

Iron deficiency and excess: diagnosis, management and impact on human health

Edited by

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Iron deficiency and excess: diagnosis, management and impact on human health

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Editorial: Iron deficiency and excess: diagnosis, management and impact on human health

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KEYWORDS

diet, iron, deficiency, overload, micronutrient

Editorial on the Research Topic

Iron deficiency and excess: diagnosis, management and impact on human health

Introduction

This editorial summarizes the main findings published within the scope of the Research Topic *Iron deficiency and excess: diagnosis, management and impact on human health*, available in Frontiers in Nutrition. The selected articles address recent advances in understanding, diagnosing, and treating iron-related disorders, with a focus on vulnerable populations and innovative therapeutic strategies.

Iron is an essential micronutrient that plays a pivotal role in numerous physiological processes, such as oxygen transport via hemoglobin, nucleic acid and protein synthesis, and energy production through oxidative phosphorylation. Disruptions in iron homeostasis—manifesting as either iron deficiency (ID) or iron overload—represent two extremes of the metabolic spectrum, each with significant implications for human health. Iron deficiency is one of the most widespread nutritional disorders globally and is primarily associated with iron-deficiency anemia (IDA). Clinical manifestations of IDA include fatigue, dizziness, dyspnea, tachycardia, and, in chronic cases, heart failure. In addition to anemia, iron deficiency contributes to a range of adverse outcomes, such as headaches in adults, cognitive and developmental delays in children, pregnancy-related complications, and an increased susceptibility to infections due to impaired innate and adaptive immunity. Conversely, iron overload can induce oxidative stress and organ dysfunction, primarily affecting the liver, heart, and pancreas. This can lead to conditions such as diabetes mellitus, infertility, and thyroid dysfunction. In recent decades, advances in diagnostic techniques and therapeutic strategies have significantly shaped the management of iron-related disorders. This editorial condenses the findings from nine recent scientific articles, highlighting progress in understanding, diagnosing, and treating iron deficiency and overload.

Iron deficiency: global burden and vulnerable populations

Iron deficiency disproportionately affects vulnerable groups, including women of reproductive age, children, and the elderly. Zhang et al. reported that despite a global decline in nutritional deficiencies between 1990 and 2021, ID continues to affect women and low-income populations, particularly in countries with low social development indices. In India, Hashmi and Singh documented a concerning rise in anemia among Muslim women—from 48.8% to 55.6% over 23 years—with higher prevalence observed among adolescents, rural residents, and individuals with limited education. Similarly, Wang et al. identified dietary iron deficiency as the predominant cause of anemia in elderly Chinese populations, especially in rural and underdeveloped regions.

Iron overload: emerging associations and risks

Recent studies have expanded the clinical spectrum of iron overload, linking it to conditions such as fecal incontinence (FI) and human papillomavirus (HPV) infection. Li et al., using NHANES data from 2007 to 2010, identified an inverted U-shaped relationship between iron intake and FI, and a negative association between serum iron levels and solid FI, particularly in elderly women—suggesting that both iron deficiency and excess may impair gastrointestinal function. Chen et al. reported an L-shaped association between iron intake and HPV infection, with adequate iron levels reducing the risk of infection by 23.2%. In Ethiopia, Endrias et al. found a 60.93% prevalence of anemia among chronic kidney disease patients, a condition exacerbated by comorbid diabetes and advanced disease stages.

Innovations in iron deficiency treatment

Several novel and cost-effective interventions have been proposed to address iron deficiency. For example, Elmraged et al. demonstrated that fortified infant cereals significantly reduced anemia prevalence by 32% among Egyptian children, while also lowering public health expenditures—underscoring their potential as a scalable intervention. Huang et al. introduced a new iron supplement, iron-chelated leek skin peptides (PSP-Fe), which not only restored hematological parameters more effectively than ferrous sulfate in anemic rats but also positively influenced gut microbiota composition. Additionally, Wagiu Basrowi et al. explored the regulatory role of microRNAs (miR-15a, miR-24)

in erythropoiesis, proposing protein-based therapies to modulate their expression as a promising avenue for IDA treatment.

Conclusion

Iron is indispensable for human health, and maintaining its homeostasis is crucial to preventing the deleterious effects of both deficiency and overload. Iron deficiency remains a pressing global health concern, particularly among high-risk populations. Current research emphasizes the importance of early screening, continuous clinical monitoring, and implementing cost-effective, nutritionally adequate, and culturally tailored interventions. Continued scientific inquiry and innovation are essential to mitigating the burden of iron-related disorders and improving population health outcomes.

Author contributions

PM: Writing – original draft, Writing – review & editing. PC: Writing – review & editing, Writing – original draft. GR: Writing – review & editing, Writing – original draft.

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Harnessing the power of proteins in modulation of miRNAs for targeting Iron deficiency Anemia: Opinion for future implications and strategies

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Iron Deficiency Anemia (IDA) remains a pervasive global health challenge, disproportionately affecting vulnerable populations such as women and children. This review explores the cutting-edge interplay between microRNAs (miRNAs) and proteins in erythropoiesis, highlighting novel therapeutic strategies for IDA. Emerging evidence underscores the pivotal role of miRNAs—such as miR-15a, miR-24, miR-150, and miR-223—in regulating erythropoiesis, with dysregulation linked to hematologic and systemic diseases. Proteins, acting as modulators of miRNA activity, present innovative pathways for intervention by influencing erythropoiesis at multiple stages, from stem cell proliferation to red blood cell maturation. Our synthesis highlights key molecular mechanisms: miR-15a suppresses erythropoiesis by inhibiting c-Myb, miR-24 impairs heme biosynthesis through ALK4 regulation, while miR-150 and miR-223 modulate critical hematopoietic pathways affecting cell differentiation and apoptosis. These miRNA-protein interactions suggest targeted therapies such as protein-based miRNA modulators could optimize erythropoiesis, advancing IDA management. Additionally, the review emphasizes the potential of leveraging protein-miRNA interactions for precision medicine, especially in resource-limited settings where anemia's burden is profound. By bridging current knowledge gaps, our proposed strategies offer personalized and scalable therapeutic solutions. This comprehensive perspective lays the groundwork for future interventions addressing one of the world's most widespread public health crises.

KEYWORDS

miRNA, microRNA, anemia, erythropoiesis, protein, iron deficiency anemia, erythrocyte

1 Introduction

Anemia is characterized by hemoglobin levels falling below the threshold required to support the physiological demand for oxygen transport by circulating red blood cells. It remains a significant global health challenge. In 2021, the global prevalence of anemia across all age groups was 24.3%, equating to approximately 1.92 billion people worldwide (1). This prevalence varies across countries, with the highest rates in South Asia, Western Sub-Saharan Africa, and Central Sub-Saharan Africa (2). Anemia affects approximately one-third of the global population and is linked to increased morbidity and mortality, particularly among women and children. It contributes to adverse birth outcomes, reduced work productivity in adults, and impaired cognitive and behavioral development in children. Preschool children (PSC) and women of reproductive age (WRA) are especially vulnerable to the effects of anemia (3). Despite extensive public health efforts, the global burden of anemia has persisted, prompting a deeper exploration of its underlying biological mechanisms. While iron deficiency remains the most recognized cause, emerging research points to complex molecular processes driving impaired red blood cell production. Recent scientific advances have identified the regulatory role of microRNAs (miRNAs) in erythropoiesis, highlighting them as key molecular players in anemia pathogenesis.

Managing anemia continues to be a considerable challenge, particularly in Indonesia. Despite various interventions, such as iron and folic acid supplementation, the prevalence of anemia has not significantly declined (4). One of the key factors contributing to anemia is nutritional deficiency, particularly protein. During protein malnutrition, structural and cellular changes in the hematopoietic microenvironment contribute to bone marrow atrophy and impair hematopoietic stem cell formation, disrupting hematopoietic homeostasis and leading to anemia (5). Furthermore, animal studies have demonstrated that protein deficiency can induce anemia by inhibiting effective erythropoiesis through reduced protein synthesis in erythroid cells and decreased erythropoietin production, even when iron stores are sufficient (6).

Proteins, serving as modulators or co-factors of miRNA activity, further shape this regulatory network. Understanding these intricate miRNA-protein interactions opens new avenues for therapeutic interventions aimed at correcting dysregulated erythropoiesis. At the biomolecular level, the red blood cell formation process, or erythropoiesis, is regulated by various molecular mechanisms, including microRNA (miRNA) modulation. Notably, miRNAs such as miRNA-15a, miRNA-24, miRNA-150, and miRNA-223 play critical roles in this process. Dysregulation of these miRNAs has been linked to diseases like thalassemia, leukemia, sickle cell disease, and cancer. Moreover, miRNAs are crucial in erythropoiesis as they regulate gene expression in erythroid progenitor cells' proliferation, differentiation, and apoptosis. For example, miRNA-15a suppresses erythropoiesis by inhibiting the expression of the MYB protein, which is essential for the kinetics of mature erythroid cells, while miRNA-24 impedes terminal differentiation by regulating the ALK4 gene (7). By shifting the focus from traditional iron supplementation to biomolecular targets such as miRNAs and their associated protein regulators, innovative strategies can be developed to combat anemia more effectively. This molecular perspective not only enhances the understanding of anemia's root causes but also paves the way for precision medicine applications tailored to individual patient profiles.

In recent years, scientific research has increasingly focused on biomolecular aspects to understand the fundamental mechanisms behind red blood cell formation and anemia. A key area of interest is the role of proteins in this process, as proteins play a pivotal role in regulating various biochemical pathways involved in red blood cell production and maintenance. For instance, proteins such as erythropoietin promote cell survival and drive terminal erythroid maturation (8), while transcription factors like GATA1 regulate all aspects of erythroid maturation at the transcriptional and functional levels (9). However, comprehensive studies exploring the relationship between proteins and miRNAs in anemia are still lacking despite the potential of this area in developing anemia management strategies. This review aims to bridge this gap by examining the modulation of miRNAs by proteins and exploring how these interactions could offer novel therapeutic strategies to combat anemia, particularly in populations most affected by this condition.

This opinion paper uniquely explores the interplay between proteins and miRNAs in erythropoiesis, offering a novel biomolecular perspective on combating iron deficiency anemia. By integrating recent evidence and highlighting therapeutic potentials, this discussion not only advances the scientific understanding of anemia management but also paves the way for globally scalable interventions aimed at mitigating one of the most prevalent and impactful health challenges worldwide.

2 Search strategy and study selection criteria

To identify relevant studies, a comprehensive literature search was conducted using major scientific databases including PubMed, Scopus, Google Scholar and Web of Science. The search strategy employed combinations of key terms such as “microRNA,” “miRNA modulation,” “iron deficiency anemia,” “erythropoiesis,” and “protein regulation.” Only peer-reviewed articles published in English were included to ensure up-to-date insights.

The inclusion criteria were: (1) original research or systematic reviews focused on miRNA and protein interactions in erythropoiesis; (2) studies highlighting therapeutic applications for IDA; and (3) articles reporting molecular mechanisms with experimental or clinical evidence. Exclusion criteria included non-English publications, studies lacking relevance to IDA, and conference abstracts or editorial pieces. Titles and abstracts were screened for eligibility, followed by full-text evaluations of selected articles. Data extraction focused on molecular pathways, key findings, and potential therapeutic implications. This systematic approach ensured a robust and focused analysis of the current landscape of miRNA and protein-based interventions in IDA management.

3 The health beneficial of protein

3.1 The fundamental role of protein in anemia

Proteins are biopolymers made up of amino acids. In the human body, they serve many crucial roles, such as providing the body's building blocks, function as hormones, enzymes, precursors of several

biologically relevant molecules, and initiators of cellular death (10). One of the key roles of protein is its involvement in the development of anemia. Hemoglobin (Hb), an important protein responsible for transporting oxygen to body tissues, serves as one of the markers for diagnosing anemia in humans (11). A reduction in hemoglobin levels below 13.5 g/dl in men and 12.0 g/dl in non-pregnant women, regardless of the underlying cause, is indicative of anemia. Structural abnormalities in hemoglobin, such as those seen in conditions like thalassemia and sickle cell disorders, can lead to anemia because of premature destruction of red blood cells (12).

Iron is an essential component of hemoglobin and its metabolism is regulated by various proteins, including ferritin (which stores iron), transferrin (which transports iron), and hepcidin (a negative regulator of iron absorption and recycling), along with other proteins that modulate iron metabolism at different physiological levels (13, 14). Disruptions in iron metabolism can result in iron deficiency anemia (IDA), the most common form of anemia worldwide (15). The involvement of proteins in anemia is particularly evident in anemia of inflammation (AI), also called anemia of chronic disease (ACD), the second most prevalent form of anemia globally (16). Inflammatory cytokines, key proteins in this process, contribute to a reduced lifespan of red blood cells, likely by activating macrophages, while also disrupting erythropoietin (EPO) production and function, and inhibiting the proliferation and differentiation of normal erythroid progenitor cells (3). Erythropoietin (EPO) is a glycoprotein hormone synthesized by the kidneys that is essential for stimulating red blood cell production in response to low partial pressure of oxygen (pO_2),

making it important in the context of anemia. A reduction in erythropoietin levels, commonly seen in individuals with chronic kidney disease, can also lead to the development of anemia (17). Figure 1 shows the involving of protein in anemia.

Protein deficiencies disrupt erythropoietic pathways by impairing key molecular processes essential for red blood cell formation (18). Specifically, inadequate protein intake compromises the synthesis of hemoglobin, the main oxygen-carrying protein in erythrocytes, and reduces the availability of erythropoietin, a hormone critical for stimulating erythroid progenitor proliferation (19). Furthermore, protein-related transcription factors like GATA1 are essential for the differentiation of erythroid cells, while transporter proteins such as transferrin and ferritin regulate iron homeostasis required for effective erythropoiesis (20). These disruptions collectively impair red blood cell production, leading to various anemia-related conditions.

3.2 Differences in protein types for Anemia management

Anemia has numerous etiologies which fundamentally revolve around two core issues: inadequate production of red blood cells or hemoglobin (supply) and excessive demand for them (either physiological or pathological). The management strategy for anemia focuses on addressing the specific etiology in each patient, with the goal of achieving a balance between the two (3). In cases of iron deficiency, the most common cause of anemia, therapy focuses on

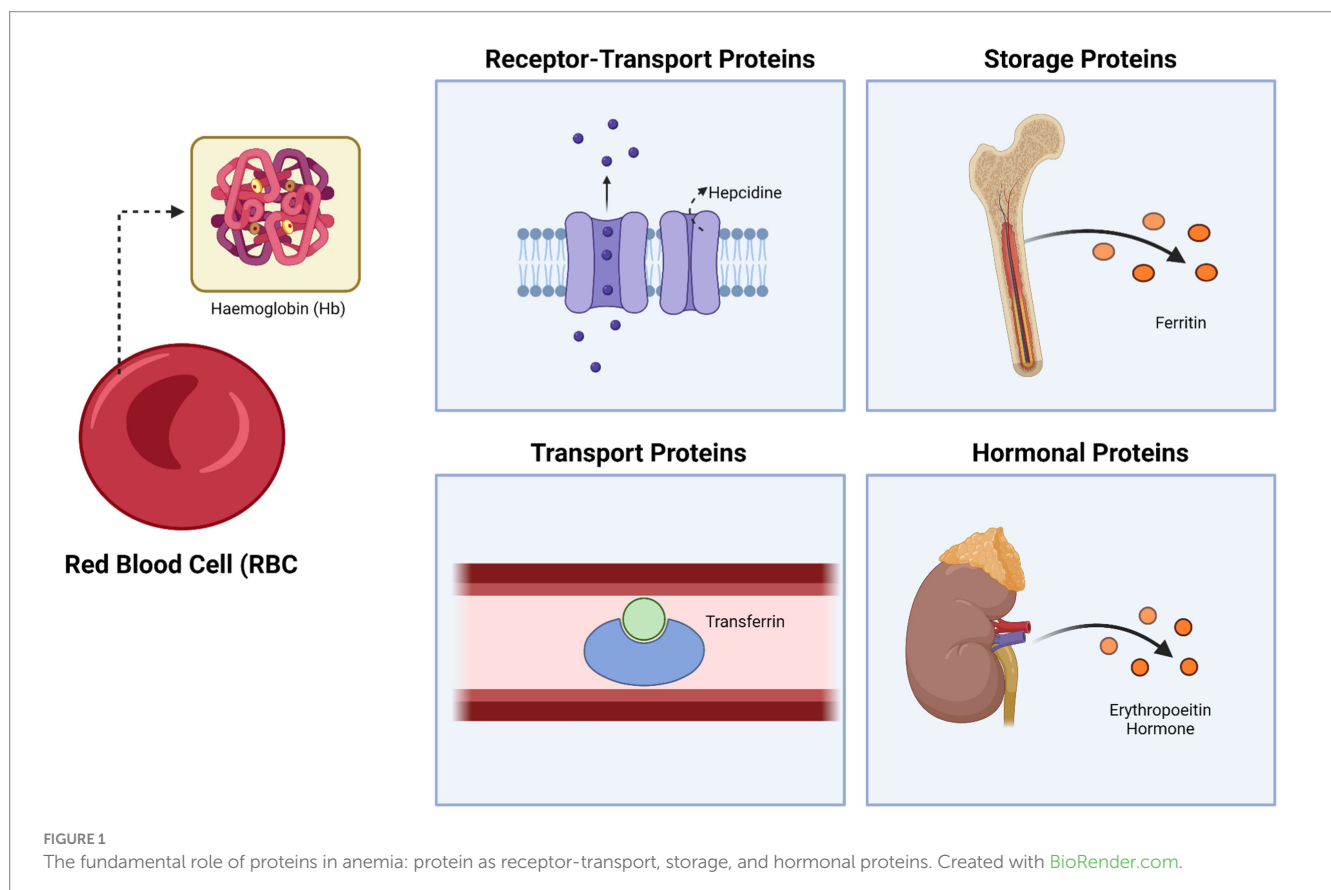


TABLE 1 How diet influences biomolecular aspects of iron deficiency anemia (IDA)?

Biomolecules	Role in iron deficiency anemia (IDA)
Iron	- Absorption and Transport: Absorbed in the duodenum as heme (from animal products) and non-heme iron (from plants). Heme iron is more readily absorbed. - Incorporation in Hemoglobin: Essential for hemoglobin synthesis, which carries oxygen in red blood cells. Low iron leads to microcytic anemia. - Storage: Stored as ferritin in liver, spleen, and bone marrow, providing a reserve for low dietary intake periods.
Protein	- Iron Transport and Storage: Proteins such as transferrin and ferritin transport and store iron for hemoglobin production. - Hemoglobin and Myoglobin Production: Hemoglobin is a protein, and amino acids from dietary protein are essential for its synthesis. - Enzymatic Functions: Proteins involved in the electron transport chain help in cellular energy production, important in IDA to prevent fatigue.
Interactions and dietary synergy	- Enhancers of Iron Absorption: Certain proteins (like from meat) enhance non-heme iron absorption. Vitamin C also boosts non-heme iron absorption. - Inhibitors of Iron Absorption: Phytates, polyphenols, and calcium can hinder absorption. Balanced intake is key to ensuring maximum absorption.
Dietary sources and recommendations	- Iron-Rich Foods: Red meat, poultry, fish (heme iron), and fortified cereals, lentils, spinach (non-heme iron). - Protein Sources: Eggs, lean meat, legumes, nuts, and dairy for necessary amino acids supporting hemoglobin synthesis and iron utilization.

restoring normal iron levels in the body, through pharmacological iron supplementation (oral or IV, if necessary) (Table 1) (21). In more complex cases, such as anemia due to chronic diseases like cancer, management options are more varied, including vitamin B12, folic acid, erythropoiesis-stimulating agents, and even blood transfusions, taking into account the appropriateness of each option, as well as their respective risks and benefits. While it is important to manage anemia as an individual disease case, this disease can cause significant burden throughout the world. Thus, WHO has formulated key strategies for global anemia prevention and control. These strategies require collaboration from various sectors, including the nutrient and food sector which encompass the provision, utilization, and education about healthy and nutrient-rich foods. The goal is enhancing the intake of essential micronutrients, particularly iron, folate, vitamin B12, vitamin A, and riboflavin, along with other micronutrients, that can be achieved through dietary diversification, food fortification, and supplementation strategies (22).

The consumption of nutritious food should not only meet daily requirements but also provide high-quality nutrients (Table 1). One of the most important components is protein, particularly for obtaining various essential amino acids (23). There are numerous types of protein sources, generally classified as animal-based and plant-based proteins. Animal-based proteins tend to contain complete

proteins and essential amino acids, which are well absorbed by the digestive system (24). High consumption of animal-based protein plays a role in anemia management due to its positive effects on iron status in the body, including blood hemoglobin levels. On the other hand, plant-based proteins typically lack complete essential amino acids in one type source and are less efficiently absorbed due to their fibrous structure, which is more difficult to digest. Therefore, individuals who follow a plant-based protein diet require a more diverse range of sources and larger quantities to meet their nutritional needs (25).

Several micronutrients required for normal erythropoiesis, such as iron, folic acid (Vitamin B9), and Vitamin B12, can be obtained from various protein sources, with their content differing across these sources. Animal-based protein sources, such as meat from four-legged animals and two-legged animals, contain good amounts of iron, particularly in the form of heme iron, which is readily absorbed by the body. Fish meat is also rich in iron, and a diet high in such sources can fully meet the daily dietary iron requirements. Plant-based protein sources (beans, nuts, peas, legumes, etc.) tend to contain non-heme iron, which has lower bioavailability. Moreover, these sources often contain iron absorption inhibitors, such as phytates (26). Folic acid, a vital micronutrient, is primarily found in animal-based protein sources like liver and kidney. In contrast, among plant-based foods, it is most abundant in leafy green vegetables, which are not typically recognized as significant sources of protein (27). Lastly, in the case of vitamin B12, there are no plant-based sources. This vitamin can be absorbed by the digestive system after being separated from the protein in meat. It is abundant in red meat (beef, lamb), all types of fish, and to a lesser extent in poultry products (28).

4 The role of miRNA in biomolecular process

The single-stranded structure of short nonprotein coding RNA or microRNA (miRNA), transcribed from DNA sequences, plays an essential role in gene expression. The miRNA interacts with messenger RNA (mRNA) to regulate transcription through the coding sequence as an independent gene promoter, producing a pattern that alters overall cellular processes. In this way, intracellular and extracellular miRNA act as messengers to cells, organs and systems that could alter the physiological and pathological homeostasis (29). A possible pathway to alter metabolism or paradoxically reprogramming the metabolism involves directly or indirectly diverse mechanisms, such as targeting key enzymes, transporters, transcription factors, and signalling pathways (30).

However, in order to interact with distant cells/organs, miRNA should remain stable and be able to interact and modulate transporters, enzymes and receptors. Exosomes or extracellular vesicles are mediators protecting miRNA stability and integrity. The miRNA exerts an influence on the modulation and inhibition of the inflammatory state, may result in alterations to physiological conditions in hematoimmunological processes. Furthermore the miRNA influences the modulation and inhibition of the inflammatory state (i.e., polarization of macrophages to two phenotypes M1 and M2), signalling pathway (NF- κ B, TLR, STAT-3), and regulates the function of receptor cells (closely related to hypoxia and inflammation) (31). The hitchhiking of miRNA affects the motility of the transporter

(vesicle), thereby enabling it to be exploited in an inactive form for the purpose of transporting from the post-synaptic (32).

MiRNA genes are transcribed by RNA polymerase II, producing a primary transcript known as pri-miRNA. These pri-miRNAs are long sequences that contain multiple miRNA segments, which fold into characteristic hairpin structures. Within the nucleus, the pri-miRNA is processed by the Drosha enzyme, in collaboration with the Dgcr8 protein, resulting in a double-stranded precursor miRNA (pre-miRNA) approximately 70 nucleotides in length. This pre-miRNA is then exported to the cytoplasm through the XPO5-GTP complex. Once in the cytoplasm, the enzyme Dicer, assisted by the cofactor TRBP, further processes the pre-miRNA into a miRNA duplex, which is about 21–24 nucleotides long. The miRNA duplex is subsequently incorporated into the RNA-induced silencing complex (RISC), where one strand is selected to become the mature miRNA, typically around 22 nucleotides in length. This mature miRNA contains a critical “seed region” of approximately 7 nucleotides at the 5′-end, essential for recognizing target mRNAs. The miRNA binds to partially complementary sites, usually located in the 3′ untranslated regions (3′UTRs) of target mRNAs, leading to either mRNA degradation or suppression of its translation. Figure 2 illustrates the miRNA processing pathway (33).

5 Proteins targeting miRNA modulation in red blood cell formation

The clinical evidence of targeting miRNA in anemia for the past 10 years is summarized in Table 2. Proteins influence miRNA biogenesis, stability, and functionality through various mechanisms (34), including transcriptional regulation of miRNA-encoding genes, post-transcriptional processing by key enzymes such as Drosha and Dicer, modulation of miRNA maturation through co-factors, and interactions with RNA-binding proteins that can alter miRNA stability, localization, and target recognition (35, 36). Table 2 presents a summary of several studies conducted over the past 10 years on related to miRNA

in erythropoiesis. The majority of several studies demonstrate that various types of miRNAs influence anemia through erythropoiesis. Erythropoiesis is part of hematopoiesis that plays a crucial part in erythrocyte equilibrium to maintain the physiological term. The origin of erythrocyte or red blood cell (RBC) results from the differentiation of hematopoietic stem cells (HSC) into multi-potent progenitor cells (MPP) and identified erythroid lineage called burst-forming unit erythroid (BFU-E). The BFU-E proliferates into several types of cells, such as proerythroblast. Below the regulation of erythropoietin hormone (Epo), the proerythroblast is amplified into a reticulocyte with erythrocyte as the final form of erythropoiesis. Several miRs contribute significantly to erythropoiesis, such as miR-15a, miR-24, miR-150, and miR-223. However, our tabulated studies also report several alteration targets, such as c-Myb, FOXP-1, and LMO2. Relative of several miRs (miR-15a, miR-24, miR-223) deregulated the erythropoiesis that can be found in several diseases such as thalassemia, leukemia, sickle cell disease, and cancer. The impaired erythropoiesis causes the common phenomenon of megakaryocytic cells that, in some cases, could alleviate the severity of the disease or be implicated with disease progressivity that leads to anemia condition.

6 Future directions of using protein in anemia

Previous research has identified the potential of miRNA targeting in the management of anemia, but the number of studies in this field remains limited. Moreover, variations in the markers used to assess reductions in disease incidence across studies complicate the ability to detect significant effects of miRNA specifically on anemia. Despite consistent findings over the past decade showing a strong correlation between miRNA and hematological processes such as erythropoiesis, the efficacy and efficiency of miRNA in modulating anemia-specific outcomes require further investigation.

This article underscores the need for additional clinical research to explore the connection between miRNA and anemia, particularly in identifying specific miRNAs involved in this condition. Although

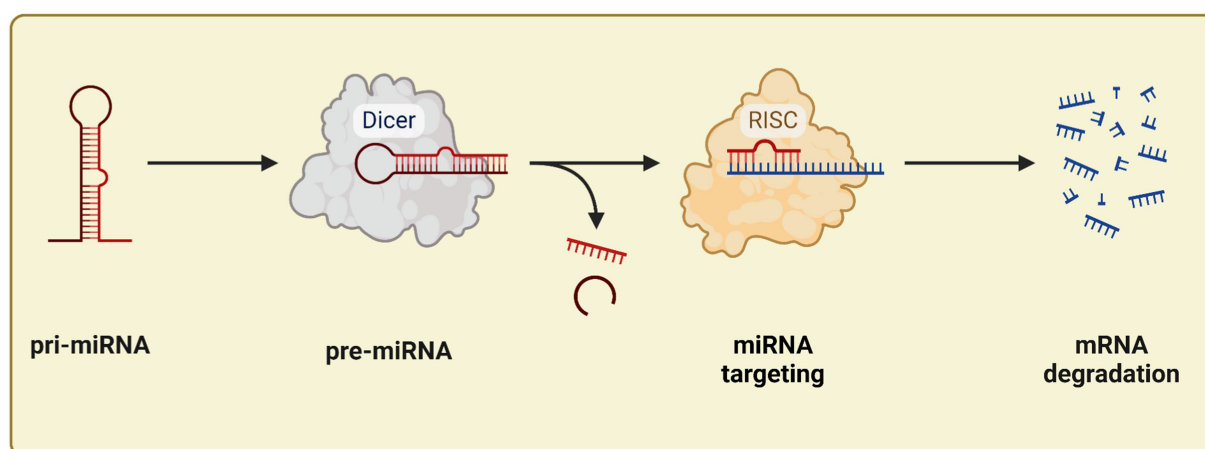


FIGURE 2
miRNA processing: start from pri-miRNA until mRNA degradation. Created with BioRender.com.

TABLE 2 Summarize of included studies.

No	Author, Year	miRNA	Biological effects	Disease related of anemia	Erythropoiesis function
1	(7, 37, 38)	miR-15a	↑ suppress the expression of c-Myb - blocks cell cycle progression (tumor suppressor of CLL) - inhibits erythropoiesis (differentiation of erythroid and myeloid) ↑ HbF expression ameliorates the severity of sickle cell disease and β-thalassemia ↓ enhances expression of IL-7 receptor (IL7R) blocks the B-cell maturation accumulation of B1 and T cells development of CLL	Sickle Cell Disease, β-thalassemia, CLL	Deregulates (suppresses) the erythropoiesis of erythroid and myeloid (Proliferation Stage)
2	(39–43)	miR-24	↑ delays the activin pathway - attenuates the erythroid differentiation reduces the formation of CFU-E and BFU-E - reduces angiogenesis and prevents the migration of cancer - inhibits heme biosynthesis accumulation of succinate transition defect blocks the heme synthesis ↓ induces the activin A pathway - enhances EV-mediated B cell increases the survival rate of B cell acute lymphoblastic leukemia - enhances terminal erythroid differentiation (erythropoiesis) - induces B cell enhances the IgA secretion prevents infection	Thalassemia, cancer	Deregulates (suppresses) the erythropoiesis of progenitor proliferation and differentiation of BFU-E and CFU-E (Proliferation and Differentiation Stage)
3	(44–47)	miR-150	↑ related to the increased proliferation of megakaryocytic cells (c-Myb) inhibits early development of B cells - increase platelet formation - promote caspase-3 activation leads to apoptosis in pathologic cells (B cell lymphoma) ↓ related to deletion of c-Myb (terminal erythropoiesis) - B1 cell proliferation IgM ↑ enhances humoral immune response - interacts with FOXP1 restrict the activity of caspase-3 reduces apoptosis and influences the cell growth B cell lymphoma	B cell lymphoma, hepatocellular carcinoma	Stimulates the differentiation of MEP into BFU-E (Differentiation Stage)
4	(48–50)	miR-221/222	↑ - reduces the erythropoiesis (granulopoiesis) by suppressing the activity of HSC - reduces the chemosensitivity of cisplatin therapy in cancer (leukemia) ↓ Increases FOS expression drives the proliferation, differentiation, and maturation process	Cancer (Leukemia)	Suppress the terminal phase of erythropoiesis (Differentiation and Maturation stage)
5	(51)	miR-223	↑ impairs the LMO2 expression blocks the erythropoiesis process inhibits the erythroid differentiation increase the development of megakaryocytic cell increase platelet formation ↓ stimulates the LMO2 expression - induces the erythroid development - inhibits the E-cadherin expression induces tumor proliferation and migration - induces angiogenesis increases the cancer survivability	B-thalassemia, lymphoblastic leukemia, cancer	Regulates the erythropoiesis (HSC, erythroid cells, and granulocyte-monocyte development) (Proliferation stage)

BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; CLL, chronic lymphocytic leukemia; EV, extracellular vesicle; FOS, Fos proto-oncogene; FOXP1, forkhead box P1; HbF, Fetal Hemoglobin; HSC, hematopoietic stem cell; IgA, immunoglobulin A; IL, interleukin; JUN, Jun proto-oncogene; LMO2, LIM domain only 2 MEP, megakaryocyte-erythroid progenitor.

this paper provides indirect evidence of miRNA's involvement in anemia through its regulation of erythropoiesis, future studies should aim to clarify its role in anemic patients, ultimately enhancing therapeutic efficacy.

6.1 Future directions

Advancing research into miRNA-protein interactions for IDA treatment necessitates a multidisciplinary approach involving molecular biology, bioinformatics, and clinical sciences. Proposed methodologies include:

1. Preclinical studies: conduct *in vitro* studies using erythroid progenitor cell lines to explore the effects of specific miRNA inhibitors or protein modulators. CRISPR-Cas9 technology could be employed to knock out target miRNAs, enabling precise mechanistic evaluations.
2. Animal models: utilize transgenic mouse models with altered expression of relevant miRNAs and proteins to assess phenotypic changes in erythropoiesis. Longitudinal studies could measure red blood cell counts, hemoglobin levels, and survival outcomes.
3. Omics integration: employ multi-omics approaches, including transcriptomics, proteomics, and metabolomics, to uncover new molecular targets and validate miRNA-protein interaction networks.
4. Clinical trial designs:
 - o Phase I/II trials: design early-phase clinical trials focusing on safety, dosage optimization, and proof-of-concept efficacy of miRNA-targeting therapeutics.
 - o Randomized controlled trials (RCTs): conduct RCTs comparing standard iron supplementation with novel protein-based miRNA modulators.
 - o Biomarker-driven studies: incorporate predictive biomarkers such as circulating miRNA signatures to stratify patient populations and personalize treatments.
5. Implementation science: evaluate the scalability and cost-effectiveness of miRNA-based therapies in low-resource settings. This could involve partnership models with global health organizations.

By integrating these strategies, future research can accelerate the development of transformative treatments for IDA, bridging the gap between molecular discoveries and clinical applications.

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RW: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft. TS: Funding acquisition, Supervision, Validation, Writing – review & editing. DP: Data curation, Funding acquisition, Resources, Writing – review & editing. NA: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. YT: Data curation, Investigation, Software, Writing – original draft. MS'a: Formal analysis, Methodology, Software, Writing – original draft. GU: Data curation, Writing – review & editing. MA: Data curation, Formal analysis, Investigation, Writing – original draft. FN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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L-shaped association between dietary iron intake and HPV infection: a cross-sectional analysis based on national health and nutrition examination survey 2005–2016

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Background: Human Papillomavirus (HPV) infection is a ubiquitous sexually transmitted infection globally, frequently associated with sexual behaviors characterized by increased frequency and multiple partnerships. The relationship between varying levels of dietary iron intake and the occurrence of Human Papillomavirus (HPV) infection remains an unresolved question in the scientific community. The objective of our study was to investigate the potential relationship between the consumption of dietary iron and HPV infection.

