the primary contributors to anemia-related YLDs. Hemoglobinopathies are genetic disorders that affect the structure or production of hemoglobin and are notably significant in the first days of life (24). Conditions such as thalassemia and sickle cell disease often appear shortly after birth, imposing a greater disease burden in this age group than iron-deficiency anemia, which typically manifests later (25). Due to the genetic basis of hemoglobinopathies, prenatal screening and genetic counseling are vital. These services are particularly beneficial for parents with known risk factors or those who carry genes associated with hemoglobinopathies, enabling them to make informed decisions regarding their child's health (26, 27).

The primary role of dietary iron deficiency in childhood anemia highlights the critical importance of nutrition in public health (7, 28). This emphasizes the need for global initiatives aimed at enhancing dietary quality, particularly with respect to iron consumption, which is essential for both preventing and treating anemia in children (29). Although iron deficiency is a principal cause, the considerable influence of hemoglobinopathies and hemolytic anemias also demands attention. In regions such as Africa, infectious diseases like malaria notably contribute to the anemia burden (12). This suggests that, in addition to nutritional interventions, disease control is a vital component of anemia management in these areas. Comprehensive health policies are therefore necessary to address the multifaceted causes of anemia. Such policies should include food fortification with iron, the expansion of maternal and child health programs, and improved healthcare access (30–32). Specifically in regions like Africa, where certain diseases exacerbate anemia, efforts should focus not only on improving nutrition but also on managing diseases that significantly contribute to anemia, including strategies to reduce the prevalence of malaria and other neglected tropical diseases (11, 33).

Limitations

This study underscores the multifaceted nature of anemia in children under five. Nonetheless, the current study is subject to certain limitations. Although the GBD study provides a comprehensive dataset, it predominantly depends on extant public health records and research, which may be incomplete or less accurate in specific regions. Consequently, estimates of the anemia burden in particular areas or among distinct demographic groups might not be entirely precise. Moreover, the inherent focus of this epidemiological methodology on quantitative metrics, such as incidence rates and YLDs, may result in the underestimation of the qualitative impact of anemia on individual and community well-being. This limitation could obscure significant socio-economic and psychological dimensions of anemia, thereby restricting the scope of the analysis and its implications for public health interventions.

Conclusion

This analysis delineates the global burden of anemia in children under five as a complex issue influenced by genetic, nutritional, socio-economic, and region-specific factors. A targeted, multi-faceted strategy is essential, incorporating early detection, nutritional supplementation, improved healthcare access, and regionally tailored interventions. Such approaches are crucial for alleviating the anemia burden and fostering optimal developmental outcomes in this vulnerable demographic, highlighting the necessity of prioritizing childhood anemia in global public health agendas.

Data availability statement

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

Author contributions

YL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. WR: Investigation, Software, Writing – original draft. SW: Data curation, Investigation, Software, Writing – review & editing. MX: Data curation, Software, Supervision, Writing – original draft. SZ: Data curation, Investigation, Methodology, Writing – review & editing. FZ: Software, Supervision, Validation, Writing – original draft.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1474664/full#supplementary-material

References

1. Newhall DA, Oliver R, Lugthart S. Anaemia: a disease or symptom. *Neth J Med*. (2020) 78:104–10.

2. McWilliams S, Singh I, Leung W, Stockler S, Ipsiroglu OS. Iron deficiency and common neurodevelopmental disorders-a scoping review. *PLoS One*. (2022) 17:e0273819. doi: 10.1371/journal.pone.0273819

3. Szajewska H, Ruszczynski M, Chmielewska A. Effects of iron supplementation in nonanemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials. *Am J Clin Nutr*. (2010) 91:1684–90. doi: 10.3945/ajcn.2010.29191

4. Sankaran VG, Weiss MJ. Anemia: progress in molecular mechanisms and therapies. *Nat Med*. (2015) 21:221–30. doi: 10.1038/nm.3814