Methods: Our investigation drew upon comprehensive datasets from 7,819 participants enrolled in the National Health and Nutrition Examination Survey (NHANES) from 2005 to 2016. Employing a cross-sectional analytical framework, we delved into the potential correlation between dietary iron consumption and Human Papillomavirus infection. To statistically assess this relationship, we utilized weighted multivariate logistic regression models. Additionally, we implemented smooth curve fitting and threshold effect analysis, to delineate the complex, nonlinear association between iron intake and HPV infection. Furthermore, we conducted subgroup analyses.

Results: After adjusting for multiple confounding variables, our results demonstrated a statistically significant inverse association between iron intake and HPV infection (OR = 0.988, 95% CI: 0.979–0.998, $p = 0.018$). It's worth noting that, in comparison to individuals in the quartile with the lowest iron intake, those in the highest quartile exhibited a 23.2% reduction in the odds of HPV infection for each incremental unit of iron intake (OR = 0.768, 95% CI: 0.634 to 0.930, $p = 0.009$). A refined analysis employing smoothing curve fitting techniques unveiled an L-shaped correlation, delineating a specific relationship between dietary iron intake and the incidence rate of Human Papillomavirus infection. When iron intake was <16.99 mg, a higher incidence of HPV infection was associated with lower levels of iron intake. (OR = 0.968, 95% CI: 0.956–0.980, $p < 0.001$).

Conclusion: The presence of an L-shaped association between iron intake and HPV infection underscores and emphasizes the possible beneficial effect of sufficient iron intake in reducing the likelihood of HPV infection.

KEYWORDS

human papillomavirus, iron intake, nutrition, female reproductive diseases, infectious diseases

1 Introduction

Human Papillomavirus (HPV) infection constitutes a prevalent sexually transmitted infection on a global scale, posing a significant public health challenge. Structurally, HPV is characterized by its small, circular double-stranded DNA genome, classified into high-risk and low-risk genotypes. The low-risk genotypes, notably HPV-6 and HPV-11, are commonly associated with benign conditions such as genital warts (1). Chronic infection with high-risk HPV genotypes has been conclusively linked to a significant rise in cervical cancer cases among women (2). Currently, twelve specific HPV subtypes—HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59—are recognized as high-risk and are implicated in the oncogenesis of cervical cancer in women (3). Of particular significance, HPV16 and HPV18 have been identified as the primary subtypes that are closely associated with the onset of cervical cancer in a substantial proportion, exceeding 70%, of women worldwide (4, 5). Despite notable advancements achieved through HPV vaccination in mitigating the risk of infection and consequently reducing cervical cancer incidence, the vaccine's efficacy remains confined in terms of its ability to cover high-risk HPV strains and specific variant subtypes (6, 7). Consequently, in the contemporary post-vaccination context, a more expansive and multifaceted approach to HPV infection prevention may necessitate interventions that prioritize the alteration of individuals' lifestyles and dietary practices.

An indispensable trace element, iron occupies a pivotal role in a multitude of bodily functions. Within human physiology, it is particularly crucial for the biosynthesis of deoxyribonucleic acid (DNA) and the facilitation of oxygen transportation mechanisms (8, 9). Approximately 65% of the total body iron is sequestered within the hemoglobin molecules of red blood cells. Conversely, a minor proportion, constituting about 10% of the total iron pool, is allocated to myoglobin in muscular tissues and is also incorporated into a variety of enzymes and cytochromes present in diverse tissue compartments (10). Typically, adults experience a daily excretion of iron in the range of 1 to 2 milligrams (1–2 mg). In adult females, an augmented amount of iron is lost during the menstrual cycle. Non-menstrual iron loss primarily stems from the desquamation of the gastrointestinal mucosa and epidermal cells, alongside minor hemorrhagic occurrences (9). Given the absence of an endogenous excretory mechanism for iron, the human organism relies on dietary management to sustain iron homeostasis (11). The preservation of iron concentrations within a tightly controlled physiological range is paramount. Deviations from this optimal range, whether from iron deficiency or iron overload, can significantly impair physiological integrity, potentially precipitating a spectrum of pathological conditions and adverse health sequelae (10, 12).

Recent research has highlighted the paramount importance of maintaining an optimal iron balance, which is indispensable for cancer initiation, progression, therapeutic responsiveness, and immune system competence (13, 14). A substantial body of research has indicated that increased iron levels may promote the formation of free radicals, augmenting oxidative stress responses and potentially accelerating the development of oncogenic transformations (15–17).

Dietary iron-laden foods, such as red and processed meats, have been correlated with a heightened cancer risk in several epidemiological studies (18–21). Specifically, dietary iron overload leading to hyperferremia has been identified as a significant predictor of breast cancer, especially in postmenopausal women (22). Conversely, iron deficiency within the body has also been implicated in accelerating cancer progression (23, 24). This may be attributed to iron deficiency impeding ferroptosis, a form of iron-dependent regulated cell death induced by lipid peroxide accumulation, which subsequently disrupts immune surveillance mechanisms (25–28). Currently, there is an active pursuit of anticancer therapies targeting ferroptosis-related pathways in cancer cells (14). In light of iron's dual impact, establishing an optimal dietary iron intake is crucial for systemic iron regulation.

While both iron excess and deficiency have been implicated in various cancers, the relationship between iron intake and cervical cancer caused by human papillomavirus (HPV) infection remains an area of ongoing investigation. Research undertaken by Elham Nazari unveiled a beneficial impact of consuming iron through diet on mitigating the incidence and advancement of cervical cancer among Iranian females (29). Conversely, a recent observational investigation suggested that females exhibiting elevated ferritin levels demonstrated a reduced likelihood of experiencing resolution of oncogenic human papillomavirus (HPV) infections, in comparison to those with lower ferritin concentrations (30).

Notwithstanding these discoveries, the present body of evidence linking iron intake to HPV infection remains unresolved and lacks definitive conclusions (31). Therefore, we conducted an analysis of data from the National Health and Nutrition Examination Survey (NHANES) to evaluate the potential correlation between iron intake and HPV infection. Our objective was to identify a balanced iron intake level that could provide dietary guidance for preventing the onset of HPV infection in women.

2 Materials and methods

2.1 Data provenance and examined population

Our research endeavor employed cross-sectional data obtained from six sequential iterations of the National Health and Nutrition Examination Survey (NHANES), encompassing the timeframes from 2005–2006 to 2015–2016, with a comprehensive sample of 60,936 participants. NHANES represents a substantial, population-representative cross-sectional study executed by the National Center for Health Statistics (NCHS), a division of the United States Centers for Disease Control and Prevention (CDC). Rigorous adherence to the NHANES' prescribed guidelines and protocols was maintained throughout all analytical procedures conducted within the scope of our investigation. Upon the conclusion of an exhaustive and rigorous exploration and screening protocol applied to the NHANES database, a cohort of 60,936 subjects spanning the years 2005 to 2016 was initially deemed eligible for inclusion in this investigative study. After

this initial identification phase, the sample was refined to encompass exclusively 12,896 female participants aged 18 to 59 years. Exclusion criteria were applied, resulting in the removal of participants with missing data on HPV infection status (1820 subjects), iron intake (539 subjects), or incomplete covariate information (2,718 subjects). Ultimately, the resultant analytical sample consisted of 7,819 subjects. A schematic diagram, presented in [Figure 1](#), illustrates the systematic process of participant screening and inclusion in this study.

2.2 Variables

The research endeavor was centered on two key exposure variables: iron intake and human papillomavirus (HPV) infection. Data on dietary intake were obtained through a two-day, non-consecutive dietary recall protocol employed by NHANES. Specifically, the first dietary recall interview took place face-to-face at a mobile examination center (MEC), whereas the subsequent interview was administered telephonically within a window of 3 to 10 days thereafter. Owing to the shortcomings observed in the second phase of interviews, our analysis relied on iron intake figures sourced from the first phase of food consumption data. To estimate dietary iron nutrition, NHANES referred to the authoritative Dietary Research Food and Nutrition Database maintained by the United States Department of Agriculture. It should be clarified that the dietary iron intake information gathered in this study exclusively reflected the amounts ingested from food sources,

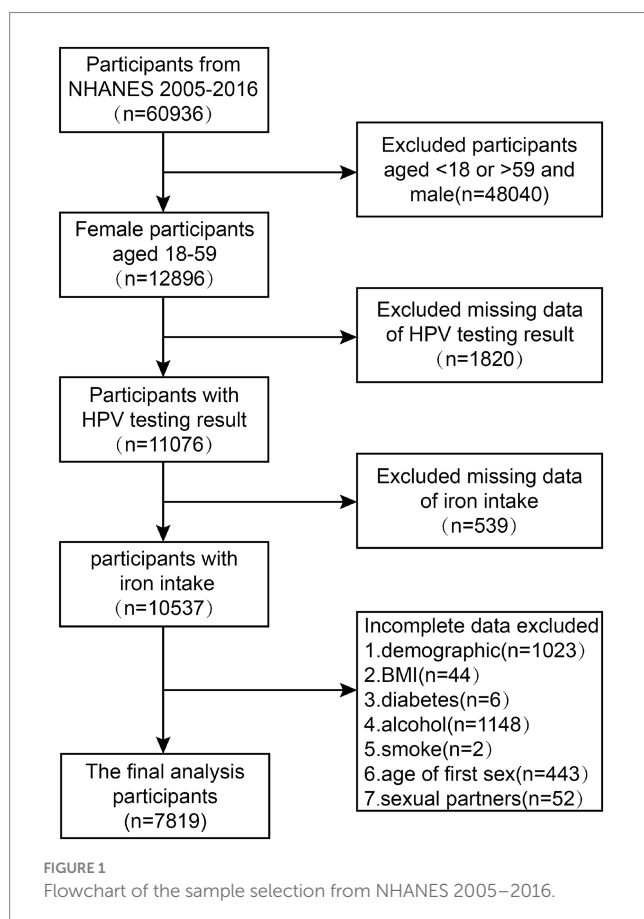
excluding any contribution from iron supplements. For the conduct of statistical analysis, the iron intake values were stratified into quartiles: Q1 spanning 0 to 8.16 milligrams per deciliter (mg/dL), Q2 ranging from 8.17 to 11.73 mg/dL, Q3 encompassing 11.74 to 16.39 mg/dL, and Q4 extending from 16.4 to 82.99 mg/dL. In the context of the NHANES repository, the ascertainment of HPV infection status necessitated the collection of vaginal cellular material using a swab, followed by the isolation and analysis of DNA from the swab sample with the aid of the Roche Prototype Line Blot Test and the Roche Linear Array (LA) Human Papillomavirus Genotyping Assay. Participants whose test results were “positive” were classified as HPV-positive, whereas those yielding “negative” results were deemed HPV-negative.

2.3 Other covariates

Utilizing existing literature and clinical insights, we incorporated a range of covariates that might modulate the correlation between iron intake and HPV infection. The continuous variables integrated into this investigation encompassed age, poverty-to-income ratio (PIR, Household or individual income divided by poverty guidelines for a given survey year), and body mass index (BMI, Divide the weight of an individual by the square of its height, kg/m²). The categorical variables included race/ethnicity, education level, marital status, alcohol consumption (at least 12 drinks per year?), diabetes history (have you been told by a doctor or health professional that you have diabetes?), history of smoking (at least 100 cigarettes in a lifetime?), age of first sex, and number of sexual partners in the past year. Comprehensive details regarding the aforementioned covariates can be accessed on the NHANES website.

2.4 Analytical statistics

We performed a descriptive statistical analysis of the variables in our study by computing metrics such as the mean, median, and standard deviation for each variable listed in the baseline table. This approach facilitated the identification of trends in data concentration and dispersion. To elucidate the demographic characteristics of patients with and without HPV infection, we utilized ANOVA to assess the statistical significance of differences in continuous variable means and the chi-square test to compare differences in categorical variable frequency distributions. Continuous variables were reported using mean $\pm$ standard deviation (SD), while categorical variables were presented as frequency (percentage). Notably, given the complex sampling design of the NHANES database, we employed weighted univariate analysis to correct for sampling bias and differences in sampling probabilities across populations, thereby minimizing sampling error and enhancing the precision and external validity of our estimates. Adopting statistical techniques involving weighted univariate and multivariate logistic regression frameworks, we embarked on an investigation to scrutinize the connection between iron ingestion and the occurrence of HPV infection. In the multivariate logistic regression analyses, we constructed three models to evaluate the statistical relationships of various factors with HPV infection. Specifically, we developed a binary logistic model with HPV infection status (infected/uninfected) as the dependent variable



and iron intake and other demographic characteristics as independent variables. We calculated the adjusted odds ratios (AORs) for the following models: (1) Model 1 (unadjusted for covariates), (2), Model 2 (adjusted for age and race), and (3) Model 3 (adjusted for all covariates) to ascertain the link between iron intake and HPV infection. Furthermore, based on the fully adjusted models, we conducted subgroup analyses using multilevel multivariate logistic regression to identify stratified associations between iron intake and HPV infection. Forest plots were generated to facilitate interaction tests among subgroups. To analyze the relationship between iron intake and HPV infection, we considered threshold and saturation effects and incorporated a threshold variable to categorize iron intake into two groups: below and above the threshold. Logistic regression models were employed to assess the impact of iron intake on HPV infection. By comparing the odds ratios (ORs) and 95% confidence intervals (CIs) between these two groups, we observed changes in the risk of HPV infection. Additionally, smooth curve fitting was applied in the fully adjusted model to visually estimate the association between iron intake and HPV infection. In our study, statistical significance was determined at a p -value of <0.050 .

3 Results

3.1 Baseline features

A sample of 7,819 adults aged 18 to 59 years was enrolled in this study, with 3,481 individuals (constituting 44.52% of the cohort) diagnosed with HPV infection. Table 1 presents a detailed demographic and behavioral profile of the participants, stratified by their HPV infection status. Notably, non-Hispanic Black women who were divorced, older, economically disadvantaged, less educated, sexually active at an earlier age, and with histories of smoking, alcohol abuse, and diabetes mellitus exhibited an elevated prevalence of HPV infection. The mean dietary iron intake among participants with a confirmed diagnosis of HPV infection was 12.68 ± 0.18 milligrams per day, which was statistically significantly lower ($p < 0.050$) compared to the mean intake of 13.67 ± 0.17 milligrams per day observed in those without HPV infection. When the cohort was stratified into quartiles based on their dietary iron intake levels, a statistically significant difference ($p < 0.050$) in HPV infection status persisted between the quartiles.

3.2 Correlation between iron intake and HPV infection

The outcomes of the multivariate regression analysis, which investigates the relationship between iron intake and HPV infection, are displayed in Table 2. Across three distinct models—an unadjusted model, a partially adjusted model, and a fully adjusted model—a significant inverse relationship was identified between elevated levels of iron consumption and the likelihood of HPV infection, with statistical significance. In the unadjusted model, OR was 0.981 (95% CI: 0.972–0.991, $p < 0.001$), indicating a decrement in the risk of HPV infection with each incremental increase in iron intake. This inverse relationship persisted in both the partially adjusted model

(OR = 0.983, 95% CI: 0.973–0.992, $p < 0.001$) and the fully adjusted model (OR = 0.988, 95% CI: 0.979–0.998, $p = 0.018$).

When iron intake was stratified into quartiles, Q3 and Q4 demonstrated a statistically significant negative correlation with HPV infection compared to Q1 in all three models ($p < 0.05$). In the fully adjusted model, a comparison of the highest quartile to the lowest quartile revealed a 23.2% decrement in the risk of HPV infection for each incremental unit of iron intake (OR = 0.768, 95% CI: 0.634–0.930, $p = 0.009$).

To conduct a more thorough examination of the potential association between iron consumption and human papillomavirus (HPV) infection, we conducted an analysis utilizing smooth curve fitting techniques. The statistical model employed in this analysis incorporated comprehensive adjustments for a multitude of covariates, including age, race, poverty-to-income ratio, education level, marital status, body mass index, diabetes history, alcohol consumption, history of smoking, age of first sex, and number of sexual partners. The findings from this analysis revealed a significant L-shaped correlation between increasing levels of iron intake and the risk of HPV infection (Figure 2). The results from an augmented threshold effect analysis, as detailed in Table 3, indicate the presence of a saturation threshold for iron intake at an inflection point of 16.99 milligrams. Below this threshold, a statistically significant inverse relationship is observed, where each incremental unit of iron intake is associated with a 3.2% decrement in the odds of HPV infection (OR = 0.968, 95% CI: 0.956–0.980, $p < 0.001$). Conversely, above this inflection point, no significant correlation is discernible between further increments in iron intake and the prevalence of HPV infection (OR = 1.006, 95% CI: 0.993–1.018, $p = 0.372$), suggesting a ceiling effect on the protective benefits of iron against HPV infection.

3.3 Subgroup analysis

Ultimately, we conducted an extensive series of subgroup analyses. Our cohort was stratified based on a multitude of demographic and behavioral characteristics, to further elucidate the interplay between iron intake and HPV infection, we conducted an extensive series of subgroup analyses. Our cohort was stratified based on a multitude of demographic and behavioral characteristics, age, race, poverty-to-income ratio, education level, marital status, body mass index, diabetes history, alcohol consumption, history of smoking, age of first sex, and number of sexual partners (Figure 3). The results of these analyses revealed that marital status served as a notable modifier of the association between iron intake and HPV infection (p for interaction = 0.038). Conversely, the interaction tests performed that no significant statistical differences emerged in the connection between iron consumption and HPV infection among the other subgroups, suggesting that the differences noted among these subgroups did not have a notable impact on this inverse relationship. (p for interaction >0.050).

4 Discussion

Our cross-sectional study, the first to explore the correlation between iron intake and human papillomavirus (HPV) infection, has elucidated a pronounced L-shaped correlation, indicative of an

TABLE 1 Baseline characteristics of the study population.

Characteristics	HPV infection		<i>p</i> -value
	Yes (<i>n</i> = 3,481)	No (<i>n</i> = 4,338)	
Age, years, Mean (SD)	38.38 (0.37)	41.37 (0.27)	<0.001
Poverty-to-income ratio, Mean (SD)	2.69 (0.05)	3.24 (0.05)	<0.001
BMI, kg/cm ² , Mean (SD)	28.98 (0.19)	28.91 (0.18)	0.755
RACE, <i>n</i> (%)			<0.001
Mexican American	495 (7.80%)	768 (8.62%)	
Other Hispanic	351 (6.32%)	418 (5.00%)	
Non-Hispanic White	1,378 (62.63%)	1984 (70.97%)	
Non-Hispanic Black	1,033 (18.02%)	670 (7.62%)	
Other Race - Including Multi-Racial	224 (5.23%)	498 (7.78%)	
Education level, <i>n</i> (%)			<0.001
Less than high school	1,472 (36.93%)	1,541 (29.62%)	
High school and above	2,009 (63.07%)	2,797 (70.38%)	
Marital status, <i>n</i> (%)			<0.001
Married	1952 (58.16%)	3,309 (78.66%)	
Divorce	567 (17.58%)	373 (8.76%)	
Separated	187 (4.34%)	138 (2.28%)	
Never married	775 (19.92%)	518 (10.30%)	
The doctor told you have diabetes, <i>n</i> (%)			0.014
Yes	231 (4.69%)	316 (6.16%)	
No	3,250 (95.31%)	4,022 (93.84%)	
Smoked at least 100 cigarettes in life, <i>n</i> (%)			<0.001
Yes	1,541 (47.26%)	1,394 (35.90%)	
No	1,940 (52.74%)	2,944 (64.10%)	
Had at least 12 alcoholic drinks 1 year, <i>n</i> (%)			<0.001
Yes	2,549 (79.31%)	2,780 (71.99%)	
No	932 (20.69%)	1,558 (28.01%)	
How old when first had sex, <i>n</i> (%)			<0.001
<18	2,321 (67.73%)	2,185 (51.18%)	
>= 18	1,160 (32.27%)	2,153 (48.82%)	
Male sex partners/year, <i>n</i> (%)			<0.001
0–1	2,706 (78.16%)	4,089 (94.61%)	
2–5	714 (20.44%)	236 (5.08%)	
>5	61 (1.40%)	13 (0.30%)	
Iron intake, mg, Mean (SD)	12.68 (0.18)	13.67 (0.17)	0.001
Iron intake (quartile), <i>n</i> (%)			<0.001
Q1 (0–8.16)	991 (27.91%)	957 (21.51%)	
Q2 (8.17–11.73)	905 (27.92%)	1,050 (23.84%)	
Q3 (11.74–16.39)	801 (21.59%)	1,159 (27.58%)	
Q4 (16.4–82.99)	784 (22.58%)	1,172 (27.07%)	

Mean (SD) for continuous variables, % for categorical variables. BMI, body mass index.

inverse trend between the two variables. Utilizing a rigorous threshold effect analysis, we identified a pivotal inflection point at an iron intake of 16.99 milligrams. Below this threshold, we observed a statistically significant 3.2% decrease in the odds ratio (OR) of HPV infection for

every incremental unit of iron consumed (OR = 0.968, 95% CI: 0.956–0.980, $p < 0.001$). Conversely, above this inflection point, the statistical significance of the correlation between iron intake and HPV infection diminished. Furthermore, our subgroup analyses

TABLE 2 Association between iron intake and HPV infection.

	Model 1		Model 2		Model 3	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Iron intake (mg)	0.981 (0.972, 0.991)	<0.001	0.983 (0.973, 0.992)	<0.001	0.988 (0.979, 0.998)	0.018
Iron intake quartile						
Q1 (0–8.16)	1.00 (1.000, 1.000)	1.000	1.000 (1.000, 1.000)	1.000	1.000 (1.000, 1.000)	1.000
Q2 (8.17–11.73)	0.903 (0.772, 1.055)	0.201	0.919 (0.784, 1.078)	0.303	0.974 (0.822, 1.154)	0.760
Q3 (11.74–16.39)	0.603 (0.509, 0.715)	<0.001	0.627 (0.530, 0.743)	<0.001	0.712 (0.597, 0.850)	<0.001
Q4 (16.4–82.99)	0.643 (0.542, 0.762)	<0.001	0.663 (0.559, 0.787)	<0.001	0.768 (0.634, 0.930)	0.009
p for trend		<0.001		<0.001		<0.001

Model 1 adjusted for no covariates. Model 2 adjusted for age and race. Model 3 adjusted for age, race, poverty-to-income ratio, education level, marital status, body mass index, diabetes history, alcohol consumption, history of smoking, age of first sex, and number of sexual partners.

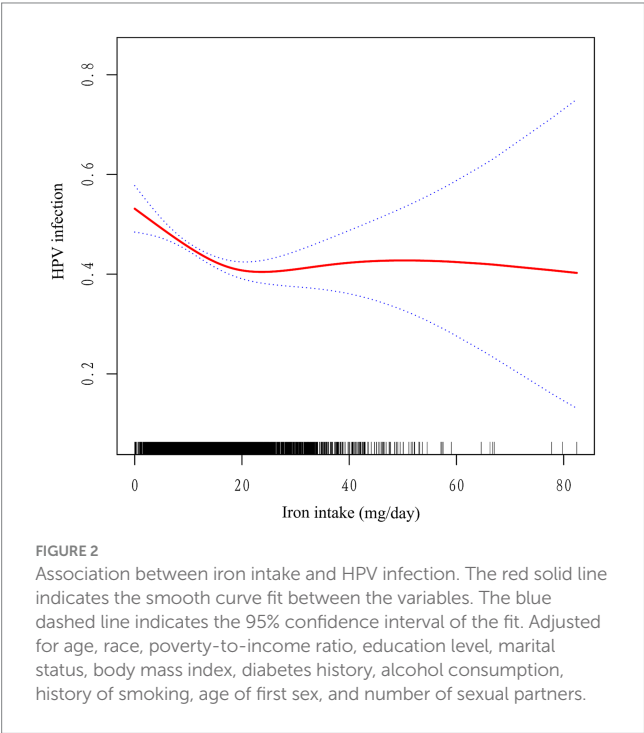


FIGURE 2 Association between iron intake and HPV infection. The red solid line indicates the smooth curve fit between the variables. The blue dashed line indicates the 95% confidence interval of the fit. Adjusted for age, race, poverty-to-income ratio, education level, marital status, body mass index, diabetes history, alcohol consumption, history of smoking, age of first sex, and number of sexual partners.

provided additional evidence supporting the robustness of our findings. In conclusion, our results demonstrate a clear L-shaped correlation between iron intake and HPV infection, emphasizing that maintaining an adequate intake of iron offers protection against HPV infection.

Despite the paucity of studies investigating the association between dietary iron intake and human papillomavirus (HPV) infection, available scientific research hints at a relationship between serum iron concentrations and the underlying causes of diseases that affect the female reproductive system. Specifically, alterations in iron metabolism have been implicated in the pathogenesis of endometriosis, with ferroptosis potentially serving as a natural regulatory mechanism for the clearance of ectopic endometrial cells in healthy individuals. Perturbations in this process may contribute to the initiation and progression of endometriomas (EMs), thereby promoting their proliferation and metastatic behavior (32). Optimal maintenance of serum iron concentrations in gestational females has

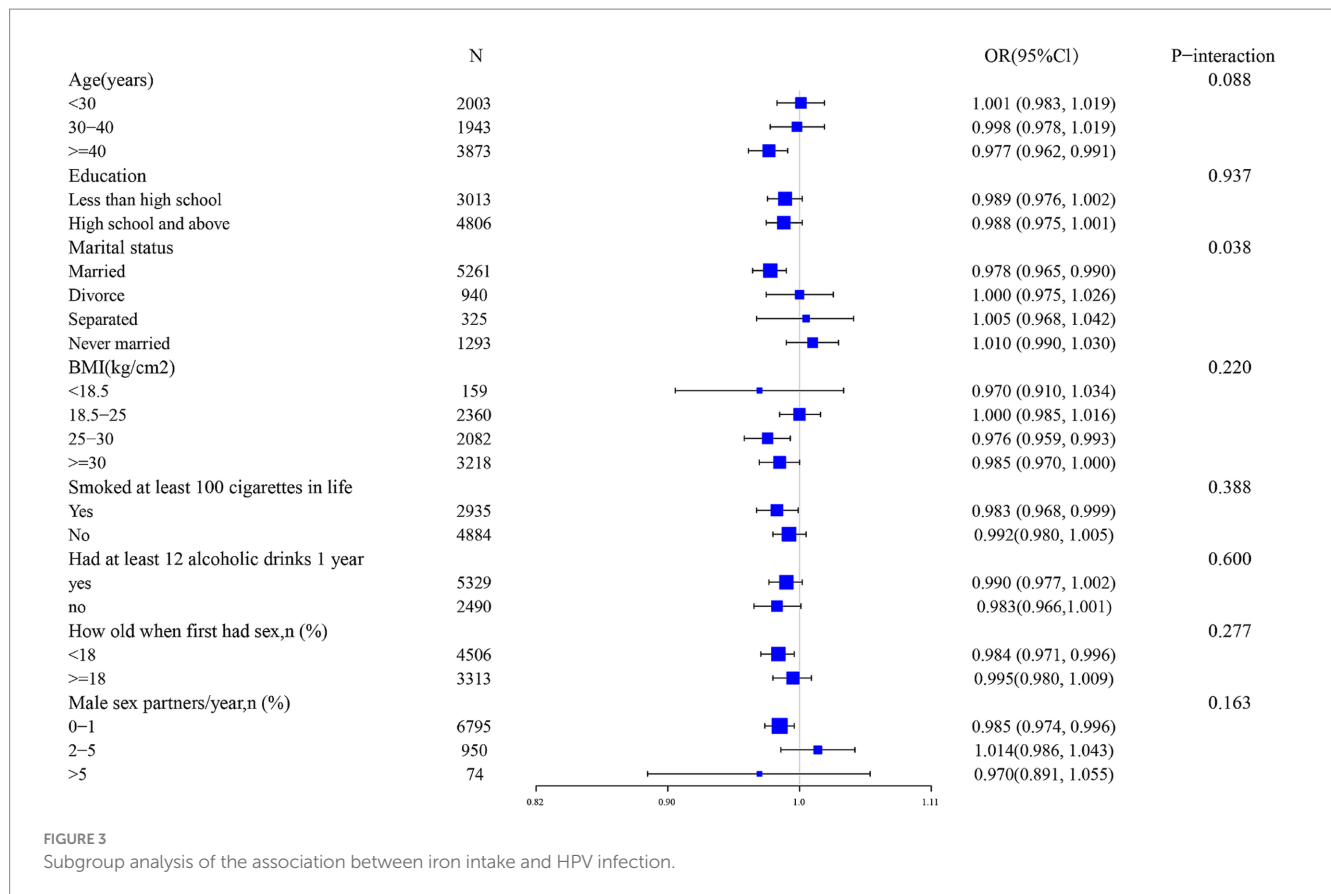
TABLE 3 Threshold effect analysis of iron intake and HPV infection.

	Adjusted OR (95% CI)	p value
Fitting by the 2-piecewise linear model		
Inflection point	16.99	
Iron intake<16.99	0.968 (0.956, 0.980)	<0.001
Iron intake>= 16.99	1.006 (0.993, 1.018)	0.372
p for Log-likelihood ratio	<0.001	

Adjusted for age, race, poverty-to-income ratio, education level, marital status, body mass index, diabetes history, alcohol consumption, history of smoking, age of first sex, and number of sexual partners.

been established to diminish the incidence of an array of pregnancy-associated complications, encompassing anemia, hypertensive disorders of pregnancy, fetal growth restriction leading to low birth weight, preeclampsia, and various postpartum complications (33). Moreover, an investigation encompassing 2088 Iranian subjects examining dietary iron intake and cervical neoplasia suggested that adequate iron ingestion may confer a protective effect against cervical cancer incidence and potentially attenuate its progression (29). These observations are consonant with our research findings, which demonstrate an inverse relationship between dietary iron intake and HPV infection. In contrast to prior scholarly endeavors, our investigation offers nuanced elucidation of the L-shaped association between dietary iron ingestion and human papillomavirus (HPV) infection, disclosing the presence of a saturation threshold in the relationship between iron intake and HPV acquisition.

The existing body of evidence concerning the correlation between iron intake and HPV infection remains inconclusive. Prior investigations have emphasized the pivotal role of iron homeostasis in immune modulation. Initially, iron is indispensable for the differentiation and growth of epithelial tissue, which acts as the primary line of defense for the cervical mucosa against HPV infection (34). Furthermore, iron may inhibit HPV viral replication by enhancing the synthesis of interferon-gamma (IFN- γ), thereby amplifying IFN- γ -induced innate immune responses and mitigating the susceptibility to intracellular infection (34–36). In the realm of innate immunity, neutrophils rely on iron for the generation of reactive oxygen species (ROS) to eradicate pathogens (37). Additionally, iron plays a crucial role in promoting macrophage polarization, which is essential for



cytotoxic and pro-inflammatory functions (38–40). Moreover, iron is closely associated with natural killer (NK) cells, and suboptimal iron levels impair NK cell activation and antiviral activity (41). In adaptive immunity, T cells stimulate the production of transferrin receptor (TFR) through the interleukin-2 (IL-2) signaling pathway, thereby accelerating cellular iron uptake (42–45).

We hypothesize that as iron uptake increases, T cells accumulate more iron, leading to enhanced differentiation and proliferative activity of T helper (Th) cells. This occurs by fostering cytokine production and activating the phosphorylation of cell proliferation-regulating factors via protein kinase C. Th cells further differentiate into Th1 and Th2 subsets. Th1 cells are inclined to initiate a response against intracellular viruses, and their production of IL-2 promotes lesion cell apoptosis, exerting antiviral and antitumor effects. High concentrations of these cytokines should facilitate the clearance of HPV infection and prevent lesion progression. Conversely, Th2 cells secrete various other interleukins (such as IL-4 and IL-10) and trigger immune responses against extracellular microbes (34, 36). Notably, inadequate iron intake inhibits DNA synthesis in Th1 cells, which are more sensitive to iron than Th2 cells, suggesting that insufficient iron intake has a greater inhibitory impact on Th1 than on Th2 (45). Thus, adequate iron intake supports pro-inflammatory Th1 cytokine-mediated immune responses, maintains effective immunity, and avoids a shift to anti-inflammatory Th2 cell-mediated immune responses, which could elevate the risk of extracellular infections (35, 36).

Another plausible mechanism proposes that iron possesses antiviral properties. Previous studies have demonstrated that iron can

inhibit HCV viral RNA and protein expression by suppressing the enzymatic activity of RNA polymerase NS5B (47–49). However, no specific studies have examined the role of iron in HPV infection. The life cycle of HPV begins with infection of the basal cell layer following microtrauma to the epithelial barrier (50). Following the establishment of infection, the HPV genome maintains a low copy number. Upon differentiation of the host cell, HPV replicates extensively and expresses the capsid-forming genes, L1 and L2, enabling the creation and extrusion of novel viral particles from the epithelial barrier (51). To sustain a chronic infection, HPV requires the colonization of basal cells exhibiting stem-cell-like attributes (52). Therefore, cervical migratory epithelial cells become crucial targets for HPV infection, and infected cervical cells must enter the mitotic (M) phase for persistent HPV infection to occur (53). Iron-mediated ROS generation blocks the PI3K/AKT and JAK/STAT3 pathways and activates P38 MAPK, resulting in failed sustained cell division through G1 phase blockade and autophagy induction, thereby reducing the ability of HPV to sustain replication in recipient cells (54). Previous studies have also shown that ferritin can inhibit G1 cell cycle protein-dependent kinases, thus inhibiting cell division and tumor progression during growth arrest in human breast cancer cells (55). Furthermore, ingested iron is absorbed and chelated with lactoferrin or other transferrin proteins, potentially blocking viral entry into the host cell by inhibiting cellular receptors or directly binding to viral particles, which may play a role in blocking early stages of infection (56–58).

Employing the L-shaped curve model, we ascertained that 16.99 milligrams represented the inflection point in iron intake. Beyond this level, the reduction in HPV infection did not continue to increase with

higher iron intake. Iron overload suppressed the proliferation rate, cell number, and active state of helper T cells (CD4+), while elevating the CD8 to CD4 ratio, promoting the production of cytotoxic T cells, and disrupting the immunoglobulin secretion pattern. This further led to an increase in the concentrations of the cytokines IL-4, IL-6, and IL-10 (59). IL-6 enhances the ability of HPV-infected cells to evade T-cell immune responses, and at high concentrations, may be crucial for the persistence of high-risk HPV (HR-HPV) infection and disease progression (45). Increased secretion of the anti-inflammatory cytokine IL-10 in the Th2 response has been associated with impaired innate and adaptive immune defenses and the progression of cervical lesions during high-risk HPV infection (60). Iron modulates macrophage activity, resulting in a reduction of TNF- α production, a key pro-inflammatory cytokine, which subsequently dampens the immune defense against HPV in the skin and mucosa (61).

Therefore, chronic iron supplementation and iron overload states may attenuate Th1 cytokine-mediated pro-inflammatory responses, increase Th2 cytokine-mediated anti-inflammatory responses, impair macrophage killing of intracellular pathogens, and promote the growth of pathogens due to the growth-promoting effects of iron, leading to increased susceptibility to infection. Continued excessive iron intake may disrupt the balance between Th1 and Th2, resulting in alterations in cytokine concentration and quantity, which may weaken the body's immune response to HPV. These changes correspond to a turning point in the L-shaped curve, and the protective effect against HPV infection gradually plateaus with further increases in iron intake.

Our survey possesses several notable strengths. Foremost among these is its pioneering utilization of the NHANES database to examine the correlation between iron intake and HPV infection. The NHANES database annually enrolls a demographically diverse and representative cohort of approximately 5,000 participants from all regions of the United States, spanning various socioeconomic statuses, age strata, and ethnic groups. By harnessing nearly 12 years of extensive research data, our study encompasses a substantial sample of 7,819 women, thereby greatly enhancing the statistical power and reliability of our findings. Additionally, our investigation incorporated subgroup analyses to offer an enhanced understanding of the interplay between iron intake and HPV infection across various subgroups. Hence, our findings gain further credibility. Our investigation encounters several constraints that necessitate careful consideration. Primarily, the cross-sectional methodology employed hinders our ability to conclusively determine the temporal dynamics between iron consumption and HPV infection. Consequently, our findings can only indicate a simultaneous association between these variables, which could be affected by a wide array of additional factors. Furthermore, the cross-sectional nature of our study limits the extent to which we can capture the extended impacts of iron intake on HPV infection over time. Secondly, the exclusive focus on the U.S. population introduces potential limitations to the generalizability of our results. Significant variations in dietary patterns, HPV prevalence rates, and genetic compositions within this cohort may restrict the applicability of our findings to other demographic groups. Thus, when disseminating our results, caution should be exercised in relation to other populations. Moreover, despite adjusting for numerous covariates, limitations in the available data prevented us from comprehensively excluding other potential confounding variables, such as genetic polymorphisms and lifestyle factors, which may independently affect both iron intake and

HPV infection. These unaccounted confounders may obscure or distort the true underlying relationship between the two variables. Additionally, while the NHANES data are collected through standardized personal interviews and examinations, the use of dietary recall interviews inherently involves the risk of recall bias. Factors such as participants' memory accuracy, cognitive functioning, cultural influences, and individual dietary practices can all impact the reliability of dietary information obtained through such interviews.

To further elucidate the causal relationship between iron intake and HPV infection, future research endeavors should consider adopting longitudinal designs or cohort studies. These methodologies can provide a more precise understanding of the temporal sequence and causal links between these variables, thereby advancing our knowledge in this area.

5 Conclusion

Upon completion of our study, we revealed an L-shaped link between iron intake and HPV infection, with <16.99 mg/day associated with higher HPV risk. Adequate iron intake emerged as a protective factor. These findings pave the way for future research in HPV management and treatment, emphasizing iron's crucial role in HPV pathogenesis.

Data availability statement

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

Ethics statement

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

XC: Conceptualization, Data curation, Formal analysis, Project administration, Writing – original draft. HC: Data curation, Formal analysis, Writing – original draft. YC: Investigation, Methodology, Writing – original draft. LT: Investigation, Writing – original draft. JL: Investigation, Writing – original draft. Y-HO: Funding acquisition, 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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Global, regional, and national burden of nutritional deficiencies spanning from 1990 to 2021, with a focus on the impacts observed during the COVID-19 pandemic

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Background: The United Nations has recognized nutritional deficiencies as a critical health issue that necessitates urgent eradication. This study aimed to provide a comprehensive analysis of the spatial distribution and temporal trends of the global disease burden associated with nutritional deficiencies and their four subtypes from 1990 to 2021, with a particular focus on the impact of the COVID-19 pandemic.

Methods: This study primarily employs the most recent data from the Global Burden of Disease (GBD) 2021 to conduct a thorough analysis of the distribution trends of incidence, mortality, and disability-adjusted life years (DALYs) associated with nutritional deficiencies and their four subtypes from 1990 to 2021, incorporating detailed subgroup analyses categorized by sex, age, and region. In comparison to the GBD 2019, the GBD 2021 update places a particular emphasis on supplementing disease burden data for the period of the COVID-19 pandemic (2019–2021). Furthermore, this study investigates the primary risk factors contributing to disability-adjusted life years (DALYs) linked to nutritional deficiencies.

Results: Between 1990 and 2021, the global burden of nutritional deficiencies experienced a substantial decline, evidenced by a 54.9% reduction in the age-standardized incidence rate (ASIR), a 72.2% decrease in the age-standardized death rate (ASDR), and a 51.9% reduction in the age-standardized DALY rate. However, it is noteworthy that the burden of iodine deficiency (ASIR: 137.72 vs. 75.49; Age-standardized DALY rate: 35.43 vs. 19.98) and dietary iron deficiency (Age-standardized DALY rate: 597.97 vs. 253.05) is considerably greater in women than in men. Moreover, in regions characterized by a low social demographic index (SDI) and lower income levels, the burden of diseases associated with nutritional deficiencies remains substantial. In contrast, the COVID-19 pandemic has not markedly changed the epidemiological profile of nutritional deficiencies compared to the pre-2019 period, and the global burden of nutritional deficiencies has continued its gradual decline.

Conclusions: Despite a decline in the global burden of nutritional deficiencies over time, significant disparities related to gender, region, and age persist.

Fortunately, the COVID-19 pandemic has had a relatively limited impact on the global burden of nutritional deficiencies. Healthcare institutions must formulate more targeted strategies aimed at alleviating the adverse effects of nutritional deficiencies on global public health.

KEYWORDS

nutritional deficiencies, global burden, dietary iron deficiency, vitamin A deficiency, protein-energy malnutrition, iodine deficiency

Introduction

Nutritional deficiencies are typically defined as a pathological state resulting from inadequate intake, poor absorption, or excessive loss of essential nutrients (including vitamins, minerals, and proteins); chronic malnutrition can result in multi-system damage, encompassing neurological, endocrine, and cardiovascular complications, and may ultimately lead to death (1–4). A report from the World Health Organization (WHO) indicates that nutritional deficiencies are widespread in both developed and developing countries, significantly affecting public health, particularly among vulnerable populations, including children, pregnant women, and the elderly (5, 6). The epidemiological profile of nutritional deficiencies highlights the intricate interactions among various risk factors. The 2021 Global Nutrition Report indicates that nearly 2 billion adults are classified as overweight or obese, yet one in three adults experiences at least one form of micronutrient deficiency. Among children, stunting and wasting continue to pose significant challenges, especially in low- and middle-income countries (7, 8). Consequently, the United Nations General Assembly proclaimed the period from 2016 to 2025 as the United Nations Decade of Action on Nutrition, with the objective of eradicating hunger and preventing all forms of malnutrition (9).

Nutritional deficiencies are typically classified into two primary categories: macronutrient deficiencies and micronutrient deficiencies (10). Macronutrient deficiencies specifically pertain to inadequate intake of proteins, carbohydrates, and fats (11). Among these, protein-energy malnutrition represents a critical concern in numerous low-income countries, often resulting in diseases such as kwashiorkor and marasmus (12, 13). Micronutrient deficiencies primarily denote a lack of essential vitamins and minerals; although the human requirement for these nutrients is relatively minimal, they play a vital role in various physiological functions (14). For instance, iodine deficiency may result in goiter and cognitive impairment, particularly among children (15). Vitamin A deficiency can adversely impact vision and immune function, and it is recognized as one of the leading causes of blindness (16).

Existing research indicates that the global burden of nutritional deficiencies remains prevalent as of 2019, highlighting the need for focused attention on the more pronounced burden in economically disadvantaged regions due to disparities in economic development (17). Unfortunately, it is well established that the COVID-19 pandemic has rendered the global nutritional landscape more complex, disrupting existing food supply chains and exacerbating disparities in nutritional health (18, 19). However, the extent to

which the COVID-19 pandemic has influenced the global burden of nutritional deficiencies and whether it has altered the pre-existing epidemiological patterns remains unclear. No studies have yet reported changes in the global burden of nutritional deficiencies during the COVID-19 pandemic. To address this gap, this study utilizes the most recent Global Burden of Disease (GBD) 2021 data to evaluate the burden of nutritional deficiencies across 204 countries and territories worldwide from 1990 to 2021, thereby expanding on previous research and filling the data void caused by the pandemic of COVID-19. Furthermore, this study examines the impact of various risk factors on disability-adjusted life years (DALYs) associated with nutritional deficiencies. Compared to previous GBD studies, GBD 2021 represents a significant advancement by incorporating updated data from the COVID-19 pandemic, enabling researchers to assess epidemiological trends and explore the potential impact of COVID-19 on disease burden.

Method

Data source

The GBD 2021 comprehensively evaluated over 370 diseases and injuries across 204 countries and regions globally, delivering detailed data on incidence, death, DALYs, and their corresponding age-standardized rates (20, 21). All data were derived from publicly available databases established by national institutions, with numerous published studies integrated and subjected to rigorous quality control measures to ensure data accuracy and reliability (22, 23).

Data definitions

Total incidence and age-standardized incidence rates (ASIR) were evaluated using Bayesian meta-regression models, whereas total mortality and age-standardized mortality rates (ASDR) were predominantly estimated utilizing the Cause of Death Ensemble Model (CODEm). Disability weights quantify the severity of health loss or non-fatal disabilities, while DALYs reflect the total years lost due to health impairment, from the onset of disease to death, serving as a crucial indicator for evaluating disease burden (24, 25).

The Socio-Demographic Index (SDI) is a composite measure that reflects a country's level of development, incorporating per capita income, the average education level of individuals aged 15 and older, and total fertility rates among individuals under 25

years. This index is closely associated with health outcomes, with SDI values ranging from 0 to 1, where 0 signifies the lowest level of development and 1 denotes the highest level of development (26, 27).

Based on SDI values, the 204 countries and regions were categorized into five distinct groups: high SDI, medium-high SDI, medium SDI, medium-low SDI, and low SDI regions. Patients were stratified into twelve age groups: <5 years, 5–9 years, 10–14 years, 15–19 years, 20–24 years, 25–29 years, 30–34 years, 35–39 years, 40–44 years, 45–49 years, 50–69 years, and 70+ years.

Definition of nutritional deficiencies

In the GBD 2021 study, the definition of nutritional deficiencies is grounded in the codes for nutritional deficiencies outlined in the 10th edition of the International Classification of Diseases (ICD), encompassing protein-energy malnutrition (ICD-10 codes E40–E46.9, E64.0), iodine deficiency (E00–E02), vitamin A deficiency (E50–E50.9, E64.1), and dietary iron deficiency (D50–D50.9) (28).

Estimation of risk factor

We assessed 70 specific attributable risk factors identified in GBD 2021, including particulate pollution, extreme temperatures, lead exposure, smoking, secondhand smoke exposure, high consumption of red meat and sodium, low intake of fiber, fruits, and vegetables, as well as elevated fasting blood glucose levels. However, owing to limitations in data availability, the final attribution analysis predominantly concentrated on iron deficiency, vitamin A deficiency, child underweight, and child wasting.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Statistical analyses

We evaluated the global burden of nutritional deficiencies by analyzing annual incidence, total death, DALYs, and the corresponding ASRs. Age-standardized rates serve to mitigate the effects of disparate age distributions and demographic shifts across various regions, thereby ensuring the comparability of study metrics. Utilizing the ASIR, ASDR, and age-standardized DALYs, we calculated estimates of the annual percentage change (EAPC) to retrospectively analyze the trends in the burden of nutritional deficiencies over the past 32 years. In the equation $y = \alpha + \beta x$, y denotes the log₁₀ (ASR) value, while x signifies the year. The formula for calculating EAPC is given by $EAPC = 100 * (10^{\beta} - 1)$. If the EAPC value and its 95% confidence interval (CI) exceed zero, this indicates an increasing trend in ASR; conversely, it signifies a decreasing trend (23).

Furthermore, to investigate the correlation between ASIR, ASDR, age-standardized DALY rates, and levels of social development, we computed the Pearson correlation coefficients alongside the SDI values. Statistical analyses were performed utilizing GraphPad Prism (version 10.0.0 for Windows), encompassing *t*-tests and analysis of variance (ANOVA) to compare incidence rates and disability-adjusted life years across diverse genders, age groups, and regions.

All hypothesis tests conducted in this study were two-tailed, with a significance threshold established at $P < 0.05$ (29).

Result

Global trends in the burden of nutritional deficiencies

From 1990 to 2021, the global burden of nutritional deficiencies exhibited a consistent decreasing trend. The ASIR decreased by 54.9% (from 17,112.55 per 100,000 in 1990 to 7,725.10 per 100,000 in 2021, EAPC = −2.52), the ASDR declined by 72.2% (from 10.90 per 100,000 in 1990 to 3.03 per 100,000 in 2021, EAPC = −4.41), and the age-standardized DALY rate decreased by 51.9% (from 1,367.15 per 100,000 in 1990 to 657.62 per 100,000 in 2021, EAPC = −2.52). Furthermore, the ASIR for males was generally higher than that for females, whereas the age-standardized DALY rate was elevated for females; however, the difference in ASDR between genders was not statistically significant (Table 1, Figure 1A).

At the regional level, the burden of nutritional deficiencies in areas classified with a medium SDI and above continued to decline, with the lowest burden observed in high SDI regions. In low SDI regions, the ASDR and age-standardized DALY rate for nutritional deficiencies witnessed a significant increase between 2010 and 2012, peaking at 36.12 per 100,000 and 3,033.25 per 100,000, respectively; however, a subsequent consistent decreasing trend was observed (Table 1, Figure 1B).

Regionally, although the burden of nutritional deficiencies in Sub-Saharan Africa has diminished relative to 1990, it continues to be higher than in other regions. The ASIR in this region stands at 18,022.35 per 100,000, the ASDR is recorded at 9.56 per 100,000, and the age-standardized DALY rate is measured at 1,120.50 per 100,000. According to the World Bank's regional income statistics, as income levels increase, nutritional deficiencies diminish; the ASIR, ASDR, and age-standardized DALY rates in low-income areas are 11 times, 11 times, and over 10 times higher than those in high-income regions, respectively (Table 1).

Among the 204 countries analyzed, Somalia, Niger, and Chad exhibit the highest ASIR, with values of 83,940.53, 49,780.97, and 36,706.18, respectively. The highest ASDR values are reported in Sierra Leone, South Sudan, and Somalia, at 39.82, 33.90, and 32.07, respectively. The highest age-standardized DALY rates are recorded in Sierra Leone (2,901.99), Mali (2,742.30), and South Sudan (2,426.55). Furthermore, India ranks highest in total incidence, total mortality, and DALYs (Supplementary Tables S1–6, Figures 1C, D).

We further assessed the trends in the SDI across 21 regions worldwide from 1990 to 2021 and explored the potential associations with trends in ASIR, ASDR, and age-standardized

TABLE 1 The incidence of nutritional deficiencies in 1990/2021.

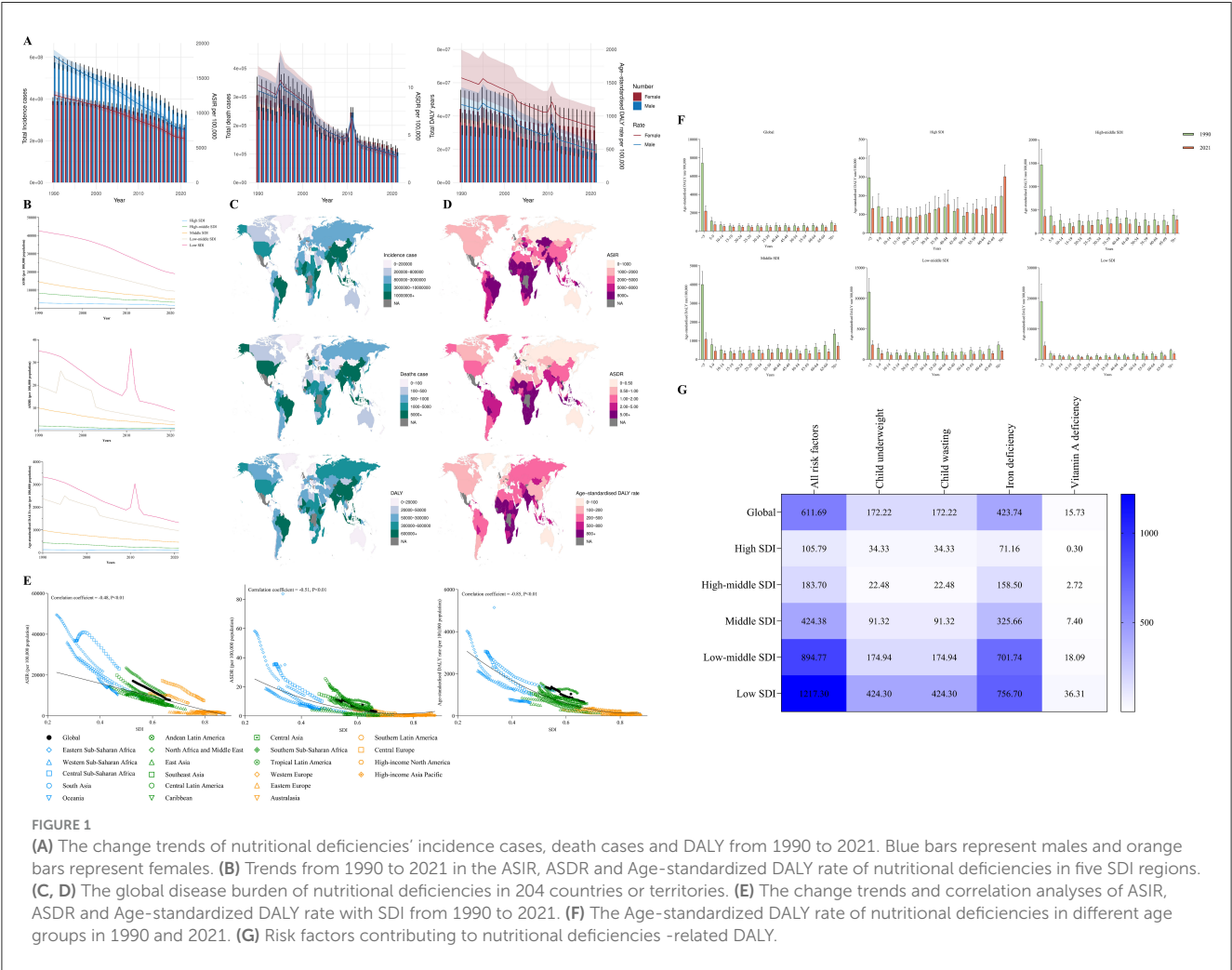
Variables	1990			2021		
	ASIR/100,000 No. (95% UI)	ASDR/100,000 No. (95% UI)	Age- standardized DALY rate/100,000 No. (95% UI)	ASIR/100,000 No. (95% UI)	ASDR/100,000 No. (95% UI)	Age- standardized DALY rate/100, 000 No. (95% UI)
Overall	17,112.55 (16,470.31, 17,731.43)	10.90 (9.44, 12.97)	1,367.15 (1,126.30, 1,708.49)	7,725.10 (7,404.01, 8,109.01)	3.03 (2.69, 3.40)	657.62 (489.93, 869.58)
EAPC-ASIR		−2.52 (−2.67, −2.38)				
EAPC-ASDR		−4.41 (−4.84, −3.98)				
EAPC-Age-standardized DALY rate		−2.52 (−2.71, −2.32)				
Sex						
Male	20,170.14 (19,158.18, 21,173.10)	10.71 (9.28, 12.56)	1,171.63 (967.32, 1,430.87)	8,423.99 (7,987.09, 8,934.09)	3.16 (2.80, 3.58)	484.62 (372.00, 631.78)
Female	14,000.41 (13,368.81, 14,655.15)	11.40 (9.51, 13.61)	1,573.03 (1282.74, 1,994.48)	7,006.41 (6,700.12, 7,383.65)	2.96 (2.63, 3.33)	834.92 (598.63, 1,127.32)
SDI						
High SDI	2,909.50 (3,180.88, 2,668.01)	0.78 (0.70, 0.84)	131.07 (92.53, 189.66)	1,671.71 (1,484.68, 1,885.64)	0.95 (0.80, 1.03)	118.26 (83.05, 165.11)
High-middle SDI	8,339.40 (7,781.30, 8,950.07)	2.06 (1.86, 2.26)	429.22 (326.50, 575.07)	3,305.31 (3,046.36, 3,589.94)	0.8 (0.7, 0.89)	203.41 (140.77, 288.96)
Middle SDI	14,567.32 (13,862.76, 15,328.32)	9.89 (9.09, 10.56)	972.77 (797.32, 1,229.51)	5,016.29 (4,705.26, 5,381.24)	2.62 (2.34, 2.82)	459.47 (331.59, 624.72)
Low-middle SDI	27,944.93 (26,526.37, 29,160.78)	19.60 (16.61, 22.99)	2,374.12 (1,965.67, 2,980.94)	9,389.32 (8,870.92, 9,951.09)	3.73 (3.33, 4.12)	971.02 (706.71, 1,308.72)
Low SDI	35.05 (28.92, 43.14)	35.05 (28.92, 43.14)	334.19 (2,727.78, 4,117.71)	19,047.59 (18,448.14, 19,697.06)	8.73 (7.44, 10.02)	1,319.28 (1,002.38, 1,722.91)
Region						
Central Asia	8,894.18 (8,339.60, 9,462.22)	1.23 (1.13, 1.33)	845.24 (595.7, 1,176.90)	4,927.20 (4,597.18, 5,281.99)	0.32 (0.28, 0.36)	585.12 (399.86, 837.14)
East Asia	11,535.28 (10,239.38, 12,956.89)	5.45 (4.78, 6.10)	570.83 (462.24, 725.04)	3,450.57 (3,075.48, 3,859.46)	1.13 (0.93, 1.32)	160.76 (110.46, 228.34)
South Asia	28,292.51 (26,126.08, 30,300.72)	19.49 (16.02, 23.18)	2,796.88 (2,246.58, 3,574.34)	9,171.97 (8,326.93, 10,138.25)	2.56 (2.16, 3.00)	1,187.87 (840.68, 1,627.64)
Southeast Asia	19,895.08 (18,775.33, 21,118.15)	15.04 (12.61, 17.05)	1,104.99 (899.41, 1,380.21)	5,892.56 (5,501.48, 6,347.38)	5.96 (5.10, 6.65)	505.55 (384.30, 664.82)
Central Europe	17,286.89 (16,504.76, 18,203.53)	0.17 (0.15, 0.18)	360.34 (237.09, 521.60)	7,479.70 (7,086.41, 7,915.69)	0.36 (0.33, 0.39)	200.85 (134.94, 293.55)
Eastern Europe	1,891.24 (1,660.15, 2,160.48)	0.48 (0.46, 0.50)	352.98 (242.19, 508.14)	1,146.35 (947.99, 1,355.48)	0.29 (0.27, 0.31)	236.53 (163.10, 337.20)
Sub-Saharan Africa	38,774.88 (38,136.38, 39,441.60)	33.92 (28.39, 41.91)	2,917.80 (2,401.44, 3,613.64)	18,022.35 (17,539.945, 18,491.50)	9.56 (8.11, 11.03)	1,120.50 (871.92, 1,444.32)
Oceania	18,443.43 (17,325.88, 19,591.87)	7.58 (6.30, 9.10)	776.54 (583.49, 1,037.62)	10,329.04 (9,522.33, 11,202.87)	4.36 (3.57, 5.42)	610.45 (433.75, 897.37)
Latin America and Caribbean	16,808.45 (15,883.74, 17,764.75)	17.61 (16.72, 18.30)	1,121.27 (980.98, 1,319.18)	7,346.28 (6,882.37, 7,924.83)	3.94 (3.51, 4.37)	399.33 (305.24, 515.72)
World Bank Income Level						
World Bank High Income	3,638.71 (3,401.69, 3,912.01)	0.77 (0.70, 0.83)	142.22 (99.89, 203.76)	1,960.85 (1,778.65, 2,169.05)	0.91 (0.78, 0.98)	120.48 (85.04, 168.70)
World Bank Upper Middle Income	12,110.18 (11,292.02, 12,987.79)	6.45 (5.98, 6.85)	671.03 (555.08, 832.94)	4,393.77 (4,115.72, 4,714.37)	1.66 (1.48, 1.82)	259.38 (188.68, 351.68)

(Continued)

TABLE 1 (Continued)

Variables	1990			2021		
	ASIR/100, 000 No. (95% UI)	ASDR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)	ASIR/100, 000 No. (95% UI)	ASDR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)
World Bank Lower Middle Income	24,340.90 (23,025.33, 25,513.18)	17.16 (14.60, 19.92)	2,143.96 (1,765.05, 2,715.71)	8,420.95 (7,932.39, 8,997.21)	1.66 (1.48, 1.82)	259.38 (188.68, 351.68)
World Bank Low Income	44,320.10 (43,510.02, 45,120.47)	38.30 (31.58, 47.90)	3,170.54 (2,571.98, 3,998.78)	22,224.09 (21,569.43, 22,834.05)	10.45 (8.80, 12.18)	1,206.68 (938.33, 1,540.59)

UI, uncertainty intervals.



DALYs. The results demonstrated that SDI exhibited a significant negative correlation with the ASIR of nutritional deficiencies (correlation coefficient = -0.48 , $P < 0.01$), the ASDR (correlation coefficient = -0.51 , $P < 0.01$), and the age-standardized DALY rate (correlation coefficient = -0.85 , $P < 0.01$) (Figure 1E).

Figure 1F illustrates the results of a subgroup analysis stratified by age. The results revealed that except for high SDI regions, the age-standardized DALY rate for nutritional deficiencies among the

<5 years age group was consistently the highest. Conversely, in high SDI regions, the age-standardized DALY rate was highest among the 70+ years age group.

In 2021, the age-standardized DALY rate attributable to all risk factors associated with nutritional deficiencies was recorded at 611.69 per 100,000. The primary risk factors identified globally and across different SDI regions included iron deficiency, vitamin A deficiency, child underweight, and child wasting. Among these factors, iron deficiency emerged as the predominant risk

factor contributing to disabilities and mortality associated with nutritional deficiencies (Figure 1G).

Global trends in the burden of protein-energy malnutrition

From 1990 to 2021, the global burden of protein-energy malnutrition demonstrated a generally fluctuating decreasing trend. The most substantial reductions were observed in ASDR, which decreased from 9.44 per 100,000 in 1990 to 2.61 per 100,000 in 2021 (with an EAPC of -4.47 , indicating a decline of 72.4%), and in the age-standardized DALY rate, which fell from 699.3 per 100,000 in 1990 to 172.38 per 100,000 in 2021 (with an EAPC of -4.64 , reflecting a decline of 75.3%). In contrast, the ASIR exhibited considerable fluctuations, with a significant decline occurring primarily after 2014. Furthermore, no significant differences were observed in the ASDR and age-standardized DALY rates for protein-energy malnutrition between males and females; however, the ASIR for males was notably higher than that for females (Table 2, Figure 2A).

At the SDI's regional level, the ASIR in middle SDI and higher regions displayed an upward trend before 2014, followed by a marked decline thereafter. By 2021, the ASIR in middle SDI regions was the highest, whereas that in high SDI regions was the lowest. Notably, in low SDI regions, the ASDR and age-standardized DALY rate for protein-energy malnutrition experienced a significant increase from 2010 to 2012, peaking at values of 34.39 per 100,000 and 2,037.19 per 100,000, respectively, before continuing to decline thereafter (Table 2, Figure 2B).

Regionally, in 2021, South Asia reported the highest ASIR for protein-energy malnutrition at 2,044.84 per 100,000, while Sub-Saharan Africa recorded the highest ASDR and age-standardized DALY rates at 9.14 per 100,000 and 451.59 per 100,000, respectively. According to the regional income classifications established by the World Bank, the burden of protein-energy malnutrition has diminished in both the World Bank Lower Middle Income and World Bank Low Income regions compared to 1990. Surprisingly, in 2021, both the ASDR and age-standardized DALY rates for protein-energy malnutrition increased in the World Bank High Income and World Bank Upper Middle-Income regions, while the ASIR in the World Bank High-Income region experienced a slight rise as well (Table 2).

Among the 204 countries, the highest ASIR was reported in the United Arab Emirates (4,688.09), followed by Bulgaria (2,563.81) and India (2,226.25). In contrast, the highest ASDR was observed in Sierra Leone (38.98), South Sudan (32.77), and Somalia (30.89), while the highest age-standardized DALY rates were recorded in Sierra Leone (2,200.17), South Sudan (1,891.77), and Mali (1,533.51). Additionally, India recorded the highest total incidence and total DALY years globally, with 29,505,320.45 cases and 2,035,962.502 years. Meanwhile, Indonesia ranked first in terms of mortality, with 18,148.40 deaths attributed to protein-energy malnutrition (Supplementary Tables S7–12, Figures 2C, D).

Figure 2E illustrates the potential correlation between the trends in changes of the SDI and the ASIR, ASDR, and age-standardized DALYs of protein-energy malnutrition across 21

global regions from 1990 to 2021. The results indicate a significant negative correlation between the SDI and the ASIR of protein-energy malnutrition (correlation coefficient = -0.14 , $P < 0.01$), ASDR (correlation coefficient = -0.51 , $P < 0.01$), and age-standardized DALY rate (correlation coefficient = -0.73 , $P < 0.01$). Figure 2F presents the results of subgroup analyses categorized by age groups. The findings indicate that except for high SDI regions, the age-standardized DALY rate for protein-energy malnutrition in the under-5 age group is consistently the highest. Conversely, in high SDI regions, the age-standardized DALY rate for the 70+ age group is the highest.

Global trends in the burden of iodine deficiency

From 1990 to 2021, the global burden of iodine deficiency exhibited a consistent year-on-year decline. The ASIR decreased from 126.14 per 100,000 in 1990 to 105.93 per 100,000 in 2021, reflecting a reduction of 16.2%, with an EAPC of -0.40 (-0.51 , -0.29). The decline in the age-standardized DALY rate is even more pronounced, decreasing from 46.19 per 100,000 in 1990 to 27.66 per 100,000 in 2021, representing a decrease of 40.1%, with an EAPC of -1.56 (-1.66 , -1.45). Notably, the burden of iodine deficiency is more pronounced in females compared to males (Table 3, Figure 3A).

At the regional level, the burden of iodine deficiency has decreased to varying degrees across all SDI categories, with more significant reductions observed in low-middle SDI and low SDI regions. Nevertheless, in 2021, the ASIR for iodine deficiency in low SDI regions was 9.71 times higher than that in high SDI regions (Low SDI: 20.54 per 100,000; High SDI: 199.41 per 100,000), while the age-standardized DALY rate was more than 15.95 times higher (Low SDI: 4.25 per 100,000; High SDI: 67.8 per 100,000) (Table 3, Figure 3B).

Regionally, most areas experienced a decline in the burden of iodine deficiency compared to 1990; however, a slight increase was noted in Eastern Europe, with the ASIR rising from 11.38 per 100,000 in 1990 to 11.52 per 100,000 in 2021, and the age-standardized DALY rate increasing from 4.42 per 100,000 in 1990 to 4.56 per 100,000 in 2021. Furthermore, in 2021, the burden of iodine deficiency in South Asia remains the highest, with an ASIR of 206.99 per 100,000 and an age-standardized DALY rate of 62.86 per 100,000.

According to the World Bank's classification of regional income statistics, areas with lower income levels also bear a relatively heavier burden of iodine deficiency (Table 3). Among 204 countries, Somalia, the Democratic Republic of the Congo, and Djibouti rank as the top three in terms of the burden of iodine deficiency, with Somalia's ASIR reaching 725.91 per 100,000. Additionally, India has the highest total number of cases and DALY associated with iodine deficiency globally, with 3,339,819.66 cases and 953,546.19 years (Supplementary Tables S13–16, Figures 3C, D).

Figure 3E illustrates the potential association between the trends of changes in the SDI and the ASIR and age-standardized DALYs of iodine deficiency across 21 global regions from 1990

TABLE 2 The incidence of protrin-energy malnutrition in 1990/2021.