5. Tanih NF, Belinga KI, Nyasa R, Tanih GN, Cho JF, Samie A, et al. Co-infection with malaria and intestinal parasites and its association with Anemia in children (zero to ten years old) in Tiko subdivision, Cameroon. *J Parasitol*. (2023) 109:615–21. doi: 10.1645/23-48

6. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. *Lancet*. (2016) 387:907–16. doi: 10.1016/S0140-6736(15)60865-0

7. Safiri S, Kolahi AA, Noori M, Nejadghaderi SA, Karamzad N, Bragazzi NL, et al. Burden of anemia and its underlying causes in 204 countries and territories, 1990-2019: results from the global burden of disease study 2019. *J Hematol Oncol*. (2021) 14:185. doi: 10.1186/s13045-021-01202-2

8. Gallagher PG. Anemia in the pediatric patient. *Blood*. (2022) 140:571–93. doi: 10.1182/blood.2020006479

9. Keya TA. Prevalence and predictors of Anaemia among women of reproductive age in south and Southeast Asia. *Cureus*. (2023) 15:e50090. doi: 10.7759/cureus.50090

10. Awe OO, Dogbey DM, Sewpaul R, Sekgala D, Dukhi N. Anaemia in children and adolescents: a bibliometric analysis of BRICS countries (1990-2020). *Int J Environ Res Public Health*. (2021) 18:5756. doi: 10.3390/ijerph18115756

11. Magalhães RJ, Clements AC. Mapping the risk of anaemia in preschool-age children: the contribution of malnutrition, malaria, and helminth infections in West Africa. *PLoS Med*. (2011) 8:e1000438. doi: 10.1371/journal.pmed.1000438

12. Halterman JS, Kaczorowski JM, Aligne CA, Auinger P, Szilagyi PG. Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. *Pediatrics*. (2001) 107:1381–6. doi: 10.1542/peds.107.6.1381

13. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr*. (2007) 85:931–45. doi: 10.1093/ajcn/85.4.931

14. Bruzzese JM, Usseglio J, Iannacci-Manasia L, Diggs KA, Smaldone AM, Green NS. Mental and emotional health of caregivers of youth with sickle cell disease: a systematic review. *J Health Care Poor Underserved*. (2023) 34:1070–104. doi: 10.1353/hpu.2023.a903064

15. Aapro MS, Cella D, Zagari M. Age, anemia, and fatigue. *Semin Oncol*. (2002) 29:55–9. doi: 10.1053/sonc.2002.33534

16. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019. Seattle. United States: Institute for Health Metrics and Evaluation (IHME) (2020).

17. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet*. (2011) 378:2123–35. doi: 10.1016/S0140-6736(10)62304-5

18. Zhu Z, Sudfeld CR, Cheng Y, Qi Q, Li S, Elhoumed M, et al. Anemia and associated factors among adolescent girls and boys at 10-14 years in rural western China. *BMC Public Health*. (2021) 21:218. doi: 10.1186/s12889-021-10268-z

19. Joo EY, Kim KY, Kim DH, Lee JE, Kim SK. Iron deficiency anemia in infants and toddlers. *Blood Res*. (2016) 51:268–73. doi: 10.5045/br.2016.51.4.268

20. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. (2014) 123:615–24. doi: 10.1182/blood-2013-06-508325

21. Kassebaum NJ. The global burden of Anemia. *Hematol Oncol Clin North Am*. (2016) 30:247–308. doi: 10.1016/j.hoc.2015.11.002

22. Hay G, Refsum H, Whitelaw A, Melbye EL, Haug E, Borch-Iohnsen B. Predictors of serum ferritin and serum soluble transferrin receptor in newborns and their associations with iron status during the first 2 y of life. *Am J Clin Nutr*. (2007) 86:64–73. doi: 10.1093/ajcn/86.1.64

23. Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Rivera LL, Hernell O. Sex differences in iron status during infancy. *Pediatrics*. (2002) 110:545–52. doi: 10.1542/peds.110.3.545