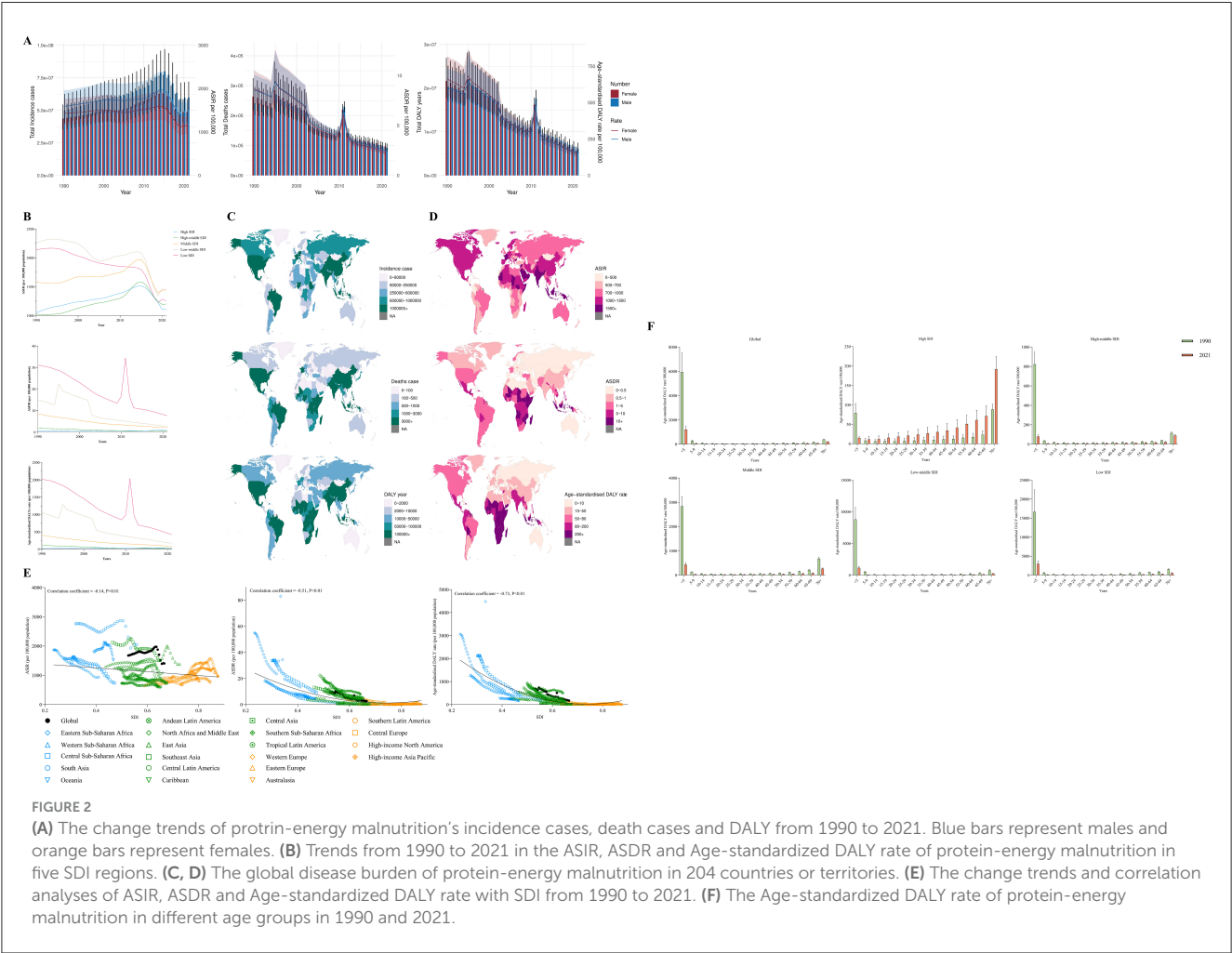
Variables	1990			2021		
	ASIR/100, 000 No. (95% UI)	ASDR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)	ASIR/100, 000 No. (95% UI)	ASDR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)
Overall	1,677.02 (1,363.96, 2,070.23)	9.44 (8.16, 11.32)	699.3 (594.81, 869.67)	1,406.21 (1,177.88, 1,691.07)	2.61 (2.29, 2.98)	172.38 (142.75, 204.1)
EAPC-ASIR		−0.20 (−0.52, 0.13)				
EAPC-ASDR		−4.47 (−4.94, −3.99)				
EAPC-Age-standardized DALY rate		−4.64 (−5.11, −4.17)				
Sex						
Male	1,766.52 (1,439.35, 2,183.16)	9.49 (8.14, 11.21)	667.59 (564.84, 817.18)	1,549.56 (1,293.16, 1,860.79)	2.77 (2.43, 3.18)	178.38 (146.79, 215)
Female	1,586.98 (1,287.79, 1,964.66)	9.65 (8.07, 11.72)	736.13 (608.81, 912.28)	1,261.43 (1,053.8, 1,509.84)	2.51 (2.21, 2.86)	166.96 (138.67, 196.18)
SDI						
High SDI	1,052.68 (828.38, 1,301.59)	0.63 (0.57, 0.68)	21.66 (17.72, 28.31)	1,118.15 (931.89, 1,329.48)	0.88 (0.74, 0.95)	34.33 (25.63, 48.18)
High-middle SDI	1,026.36 (808.06, 1,286.82)	1.81 (1.63, 1.99)	110.29 (98.33, 124.3)	1,202.77 (996.16, 1,464.96)	0.63 (0.55, 0.69)	22.49 (19.18, 26.71)
Middle SDI	1,584.92 (1,292.55, 1,965.1)	8.45 (7.76, 9.03)	402.7 (368.31, 446.62)	1,260.03 (1,043.98, 1,516.13)	7.68 (6.44, 8.91)	424.78 (333.52, 512.5)
Low-middle SDI	1,058.61 (892.98, 1,279.44)	14.96 (12.54, 17.93)	2,260.85 (1,824.5, 2,778.4)	1,447.85 (1,198.87, 1,736.67)	2.74 (2.4, 3.07)	175.06 (148.99, 204.94)
Low SDI	2,134.82 (1,752.88, 2,595.12)	31.07 (25.8, 38.1)	2,019.51 (1,602.83, 2,637.16)	1,445.72 (1,205.61, 1,752.87)	2.17 (1.94, 2.35)	91.34 (79.47, 105.43)
Region						
Central Asia	859.35 (650.88, 1,106.48)	0.66 (0.6, 0.72)	66.73 (57.4, 77.58)	741.53 (585.62, 924.03)	0.18 (0.16, 0.21)	12.71 (10.59, 15.29)
East Asia	1,243.43 (992.17, 1,558.93)	5.15 (4.54, 5.75)	241.67 (207.45, 279.94)	1,379.46 (1,129.1, 1,698.95)	0.91 (0.75, 1.06)	17.67 (15.15, 20.36)
South Asia	2,762.86 (2,178.39, 3,458.38)	12.73 (10.14, 15.77)	1,083.59 (891.43, 1,310.04)	2,044.84 (1,655.49, 2,494.51)	1.15 (0.9, 1.44)	165.27 (128.86, 207.35)
Southeast Asia	2,128.86 (1,764.35, 2,623.18)	13.23 (11.16, 15.08)	534.62 (451.28, 654.6)	1,502.74 (1,293.1, 1,744.65)	5.22 (4.44, 5.87)	153.01 (134.56, 173.4)
Central Europe	697.64 (529.02, 886.7)	0.11 (0.1, 0.13)	10.75 (8.87, 13.07)	893.05 (714.88, 1,111.43)	0.32 (0.29, 0.35)	9.7 (8.8, 10.65)
Eastern Europe	934.12 (713.25, 1,182.54)	0.23 (0.21, 0.24)	32.25 (24.89, 40.89)	815.12 (623.72, 1,024.76)	0.15 (0.14, 0.15)	14.1 (10.92, 18.11)
Sub-Saharan Africa	1,604.73 (1,333.11, 1,938.82)	32.42 (27.32, 39.83)	2,029.43 (1,611.67, 2,650.05)	889.44 (740.94, 1,054.76)	9.14 (7.74, 10.61)	451.59 (352.84, 547.78)
Oceania	1,789.31 (1,489.6, 2,115.97)	7.56 (6.28, 9.08)	268.03 (222.04, 322.61)	1,515.6 (1,278.41, 1,752.19)	4.35 (3.56, 5.4)	167.02 (131.17, 209.58)
Latin America and Caribbean	1,147.93 (976.38, 1,375.89)	15.94 (15.13, 16.56)	667.77 (633.36, 706.64)	824.46 (720.82, 947.8)	3.65 (3.25, 4.06)	119.18 (103.42, 139.56)
World bank income level						
World bank high income	1,004.88 (789.84, 1,248.63)	0.62 (0.56, 0.66)	23.04 (19.47, 28.86)	1,104.7 (918.77, 1,322.48)	0.83 (0.71, 0.9)	33.42 (25.39, 45.69)
World bank upper middle income	1,138.63 (922.98, 1,418.15)	5.93 (5.51, 6.3)	294.19 (269.6, 321.66)	1,054.21 (883.93, 1,235.64)	9.93 (8.35, 11.56)	512.64 (396.77, 628.22)

(Continued)

TABLE 2 (Continued)

Variables	1990			2021		
	ASIR/100, 000 No. (95% UI)	ASDR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)	ASIR/100, 000 No. (95% UI)	ASDR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)
World Bank Lower Middle Income	2,246.29 (1,797.05, 2,793.42)	12.94 (10.96, 15.32)	921.1 (774.49, 1,117.99)	1,601.34 (1,318.24, 1,938.5)	2.8 (2.48, 3.14)	177.2 (149.99, 209.86)
World Bank Low Income	1,929.68 (1,633.67, 2,286.46)	36.4 (30.25, 45.35)	2,248.03 (1,764.79, 2,995.97)	1,150.26 (963.98, 1,394.41)	1.45 (1.29, 1.59)	48.56 (43.3, 54.14)

UI, uncertainty intervals.



to 2021. The results indicate a significant negative correlation between SDI and the ASIR of iodine deficiency (correlation coefficient = -0.40 , $P < 0.01$) and the age-standardized DALY rate (correlation coefficient = -0.62 , $P < 0.01$). Figure 3F presents the results of subgroup analyses based on age groups. The findings demonstrate that the DALYs related to iodine deficiency are primarily concentrated in the population aged over 5 years in both global and various SDI regions. Notably, in comparison to 1990, there was a slight increase in iodine deficiency-related DALYs for the 50–69 age group in high-middle SDI regions in 2021.

Global trends in the burden of vitamin A deficiency

From 1990 to 2021, the global burden of vitamin A deficiency demonstrated a consistent downward trend annually. The ASIR decreased from 15,309.38 per 100,000 in 1990 to 6,212.97 per 100,000 in 2021, indicating a reduction of 59.4%, with an EAPC of -2.94 (-3.09 , -2.79). The decline in the age-standardized DALY rate was even more pronounced, decreasing from 32.56 per 100,000 in 1990 to 15.73 per 100,000 in 2021, a reduction of 51.7%, with

TABLE 3 The incidence of iodine deficiency in 1990/2021.

Variables	1990		2021	
	ASIR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)	ASIR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)
Overall	126.14 (106.85, 149.86)	46.19 (27.96, 74.29)	105.93 (86.16, 128.28)	27.66 (14.72, 49.47)
EAPC-ASIR	−0.40 (−0.51, −0.29)			
EAPC-Age-standardized DALY rate	−1.56 (−1.66, −1.45)			
Sex				
Male	103.28 (87.48, 121.43)	39.98 (24.32, 63.78)	75.49 (61.12, 92.17)	19.98 (10.57, 35.14)
Female	149.69 (127.26, 176.98)	52.52 (32.29, 84.9)	137.72 (112.67, 166.66)	35.43 (18.37, 63.81)
SDI				
High SDI	22.26 (17.81, 27.15)	4.7 (2.11, 8.93)	20.54 (16.38, 25.01)	4.25 (1.92, 7.95)
High-middle SDI	61.11 (50.64, 73.78)	16.5 (8.6, 29.11)	50.4 (40.83, 61.26)	14.03 (6.93, 25.72)
Middle SDI	88.84 (73.75, 107.29)	35.85 (21.72, 59.08)	75.31 (60.21, 92.44)	19.69 (9.62, 36.81)
Low-middle SDI	224.89 (191.23, 266.24)	107.5 (67.6, 167.85)	135.87 (108.85, 166.15)	41.95 (22.79, 73.82)
Low SDI	269.21 (229.96, 316.2)	269.21 (229.96, 316.2)	199.41 (163.48, 241.93)	67.8 (36.73, 117.79)
Region				
Central Asia	27.54 (22.22, 33.79)	9.82 (5.18, 16.52)	18.43 (14.48, 22.35)	5.94 (3.23, 10.01)
East Asia	65.08 (51.67, 81.34)	18.59 (9.98, 33)	67.52 (53.27, 82.61)	17.1 (7.9, 32.75)
South Asia	323.19 (272.79, 385.97)	158.58 (100.23, 246.88)	206.99 (164.96, 255.03)	62.86 (33.98, 112.41)
Southeast Asia	72.99 (59.63, 88.7)	27.77 (16.67, 43.86)	40.62 (31.93, 49.82)	11.28 (6.16, 19.26)
Central Europe	15.55 (12.81, 18.66)	3.72 (1.83, 6.55)	11.48 (9.01, 13.99)	2.38 (1.06, 4.44)
Eastern Europe	11.38 (8.9, 13.94)	4.42 (2.11, 7.29)	11.52 (8.97, 14.06)	4.56 (2.13, 7.59)
Sub-Saharan Africa	200.61 (173.48, 233.79)	63.62 (32.77, 114.28)	151.26 (124.98, 182.69)	44.98 (22.92, 81.28)
Oceania	7.33 (5.77, 9.09)	2.89 (1.65, 4.49)	4.35 (3.44, 5.45)	1.61 (0.9, 2.53)
Latin America and Caribbean	18.72 (15.16, 22.66)	5.47 (2.86, 9.5)	17.63 (14.21, 21.39)	4.8 (2.47, 8.51)
World Bank Income Level				
World Bank High Income	31.63 (25.82, 38.22)	7.42 (3.37, 14.08)	25.31 (20.41, 30.62)	5.7 (2.58, 10.79)
World Bank Upper Middle Income	53.66 (43.6, 65.59)	15.64 (8.46, 27.41)	45.9 (37.49, 55.39)	12.99 (6.45, 24.04)
World Bank Lower Middle Income	207.42 (174.84, 246.56)	98.21 (61.96, 153.86)	136.12 (108.61, 167.47)	40.67 (21.99, 72.4)
World Bank Low Income	258.43 (225.3, 300.15)	86.77 (46.37, 153.23)	201.72 (167.83, 242.68)	64.18 (33.73, 113.64)

UI: uncertainty intervals.

an EAPC of −2.43 (−2.63, −2.24). Furthermore, the burden of vitamin A deficiency is significantly greater in males than in females (Table 4, Figure 4A).

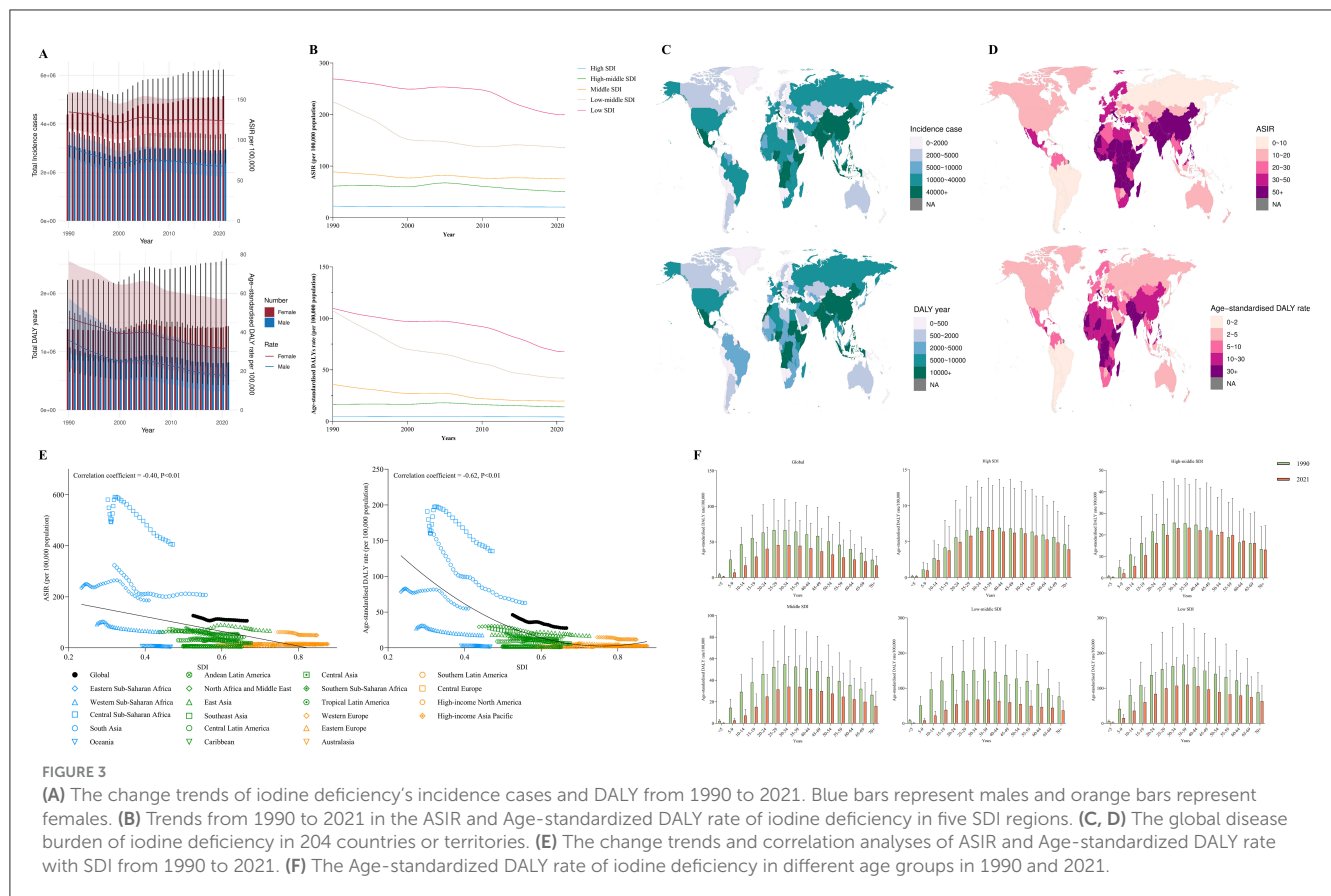
At the regional level, the burden of vitamin A deficiency has diminished to varying degrees across all SDI categories, with reductions, particularly notable in the low-middle and low SDI regions. Nonetheless, in 2021, the ASIR of vitamin A deficiency in low SDI regions remained 33 times higher than that in high SDI regions (Low SDI: 533.02 per 100,000; High SDI: 17,588.14 per 100,000), while the age-standardized DALY rate was over 121.03 times higher (Low SDI: 0.3 per 100,000; High SDI: 36.31 per 100,000) (Table 4, Figure 4B).

Regionally, in comparison to 1990, the burden of vitamin A deficiency has decreased across all regions. In 2021, the burden of

vitamin A deficiency was highest in the Sub-Saharan Africa region, with an ASIR of 16,981.65 per 100,000 and an age-standardized DALY rate of 32.73 per 100,000.

According to regional income statistics from the World Bank, lower-income areas also bear a relatively greater burden of vitamin A deficiency (Table 4). Among 204 countries, Somalia exhibits the highest burden of vitamin A deficiency, with an ASIR of 81,451.76 per 100,000 and an age-standardized DALY rate of 108.60 per 100,000. Additionally, India holds the highest total incidence and DALY years for vitamin A deficiency globally, with 112,700,475 cases and 321,890.3 years (Supplementary Tables S17–20, Figures 4C, D).

Figure 4E illustrates the potential association between the trends of changes in the SDI and the ASIR and age-standardized



DALYs of vitamin A deficiency across 21 global regions from 1990 to 2021. The results indicate a significant negative correlation between SDI and the ASIR of vitamin A deficiency (correlation coefficient = -0.49 , $P < 0.01$) and the age-standardized DALY rate (correlation coefficient = -0.88 , $P < 0.01$). Figure 4F presents the results of subgroup analyses based on age groups. The findings indicate that the DALYs associated with vitamin A deficiency are primarily concentrated in the population aged under 15 years in both global and various SDI regions.

Global trends in the burden of iron deficiency

From 1990 to 2021, the global burden of iron deficiency exhibited a consistent downward trend each year. The age-standardized DALY rate decreased from 517.98 per 100,000 in 1990 to 423.74 per 100,000 in 2021, representing a decline of 18.2%, with an EAPC of -0.68 (-0.72 , -0.64). The burden of iron deficiency is significantly greater in females than in males (Table 5, Figure 5A).

At the regional level, although the burden of iron deficiency has diminished to varying degrees across all SDI regions, in 2021, the age-standardized DALY rate in low SDI regions was still more than 10.63 times higher than that in high SDI regions (Low SDI: 71.16 per 100,000; High SDI: 756.7 per 100,000) (Table 5, Figure 5B). Regionally, in comparison to 1990, the burden of iron deficiency has decreased across all regions. In 2021, the burden

of iron deficiency was highest in the South Asia region, with an age-standardized DALY rate of 885.49 per 100,000.

According to regional income classifications by the World Bank, the lower-middle income region had the highest age-standardized DALY rate (674.98 per 100,000), whereas the high-income region had the lowest (72.89 per 100,000) (Table 5). Among 204 countries, Yemen exhibited the highest burden of iron deficiency, with an age-standardized DALY rate of 1,405.23 per 100,000. India reported the highest total DALY years attributable to iron deficiency globally, reaching 12,022,677.85 years (Supplementary Tables S21, 22, Figures 5C, D).

Figure 5E illustrates the potential association between the trends of changes in the SDI and age-standardized DALY rates for iron deficiency across 21 global regions from 1990 to 2021. The results indicated a significant negative correlation between SDI and the age-standardized DALY rate for iron deficiency (correlation coefficient = -0.75 , $P < 0.01$). Figure 5F presents the results of subgroup analyses based on age groups. The findings indicated that the DALYs associated with iron deficiency were relatively evenly distributed across all age groups globally and within various SDI regions.

Discussion

This study employed the latest GBD 2021 data to conduct a comprehensive analysis of the disease burden associated with malnutrition and its four subtypes from 1990 to 2021. By

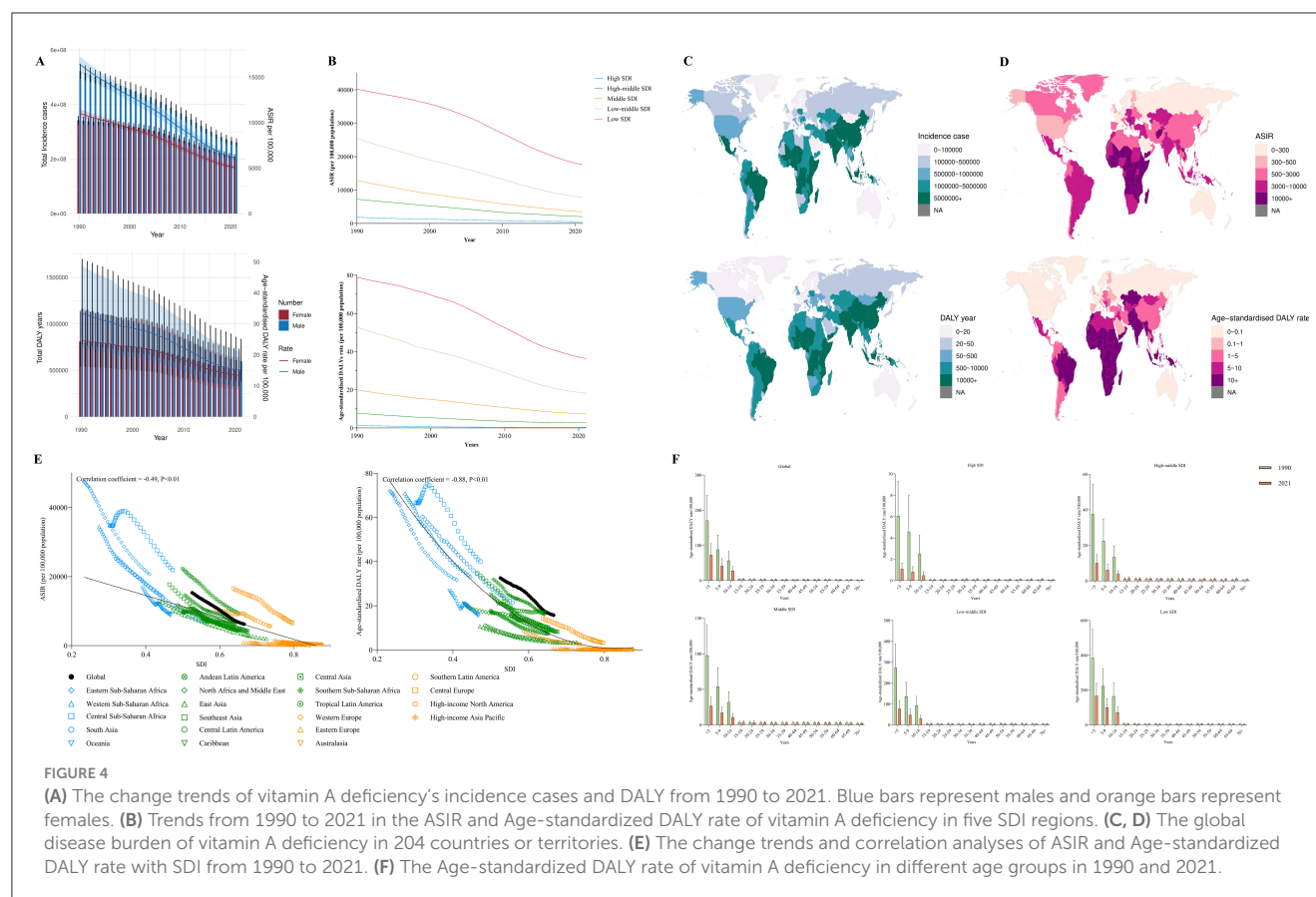
TABLE 4 The incidence of vitamin A deficiency in 1990/2021.

Variables	1990		2021	
	ASIR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)	ASIR/100, 000 No. (95% UI)	Age-standardized DALY rate/100, 000 No. (95% UI)
Overall	15,309.38 (14,795.26, 15,849.38)	32.56 (21.77, 46.45)	6,212.97 (5,995.41, 6,451.23)	15.73 (10.09, 22.28)
EAPC-ASIR	−2.94 (−3.09, −2.79)			
EAPC-Age-standardized DALY rate	−2.43 (−2.63, −2.24)			
Sex				
Male	18,300.34 (17,356.99, 19,260.4)	37.29 (24.94, 54.38)	6,798.94 (6,436.75, 7,190.76)	16.6 (10.58, 23.33)
Female	12,263.74 (11,738.46, 12,824.22)	27.55 (18.35, 38.74)	5,607.26 (5,364.31, 5,864.34)	14.8 (9.59, 21.42)
SDI				
High SDI	1,834.56 (1,731.57, 1,950.67)	1.32 (0.84, 1.99)	533.02 (500.63, 567.45)	0.3 (0.19, 0.46)
High-middle SDI	7,251.94 (6,744.03, 7,829.66)	7.78 (5.17, 11.16)	2,052.13 (1,920.15, 2,185.48)	2.72 (1.69, 3.95)
Middle SDI	12,893.56 (12,204.12, 13,560.94)	20.00 (13.18, 28.22)	3,495.26 (3,311.64, 3,685.43)	7.4 (4.83, 10.66)
Low-middle SDI	25,459.19 (24,136.77, 26,659.33)	52.67 (35.03, 75.39)	7,805.61 (7,338.17, 8,319.93)	18.09 (11.61, 26.15)
Low SDI	40,183.53 (39,126.94, 41,255.53)	78.9 (53.42, 113.22)	17,588.14 (17,081.49, 18,160.63)	36.31 (23.71, 51.36)
Region				
Central Asia	8,007.29 (7,482.21, 8,568.86)	15.76 (10.21, 24.25)	4,167.24 (3,900.84, 4,475.56)	8.21 (5.32, 11.94)
East Asia	10,226.78 (8,955.07, 11,653.73)	10.66 (6.83, 15.59)	2,003.59 (1,730.21, 2,311.42)	3.45 (2.15, 5.1)
South Asia	25,206.45 (23,113.6, 27,092.84)	60.3 (40.14, 86)	6,920.14 (6,167.34, 7,776.39)	21.49 (13.03, 30.68)
Southeast Asia	17,693.23 (16,665.65, 18,792.08)	34.69 (22.53, 49.8)	4,349.2 (4,016.04, 4,766.59)	8.98 (5.92, 13.27)
Central Europe	16,573.7 (15,835.42, 17,431.9)	14.25 (9, 21.2)	6,575.17 (6,205.13, 6,966)	3.27 (1.99, 4.98)
Eastern Europe	945.74 (869.42, 1025.71)	0.48 (0.28, 0.72)	319.71 (288.44, 351.06)	0.1 (0.06, 0.16)
Sub-Saharan Africa	36,969.54 (36,382.03, 37,522.32)	67.3 (46.15, 94.37)	16,981.65 (16,532.87, 17,387.56)	32.73 (21.6, 46.13)
Oceania	16,646.79 (15,589.96, 17,753.65)	26.67 (17.32, 39.38)	8,809.09 (8,054.27, 9,624.69)	15.62 (9.67, 24.66)
Latin America and Caribbean	15,641.8 (14,739.01, 16,564.8)	17.8 (11.79, 25.3)	6,504.18 (6,048.12, 7,052.52)	7.73 (4.99, 11.1)
World Bank Income Level				
World Bank High Income	2,602.21 (2,502.39, 2,718.39)	1.89 (1.2, 2.82)	830.85 (796.64, 868.17)	0.41 (0.26, 0.63)
World Bank Upper Middle Income	10,917.89 (10,124.87, 11,792.92)	12.65 (8.27, 18.17)	3,197.61 (2,984.25, 3,399.66)	4.61 (2.99, 6.56)
World Bank Lower Middle Income	21,887.19 (20,686.2, 22,925.34)	48.66 (32.54, 69.07)	6,683.49 (6,294.25, 7,165.24)	17.99 (11.46, 25.54)
World Bank Low Income	42,131.98 (41,384.63, 42,920.39)	74.32 (50.77, 105.32)	20,968.15 (20,411.02, 21,550.7)	39.51 (26.28, 55.78)

UI, uncertainty intervals.

calculating the EAPC values for indicators related to the burden of malnutrition over the past 32 years, this study explored the epidemiological trends. The results reveal that significant changes have occurred in the global burden of malnutrition when compared to 1990.

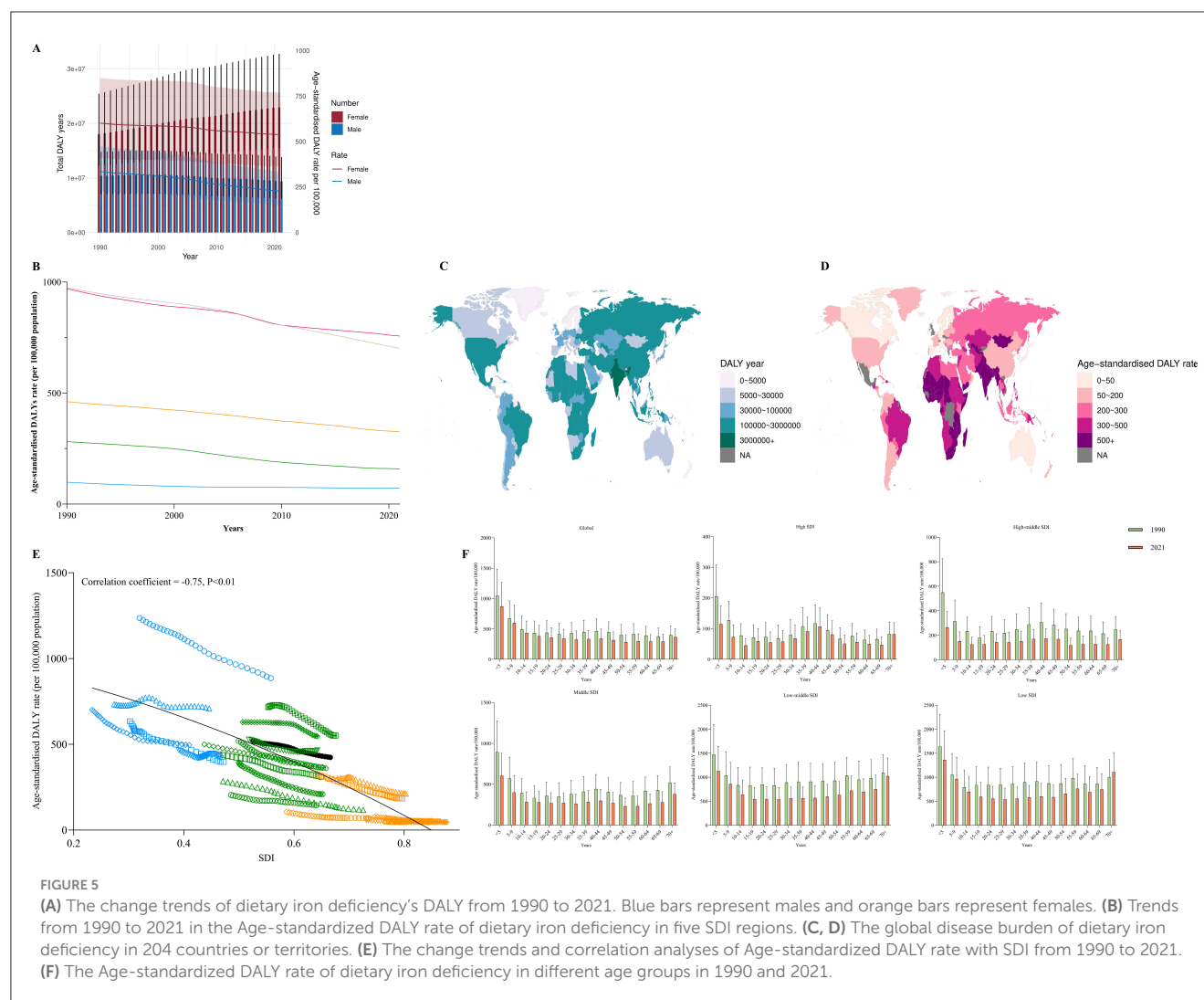
From 1990 to 2021, the global burden of malnutrition and its four subtypes has diminished to varying extents, notably in cases of general malnutrition and vitamin A deficiency. This phenomenon reflects, to a considerable degree, the effective interventions implemented by global public health organizations over the past



three decades aimed at promoting disease prevention and control. However, it is concerning that substantial disparities exist in the burden of malnutrition attributable to gender, region, country, socioeconomic status, and age. Firstly, although the global burden of malnutrition exhibits an overall downward trend, significant spatial disparities in disease burden persist across geographical regions, reflecting variances in social and cultural factors. Low-income and low-SDI regions, such as many African countries, continue to bear a substantial burden of disease (30). This situation may be attributed to various factors, including poverty, climate change, educational attainment, and government policies (31). Food security remains a critical global issue, as population growth and increasing consumption continue to drive demand for food. The competition for essential resources such as land, water, and energy further impacts the capacity of nations to produce adequate food supplies (32). A cross-sectional study in Gaza highlighted that chronic food insecurity, exacerbated by war, has led to widespread malnutrition among children, manifesting as stunted growth, wasting, and anemia (33). Similarly, in Kenya, challenges such as limited access to quality seeds, inadequate transportation infrastructure, low value addition, and climate-related food security issues have resulted in nearly 30% of children suffering from nutritional deficiencies, 35% experiencing stunted growth, and approximately 4 million people requiring long-term emergency food assistance (34). Given these challenges, a more effective global strategy is urgently needed to ensure food security and alleviate the burden of nutritional deficiencies. Additionally, infectious

diseases play a crucial role in shaping the burden of nutritional deficiencies, creating a vicious cycle that should not be overlooked (35). For instance, nutritional deficiencies have been strongly linked to tuberculosis incidence, disease severity, prognosis, and mortality. Given these associations, further research is needed to explore the mechanisms by which nutritional status influences the effectiveness of tuberculosis vaccines and treatment, particularly in high-burden TB countries (36). Fortunately, countries with low SDI and low-middle SDI have experienced a markedly greater reduction in the burden of malnutrition compared to their counterparts. This is likely primarily due to these countries undergoing a critical period of nutritional transition, coupled with rapid global economic growth and various effective targeted interventions executed by relevant organizations to combat malnutrition (37–39). Some scholars contend that this change signifies that these countries are transitioning to the second phase of the nutritional change model, moving from a decline in famine to the emergence of degenerative diseases; economic growth, urbanization, and technological innovation have rendered obesity and its associated non-communicable diseases increasingly prevalent (40).

Secondly, the global ASIR of protein-energy malnutrition has fluctuated over these 32 years, with even the ASIR in high-middle SDI and high SDI regions witnessing an increase compared to 1990. This shift may be attributed to the dietary habits of populations in these countries gradually transitioning from high-calorie foods to more nutritious alternatives (41). The



Mediterranean diet serves as a quintessential example, advocating for the reduction of sugar and red meat intake while promoting the consumption of grains, bread, and legumes to mitigate the risk of cardiovascular diseases (42). However, such a low-calorie dietary pattern may render individuals more susceptible to protein-energy malnutrition. Additionally, it is noteworthy that during the COVID-19 pandemic, the ASIR of protein-energy malnutrition markedly increased in Middle SDI and three lower SDI regions. Previous studies have attributed this phenomenon to the more severe clinical symptoms associated with COVID-19. The inadequacy of prevention and control measures for COVID-19 in lower SDI regions has resulted in many patients being hospitalized, consequently increasing the ASIR of protein-energy malnutrition (43).

Thirdly, the burden of iodine deficiency and dietary iron deficiency is considerably greater among women than men, with a more even age distribution observed for both conditions. This phenomenon may be ascribed to the necessity for pregnant women to provide iodine to their infants, potentially resulting in iodine deficiency for both themselves and their children (44). It is well established that iodine

deficiency is linked to a range of diseases, including goiter and hypothyroidism, while adequate thyroid hormone levels are crucial for optimal brain development. Severe iodine deficiency during pregnancy can result in significant neurological and cognitive impairments in children, potentially elevating the risk of infant mortality (45, 46). It is widely acknowledged that during a normal-term pregnancy, a mother requires an intake of 500–800 milligrams of iron (47). It is estimated that the daily dietary iron requirement escalates from 0.8 milligrams per day in early pregnancy to 7.5 milligrams per day in late pregnancy, with an average daily requirement of 4.4 milligrams throughout the pregnancy (48). Consequently, dietary iron deficiency is markedly more prevalent among women than men.

Finally, although the global burden of vitamin A deficiency has markedly decreased over the years, its adverse effects should not be underestimated, particularly in regions with low SDI. The primary causes of vitamin A deficiency encompass insufficient intake of vitamin A-rich foods, inadequate absorption of vitamin A, and losses attributable to various diseases (49). Some scholars posit that pervasive poverty in low SDI regions is a significant

TABLE 5 The incidence of dietary iron deficiency in 1990/2021.

Variables	1990	2021
	Age-standardized DALY rate/100,000 No. (95% UI)	Age-standardized DALY rate/100,000 No. (95% UI)
Overall	517.98 (353.99, 730.97)	423.74 (285.27, 610.83)
EAPC-Age-standardized DALY rate	−0.68 (−0.72, −0.64)	
Sex		
Male	372.14 (252.28, 528)	253.05 (167.26, 370.92)
Female	668.26 (456.63, 943.34)	597.97 (402.63, 854.44)
SDI		
High SDI	98.22 (63.9, 149.04)	71.16 (46.66, 107.1)
High-middle SDI	281.54 (186.61, 410.01)	158.5 (105.24, 230.73)
Middle SDI	461.41 (313.83, 657.08)	325.66 (217.33, 471.71)
Low-middle SDI	975.03 (668.99, 1,368.68)	701.74 (473.82, 995.6)
Low SDI	969.65 (658.73, 1,360.15)	756.7 (507.93, 1,078.97)
Region		
Central Asia	716.39 (480.61, 1,034.62)	550.96 (371.78, 796.2)
East Asia	284.97 (192.07, 407.7)	117.23 (77.42, 168.51)
South Asia	1,235.64 (849.62, 1,732.89)	885.49 (600.29, 1,271.36)
Southeast Asia	440.81 (288.92, 632.81)	310.51 (206.2, 450.38)
Central Europe	329.43 (214.17, 480.96)	184.56 (120.76, 272.99)
Eastern Europe	299.72 (196.21, 448.3)	212.2 (143.05, 307.68)
Sub-Saharan Africa	696.16 (473.66, 981.52)	577.35 (385.02, 826.3)
Oceania	466.02 (291.47, 688.36)	419.21 (260.76, 664.25)
Latin America and Caribbean	375.47 (249.35, 533.28)	258.96 (171.4, 370.87)
World bank income level		
World bank high income	104.39 (67.43, 157.16)	72.89 (47.5, 109.42)
World bank upper middle income	327.25 (220.53, 469.79)	187.71 (126.46, 270.46)
World bank lower middle income	909.51 (622.89, 1,277.49)	674.98 (456.33, 971.82)
World bank low income	682.28 (464.66, 967.47)	572.49 (385, 816.94)

UI, uncertainty intervals.

factor contributing to the prevalence of vitamin A deficiency, with lower cultural levels further exacerbating the issue (50). A study conducted among the Chinese population revealed that individuals with lower educational attainment exhibit a higher risk of vitamin A deficiency (51). A meta-analysis indicated that in regions characterized by limited income and education levels, enhancing the supply and utilization of vitamin A-rich foods may yield greater benefits than the widespread administration of vitamin A supplements (52).

Strengths and limitations

In comparison to existing studies, this research possesses several notable advantages. First, leveraging high-quality evidence and data frameworks from the latest GBD 2021 study, we offer a comprehensive analysis of the global trends in the burden of nutritional deficiencies and their four subtypes from 1990 to 2021. This study emphasizes the epidemiological characteristics of nutritional deficiencies across sex, country, region, and age group. Second, our findings provide crucial additional insights into the potential impact of the COVID-19 pandemic on the global burden of nutritional deficiencies, extending, and updating previous research over time.

Nonetheless, this study is not without its limitations. Firstly, the study results are contingent upon aggregated data from the GBD study, with their accuracy reliant on the quality of reporting from various countries. This reliance may lead to a significant number of undiagnosed cases of nutritional deficiencies in certain regions, thereby potentially impacting the accuracy of our findings (53). Secondly, there may be potential data quality issues arising from challenges in accurately determining the number of deaths and DALYs attributable to specific causes, such as nutritional deficiencies.

Conclusions

From 1990 to 2021, the global burden of nutritional deficiencies has exhibited a general decline. However, in certain regions, particularly low SDI areas, the burden persists at significant levels. Relevant organizations must devise effective, cost-efficient, and targeted interventions tailored to specific regions, genders, ages, and disease subtypes to comprehensively mitigate the adverse impacts of nutritional deficiencies on global public health.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

Y-YZ: Data curation, Formal analysis, Methodology, Writing – original draft. B-XC: Investigation, Software, Visualization, Writing – original draft. QW: Funding acquisition, Validation, 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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Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2025.1535566/full#supplementary-material>

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Analysis of prevalence, years lived with disability, and trends of anemia burden and main causes in China

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Background: China is facing a rapidly aging population. In 2020, the number of people aged 60 and above was 264 million, accounting for 18.7% of the total population. Therefore, addressing the health issues of older adults is of great importance. This study aimed to analyze trends of anemia burden and its underlying causes in older adults aged 60 and above.

Methods: Three indicators were used to evaluate the disease burden of anemia: prevalence, Years Lived with Disability (YLDs), and Years Lived with Disability rate (YLD rate). Data on anemia prevalence were obtained from the 2002 Chinese Nutrition and Health Survey, the 2010–2013 Chinese Nutrition and Health Surveillance, and the 2015 China Adults Chronic Diseases and Nutrition Surveillance. A multi-stage stratified cluster random sampling method was adopted in these surveys. YLDs, YLD rate (per 100,000 population), and causes of anemia were sourced from the Global Burden of Disease 2021 (GBD 2021). The Cochran-Armitage trend test was used to test the trends between different ages, genders, and living areas.

Results: The prevalence and YLD rate of anemia among older adults aged 60 and above exhibited a notable decreasing trend across various demographic factors. Anemia prevalence was higher in rural areas, western areas, and southern China. The YLD rate of females was higher than that of males, except for adults over 85. Both prevalence and YLD rates increased with age. Dietary iron deficiency was the leading cause of anemia.

Conclusion: While the disease burden of anemia has shown a decreasing trend, significant age, and regional disparities persist. Anemia among older adults, particularly in rural and western regions, remains a major health concern. Special attention is needed to address dietary iron deficiency as a key factor contributing to anemia.

KEYWORDS

anemia, older adults, disease burden, trend, main causes

1 Introduction

Population aging is a global phenomenon, that has significant implications for China as well. According to the results of the Seven National Population Census, there were 264 million people aged 60 and over in 2020, accounting for 18.7% of China's total population (1). By 2040, this number is expected to reach 402 million (accounting for 28% of the total population), according to WHO estimates (2). The physiological functions of older adults begin to decline, which results in changes to the performance of different organ systems (3), and the immune function of older adults is also in decline (4). Anemia not only seriously affects the health of older adults, but also increases the social burden (5, 6). It associations with cognitive impairment, physical measures (such as mean handgrip, relative handgrip, and usual gait speed), and late recall in older adults (7). Anemia also increases the risk of heart failure, coronary heart disease, and mortality in older adults (8–11).

The burden of anemia is assessed using Years Lived with Disability (YLDs) since it does not directly cause mortality, making Years of Life Lost (YLLs) inapplicable (12). From 1990 to 2021, although the prevalence and YLD rate (per 100,000 population) of anemia decreased, the global total number of cases and YLDs of anemia still increased by 0.42 million and 3.4 million, respectively. At the national level, Mali, Zambia, Togo, and Senegal had a prevalence of anemia above 50% in 2021 (13). In China, the prevalence of anemia among adults was 8.3% in 2015, a decrease of 2.1 percentage points compared with 2012 (14). However, due to the physiological decline associated with aging and the increasing older adults population in China, anemia among older adults remains a significant concern that cannot be overlooked (1, 4).

Anemia can be caused by a variety of factors, but its root cause is an imbalance between erythrocyte production and loss at the biological level. This includes ineffective or deficient erythropoiesis and excessive erythrocyte loss (15). Ineffective or deficient erythropoiesis can result from nutritional deficiencies and inflammation, while excessive erythrocyte loss is typically caused by hemolysis and blood loss (12, 16–19). Understanding the underlying causes of anemia in older adults is crucial for developing effective strategies to prevent and control this health issue in older adults.

While most anemia research in China has focused on pregnant women and children, this study shifts its attention to older adults, a demographic that has been largely overlooked in existing literature. By integrating data from three national cross-sectional surveys and the Global Burden of Disease (GBD) database, this study provides a comprehensive and comparative analysis of the anemia burden among older adults. By examining 35 causes of anemia, this study offers a

comprehensive assessment of the diverse causes among older adults, identifying key causes to inform evidence-based interventions and policy-making. In this study, the distribution of anemia in different population characteristics and underlying causes among older adults in China were analyzed, which was critical for developing interventions suitable for the actual situation of China and reducing the burden of anemia.

2 Methods

2.1 Study design and participants

The data on the prevalence of anemia were obtained from the 2002 Chinese Nutrition and Health Survey (2002 CNHS), the 2010–2013 Chinese Nutrition and Health Surveillance (2010–2013 CNHS), and the 2015 China Adults Chronic Diseases and Nutrition Surveillance (2015 CACDNS). Multi-stage stratified cluster random sampling method was adopted in these three surveys or surveillance. Nationally representative samples covering 31 provinces, autonomous regions, and municipalities directly under the Central Government were obtained. The specific sampling methods (14, 20, 21) were shown in Table 1. The ethical approval numbers were No. 2013-018 and 201519-B, respectively. Informed consent was signed by all participants before the survey/surveillance.

The YLDs, YLD rate (per 100,000 population), and causes of anemia were obtained from the Global Burden of Disease 2021 (GBD 2021). The GBD 2021 estimated burden of 371 diseases and injuries using 100,983 data sources (22). The YLDs and YLD rate of anemia for older adults aged 60 years and above were sourced from the GBD 2021 database for anemia disease burden analysis. A total of 35 detailed causes of anemia were selected for analysis of the underlying causes of anemia. Since this data was obtained from public databases, ethical review and informed consent were not required.

2.2 Definition of anemia

In accordance with the WHO criteria, anemia was defined as a hemoglobin concentration of <130 g/L in men aged 15 years and above, and <120 g/L in non-pregnant women aged 15 years and above (23). The impact of altitude on hemoglobin concentration was adjusted using the Equation 1 (13). Participants were restricted to permanent residents (residency ≥6 months within the 12 months prior to the survey). This criterion ensured that participants had

TABLE 1 Multistage cluster sampling in three-round surveillance.

	2002	2010–2013	2015
The first stage	6 regions by economic development level, 22 counties/districts from each type of region	4 tiers: large urban, mid-sized cities, rural (typical/disadvantaged), 150 surveillance sites	302 survey sites, 3 communes/subdistricts from each site
The second stage	3 communes/subdistricts from each county/district	6 neighborhood committees from each surveillance site	2 communities/villages from each township/subdistrict
The third stage	2 communities/villages from each commune/subdistrict	about 1,000 people from 450 households in six neighborhood committees	1 village/residential group
The fourth stage	90 households	–	45 households

sufficient time to physiologically adapt to the local altitude, thereby minimizing the confounding effects of short-term population mobility.

$$\Delta Hb = -0.32 \times (\text{elevation in meters} \times 0.0033) + 0.22 \times (\text{elevation in meters} \times 0.0033)^2 \quad (1)$$

ΔHb : hemoglobin values requiring adjustment at various altitudes (g/L).

Elevation in meters: altitude of each survey point (m).

2.3 Data collection and measurements

The basic information in the survey or surveillance, including gender, age, living area, and other relevant details, was gathered through face-to-face interviews conducted by the China Center for Disease Control and Prevention (China CDC). Fasting venous blood samples were collected and analyzed using the ferrocyanide method. A rigorous quality control program was implemented, covering standardized protocols for questionnaires, training, equipment, reagent handling, and data entry.

2.4 Assessment of other variables

To assess the associations between age and anemia, all individuals were divided into six distinct age groups: 60–64 years, 65–69 years, 70–74 years, 75–79 years, 80–84 years, and ≥ 85 years. The residential areas were categorized into two groups based on urban and rural distinctions, and classified into three regional groups: the eastern region, which includes cities and provinces such as Beijing, Tianjin, Liaoning, Shandong, Hebei, Jiangsu, Zhejiang, Shanghai, Fujian, Guangdong, and Hainan; the central region, comprising Jilin, Heilongjiang, Shanxi, Henan, Anhui, Jiangxi, Hunan, and Hubei; and the western region, which encompasses Inner Mongolia, Ningxia, Gansu, Qinghai, Xinjiang, Tibet, Guizhou, Chongqing, Sichuan, Yunnan, and Guangxi. China is divided into southern and northern regions by the Qinling-Huaihe Line.

2.5 Statistical analysis

In order to improve the representativeness of the sample to the population, the sample data were adjusted by the post-stratification weighting method. And adjusted survey or monitoring results in 2002, 2010–2013, and 2015 based on national census results in 2000, 2010 and 2020, respectively. The calculation of post-stratification weighting (Weight) for each layer was shown in Equation 2 (14).

$$\text{Weight} = \frac{\text{Population at a certain level}}{\div \text{The sum of the sample weights at that level}} \quad (2)$$

SAS 9.4 software was used for basic information description and statistical analysis. R software (Version 4.3.1) was used for plotting and result visualization. Categorical data were presented as numbers (percentages). The Weighted Chi-square test was used to analyze

whether there were differences between the basic characteristics. The Weighted Cochran–Armitage trend test was used to assess trends across different characteristics. Statistical significance was determined based on two-sided p -values, with p -value less than 0.05 considered statistically significant.

3 Results

3.1 Basic characteristics of subjects

A total of 26,247, 32,820, and 48,553 individuals aged 60 and above were included in the 2002 CNHS, the 2010–2013 CNHS, and the 2015 CACDNS, respectively. In 2002, the male-to-female ratio was 1:1.01; the male-to-female ratio was 1:1.11 in 2010–2013; in 2015, the male-to-female ratio in this study was 1:0.99. There were statistically significant differences in age groups, places of residence, region, and north–south distribution of population in different years ($p < 0.0001$). Table 2 provides a detailed overview of the survey participants' demographic characteristics in 2002, 2010–2013, and 2015.

3.2 Trends in prevalence of anemia

As shown in Table 3, in 2002, 2010–2013, and 2015, the prevalence of anemia among older adults demonstrated a notable decreasing trend across various demographic factors. This decline was consistent across genders, with both males and females experiencing reductions in anemia rates. Furthermore, the trend was evident across various age groups, suggesting an overall improvement in anemia prevalence among older adults. This decreasing trend was also observed in different places of residence, regions, and the north–south divide. The anemia prevalence in rural areas was higher than that in urban areas, and southern China compared to northern China, across all 3 years (p was less than 0.05). And in 2002 and 2015 the prevalence of anemia in western areas was higher than in eastern and central areas (p was less than 0.05). The prevalence of anemia increased with age, with the prevalence in the ≥ 85 age group being twice that of the 60–64 age group ($p < 0.05$).

3.3 Trends in YLDs and YLD rate of anemia

As shown in Figure 1, in males, the YLDs for anemia showed a decreasing trend in the 60–64, 65–69, and 75–79 age groups, a decline followed by an increase in the 70–74 and 80–84 age groups, and an increasing trend in the 85 and older age group from 2002 to 2021. In females, the YLDs for anemia initially increased and then decreased in the 60–64 age group, decreased and then increased in the 65–69 and 70–74 age groups, and showed an increasing trend in the 75–79, 80–84, and 85 and older age groups from 2002–2021.

From 2002 to 2021, YLD rates (per 100,000) in males and females showed a downward trend. In the 60–64, 65–69, 70–74, 75–79, and 80–84 age groups, the YLD rates of anemia in females were higher than that in males. However, for individuals aged 85 and older, the YLD rates (per 100,000) for males were higher than for females in

TABLE 2 Demographic characteristics of older adults aged 60 and above in China in 2002, 2010–2013, and 2015 [n, %].

Characteristics	2002	2010–2013	2015	χ^2	<i>p</i>
Gender					
Male	13,069 (49.79)	15,564 (47.42)	24,354 (50.16)	1.0	0.6158
Female	13,178 (50.21)	17,256 (52.58)	24,199 (49.84)		
Age groups					
60–64	9,362 (35.67)	12,394 (37.76)	19,360 (39.87)	38.7	<0.0001
65–69	8,173 (31.14)	8,683 (26.46)	13,871 (28.57)		
70–74	5,151 (19.63)	6,262 (19.08)	8,096 (16.67)		
75–79	2,377 (9.06)	3,648 (11.12)	4,577 (9.43)		
80–84	917 (3.49)	1,391 (4.42)	1,977 (4.07)		
> = 85	267 (1.02)	442 (1.35)	672 (1.38)		
Places of residence					
Urban	11,966 (45.59)	17,387 (52.98)	20,702 (42.64)	190.0	<0.0001
Rural	14,281 (54.41)	15,433 (47.02)	27,851 (57.36)		
Region					
Eastern Region	11,921 (45.42)	13,284 (40.48)	18,810 (38.74)	98.1	<0.0001
Central Region	6,568 (25.02)	9,930 (30.26)	14,748 (30.38)		
Western Region	7,758 (29.56)	9,606 (29.27)	14,995 (30.88)		
North/South					
Northern China	12,596 (47.99)	14,916 (47.76)	19,652 (40.48)	383.5	<0.0001
Southern China	13,651 (52.01)	17,904 (54.55)	28,901 (59.52)		
Total	26,247	32,820	48,553		

Weighted Chi-square Tests was applied.

2002, 2010, and 2015. In 2021, the YLD rates for females (960.6 per 100,000) exceeded that of males (927.5 per 100,000).

In Figure 2, every year, the YLD rate of anemia showed an increasing trend with age in both males and females. In 2002, 2015, 2010, and 2021, the disease burden of anemia in males was 4.5, 6.2, 7.2, and 8.2 times higher, respectively, in the > = 85-year-old age group compared to the 60-year-old age group. The YLDs rate for males in the > = 85-year-old group was 1629.8 per 100,000 in 2002, 1195.7 in 2010, 1051.8 in 2015, and 927.5 in 2021.

Similarly, for females, the disease burden of anemia was 2.8, 3.0, 3.1, and 3.2 times higher in the > = 85-year-old age group compared to the 60-year-old age group in 2002, 2015, 2010, and 2021. The YLDs rate for females in the > = 85-year-old group was 1236.4 per 100,000 in 2002, 1060.5 in 2010, 1005.2 in 2015, and 960.6 in 2021.

Among older adults aged 60–84, the YLD rate of females was higher than that of males in 2002, 2010, 2015, and 2021. However, among older adults aged 85 and older, the YLD rate of males was higher than that of females in 2002, 2010, and 2015 (Figure 2).

3.4 The cause distribution of anemia burden in China in 2021

In Figure 3 based on the 35 causes in the GBD 2021, in China, dietary iron deficiency was the leading cause of anemia across all age groups in both males and females. However, some variations were found in the distribution of anemia causes across different age groups and

genders. In the 60–64 age group and 65–69 age group, the most common causes of anemia were dietary iron deficiency, thalassemias trait, and other hemoglobinopathies and hemolytic anemias. In the 70–74 age group, 75–79 age group, males of 80–84 age group, and > = 85 age group, the leading causes of anemia YLDs in China in 2021 were dietary iron deficiency, thalassemias trait, and chronic kidney disease due to other and unspecified causes. These three causes accounted for about 70% of all anemia cases. The main causes of anemia in females aged 80–85 were dietary iron deficiency, chronic kidney disease due to other and unspecified causes, and other hemoglobinopathies and hemolytic anemias, which account for 76.9% of all anemia causes.

4 Discussion

This study analyzed the temporal trend of the prevalence of anemia in older adults aged 60 years and above in 2002, 2010–2013, and 2015, as well as the YLDs and YLD rates of anemia. Furthermore, the causes of anemia were also investigated in this study. From 2002 to 2015, the prevalence of anemia among older adults aged 60 and above, showed a decreasing trend. Additionally, from 2002 to 2021, based on data collected in 2002, 2010, 2015, and 2021, the YLD rates caused by anemia exhibited a downward trend over time. However, age differences and regional differences in the burden of anemia cannot be ignored.

The prevalence of anemia among older adults showed a decreasing trend in both males and females, with a continuous

TABLE 3 Prevalence of anemia in older adults aged 60 and above in 2002, 2010–2013, and 2015 [95%CI].

Characteristics	2002	2010–2013	2015	Z	p
Gender					
Male	22.7 (21.2, 24.3)	13.9 (11.8, 15.9)	9.2 (7.8, 10.5)	1279.7	<0.0001
Female	23.2 (21.4, 25.1)	13.5 (11.7, 15.2)	9.1 (7.6, 10.5)	1301.3	<0.0001
Age groups					
60–64	19.3 (17.8, 20.9)	10.7 (9.1, 12.2)	6.8 (5.7, 7.9)	1031.1	<0.0001
65–69	21.2 (19.6, 22.9)	12.1 (10.3, 13.1)	7.9 (6.8, 9.1)	899.3	<0.0001
70–74	23.5 (21.5, 25.5)	14.2 (12.1, 16.2)	8.9 (7.3, 10.5)	871.9	<0.0001
75–79	27.8 (25.2, 30.4)	16.5 (14.0, 18.9)	12.2 (10.1, 14.3)	609.3	<0.0001
80–84	30.3 (26.2, 34.4)	22.0 (18.2, 25.8)	14.9 (12.3, 17.4)	514.7	<0.0001
> = 85	39.3 (32.0, 46.6)	21.1 (15.6, 26.5)	15.5 (12.7, 18.4)	412.4	<0.0001
Places of residence					
Urban	17.4 (16.1, 18.6)	12.6 (11.3, 13.8)	7.4 (6.6, 8.2)	1167.8	<0.0001
Rural	25.9 (24.7, 27.1)	14.5 (13.2, 15.9)	10.5 (9.5, 11.5)	1374.5	<0.0001
Region					
Eastern Region	19.0 (16.5, 21.6)	13.7 (10.9, 16.6)	8.1 (5.7, 10.5)	1190.0	<0.0001
Central Region	23.8 (20.8, 26.8)	14.1 (10.5, 17.7)	9.4 (7.5, 11.4)	977.2	<0.0001
Western Region	28.2 (25.6, 30.8)	13.1 (10.1, 16.1)	10.0 (7.4, 12.6)	989.7	<0.0001
North/South					
Northern China	16.3 (14.8, 17.7)	10.2 (8.8, 11.5)	6.6 (5.4, 7.8)	988.2	<0.0001
Southern China	29.0 (27.2, 30.8)	16.5 (14.5, 18.4)	10.7 (9.2, 12.3)	1591.0	<0.0001

downward trend across all age groups in China. From 1990 to 2020, the prevalence of anemia in males, females, and the overall population exhibits a significant negative correlation with the years (24). A related study also showed that the prevalence of anemia in older adults decreased from 30.61% to 18.15% ($\chi^2 = 21723.90$, $p < 0.001$), with the declining trend gradually slowing down from 2012 to 2018 (25). Females of childbearing age had a high prevalence of anemia due to pregnancy or menstrual bleeding, which has received widespread attention (26, 27). In older adults, a higher prevalence of anemia in males has been observed in the U.S. This higher anemia risk was partially explained by different dietary habits and lifestyle factors between males and females. Moreover, some cancers (such as oropharyngeal, and laryngeal) have a more significant impact on males (28–31). However, our study found no significant gender difference in anemia prevalence in China.

The prevalence of anemia among older adults showed a decreasing trend in different places of residence and regions, consistent with a related study (32). This may be because aging has become an important and pressing issue concerning national development and the well-being of the population. The health of the older adults has received significant attention. The State Council has issued a series of documents, including the “14th Five-Year Plan for National Aging Development and Older adults Care Service System” and the “Healthy China 2030 Plan” (33, 34). At the same time, the new management model based on “Internet+” healthcare platforms, using the Internet and telemedicine, has effectively integrated home, community, and medical institutions, meeting the health needs of older adults and reducing the incidence of anemia (35).

The study identified significant geographical disparities in anemia prevalence in China, characterized by higher rates in rural areas compared to urban areas, southern versus northern regions, and western versus to central-eastern areas. These differences were linked to socioeconomic, dietary, and environmental-genetic factors. Socioeconomic disparities were particularly evident in rural and western regions, where had poorer overall socioeconomic conditions, medical resources and health literacy (36–39). Additionally, although the urbanization rate in China rose 47.3% from 1978 to 2022, the dietary structures in urban and rural had significant differences. Compared to rural areas, the dietary structures in urban were closer to the recommendations of Dietary Guidelines for Chinese Residents 2022 (40). Dietary patterns in the south, with higher consumption of rice and vegetables, may affect iron absorption (41). The subtropical climate of southern regions fosters higher rates of soil-transmitted helminth infections, which induce chronic blood loss and exacerbate iron deficiency (42). Genetic predispositions, such as the higher prevalence of thalassemia traits in provinces like Guangdong and Guangxi, may impair hemoglobin synthesis and increase anemia risk (43). Therefore, enhancing socioeconomic conditions and ensuring the equitable distribution of medical resources could significantly help decrease the prevalence of anemia.

The YLD rates associated with anemia have shown a downward trend over time. However, there has been an increase in YLDs among both females and males in the 80–84 and ≥ 85 age groups. This increase may be linked to advancements in healthcare, which have led to longer life expectancies within the older adults population. Additionally, factors such as the decline in physical function and the high prevalence of underlying diseases have extended the duration of

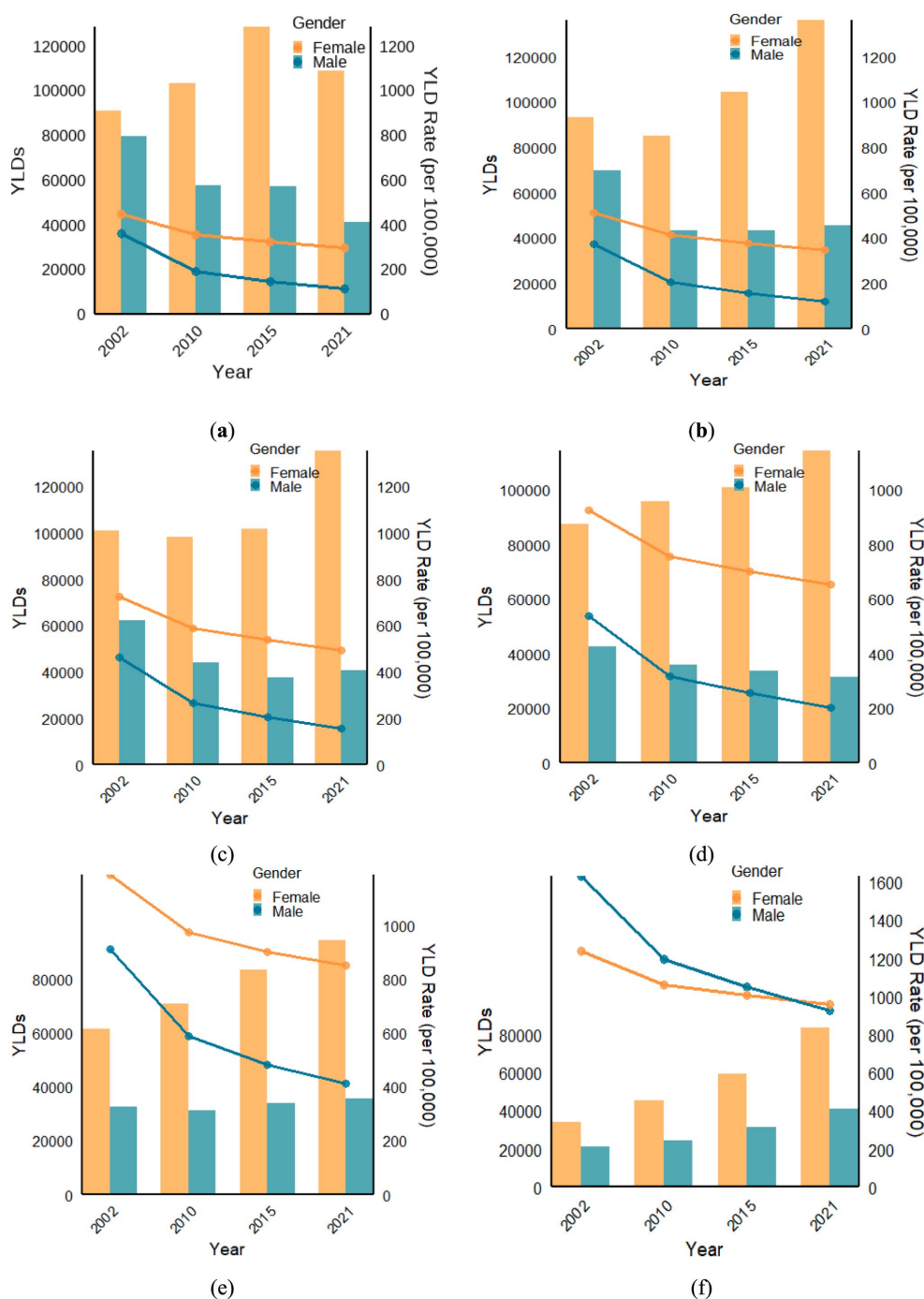


FIGURE 1

In 2002, 2010, 2015, and 2021, YLDs and YLDs Rate (per 100,000) in (a) the 60–64 age group; (b) the 65–69 age group; (c) the 70–74 age group; (d) the 75–79 age group; (e) the 80–84 age group; (f) the ≥ 85 age group.

living with chronic conditions, which may contribute to a gradual increase in the burden of anemia-related diseases over the years (24).