24. Dugdale M. Anemia. *Obstet Gynecol Clin N Am*. (2001) 28:363–82. doi: 10.1016/S0889-8545(05)70206-0

25. Weatherall DJ. Keynote address: the challenge of thalassemia for the developing countries. *Ann N Y Acad Sci*. (2005) 1054:11–7. doi: 10.1196/annals.1345.002

26. Schieve LA, Simmons GM, Payne AB, Abe K, Hsu LL, Hulihan M, et al. Vital signs: use of recommended health care measures to prevent selected complications of sickle cell Anemia in children and adolescents - selected U.S. states. *MMWR Morbidity Mortality Weekly Report 2022*. (2019) 71:1241–6. doi: 10.15585/mmwr.mm7139e1

27. Hoppe CC. Prenatal and newborn screening for hemoglobinopathies. *Int J Lab Hematol*. (2013) 35:297–305. doi: 10.1111/ijlh.12076

28. Camaschella C. Iron-deficiency anemia. *N Engl J Med*. (2015) 372:1832–43. doi: 10.1056/NEJMra1401038

29. Eussen S, Alles M, Uijterschout L, Brus F, van der Horst-Graat J. Iron intake and status of children aged 6-36 months in Europe: a systematic review. *Ann Nutr Metab*. (2015) 66:80–92. doi: 10.1159/000371357

30. Field MS, Mithra P, Peña-Rosas JP. Wheat flour fortification with iron and other micronutrients for reducing anaemia and improving iron status in populations. *Cochrane Database Syst Rev*. (2021) 18:CD011302. doi: 10.1002/14651858.CD011302.pub3

31. Das JK, Salam RA, Kumar R, Bhutta ZA. Micronutrient fortification of food and its impact on woman and child health: a systematic review. *Syst Rev*. (2013) 2:67. doi: 10.1186/2046-4053-2-67

32. Iron fortification of infant formulas. American Academy of Pediatrics. Committee on Nutrition. *Pediatrics*. (1999) 104:119–23. doi: 10.1542/peds.104.1.119

33. Korenromp EL, Armstrong-Schellenberg JR, Williams BG, Nahlen BL, Snow RW. Impact of malaria control on childhood anaemia in Africa – a quantitative review. *Trop Med Int Health*. (2004) 9:1050–65. doi: 10.1111/j.1365-3156.2004.01317.x

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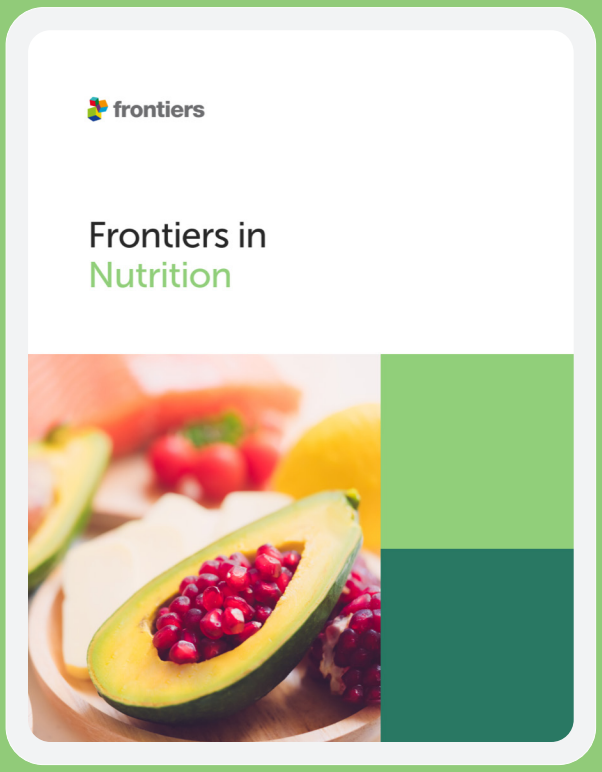

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