The YLDs and YLD rates were higher in females than males, except for the YLD rate of ≥ 85 age group. One reason may be that although the prevalence of multiple cancer types was higher among males, the mortality rate was also higher, resulting in a shorter survival period for males compared to females (30). Females may be particularly vulnerable to food insecurity within households and might have limited access to iron-rich foods. Additionally, they were

often less likely to receive health screenings and care due to domestic responsibilities, lack of autonomy, or prioritizing the needs of other family members (44–46). In females, the incidence of obesity, type 2 diabetes, and cardiovascular diseases (CVD) significantly increases after menopause (47). Obesity is accompanied by several disturbances at the endothelial, hormonal, and inflammatory levels. These disturbances induce the activation of several mechanisms that contribute to the anemic state (48). It has been reported that patients with type 2 diabetes are twice as likely to have anemia compared to

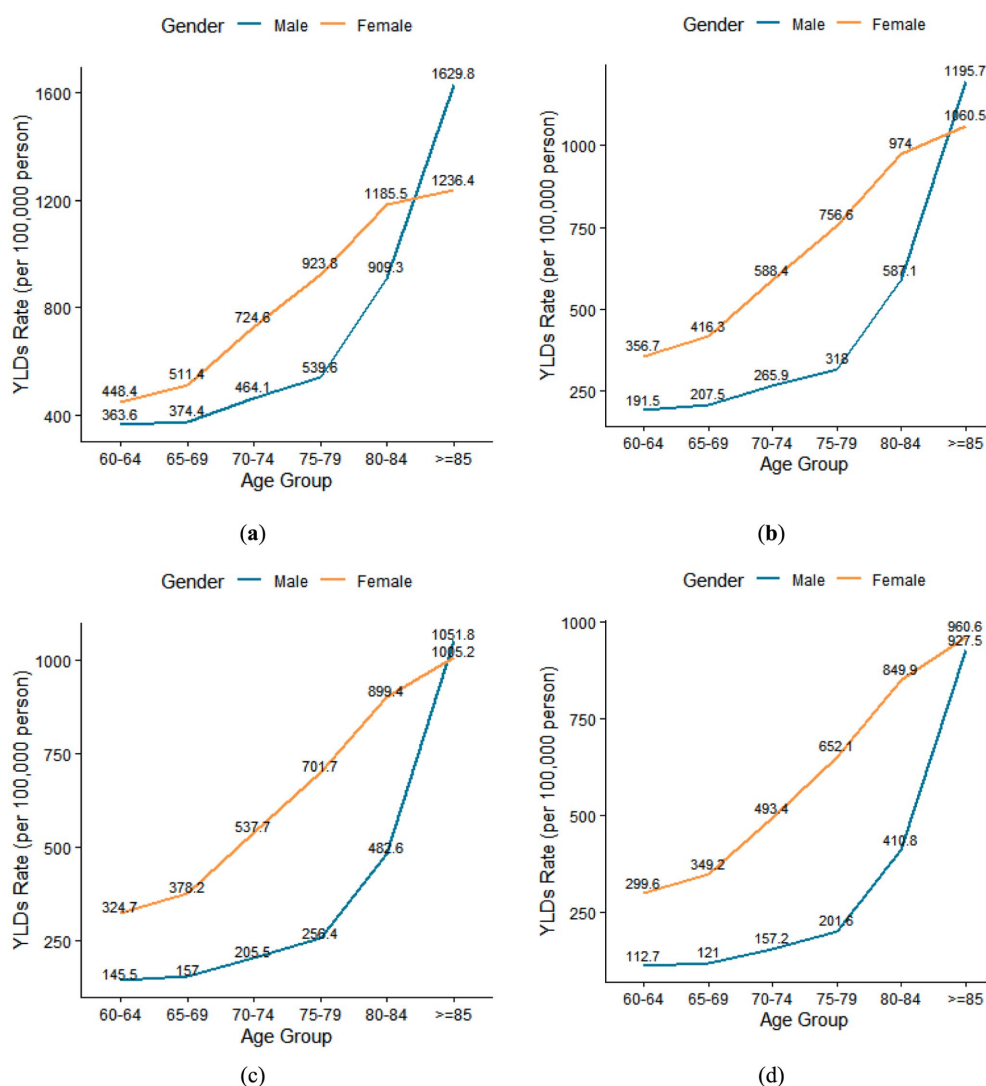


FIGURE 2

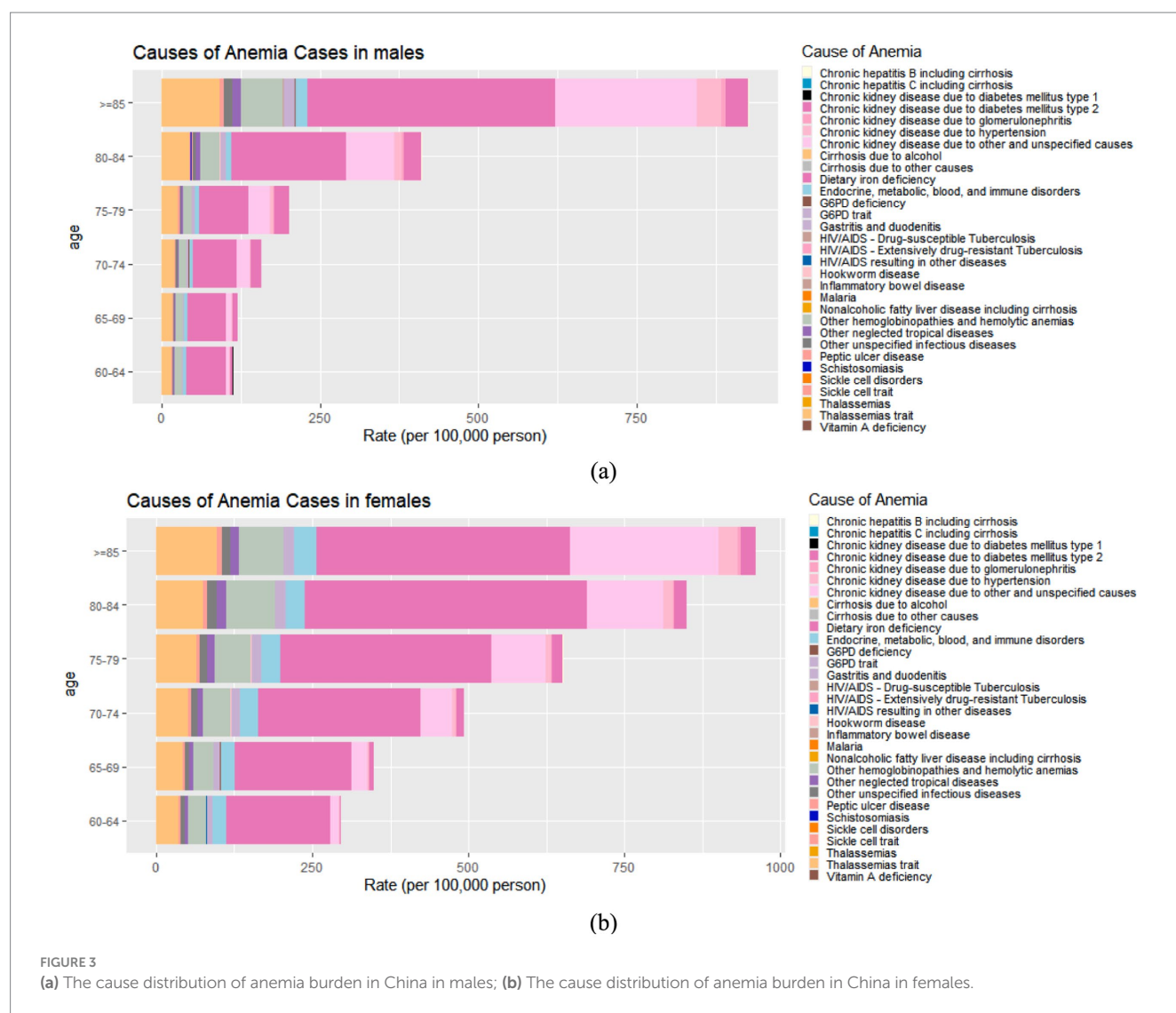
(a) Trends in YLDs Rate (per 100,000) of anemia with age in 2002; (b) Trends in YLDs Rate (per 100,000) of anemia with age in 2010; (c) Trends in YLDs Rate (per 100,000) of anemia with age in 2015; (d) Trends in YLDs Rate (per 100,000) of anemia with age in 2021.

individuals without type 2 diabetes (49). A related study showed that a higher genetically predicted risk for heart failure (HF), coronary artery disease (CAD), and ischemic stroke (AIS) was significantly positively associated with anemia risk (8). The usage rate of certain medications is higher in females than in males among older adults. For instance, one study found that 78% of females used bisphosphonates for the treatment of osteoporosis (50). There is an association between bisphosphonates and the risk of non-serious gastrointestinal adverse events ($RR = 1.16$, $CI: 1.00-1.36$), such as gastric pain, nausea, and vomiting (51). The prolonged occurrence of these adverse reactions may lead to a decrease in appetite and inadequate intake, thereby affecting the absorption of essential nutrients related to red blood cell production, such as iron, vitamin B12, and folate. Therefore, it is essential to implement targeted measures to address the gender inequalities that contribute to anemia.

The prevalence and YLD rate of anemia consistently increased with age, although there were variations between years. The prevalence

of anemia in the 60–64 age group compared to the ≥ 85 age group, increased by 20.0, 10.4, and 8.7% in 2002, 2010, and 2015, respectively. The rate of increase gradually decreased. In 2021, the YLD rate was 3.2 times higher in the 85-year-old age group compared to the 60-year-old age group in females and was 8.2 times higher in males. This was consistent with related studies (28, 52). China is experiencing population aging, which indicates that anemia among older adults is a common health concern for us.

In this study, we found that dietary iron deficiency was the leading cause of anemia across all age groups in both genders. Thalassemias trait, other hemoglobinopathies and hemolytic anemias, and chronic kidney disease due to other and unspecified causes were also common causes of anemia in older adults aged 60 and above. The etiology of anemia in older age is complex and ranges from bone marrow failure syndromes to chronic kidney disease, and from nutritional deficiencies to inflammatory processes (16). Iron Deficiency Anemia (IDA) is the most common nutritional deficiency disorder (53). Although it has primarily been regarded as a public health concern affecting growing



children, premenopausal women, and pregnant females, it is increasingly recognized as a clinical condition that can affect patients presenting to various medical and surgical specialties, especially those with chronic conditions and the older adults (54). Thalassemia is a recessive monogenic hematological disorder, which accounts for the largest share of this burden. Previous studies have indicated that it affects around 10% of the population in certain southern regions of China (55). A related study showed that hemoglobinopathies accounted for 20% of all anemia cases, ranking second in all cause-specific anemia in China (56). As individuals age, the older adults population often experiences multiple chronic conditions. One of the significant complications of chronic kidney disease, for example, is anemia (57). The analysis of the causes of anemia is essential to prevent and control anemia and reduce the burden of anemia in older adults. For example, iron deficiency can be controlled through iron supplementation, and iron fortification is an effective strategy to control dietary iron deficiency (58). China has the highest number of thalassemia cases in the world, prenatal screening can reduce the incidence of thalassemia (59).

Additionally, to reduce the burden of anemia in older adults aged 60 and above, it is crucial to monitor and prevent the onset and progression of other hemoglobinopathies hemolytic anemias, and chronic kidney disease due to other and unspecified causes.

Furthermore, due to the varying causes of anemia across different age groups, the focus of concern among older adults individuals also differs by age group. For older adults aged 60–69, the focus should be on dietary iron deficiency, thalassemia traits, other hemoglobinopathies, and hemolytic anemias. In the 70–74 age group, 75–79 age group, males in the 80–84 age group, and those aged ≥85, the focus should shift to dietary iron deficiency, thalassemia traits, and chronic kidney disease due to other and unspecified causes. For females aged 80–85, attention should be given to iron deficiency, chronic kidney disease due to other and unspecified causes, and other hemoglobinopathies and hemolytic anemias.

To effectively reduce the anemia burden among China's older adults population, targeted strategies should focus on high-risk groups and dietary optimization. First, priority should be given to vulnerable subgroups, including females, rural areas, western region, southern China, and the high-age older adults. Second, the causes of anemia in the older adults were complex. Dietary iron deficiency was the leading cause and one that can be directly and effectively controlled to prevent and treat anemia in older adults. Therefore, older adults should pay attention to proper dietary iron in their daily diet. At the same time, because the absorption and metabolism of different foods and their nutrients may interact and have a complex, cumulative effect on the

body, it is also necessary to consider the comprehensive effect between various foods and nutrients to achieve nutritional balance.

Several strengths were exhibited in our study. Firstly, three large and nationally representative surveys were used to obtain data on the prevalence of anemia, including the 2002 CNHS, the 2010–2013 CNHS, and the 2015 CACDNS. In addition, part of the data was obtained from the public database GBD 2021, which incorporates surveillance and survey data to provide a comprehensive overview of trends and the current burden of anemia among the older Chinese population. However, several limitations warrant attention. First, cross-sectional studies, while valuable for identifying associations, have inherent limitations when it comes to making causal inferences. These studies provide a snapshot of relationships at a single point in time, which makes it difficult to establish temporal sequences or causal direction, and the real burden of anemia in the older adults may be ignored during the interval between the three surveys. So large cohort studies are needed to determine causality in the future. Second, while various data sources were integrated by the GBD 2021 study to ensure accuracy, deviations from the actual situation were unavoidable because the data were fitted by the models. While this study provided a comprehensive assessment of anemia burden and etiology, it did not explore multivariate interactions among risk factors (e.g., effect modification by socioeconomic status). Therefore, future studies are needed to analyze the relationship with risk factors and anemia.

5 Conclusion

In 2002, 2010–2013, and 2015, the prevalence of anemia among older adults aged 60 and above, showed a decreasing trend. Meanwhile, in 2002, 2010, 2015, and 2021, YLD rates caused by anemia also demonstrated a downward trend over time. However, age and regional differences in the burden of anemia disease are still worth attention. Additionally, the prevalence, YLDs, and YLD rates of anemia increased with age. China is experiencing population aging, which indicates that anemia among older adults aged 60 and above is a common health concern. Moreover, greater attention should be paid to anemia in rural and western regions. Dietary iron deficiency is the leading cause of anemia across all age groups and both genders, highlighting the need for targeted interventions to address this nutritional deficiency.

Data availability statement

The datasets presented in this article are not readily available because the datasets from the 2002 CNHS, 2010 CNHS, and 2015 CACDNS used and/or analyzed during the current study are not publicly available according to the policy of the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. The data from the GBD 2021 is open to the public and can be obtained free of charge through website. Requests to access the datasets should be directed to <https://vizhub.healthdata.org/gbd-results/>.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the National Institute for Nutrition and Health of the

Chinese Center for Disease Control and Prevention. 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

PW: Conceptualization, Data curation, Methodology, Software, Writing – original draft, Writing – review & editing. XC: Writing – review & editing. QG: Writing – review & editing. LH: Conceptualization, Methodology, Supervision, Writing – review & editing. LJ: Investigation, Writing – review & editing. XX: Investigation, Writing – review & editing. WG: Investigation, Writing – review & editing. SL: Investigation, Writing – review & editing. LZ: Supervision, Writing – review & editing. HF: Data curation, Methodology, 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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Potential application of pig (*Sus scrofa domestica*) skin peptide-iron chelates in the treatment of iron deficiency anemia and regulation of intestinal flora metabolism

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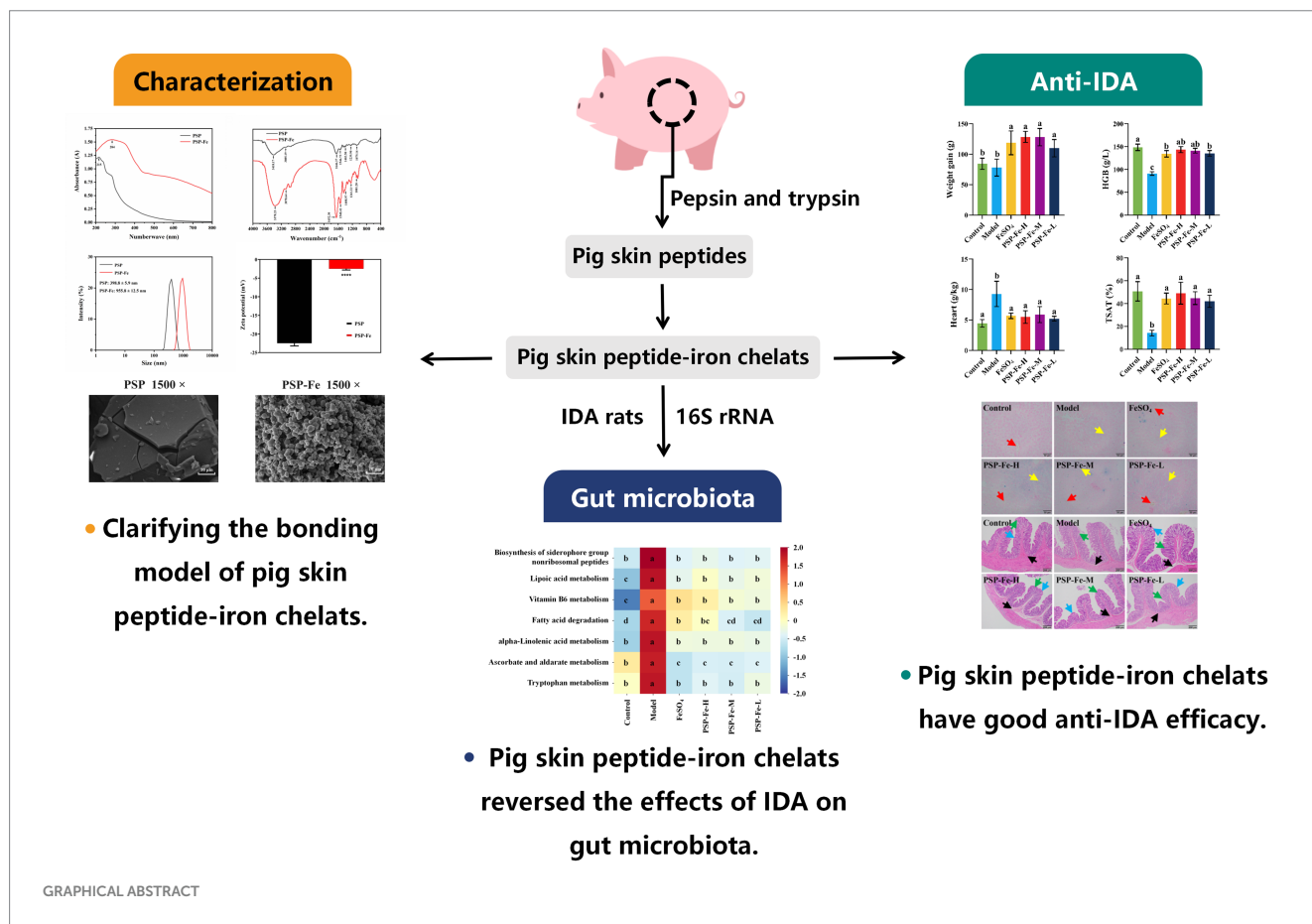
Iron deficiency is an important public health concern worldwide. Intake of iron-fortified foods has been widely used to treat iron deficiency anemia (IDA). In this study, a novel food for iron supplementation was designed: pig (*Sus scrofa domestica*) skin peptide-iron (PSP-Fe) chelates. Structural characterization demonstrated that acidic amino acids (aspartic acid, glutamic acid) and aromatic amino acids (phenylalanine, tryptophan, and tyrosine) in PSP were involved in the chelation reaction, with the carboxyl group and amino group provided the major iron binding sites. In addition, iron significantly altered the microscopic morphology of PSP. IDA rats were established and different doses of iron supplements were gavaged for 21 days to evaluate the effectiveness of PSP in treating IDA. The medium dose of PSP-Fe restored hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), serum ferritin (SF), serum iron (SI), hepcidin, total iron binding capacity (TIBC) and transferrin saturation (TSAT) to normal levels. PSP-Fe also ameliorated the abnormal changes in heart coefficients, lungs coefficients, liver coefficients and spleen coefficients caused by IDA. PSP-Fe further restored iron storage in the liver and villous damage in the colon of rats compared to FeSO₄. 16S rRNA results suggest that the 10 microbial markers in the Model group may impede iron absorption and HGB synthesis of host through biosynthesis of siderophore group nonribosomal peptides, vitamin B6 metabolism, lipoic acid metabolism, ascorbate metabolism and tryptophan metabolism. At the end, the safety of PSP-Fe was preliminarily affirmed by toxicity evaluation *in vitro* and *in vivo*. These findings suggest that PSP-Fe has potential as a novel functional food for treating IDA.

KEYWORDS

pig (*Sus scrofa domestica*) skin, iron deficiency anemia, peptide-iron chelates, iron supplementation, gut microbiota

1 Introduction

Iron is a trace element necessary for normal functioning of the human body as it is involved in basic biochemical processes such as transport of oxygen, electron transfer, DNA synthesis, and cell differentiation (1–3). The physiological range of iron content in adult body is 38–50 mg/kg, and most of the iron (>70%) is present in hemoglobin (HGB) (4, 5). Iron



deficiency (ID) is an important public health problem worldwide with a much more serious in developing countries than in developed countries, especially in areas where nutritious diverse diets are not available or affordable (6). Iron deficiency anemia (IDA) is one of the most common nutritional disorders globally, affecting >2 billion individuals, with a high prevalence in children <5 years of age, women of child-bearing age, and pregnant women (7, 8). IDA is mainly caused by a low iron intake (lack of iron-rich foods or other reasons for not absorbing iron properly), excessive iron loss (chronic gastrointestinal bleeding), and an increased iron demand (children at the stage of growth) (9). ID can cause developmental delays and cognitive impairment in young children (10). Iron imbalances in the human brain also increase the risk of neurodegenerative diseases (11). Furthermore, ID during pregnancy may lead to low birth weight and preterm delivery of the offspring (12). Therefore, prevention and effective treatment of ID is critical for these populations.

Intake of iron-fortified foods has been widely used to treat IDA worldwide. Most of the oral supplements currently available in the market, such as ferrous sulfate (FeSO₄), ferrous lactate, ferrous glycinate, and ferrous gluconate, have strong gastrointestinal adverse effects, low bioavailability, poor patient compliance, and relatively high preparation costs (9, 13). Therefore, the development of a novel functional food for iron supplementation that is safe, effective, and economical is imminent.

As one of the dietary supplements to alleviate IDA, peptide-iron chelates have several advantages, including high bioavailability, good stability, and no adverse effects (14, 15). Currently, there are three main transport pathways by which chelates promote the absorption of iron: the ferrous ion transport pathway, the peptide absorption

pathway, and peptides as metal ion transport carriers (16). In addition, chelates can prevent ferrous iron from forming insoluble complexes with phytic acid, polyphenols (e.g., tannins), and oxalic acid (17, 18). Chelates can substantially increase the absorption of iron, and iron-chelating active peptides can be obtained from different food or by-product, including oysters (19), tilapia skin (20), Alaskan pollock skin (21), whey (22), oats (23), and mung beans (24).

Pig skin is an economic by-product of pig processing. At present, pig farming is accelerating worldwide with the rapid expansion of industrial farming. In 2021, pork accounted for 34% of the global meat production, with >1.4 billion commercial pigs in slaughterhouses (25). The rich proteins contained in the dermis of pig skin may be an excellent source of ion chelating peptides; however, the study of pig skin peptide-iron (PSP-Fe) chelates has received little attention.

The aim of our study was to elucidate the binding mode of PSP to iron and to assess the efficacy of PSP-Fe in the treatment of IDA. In addition, the pathways of microbial markers affecting host hemoglobin synthesis were further screened and explored. These results provide references and bases for the comprehensive utilization of pig skin and the development of new functional food for iron supplementation.

2 Materials and methods

2.1 Materials and reagents

Pig skins were purchased from Yantai Xiwang Meat Products Co., Ltd. (Yantai, China). Pepsin (2,500 units/mg), trypsin

(2,500 units/mg), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, ascorbic acid, phenanthroline, sodium acetate buffer, hydroxylamine hydrochloride, KBr and anhydrous ethanol were purchased from Shanghai Aladdin Biochemical Science Technology Co., Ltd. (Shanghai, China). UHPLC grade acetonitrile, methanol and formic acid (FA) were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, United States), and pure water for analysis was bought from Watson Group Co., Ltd. (Guangzhou, China).

2.2 Preparation of PSP

Fresh pig skins were degreased with petroleum ether and then were placed in distilled water (12.5 g PSP per 100 mL). Subsequently, the proteins were subjected to sequential enzymatic hydrolysis with pepsin (enzyme-substrate mass ratio 1:100, pH 1.5, 1 h) and trypsin (enzyme-substrate mass ratio 1:100, pH 8.0, 3 h) at 37°C. Immediately after hydrolysis, the reaction mixture was heated at 95°C for 10 min to inactivate the enzyme, and then centrifuged at 5,000 rpm for 10 min. The supernatant was ultrafiltered using a 3 kDa cut-off membrane and the filtrate was lyophilized to obtain PSP powder.

2.3 Preparation of PSP-Fe chelates

The PSP-Fe was prepared using the method proposed by Zhao et al. (26) with some modifications. The PSP was dissolved in distilled water (3 g PSP per 10 mL), ascorbic acid was added at a mass ratio of 0.4:1 of ascorbic acid to $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, and the pH was adjusted to 5.0. Subsequently, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was added at a mass ratio of 10:1 of PSP to $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, and stirred for 40 min at 40°C. Immediately after the reaction, seven-fold anhydrous ethanol was added (volume/volume, v/v) to remove peptides not involved in the reaction. The mixture was centrifuged at 5,000 rpm for 10 min and the precipitate was lyophilized to obtain PSP-Fe.

Iron content was determined with reference to the method described by Wu et al. (27) with some modifications. PSP-Fe was dissolved in distilled water to prepare a 1 mg/mL solution. Then, 10 mL PSP-Fe solution, sodium acetate buffer, 10 mg/mL hydroxylamine hydrochloride, 1 mg/mL phenanthroline and distilled water were added sequentially into a 25 mL volumetric flask. The absorbance was recorded by an ultraviolet-visible (UV-Vis) spectrophotometer (UV-3600i Plus, Shimadzu, Japan) at 510 nm.

2.4 Characterization of the PSP and PSP-Fe chelates

2.4.1 UV-Vis absorption spectroscopy

The PSP or PSP-Fe was dissolved in distilled water to prepare a 0.1 mg/mL solution. The absorption spectra of PSP or PSP-Fe was measured in the wavelength range of 200–800 nm by the UV-Vis spectrophotometer.

2.4.2 Fourier-transform infrared spectroscopy

The PSP or PSP-Fe was mixed with 90 mg of dry KBr, thoroughly ground several times, and then pressed into transparent flakes. The Fourier-transform infrared (FTIR) spectra of PSP or PSP-Fe was

obtained by a FTIR spectrometer (Nicolet iS50, Thermo Fisher Scientific, United States) in the wave number range from 4,000 cm^{-1} to 400 cm^{-1} .

2.4.3 Particle size and zeta potential

The PSP or PSP-Fe was dissolved in distilled water to prepare a 1.5 mg/mL solution. The particle size and zeta potential of PSP or PSP-Fe was obtained by a laser particle size analyzer (Mastersizer 3000, Malvern Panalytical, United Kingdom), and the samples were tested three times.

2.4.4 Scanning electron microscopy analysis

An appropriate amount of PSP or PSP-Fe was evenly spread on the tapes on the sample rack with vacuum sprayed gold treatment, and the microstructure of the samples was observed by scanning electron microscopy (SEM) (EVO LS15, ZEISS, Germany). The magnification was 1,500 and 5,000, respectively.

2.4.5 Amino acid composition analysis

The PSP or PSP-Fe (100 mg) was hydrolyzed with 15 mL of 6 M HCl in a sealed hydrolysis tube under reduced pressure at 110°C for 24 h. Hydrolyzed samples were filtered, dried, and solubilized, and the amino acid composition was determined using an amino acid analyzer (Biochrom30+, Biochrom, United Kingdom).

2.4.6 High-performance liquid chromatography (HPLC) mass spectrometry (MS)/MS analysis

The peptide sequences of PSP-Fe were detected using EASY-nLC1200 Q Exactive Plus (Thermo Scientific, United States). The samples were desalted, dried, and dissolved, and separated on a C18 reversed-phase analytical column (75 mm $\times$ 20 cm $\times$ 3 μm). Mobile phase A was a mixture of 99.9% water and 0.1% FA, and mobile phase B was a mixture of 80% acetonitrile and 0.1% FA. The liquid phase gradients were as follows: 0–3 min, 2–6% B; 3–42 min, 6–20% B; 42–47 min, 20–32% B; 47–48 min, 32–100% B; 48–60 min, 100% B. The mobile phase flow rate was 300 nL/min. ESI⁺ assay mode was used in a data-dependent scanning mode at a resolution of 70,000 orbital trap for full-scan acquisition. An induced collision-induced dissociation MS/MS scan was then performed. Daughter ions were measured in orbitals with a resolution of 17,500. Finally, peptide sequences were obtained using software analysis and protein database comparison.

2.5 Efficacy of PSP-Fe in the treatment of IDA

2.5.1 Animals, diets and treatment

Forty-two 3-week-old male Sprague-Dawley rats (45–50 g) were purchased from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. [License No. SCXK (Lu) 2022-0006] (Jinan, China). AIN-93G standard diet (Fe = 50 mg/kg) and modified AIN-93G ID diet (5 mg Fe per kg) were purchased from Beijing Keao Xieli Animal Feed Co., Ltd. (Beijing, China). All rats were housed at standard environmental conditions (24 $\pm$ 2°C, 50 $\pm$ 5% humidity) with free access to water. Before starting the experiments, rats were allowed to acclimatize to the environment for 3 days and were fed standard diet and deionized water.

After 3 days of acclimatization, seven rats were randomly selected as the control group and fed with standard diet. Thirty-five rats were

fed with ID diet for 28 days. After 2 weeks of feeding, blood was collected weekly from the tail vein and the HGB level was monitored using an animal hematology analyzer. IDA was defined when the HGB level was lower than 100 g/L.

After IDA rats were established, the control group continued to be given a standard diet. Thirty-five IDA rats were divided into five groups (Model, FeSO₄, PSP-Fe-H, PSP-Fe-M, and PSP-Fe-L) of seven rats each and fed the ID diet. Each group was gavaged with deionized water [10 mL/kg-body weight (bw)] or the corresponding iron supplement (dissolved in deionized water, 10 mL/kg-bw) once daily at 9:00 a.m. for 21 days. The dosages for each group were as follows: Control and Model groups (deionized water), FeSO₄ group (Fe = 2 mg/kg-bw), PSP-Fe-H group (Fe = 3 mg/kg-bw), PSP-Fe-M group (Fe = 2 mg/kg-bw), and PSP-Fe-L group (Fe = 1 mg/kg-bw).

2.5.2 Sample collection

All rats were weighed weekly. At the end of the experiment, all rats were fasted for 12 h, then anesthetized with 2% sodium pentobarbital. Blood samples were collected from the abdominal aorta, and serum was separated by centrifugation at 3,000 rpm for 6 min. The heart, liver, spleen, lungs, and kidney organs were removed, rinsed, blotted and weighed. Liver and colon were stored in 4% paraformaldehyde solution. Cecal contents were collected, placed in freezing tubes, and stored at −80°C.

2.5.3 Hematological tests

Hematological parameters were obtained by an animal hematology analyzer, including red blood cell (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW). Serum ferritin (SF), serum iron (SI) and total iron binding capacity (TIBC) were obtained by a biochemical analyzer. Hepcidin was measured with an ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Transferrin saturation (TSAT) was calculated as follows:

$$\text{TSAT}(\%) = \text{SI}(\mu\text{mol/L}) / \text{TIBC}(\mu\text{mol/L}) \times 100\%$$

2.5.4 Organ coefficient

The relative weight indices of the heart, liver, spleen, lungs and kidney were calculated from the final body weight measured at the end of the experiment. The organ coefficients were calculated as follows:

$$\text{Organ coefficient (g/kg)} = \text{Organ weight (g)} / \text{Rat weight (kg)}$$

2.5.5 Histological analysis

The colonic tissues were fixed in 4% paraformaldehyde solution, and the fixed tissue blocks were dehydrated stepwise, transparent with xylene, impregnated in wax and embedded in paraffin in order to prepare 5 μm-thick sections for hematoxylin and eosin (H&E) staining. They were examined using a biomicroscope at magnification

of 100. Liver was stained using Prussian blue as described above to visualize iron storage in the liver.

2.5.6 High-throughput sequencing analysis

Microbiota analysis was performed on the Illumina NoveSeq 6000 sequencing platform (Illumina, San Diego, CA, United States). Primers 341F and 806R were used to amplify the V3 + V4 variable region of the 16S rRNA gene. Sequencing libraries were generated by NEB Next Ultra DNA Library Prep Kit (Illumina, San Diego, CA, United States), and the libraries were tested using a bioanalyzer (Agilent 5400, Agilent Technologies Co., Ltd., United States) and PCR quantification (T100PCR, Bio-Rad, United States). Once the libraries were qualified, up-sequencing was performed using the Illumina sequencing platform and 250 bp paired-end reads were generated. The Greengenes 2 database was used as the reference dataset. Raw sequences from each sample were quality filtered, and then the chimeric sequences were identified and removed by the plug-in in the QIIME2 software to obtain the operational taxonomic units (OTUs). Metabolic pathway enrichment analysis was performed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) (28).

2.6 Toxicity evaluation of PSP-Fe *in vitro*

2.6.1 Cell culture

Human renal proximal tubular epithelial cells (HK-2) were purchased from Wuhan Pricella Biotechnology Co., Ltd. (Wuhan, China) and cultured in MEM (Pricella, Wuhan, China) supplemented with 10% fetal bovine serum (Pricella, Wuhan, China) and 1% penicillin-streptomycin (Gibco, United States). HK-2 cells were cultured in a 37°C and 5% CO₂ incubator under a humidified atmosphere.

2.6.2 CCK-8 assay

HK-2 cells were seeded in 96-well plates at a concentration of 5×10^3 cells/mL. After overnight culture, medium containing different concentrations of PSP-Fe (Fe = 0, 31, 63, 125 and 250 μg/mL) was added and cultured for 24 h. Then, 10 μL CCK-8 solution (Beijing Lablead Biotechnology Co., Ltd., Beijing, China) was added to each well and cultured at 37°C for 3 h. Absorbance values of the solution were detected using a microplate reader (Thermo Scientific, United States) at 450 nm. Cell viability was calculated as follows:

$$\text{Cell viability}(\%) = \frac{\left(\frac{\text{OD of experimental group} - \text{OD of blank group}}{\text{OD of control group} - \text{OD of blank group}} \right) \times 100\%}{1}$$

2.7 Toxicity evaluation of PSP-Fe *in vivo*

2.7.1 Animals and treatment

Twenty-eight 6-week-old male Sprague–Dawley rats (190–200 g) were purchased from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. AIN-93G standard diet were purchased from Beijing Keao Xieli Animal Feed Co., Ltd. All rats were housed at standard

environmental conditions with free access to water. Before starting the experiments, rats were allowed to acclimatize to the environment for 3 days and were fed standard diet and deionized water. After 3 days of acclimatization, all rats were randomly divided into four groups (Control, PSP-Fe-H, PSP-Fe-M, and PSP-Fe-L) of seven rats each and fed the standard diet. Each group was gavaged with deionized water (10 mL/kg.bw) or the corresponding iron supplement (dissolved in deionized water, 10 mL/kg.bw) once daily for 28 days. The dosages for each group were as follows: Control groups (deionized water), PSP-Fe-H group (Fe = 3 mg/kg.bw), PSP-Fe-M group (Fe = 2 mg/kg.bw), and PSP-Fe-L group (Fe = 1 mg/kg.bw).

2.7.2 Sample collection

All rats were weighed weekly. At the end of the experiment, all rats were fasted for 12 h, then anesthetized with 2% sodium pentobarbital. Blood samples were collected from the abdominal aorta, and serum was separated by centrifugation at 3,000 rpm for 6 min. The heart, liver, spleen, lungs, and kidney organs were removed, rinsed, blotted, weighed and stored in 4% paraformaldehyde solution.

2.7.3 Hematological tests

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine (CR) were obtained by a biochemical analyzer. Serum superoxide dismutase (SOD) and glutathione (GSH) were measured with corresponding assay kits (Wuhan Servicebio Technology Co., Ltd., Wuhan, China).

2.7.4 Organ coefficient

The relative weight indices of the heart, liver, spleen, lungs and kidney were calculated from the final body weight measured at the end of the experiment.

2.7.5 Histological analysis

The heart, liver, spleen, lungs, and kidney tissues were fixed in 4% paraformaldehyde solution, and the fixed tissue blocks were

dehydrated stepwise, transparent with xylene, impregnated in wax and embedded in paraffin in order to prepare sections for H&E staining. They were examined using a biomicroscope at magnification of 100.

2.8 Statistical analysis

All data were presented as mean $\pm$ standard deviation. Statistical analyses were assessed by Student's *t*-test, one-way ANOVA and Duncan's multiple range method, statistically significant differences between the groups were considered as $p < 0.05$.

3 Results

3.1 Characterization of PSP and PSP-Fe chelates

3.1.1 UV-Vis absorption spectroscopy

As shown in Figure 1A, the maximum UV-Vis absorption peak of PSP shifted from 215 nm to 284 nm after chelating with iron. This suggests that aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine, may be involved in the chelation of PSP with iron (15). In addition, the absorption peak strength of the ferrous ions of the ring of PSP-Fe was higher than that of PSP due to the valence electron transition. Zhao et al. (29) concluded that upon combining a peptide with a metal ion, the chirality of the chromophore changes, including C=O, -COOH, -OH, and -NH₂, resulting in a change in the spectrum. A similar variation in the UV-Vis spectra of the tilapia skin collagen iron-chelating peptides was observed (20).

3.1.2 FTIR spectroscopy

When ferrous ions chelate with the amino acid residues of PSP, clear shifts in the absorption peaks can be observed in the FTIR spectrum. As shown in Figure 1B, the absorption peak of PSP at 3,418 cm⁻¹ corresponds to the stretching vibration of the N-H bond. After chelating ferrous ions, the absorption peak of PSP-Fe was shifted to 3,378 cm⁻¹,

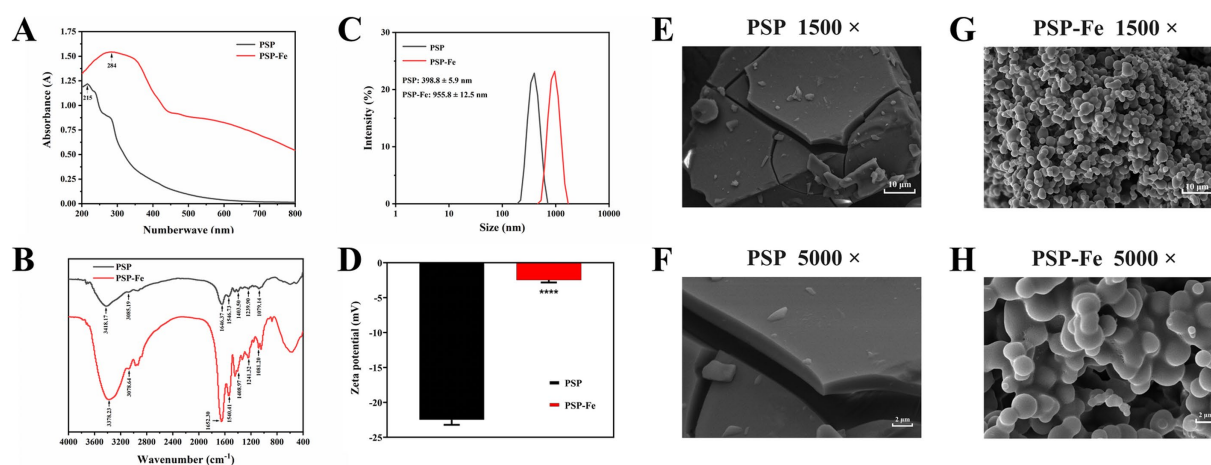


FIGURE 1

Structural characterization of PSP and PSP-Fe. (A) UV-Vis spectrum. (B) FTIR spectrum. (C) Particle size distribution. (D) Zeta potential. Microstructures of PSP at magnifications of (E) 1,500 and (F) 5,000. Microstructures of PSP-Fe at magnifications of (G) 1,500 and (H) 5,000. **** $p < 0.0001$ versus the PSP.

indicating that the N–H bond of PSP was involved in the chelating reaction. The absorption peaks of PSP at 1,646 cm^{-1} and 1,546 cm^{-1} corresponded to the amide I band and the amide II band, which were generated by the stretching vibration of the C=O bond and the bending vibration of the N–H bond, respectively. The absorption peaks corresponding to PSP-Fe were shifted to 1,652 cm^{-1} and 1,540 cm^{-1} , respectively, indicating that the C=O and N–H bonds provide coordination sites for the ferrous ions. The absorption peak of PSP at 1,403 cm^{-1} shifted to 1,408 cm^{-1} , indicating that the ferric chelating site is also related to the carboxyl group on the peptide chain. In addition, the absorption peak of PSP-Fe at 1,081 cm^{-1} may be due to the formation of C–O–Fe coordination bonds. Overall, the $-\text{NH}_2$ and $-\text{COOH}$ groups of PSP provided the major binding sites for ferrous ions. Similar results confirmed that silver carp scale collagen peptide binds to iron mainly through amino and carboxylic acid groups (26). Furthermore, Liu et al. (19) reported that ferrous ions could bind to $-\text{NH}_2$ and $-\text{COOH}$ groups of oyster protein hydrolysate to form chelates.

3.1.3 Particle size and zeta potential

The particle size of PSP and PSP-Fe are shown in Figure 1C. The results showed that the particle size of PSP ($398.8 \pm 5.9 \text{ nm}$) was significantly ($p < 0.0001$) lower than that of PSP-Fe ($955.8 \pm 12.5 \text{ nm}$). The increase in particle size distribution indicated that the chelating reaction between ferrous ions and peptides exists not only in intramolecular interactions but also in intermolecular interactions (30). A single ferrous ion may bind to multiple peptide chains at the same time. The polydispersity index of PSP and PSP-Fe ranged from 0.279 to 0.313, suggesting an acceptable homogeneity.

The zeta potential is an important indicator of the surface charge state of particles in a dispersed system. As shown in Figure 1D, the zeta potential of PSP increased significantly from $-22.5 \pm 0.72 \text{ mV}$ to $-2.49 \pm 0.34 \text{ mV}$ after chelating with ferrous ions ($p < 0.0001$). This may be due to the binding of ferrous ions to carboxyl groups during chelation neutralizing some of the negative charges. A similar conclusion was reached by Li et al. (31) for the zeta potential result of duck egg white peptide-ferrous chelate.

3.1.4 SEM analysis

SEM can visualize the object micromorphology. The microstructures of PSP and PSP-Fe were observed at magnifications of 1,500 and 5,000, respectively (Figures 1E–H). The surface of PSP was relatively smooth and dense, and was in the form of blocks. Interestingly, PSP-Fe showed loose particle aggregates. The reason for the change in microstructure may be that the carboxyl group of PSP formed a “bridging role” with ferrous ions, destroying the original dense structure of the peptide surface (32). This phenomenon is also similar to that of the tilapia skin collagen iron-chelating peptides (20).

3.1.5 Amino acid composition analysis

Since various amino acids have different binding affinities for iron, changes in amino acid composition are of interest. The amino acid composition and relative content of PSP and PSP-Fe are shown in Table 1. The relative content of aspartic acid and glutamic acid were increased after the chelation of PSP with ferrous ions. Among them, the contents of aspartic acid increased significantly from $50.48 \pm 1.05 \text{ mg/g}$ to $58.29 \pm 0.67 \text{ mg/g}$ ($p < 0.05$), and the contents of glutamic acid increased significantly from $83.82 \pm 0.25 \text{ mg/g}$ to $95.11 \pm 0.34 \text{ mg/g}$ ($p < 0.05$). This result indicated that the carboxyl

groups these two acidic amino acids contributed significantly to the chelation of iron. Through detailed experiments, Hu et al. (33) verified that ferrous iron can bind to the carboxyl group of aspartic acid and the imidazolyl group of histidine in the Antarctic krill heptapeptide through coordinate bonds and π -cation interactions, respectively. In addition, Huang et al. (29) isolated a tripeptide with calcium chelating activity in the hydrolysate of shrimp processing byproducts with the sequence “threonine-cysteine-histidine”.

3.1.6 HPLC MS/MS analysis

Two enzymes were used in the preparation of PSP. Pepsin tends to hydrolyze peptide bonds with aromatic amino acids at the amino terminus or carboxyl terminus, while the site of action of trypsin is generally after the lysine and arginine, yielding peptides with either lysine or arginine as the carboxyl terminus residue. The molecular weight of peptides is an important factor influencing metal chelating activity. Low molecular weight peptides have better metal chelating activity compared to high molecular weight peptides. Table 2 demonstrates the top 30 peptide sequences with the highest peak areas, of which 17 peptides have molecular weights located in the range of 1–2 kDa, and 10 peptides have molecular weights $< 1 \text{ kDa}$. Fan et al. (16) identified 17 peptides with iron-chelating activity from hydrolysates of sea cucumber, and their molecular weights were all $< 2 \text{ kDa}$. Guo et al. (21) identified an iron-chelating decapeptide from Alaska pollock skin; the peptide sequence was GPAGPHGPPG. Huang et al. (34) reported that small molecular weight peptides ($< 5 \text{ kDa}$) extracted from hairtail protein hydrolysates had better ferrous iron-chelating activity than large molecular weight peptides ($> 5 \text{ kDa}$).

Peptides have different binding sites for ferrous ions including terminal amino groups and carboxyl groups, nitrogen atoms of the peptide chain, and active side chains of amino acids (35). Athira et al. (15)

TABLE 1 Amino acid composition of PSP and PSP-Fe.

Amino acids	Relative content (mg/g)	
	PSP	PSP-Fe
Aspartate	50.48 ± 1.05^a	58.29 ± 0.67^b
Threonine	16.46 ± 1.41^a	16.09 ± 0.99^a
Serine	29.55 ± 0.34^a	28.81 ± 0.83^a
Glutamate	83.82 ± 0.25^a	95.11 ± 0.34^b
Glycine	116.76 ± 1.67^a	115.45 ± 1.17^a
Alanine	67.17 ± 0.10^a	62.66 ± 0.44^b
Cysteine	16.10 ± 1.22^a	16.73 ± 0.56^a
Valine	22.59 ± 0.38^a	18.88 ± 1.35^b
Methionine	9.11 ± 0.14^a	8.07 ± 0.35^b
Isoleucine	12.74 ± 0.95^a	8.43 ± 0.11^b
Leucine	29.27 ± 0.57^a	22.52 ± 0.30^b
Tyrosine	9.70 ± 0.36^a	9.37 ± 0.84^a
Phenylalanine	20.70 ± 0.73^a	18.19 ± 1.52^a
Histidine	8.36 ± 0.61^a	8.71 ± 0.30^a
Lysine	39.44 ± 0.79^a	38.05 ± 0.61^a
Arginine	71.08 ± 1.20^a	69.55 ± 1.07^b
Proline	104.29 ± 1.23^a	95.20 ± 1.88^b

Different letters (a,b) showed significant difference ($p < 0.05$).

TABLE 2 Peptide sequence identified in PSP-Fe.

Number	Identified peptide sequence	Molecular weight (Da)	Retention time (min)
1	GQAGVMGFPGPK	1144.5699	21.19
2	GFSGLDGAK	850.4185	16.76
3	GFPGPKGANGEPGK	1311.6571	31.93
4	ISVPGPMGPSGPR	1250.6440	31.42
5	SGDRGETGPAGPAGPVGPVGAR	1960.9714	19.47
6	GYEGDFY	849.3181	30.82
7	GFPGLPGSGEPGK	1295.6509	37.80
8	GVVGLPGQRGER	1223.6735	15.35
9	SAGISVPGPMGPSGPR	1465.7347	35.45
10	DIASPHELYR	1300.6411	21.77
11	GVVGLPGQR	881.5083	21.05
12	ISVPGPMGPSGPR	1250.6441	23.58
13	GFSGLDGAKGDAGPAGPK	1600.7844	20.14
14	GETGPAGPAGPVGPVGAR	1545.7899	23.31
15	SHNQMQEHVDLRDPNIR	2087.9919	19.04
16	EGLRGRGDQGPVGR	1549.8073	9.54
17	DSFQEVLR	992.4927	30.35
18	FGYEGDF	833.3231	43.42
19	AHDGGYY	937.4042	8.18
20	GHNLQGLPGLAGHHGDQAGPVGPAGPR	2777.3858	33.92
21	SFLPQQPEK	1169.6080	30.22
22	GHAGLAGAR	808.4304	7.20
23	GSEGPQGVR	885.4304	7.80
24	GEAGPQGAR	841.4042	6.75
25	VGLEHLR	822.4712	13.20
26	SFLPQQPQE	1041.5131	44.36
27	LIDSSDGVKPDGIAHIRD	1906.9749	28.43
28	LYLRNNQIDHIDDK	1755.8904	25.65
29	FSFLPQQPEK	1316.6764	50.41
30	GYDEKSAGISVPGPMGPSGPR	2057.9839	33.78

classified amino acids that provide active side chains for metal chelation into three categories: non-coordinating, weakly coordinating, and strongly coordinating side chains. Of the 30 peptides we identified, each peptide contained 1–9 coordinating amino acids, which could be the significant reason for the good iron-binding effects of PSP-Fe. In addition, although non-coordinating amino acids do not directly act as iron binding sites, they can provide stability and conformation for peptide-iron chelates through non-covalent interactions, such as hydrophobic interactions, van der Waals forces and hydrogen bonding (35, 36).

3.2 Efficacy of PSP-Fe in the treatment of IDA

3.2.1 Changes in body weight

Figures 2A,B have shown the final body weight and weight gain of the rats in each group. At the end of day 21, the weights of

Control, PSP-Fe-H and PSP-Fe-M groups were significantly higher than those of the Model group ($p < 0.05$). Although the mean weights of the PSP-Fe-L and FeSO₄ groups were higher than those of the Model group, there were no significant differences ($p > 0.05$). The weight of the PSP-Fe-H group was not significantly different from that of the Control group ($p < 0.05$), and there were no significant differences in weights between each PSP-Fe and FeSO₄ groups ($p < 0.05$). The weight gains of each PSP-Fe and FeSO₄ groups were significantly higher than that of the Model and Control groups ($p < 0.05$), while there was no significant difference between the Model and Control groups ($p > 0.05$).

3.2.2 Hematological tests

Table 3 and Figures 2C–G have shown the hematological levels of the rats in each group. The levels of HGB, RBC, HCT, MCV, MCH, MCHC, SF, SI, hepcidin and TSAT in the Model group were significantly lower than those in the Control group ($p < 0.05$),

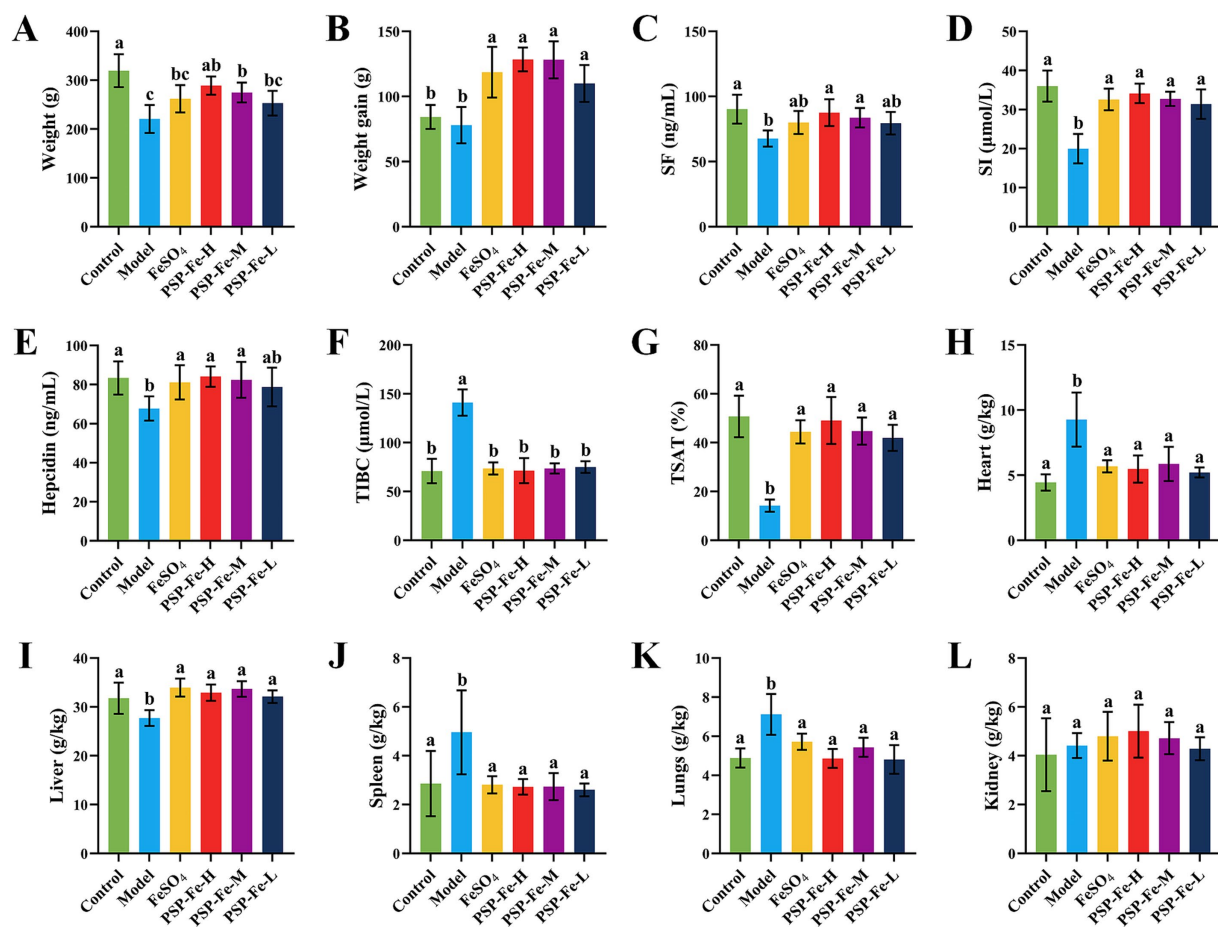


FIGURE 2

Body weight changes, hematological indicators and organ coefficients in rats fed a normal diet and in IDA rats treated with different iron supplements. (A) Body weight. (B) Body weight gain. (C) Serum ferritin. (D) Serum iron. (E) Hepcidin. (F) Total iron binding capacity. (G) Transferrin saturation. (H) Heart coefficient. (I) Liver coefficient. (J) Spleen coefficient. (K) Lungs coefficient. (L) Kidney coefficient. Different letters (a–c) showed significant difference ($p < 0.05$).

while the levels of RDW and TIBC were significantly higher than those in the Control group ($p < 0.05$). After 21 days of gavage with iron supplementation, the levels of HGB, RBC and HCT in the PSP-Fe-H and PSP-Fe-M groups were normalized ($p > 0.05$ versus the Control group). While the levels of HGB and HCT in the FeSO₄ group were incomplete recovered ($p < 0.05$ versus the Control group). In addition, the levels of MCV, MCH, MCHC and RDW in the PSP-Fe-H group, as well as the level of MCHC in the PSP-Fe-M and FeSO₄ groups were all recovered ($p > 0.05$ versus the Control group). The level of MCV in the PSP-Fe-M and FeSO₄ groups were incomplete recovered ($p < 0.05$ versus the Control group), and there was no significant difference between them ($p > 0.05$). The levels of MCH and RDW in the PSP-Fe-M and FeSO₄ groups were incomplete recovered ($p < 0.05$ versus the Control group), and there were significant difference between them ($p < 0.05$). The levels of SF, SI, hepcidin, TIBC and TSAT in each PSP-Fe and FeSO₄ groups were all recovered ($p > 0.05$ versus the Control group). These results indicated that an effective dose of PSP-Fe can effectively improve various hematological parameters in IDA rats, showing relatively excellent hematopoietic

capacity. Similar pharmacodynamic results were found for oat peptide-ferrous chelate (23).

3.2.3 Organ coefficient

Figures 2H–L demonstrated the organ coefficients of rats in each group. The heart coefficient, lungs coefficient, and spleen coefficient of Model group were significantly higher than those of the Control group ($p < 0.05$). After treatment with iron supplements, the above coefficients of each PSP-Fe and FeSO₄ groups were all recovered ($p > 0.05$ versus the Control group). Kidney coefficient were not significantly different between all groups ($p > 0.05$).

3.2.4 Histological analysis

Prussian blue staining of the liver and H&E staining of the colon in each group of rats have shown in Figures 3A,B. Hemosiderin deposition could be clearly observed in liver sections of Control, each PSP-Fe and FeSO₄ groups, whereas the presence of hemosiderin deposition was difficult to observe in the Model group. Lipid droplets were found in the liver sections of all groups except Control group. The villi of the colon were significantly damaged in the Model and

TABLE 3 Hematological parameters of rats fed standard diet and IDA rats treated with different iron supplements.

Group	N	HGB (g/L)	RBC (10 ¹² /L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/L)	RDW (%)
Control	7	148.57 ± 7.09 ^a	7.27 ± 0.40 ^a	45.90 ± 2.74 ^a	61.84 ± 1.32 ^a	20.41 ± 0.59 ^a	330.71 ± 6.50 ^a	14.56 ± 0.54 ^d
Model	7	90.86 ± 3.53 ^c	4.32 ± 0.73 ^b	25.59 ± 3.02 ^c	44.19 ± 1.95 ^d	16.64 ± 0.74 ^c	307.29 ± 6.78 ^b	25.73 ± 1.02 ^a
FeSO ₄	7	134.14 ± 7.13 ^b	7.06 ± 0.30 ^a	40.44 ± 2.40 ^b	55.46 ± 3.22 ^c	17.29 ± 0.79 ^c	323.43 ± 8.72 ^a	18.54 ± 0.84 ^b
PCP-Fe-H	7	143.57 ± 6.60 ^{ab}	7.17 ± 0.38 ^a	43.87 ± 2.69 ^{ab}	59.61 ± 1.90 ^{ab}	19.67 ± 0.66 ^a	329.86 ± 7.47 ^a	15.44 ± 0.80 ^{cd}
PCP-Fe-M	7	141.14 ± 5.21 ^{ab}	7.07 ± 0.49 ^a	42.64 ± 2.93 ^{ab}	57.71 ± 2.29 ^{bc}	18.47 ± 0.64 ^b	324.57 ± 7.41 ^a	16.63 ± 0.72 ^c
PCP-Fe-L	7	135.29 ± 6.18 ^b	7.01 ± 0.24 ^a	40.49 ± 2.19 ^b	55.04 ± 2.47 ^c	17.53 ± 0.50 ^{bc}	322.86 ± 2.79 ^a	18.40 ± 0.55 ^b

Different letters (a–d) showed significant difference ($p < 0.05$).

FeSO₄ groups. In contrast, the Control and each PSP-Fe groups had intact colonic villi with abundant numbers of goblet cell.

3.3 High-throughput sequencing analysis of the gut microbiota

3.3.1 Endemic species analysis

Linear discriminant effect size (LEfSe) analysis was used to identify microbial markers that were significant in the Model group (Figure 4). Ten statistically significant microbial markers were finally identified (Figure 5). *Alloprevotella* sp004555055, *Bacteroides zhangwenhongii*, *Bacteroides thetaiotaomicron*, *Bacteroides clarus*, *Barnesiella intestinihominis*, *Barnesiella viscericola*, *Bilophila wadsworthia*, *Clostridium ramosum*, *Desulfovibrio piger*, and *Parabacteroides merdae* had a significantly higher relative abundance in the Model group than in the other groups ($p < 0.05$). Currently, there are no reported associations between these microbial markers and IDA.

3.3.2 Metabolic pathway enrichment analysis

The metabolic pathway enrichment analysis has shown in Figure 6A. The biosynthesis of siderophore group nonribosomal peptides, vitamin B6 metabolism, lipoic acid metabolism, fatty acid degradation, α -linolenic acid metabolism, ascorbate metabolism and tryptophan metabolism were all significantly upregulated in the Model group compared to the other groups ($p < 0.05$).

The Spearman correlation analysis between the 10 microbial markers and metabolic pathways has shown in Figure 6B. All microbial markers were significantly positively correlated with the biosynthesis of siderophore group nonribosomal peptides, VB6 metabolism, and lipoic acid metabolism. All microbial markers showed a significant positive correlation with ascorbate metabolism, except *P. merdae*; and showed a significant positive correlation with tryptophan metabolism, except *B. intestinihominis*, *D. piger*, and *P. merdae*. In addition, all microbial markers were significantly positively correlated with α -linolenic acid metabolism, and six microbial markers (*A. sp004555055*, *B. zhangwenhongii*, *B. viscericola*, *B. wadsworthia*, *C. ramosum*, and *P. merdae*) were significantly positively correlated with fatty acid degradation.

3.4 Toxicity evaluation of PSP-Fe *in vitro*

Figure 7 has shown the cell viability of HK-2 treated with different concentrations of PSP-Fe. The cell viability exceeded when the Fe

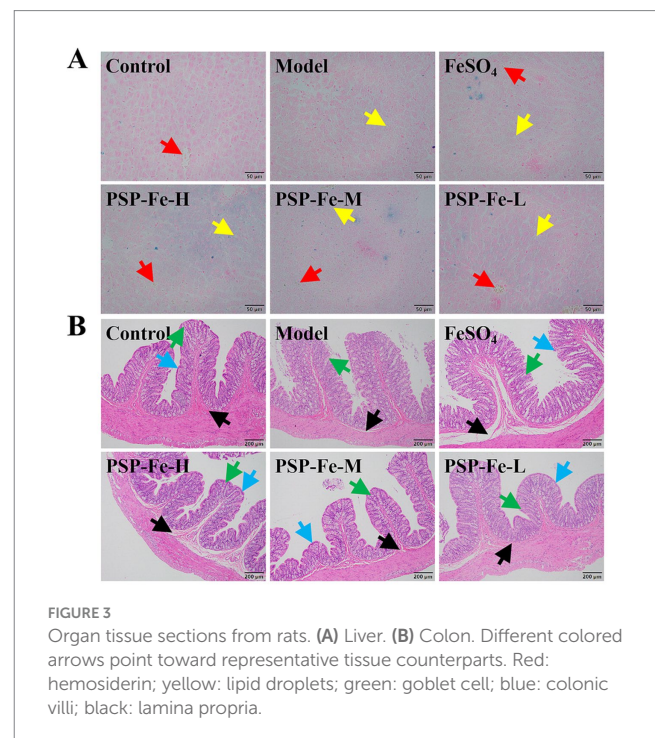


FIGURE 3

Organ tissue sections from rats. (A) Liver. (B) Colon. Different colored arrows point toward representative tissue counterparts. Red: hemolysis; yellow: lipid droplets; green: goblet cell; blue: colonic villi; black: lamina propria.

content was within 250 $\mu\text{g/mL}$. This results indicated that PSP-Fe had no significant toxicity *in vitro*.

3.5 Toxicity evaluation of PSP-Fe *in vivo*

Figures 8A,B have shown the change of the body weight of the rats in each group during the 28-day experiment. There were no significant differences ($p > 0.05$) in the final body weight and weight gain between the groups. Figures 8C–G demonstrated the organ coefficients of rats in each group. The heart coefficient, liver coefficient, spleen coefficient, lungs coefficient and kidney coefficient have no significant differences ($p > 0.05$) between the groups. Figures 8H–M have shown the hepatotoxicity (ALT and AST), nephrotoxicity (BUN and CR) and antioxidant capacity (SOD and GSH) of rats. There were no significant differences ($p > 0.05$) in these indicators between the groups. H&E staining of the heart, liver, spleen, lungs and kidney in each group of rats have shown in Figure 9. Histological observations of heart, liver, spleen, lungs, and kidney did not reveal significant differences. These results suggested that PSP-Fe had no obvious toxicity *in vivo*.

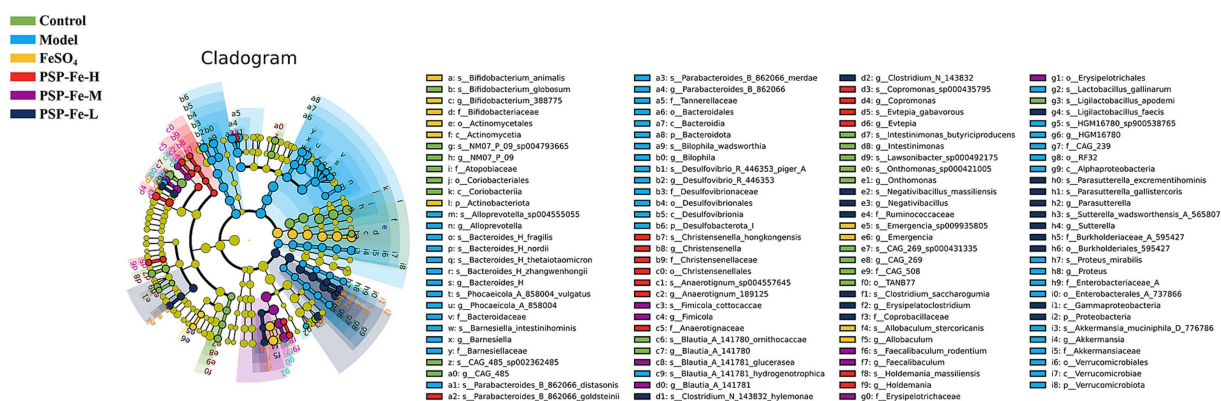


FIGURE 4
LefSe analysis (LDA significant threshold >2.0).

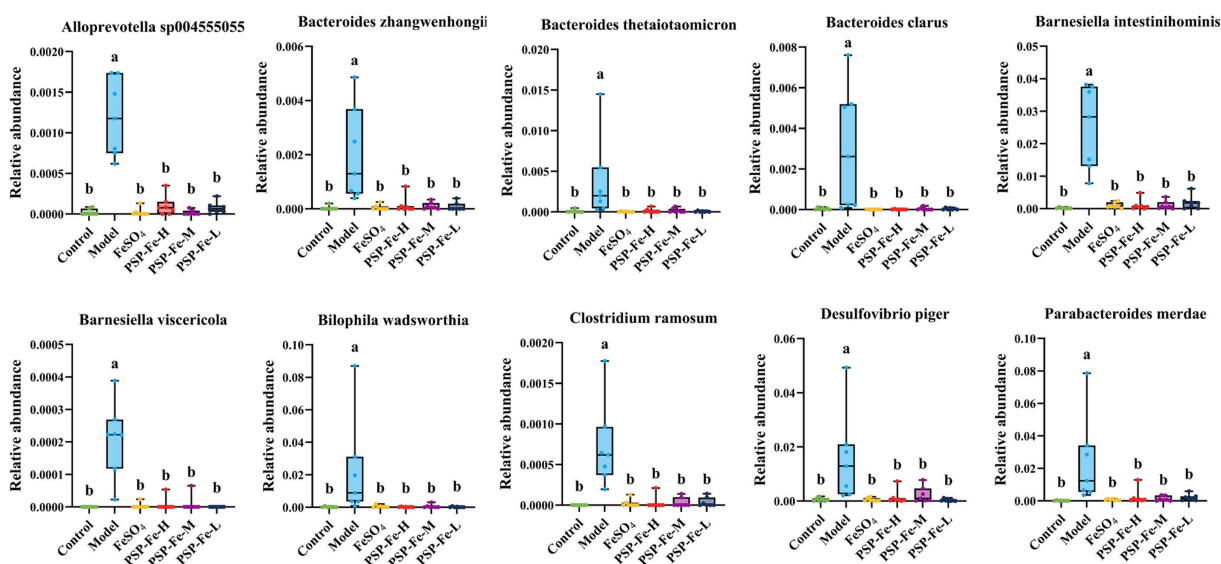


FIGURE 5
Ten statistically significant microbial markers in the Model group. Different letters (a,b) showed significant difference ($p < 0.05$).

4 Discussion

IDA usually adversely affects the normal growth of the organism. In our study, rats in the Model group showed decreased activity, light brown feces, and slightly pale pupils, ears, and paws compared to the Control group. Importantly, IDA can also cause slow weight gain. Nevertheless, after PSP-Fe treatment, the slow weight gain of the rats was alleviated, pale pupils, ears and paws became reddish, and feces change from brown to black. This indicated that PSP-Fe could alleviate the low weight caused by IDA and had a positive effect on the normal growth of rats.

One of the manifestations of IDA is significantly lower levels of HGB, RBC, HCT, SF, SI, hepcidin, and TSAT in the body. SF is the most sensitive indicator for the clinical diagnosis of IDA (37). Low levels of ferritin are only seen in patients suffering from IDA, no other possible pathology would confound this finding. Other indicators used to supplement the diagnosis may be SI, hepcidin and TSAT. SI is

reduced in absolute ID as well as in functional ID or inflammatory conditions. Hepcidin is a peptide hormone produced in the liver that negatively regulates the entry of iron into the bloodstream. The synthesis of peptide hormones is regulated at the transcriptional level controlled by serum iron concentration. Hepcidin expression is upregulated when SI levels are elevated. Hepcidin triggers tyrosine phosphorylation, internalization, and ubiquitin mediated degradation in lysosomes by binding to the ferroportin 1 (FPN1) in tissue duodenal enterocytes, liver cells, Kupffer cells, and other target cells (38). After removing the ferroportin FPN1 from the plasma membrane, the cellular iron output is shut off, resulting in a decrease in SI levels. In addition, high concentrations of iron in the liver, inflammation, and physical activity can also upregulate hepcidin expression, while iron deficiency, erythropoiesis, hypoxia, and endocrine signals (testosterone, estrogen, and growth factors) can downregulate hepcidin expression (8, 39). TSAT is a major indicator and determinant of systemic iron homeostasis. The TSAT is

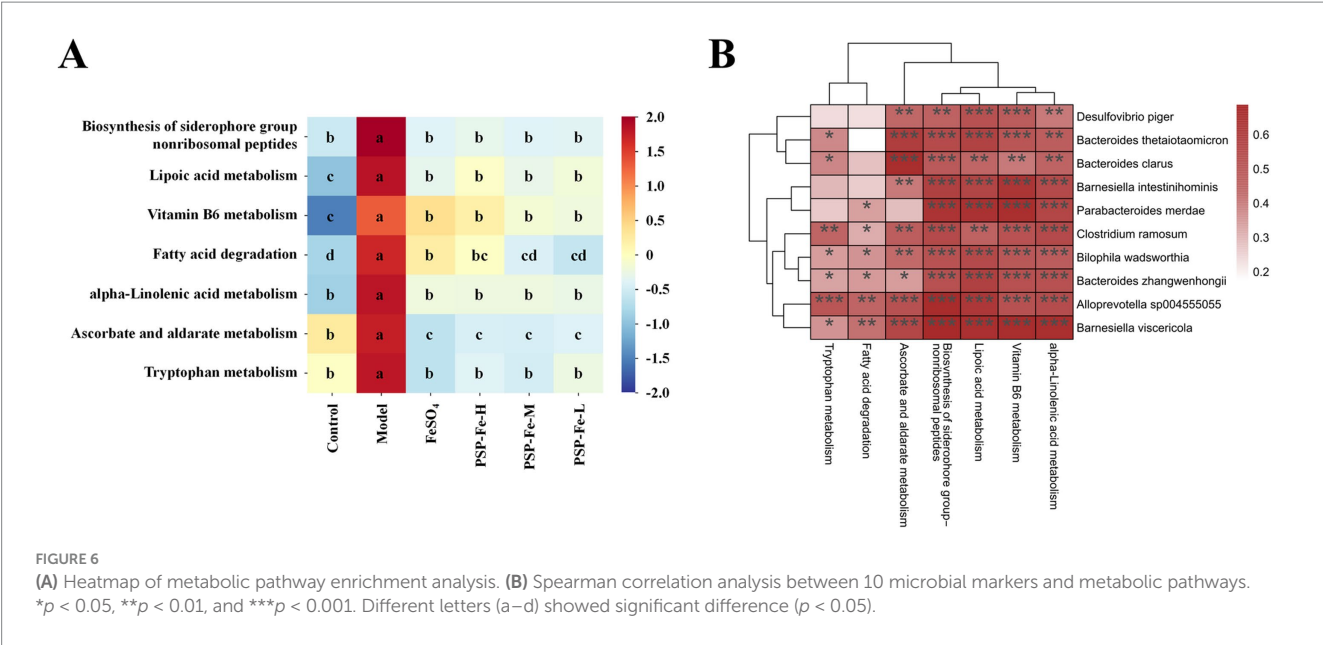


FIGURE 6 (A) Heatmap of metabolic pathway enrichment analysis. (B) Spearman correlation analysis between 10 microbial markers and metabolic pathways. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Different letters (a–d) showed significant difference ($p < 0.05$).

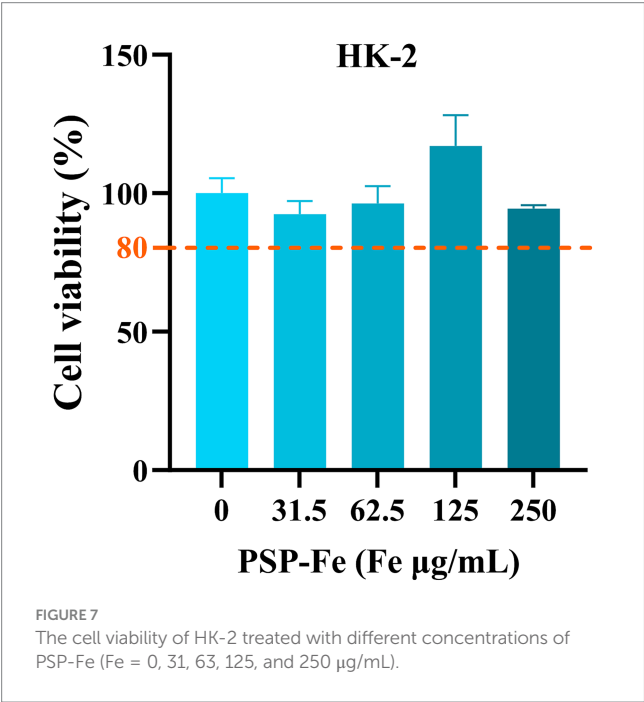


FIGURE 7 The cell viability of HK-2 treated with different concentrations of PSP-Fe (Fe = 0, 31, 63, 125, and 250 $\mu\text{g/mL}$).

determined by the amount of iron absorbed from the intestine, recycled and released by macrophages, and used for erythropoiesis. The organ coefficients could reflect, to some extent, the damage and repair effects of the disease or drugs on organs. The heart coefficient, lungs coefficient, and spleen coefficient were significantly higher in the Model group than in the Control and different iron supplementation groups. This may be due to the decrease in the concentration of erythrocytes and HGB in the blood; to meet the body's normal oxygen demand, compensatory hypertrophy and hyperplasia of the heart and lungs are required to enhance the ability to pump blood and exchange oxygen at the alveoli. This is consistent with the findings of Wang et al. (40). Kuvibidila et al. (41) has demonstrated that ID activates splenocytes

and promotes cell proliferation. In addition, IDA was observed to cause damage to the liver. After treatment with PSP-Fe, the heart coefficient, lungs coefficient, spleen coefficient and liver coefficients were normalized. The effect of IDA on the kidney was not significant, at least on the organ coefficient. This is similar to the results of Song et al. in a study on the *Lachnum* YM226 melanin-iron complex (42). The liver is one of the most important tissues for iron storage. When SI is not used for erythropoiesis, iron will be stored in the liver for later use. Hepatic iron deposition can physiologically range between 300 mg and 1 g. For patients suffering from hereditary hemochromatosis, the hepatic iron deposition can reach up to 25–30 g (43). Due to iron depletion caused by IDA, it was difficult to observe hemosiderin deposition in liver sections in the Model group. After treatment with PSP-Fe, iron storage in the liver was significantly restored. In addition, lipid droplets were found in the liver of all groups except the Control group, suggesting steatosis, and relying solely on iron supplements does not seem to cure it in the short term. This is consistent with the results observed by Pan et al. (44). Sections of colon tissue were stained with H&E to further investigate the effects of IDA, FeSO_4 , and PSP-Fe on colon morphology. Compared with the Control group, the villi were damaged in the Model group. The impaired intestinal barrier may increase the chance of toxic substances, such as lipopolysaccharides and pathogenic bacteria, entering the tissue through the body's circulation, resulting in infection. After treatment with PSP-Fe, the intestinal villi were significantly repaired, and the overall structure was more intact. However, FeSO_4 -treated colon tissues were poorly repaired, and damage to the villi remained. Similar results were reported in a study on the Ejiao peptide-iron chelates (45). Under low iron conditions, some pathogenic bacteria will secrete siderophores to increase the efficiency of iron uptake from the external environment (46). Siderophores are low molecular weight compounds with a high binding affinity for ferric iron. Siderophores can be classified into three main families based on their characteristic functional group: catecholate, hydroxamate, and carboxylate (47). They are generally synthesized by the nonribosomal peptide

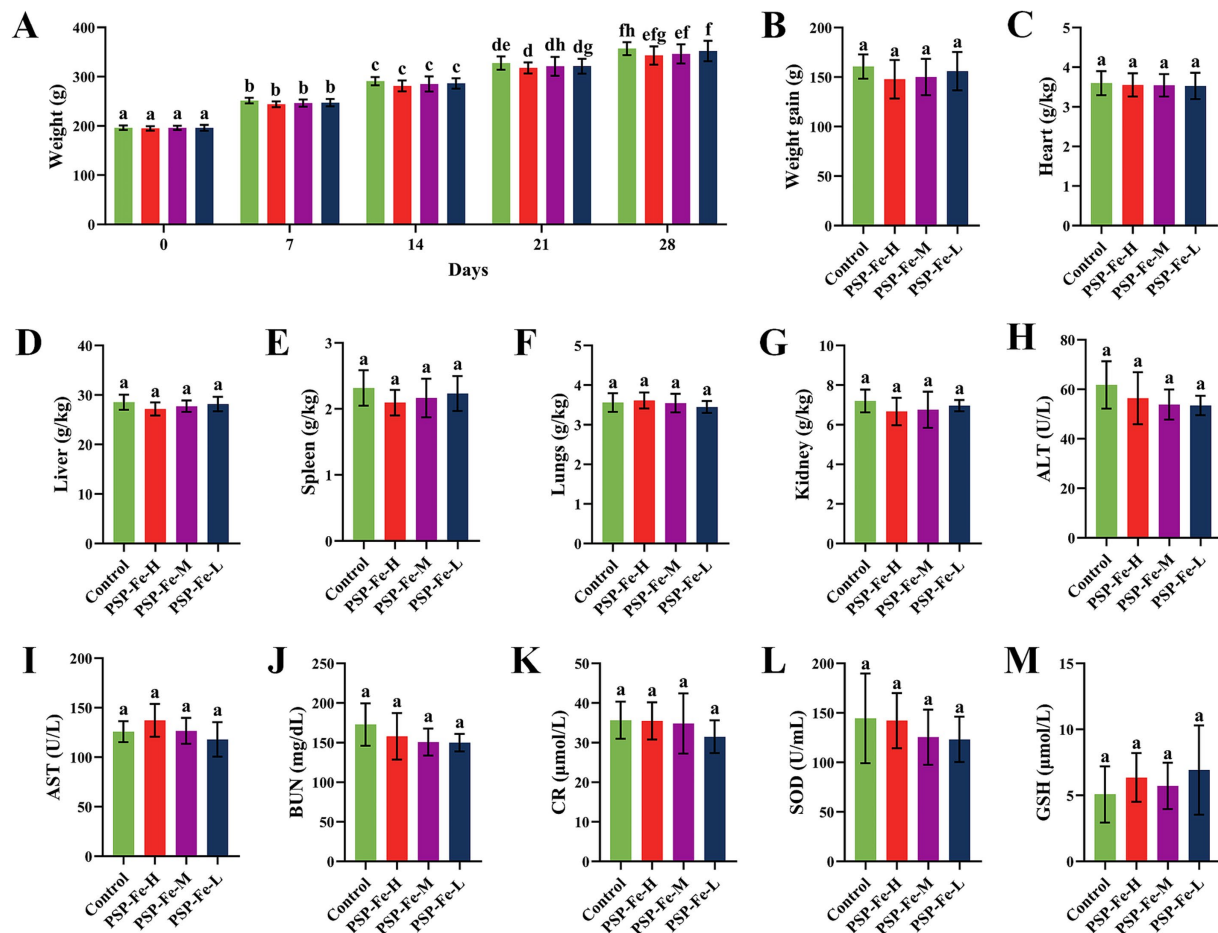


FIGURE 8

Body weight changes, organ coefficients and hematological indicators in rats fed a normal diet for toxicity evaluation. (A) The change of the body weight during the 28-day experiment. (B) Body weight gain. (C) Heart coefficient. (D) Liver coefficient. (E) Spleen coefficient. (F) Lungs coefficient. (G) Kidney coefficient. (H) Alanine aminotransferase. (I) Aspartate aminotransferase. (J) Blood urea nitrogen. (K) Creatinine. (L) Superoxide dismutase. (M) Glutathione. Different letters (a–f) showed significant difference ($p < 0.05$).

synthetase or polyketosomal synthetase domains and are secreted by the effervescent pump (48, 49). In the Model group, the biosynthesis of siderophore group nonribosomal peptides was significantly upregulated, suggesting that gut microbes compete with the host for iron sources. This may lead to the persistence of IDA.

Heme, which is involved in the composition of HGB, is synthesized primarily in the young erythrocytes and reticulocytes of the bone marrow. The basic raw materials for the synthesis of heme in the body are glycine, succinyl coenzyme A (succinyl-CoA), and Fe^{2+} . In the mitochondria, glycine, and succinyl-CoA are condensed to form δ -amino levulinic acid (ALA), which is catalyzed by ALA synthase. ALA synthase is the rate-limiting enzyme for the synthesis of heme. This reaction requires pyridoxal-5'-phosphate (PLP) as a coenzyme. Pyridoxal and PLP are the main forms of vitamin B6 (VB6) present in the body. In addition, succinyl-CoA is produced by oxidative removal of the carboxyl group from α -ketoglutaric acid by the oxoglutarate dehydrogenase complex, and lipoic acid is one of the essential coenzymes that make up the oxoglutarate dehydrogenase complex (50). VB6 metabolism and lipoic acid metabolism were significantly upregulated in the Model group, which may lead to reduced heme synthesis in the host, which further affects HGB synthesis.

According to previous reports, IDA can lead to lipid metabolism disorders (51–53). The fatty acid degradation and α -linolenic acid metabolism of the gut microbiota in the Model group were significantly upregulated compared to the Control group. Abnormalities in lipid metabolism were reversed after treatment with PSP-Fe. Iron solubility is also a limiting factor in iron absorption because iron ions precipitate readily when transitioning from the low pH environment of the stomach to the high pH environment of the duodenum. Ascorbic acid has reducing properties that allow it to form soluble iron complexes with iron ions to improve iron bioavailability and reduce the opportunity for siderophores to bind to ferric iron (54). However, ascorbate metabolism was upregulated in the Model group.

Interestingly, we found that tryptophan metabolism was also significantly higher in the Model group than in the Control group. Julian reported that in a cohort of 430 patients, 115 patients with ID had lower serum tryptophan levels than 315 patients without ID (55). As a nutritional pyrrole source, tryptophan is also essential for HGB synthesis. And there are six tryptophan residues that located in the α - and β -subunits of human HGB (56). Tryptophan availability limits HGB production, which may be one of the reasons for the positive correlation between HGB concentration and tryptophan. Weiss elucidated a possible process for degrading tryptophan under inflammatory conditions:

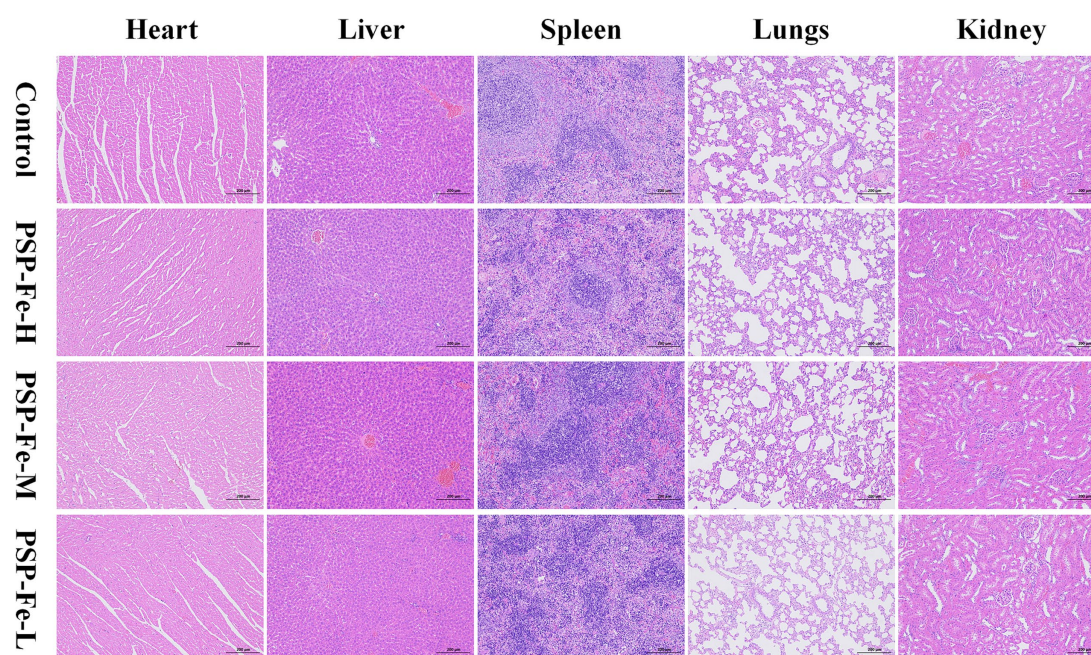


FIGURE 9
Organ tissue sections from rats for toxicity evaluation.

tryptophan is degraded by cytokine-induced (e.g., interferon- γ , tumor necrosis factor- α) indoleamine 2,3-dioxygenase to kynurenine, which affects erythroid progenitor cell growth and differentiation (57). Under inflammatory conditions, the catabolism of tryptophan to kynurenine is accelerated, resulting in a deficiency of this essential amino acid. Eleftheriadis et al. (58) suggests that kynurenine, by competing with hypoxia-inducible factor 2 α , may contribute to anemia of inflammation by decreasing erythropoietin and increasing hepcidin production.

Previous studies have shown that *B. thetaiotaomicron* does not produce siderophores (59). When pathogenic *Salmonella* was present *in vitro* or the inflammatory environment of the mouse intestine, *B. thetaiotaomicron* used siderophores produced by other species to grow under iron-restricted conditions, such as *Escherichia coli* Nissle 1917. Thus, the correlation analysis implied that these microbial markers may prey on iron through siderophores produced by themselves or other species, and also upregulate VB6 metabolism, lipoic acid metabolism, ascorbate metabolism, and tryptophan metabolism to impede HGB synthesis. They may be potential disease markers, therapeutic targets, or treatments for IDA; however, this requires further experimental validation. In addition, these microbial markers also showed significantly positively correlated with α -linolenic acid metabolism and fatty acid degradation, suggesting that they might also be involved in the regulation of lipid metabolism.

In hepatocytes, ALT is mainly distributed in the cytoplasm, while AST is distributed in both the mitochondria and the cytoplasm. When hepatocytes are damaged, AST and ALT are released into the circulatory system, resulting in elevated serum AST and ALT levels (60). BUN is the end product of protein metabolism in the body and is mainly produced by the liver. CR is a compound produced in the process of muscle energy production and is metabolized by creatine phosphate. BUN and CR are excreted in the urine through glomerular filtration, which reflects the renal function (61). As the antioxidant status indices, SOD and GSH are mainly involved in antioxidant processes in organisms (62).

5 Conclusion

In summary, the binding modes of PSP to iron were elucidated and the anti-IDA efficacy of PSP-Fe was also evaluated. The biosynthesis of siderophores, as well as the consume of VB6, lipoic acid, ascorbic acid and tryptophan may impede iron absorption and HGB synthesis of host. The low cost, easy availability, and high bioavailability of PSP-Fe make it a potential source of novel functional food for iron supplementation.

Data availability statement

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

Ethics statement

The animal study was approved by Animal Ethics Committee of Binzhou Medical University (Certificate of Approval No. 2023-047). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SH: Data curation, Investigation, Methodology, Writing – original draft. HD: Data curation, Investigation, Methodology, Writing – original draft. KL: Formal analysis, Validation, Visualization, Writing – original draft. XZ: Formal analysis, Validation, Visualization, Writing – original draft. JZ: Conceptualization, Funding acquisition, 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

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2025.1553668/full#supplementary-material>

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The association of dietary iron intake and serum iron with fecal incontinence: results from NHANES 2007–2010

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Background: The relationship between iron and fecal incontinence (FI) is unclear. This study aims to explore the association between iron intake and serum iron levels and FI subtypes.

Methods: 8,612 adults from the National Health and Nutrition Examination Survey 2007–2010 were included in the study. FI was determined by the Bowel Health Questionnaire. This study corrected for demographic characteristics, chronic diseases and so on.

Results: Compared to quartile 1, quartile 3 of dietary iron was associated with a higher risk of gas gut leakage (OR = 1.35, 95%CI:1.05–1.73), and quartile 4 of serum iron was associated with a lower risk of solid bowel leakage (OR = 0.42, 95%CI: 0.20–0.89). Restricted cubic spline (RCS) models showed an inverted U-shaped association between iron intake and the prevalence of gas gut leakage (P for nonlinear < 0.001). When iron intake is between 13.68 and 21.55 mg/day, the risk of gas gut leakage is significantly increased. However, serum iron was significantly negatively linearly correlated with solid stool leakage. Subgroup analysis suggested that there was heterogeneity in the association between iron and FI in terms of gender and age. The association is stronger in women and people aged 60 to 74 years. In exploratory analysis, higher ferritin levels in women of childbearing age were associated with a lower chance of mucus gut leakage.

Conclusion: Lower serum iron levels and moderate iron intake may be associated with an increased risk of FI in adults, with gender and age differences. Older women may need to increase their iron intake, which may be beneficial in preventing FI. However, the causal relationship still needs to be verified by prospective studies.

KEYWORDS

iron intake, serum iron, fecal incontinence, NHANES, ferritin

1 Introduction

Fecal incontinence (FI) is defined as the involuntary loss of solid or liquid feces, gases, or mucus (1, 2). It is a debilitating condition that significantly affects the quality of life. FI includes a range of symptoms, including passive leakage, emergency-related accidents, and incomplete evacuation (3, 4). According to epidemiological research, the

prevalence of FI in community adults varies between 1.4 and 19.5% worldwide, and it rises sharply with age (5–7). The risk is more than three times higher in people over the age of 65 than in younger people (5, 8). Despite the clinical and psychosocial burdens, FI continues to be underreported and undertreated due to stigma and diagnostic challenge (3). Although some progress has been made in interventions such as biofeedback therapy and sacral nerve stimulation, about 30 to 40% of patients do not respond well to existing treatments, and the long-term efficacy is not stable (1, 9). Recent high-impact reviews have highlighted the multiple factors of FI, including pelvic floor dysfunction, visceral hypersensitivity, and altered bowel motility (1, 3, 8). However, gaps remain in understanding modifiable nutritional effects.

Iron is an essential trace element in the human body, and moderate intake can promote intestinal cell growth, maintain immune and digestive functions, but excessive intake can cause oxidative damage and imbalance of microflora (10–13). According to previous studies, intestinal flora disorders or iron deficiency are linked to an imbalance in iron metabolism, which can lead to intestinal inflammation and even cancer (14–16). Notably, iron excess may affect bowel motility by causing diarrhea, which is a known risk factor for fecal incontinence (17, 18). These findings suggest a two-way relationship between iron and gut health, and further research is needed.

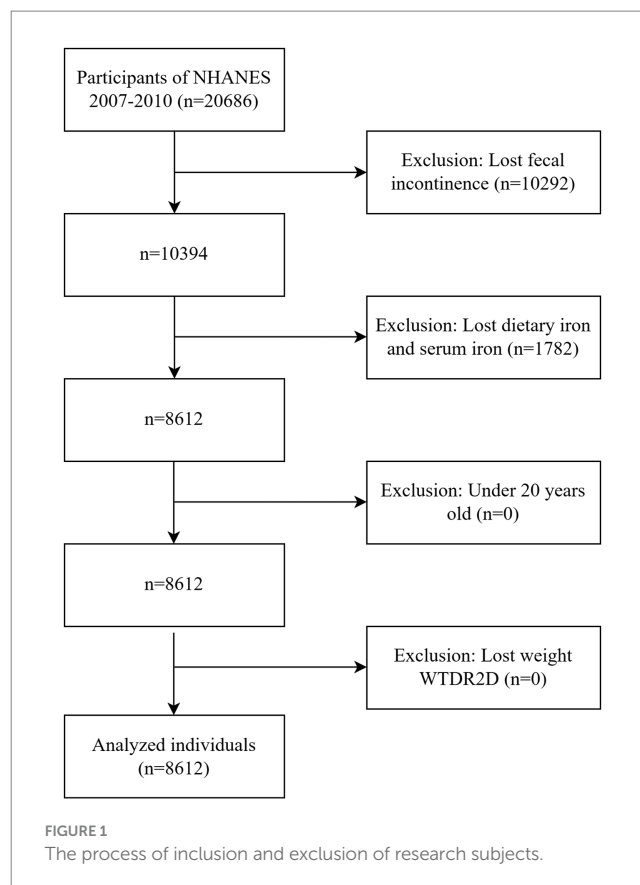
Dietary iron intake and serum iron work together to regulate iron homeostasis. Non-heme iron (plant source) and heme iron (animal source) exhibit different absorption efficiencies mediated by duodenal ferrotransporter expression under the control of the main iron-regulating hormone, hepcidin (19). Studies have found that high dietary iron intake may induce mucositis by inhibiting gut microbiota diversity (12). High serum iron levels appear to be associated with a reduced risk of colon cancer, but not significantly (20). Recent studies have found an association between unsafe diets, trace elements, and heavy metals in the blood and FI (21–24). However, the relationship of dietary iron and serum iron with FI risk has not been systematically studied to date.

Through a cross-sectional population cohort analysis of NHANES, this study investigates the connection between dietary iron intake, serum iron levels, and FI risk. The findings of this study suggest that dietary iron intake should be changed to prevent fecal incontinence. This can help identify high-risk individuals among those with abnormal iron metabolism. In addition, optimizing serum iron levels may be more beneficial for FI prophylaxis.

2 Materials and methods

2.1 Data sources and study populations

Publicly available data came from the National Health and Nutrition Survey (NHANES). Participants were invited to a Mobile Examination Centre (MEC) for a series of laboratory tests. Blood, urine, and other biological samples were collected as part of these tests. Laboratory data was collected by professionals in a controlled environment through standardized methods, ensuring data accuracy and consistency. The questionnaire was collected at the respondent's home through the computer-assisted personal interviewing (CAPI)



system by trained interviewers. They all voluntarily signed a written agreement. The data was available on the CDC website.¹

The collection of the Bowel Health Questionnaire (which includes questions related to FI) was first implemented in the 2005–2006 cycle and stopped in the 2009–2010 cycle. The 2007–2010 cycle updated the experimental assessment method for serum iron. In order to reduce measurement variability, we selected the 2007–2010 period for data analysis. NHANES 2007–2010 had a total of 20,686 participants. The study included individuals aged ≥ 20 years who had complete dietary iron intake (24-h dietary recall), serum iron data, and the Fecal Incontinence Questionnaire (via the Digestive Health module). The data were weighted according to the sampling weights provided by NHANES. Participants with missing weights were excluded. A total of 8,612 participants were included, 4,184 males and 4,428 females. Figure 1 depicts a flowchart of the inclusion process.

2.2 Fecal incontinence

Fecal incontinence (FI) includes gas leakage, mucus leakage, liquid leakage, and solid stool bowel leakage. We collected information about FI through the Bowel Health Questionnaire. In this study, participants who had 1 to 3 or more uncontrolled bowel leakage of gas (mucus/liquid/solid stool) from the intestine in a month were inferred to have a gas (mucus/liquid/solid stool) intestinal leakage. Similarly, the mucus method had been widely used in previous studies (25–27).

¹ <https://wwwn.cdc.gov/nchs/nhanes/>

2.3 Dietary iron and serum iron

NHANES' dietary data collected detailed dietary intake of participants on the first and second days, respectively. Estimate the amount of energy and nutrients ingested in them by asking about the type and amount of food and drink consumed the day before. Collected through two 24-h food recall interviews. The first interview was conducted at the Mobile Examination Center (MEC), and the second interview was conducted by telephone 3–10 days later. Dietary iron intake was based on the average (mg/day) of two 24-h dietary recalls. Serum iron data were measured by the DcX800 method, a timed endpoint method. Detailed measurement procedures could be found on the NHANES website.

2.4 Covariates

Confounding factors that may be linked to FI were included in this study based on prior research. Age, gender, race (Non-Hispanic White, Non-Hispanic Black, and others), level of education (below college, college or above), household income-to-poverty ratio (PIR), and body mass index (BMI) are examples of demographic variables. Overweight is defined as a BMI < 25 kg/m², obesity is defined as a BMI ≥ 30 kg/m², with a BMI of 25–30 kg/m² being normal. Depression, diabetes, hypertension, and lifestyle factors like alcohol and cigarette use were also included. Depression is measured by the Patient Health Questionnaire-9 (PHQ-9). A PHQ-9 score of more than 10 is thought to be significantly associated with depressive status (28). Individuals meeting any of the following criteria were diagnosed with diabetes: (1) physician-confirmed diagnosis; (2) current therapeutic regimen involving oral hypoglycemic agents or exogenous insulin administration; or (3) biochemical evidence demonstrated by either glycated hemoglobin (HbA1c) levels ≥ 6.5% or fasting plasma glucose concentrations ≥ 126 mg/dL (7.0 mmol/L). Participants were divided into recent smokers, former smokers and never smokers based on whether they had a history of smoking and whether they were smoking now. Alcohol use was determined by whether people had drunk at least 12 glasses of alcohol in their lives. The definitions of each variable are detailed in [Supplementary Table S1](#).

2.5 Statistical analysis

Weighted mean ±SD, frequency, and weighted percentages were used to describe quantitative and categorical variables, respectively. Logistic regression models were established to analyze the relationship between dietary iron and serum iron and FI (gas gut leakage, mucus gut leakage, liquid intestinal leakage, solid intestinal leakage). In addition, with cut-off points based on the distribution in participants, the levels of dietary and serum iron were stratified into quartiles (Q1, <25th; Q2, 25th–50th; Q3, 50th–75th; Q4, >75th percentile). The Model 1 adjusted for age and gender; Model 2 adjusted for race, education, and PIR from Model 1; Model 3 incorporated BMI, smoking, alcohol consumption, high blood pressure, diabetes, and depression on top of Model 2. *p* values below 0.05 were regarded as statistically significant. *P* for trend less than 0.05 was considered to have a significant trend. To further verify whether there was a dose–response relationship between iron and FI, we used a restricted cubic

spline (RCS) to fit a nonlinear relationship. Three nodes (5th, 50th, 95th) were set up, and the OR value and 95% CI were calculated with the median as the reference value. A likelihood ratio test (*P* for nonlinear) of less than 0.05 was considered to have a nonlinear relationship. The above data analysis was performed using R software. Finally, subgroup analysis was used to stratify by gender, age, BMI, etc., to further explore the association heterogeneity between iron and FI. To gain a more complete understanding of the relationship of iron status with FI, we explored the association of other biomarkers of iron homeostasis (ferritin and transferrin receptors) with FI in a subgroup of women of childbearing age.

All analysis incorporated NHANES sampling weights to ensure national representativity. Confounding factors—including age, sex, race/ethnicity, household income, and chronic diseases (diabetes, IBD)—were adjusted for in multivariable logistic regression models rather than excluding participants.

3 Results

3.1 Baseline characters of participants

A total of 8,612 participants were included in this study from the NHANES 2007–2008 and 2009–2010 cycles. Baseline characteristics of participants are presented in [Table 1](#). Among them, 3,740 people were diagnosed with gas gut leakage, 286 people with mucus gut leakage, 600 people with liquid gut leakage, and 206 people with solid fecal gut leakage. Participants with leaky gut tended to be older than those who did not. According to the age division of the WHO, the average age of participants with solid stool leakage was 60.97 years (in the elderly). The average age of the other types of leaky gut patients was between 45 and 69 years (middle-aged). There were statistically significant differences in age, ethnicity, education level, and serum iron concentration between the solid and non-solid stool leakage groups (all *p* < 0.05). The serum iron concentrations and iron intake of the gaseous, mucous, liquid, and non-leaky groups did not differ significantly. Patients with bowel leakage tended to be middle-aged and older women, non-Mexican-white, and suffer from high blood pressure, diabetes, and depression to a large extent.

3.2 Relationship between dietary iron, serum iron, and bowel leakage

The results of the logistic regression for leaky gut, serum iron, and dietary iron are displayed in [Table 2](#). The findings indicated that bowel leakage was related to dietary iron intake (*p* < 0.05). A 35% higher risk of gaseous gut leakage was linked to dietary iron in the third quartile in fully corrected model 3 than to the first quartile (95% CI: 1.05–1.73, *p* = 0.02). A positive correlation between dietary iron intake and the probability of bowel leakage was also observed in the second and fourth quartiles, but no statistical difference was found (*p* > 0.05). There was no significant trend between dietary iron and bowel leakage (*P* for trend > 0.05).

As shown in [Table 2](#), serum iron concentrations are inversely correlated with solid fecal leakage. In comparison to the first quartile, the fourth quartile of serum iron was linked to a 58% (model 1), 57% (model 2), and 58% (model 3) decreased risk of leaky bowel. The trend

TABLE 1 The basic characteristics of the participants in NHANES 2007–2008, 2009–2010.

Characteristic	Bowel leakage of gas			Bowel leakage of mucus			Bowel leakage of liquid			Bowel leakage of solid stool		
	No (N = 4,872)	Yes (N = 3,740)	<i>p</i>	No (N = 8,326)	Yes (N = 286)	<i>p</i>	No (N = 8,012)	Yes (N = 600)	<i>p</i>	No (N = 8,406)	Yes (N = 206)	<i>p</i>
Age [Mean (SD)]	45.62 (16.46)	48.83 (16.51)	<0.001	46.82 (16.55)	54.50 (14.95)	<0.001	46.41 (16.48)	57.09 (14.49)	<0.001	46.78 (16.47)	60.97 (15.00)	<0.001
Gender, <i>n</i> (%)			0.001			0.251			0.031			0.107
Male	2,501 (50.5%)	1,683 (44.0%)		4,067 (47.8%)	117 (43.5%)		3,924 (48.1%)	260 (40.8%)		4,104 (47.9%)	80 (37.1%)	
Female	2,371 (49.5%)	2,057 (56.0%)		4,259 (52.2%)	169 (56.5%)		4,088 (51.9%)	340 (59.2%)		4,302 (52.1%)	126 (62.9%)	
Race, <i>n</i> (%)			<0.001			0.527			<0.001			0.035
Non-Hispanic White	2,274 (67.4%)	2,095 (75.1%)		4,220 (70.8%)	149 (70.1%)		3,994 (70.3%)	375 (79.7%)		4,246 (70.7%)	123 (78.5%)	
Non-Hispanic Black	995 (12.1%)	523 (8.4%)		1,483 (10.5%)	35 (7.6%)		1,412 (10.5%)	106 (10.0%)		1,484 (10.4%)	34 (11.2%)	
Other	1,603 (20.5%)	1,122 (16.5%)		2,623 (18.6%)	102 (22.3%)		2,606 (19.2%)	119 (10.3%)		2,676 (18.9%)	49 (10.4%)	
Education, <i>n</i> (%)			0.696			0.569			0.749			0.006
Below college	2,520 (42.5%)	1,914 (42.0%)		4,267 (42.2%)	167 (45.3%)		4,116 (42.3%)	318 (41.2%)		4,308 (42.0%)	126 (57.5%)	
College or above	2,352 (57.5%)	1,826 (58.0%)		4,059 (57.8%)	119 (54.7%)		3,896 (57.7%)	282 (58.8%)		4,098 (58.0%)	80 (42.5%)	
PIR, <i>n</i> (%)			0.440			0.261			0.814			0.223
Not poor	2,746 (67.8%)	2,159 (68.8%)		4,756 (68.4%)	149 (62.1%)		4,585 (68.3%)	320 (67.7%)		4,798 (68.3%)	107 (63.5%)	
Poor	2,126 (32.2%)	1,581 (31.2%)		3,570 (31.6%)	137 (37.9%)		3,427 (31.7%)	280 (32.3%)		3,608 (31.7%)	99 (36.5%)	
Body mass index, <i>n</i> (%)			0.051			0.447			0.004			0.938
Normal	1,400 (32.2%)	1,006 (28.5%)		2,330 (30.5%)	76 (31.9%)		2,266 (31.0%)	140 (23.3%)		2,337 (30.5%)	69 (31.6%)	
Obese	1,787 (34.2%)	1,462 (37.4%)		3,130 (35.5%)	119 (39.7%)		2,983 (35.0%)	266 (45.8%)		3,177 (35.6%)	72 (35.4%)	
Overweight	1,685 (33.6%)	1,272 (34.1%)		2,866 (34.0%)	91 (28.4%)		2,763 (34.0%)	194 (30.9%)		2,892 (33.8%)	65 (33.0%)	
Smoke, <i>n</i> (%)			0.556			0.383			0.213			0.049
Former smoker	1,253 (25.3%)	995 (25.4%)		2,161 (25.2%)	87 (28.8%)		2,046 (25.0%)	202 (30.2%)		2,169 (25.1%)	79 (36.4%)	
Never smoker	2,577 (53.6%)	1,984 (54.6%)		4,428 (54.3%)	133 (46.7%)		4,302 (54.4%)	259 (49.0%)		4,471 (54.3%)	90 (42.4%)	
Recent smoker	1,042 (21.1%)	761 (20.0%)		1,737 (20.5%)	66 (24.5%)		1,664 (20.6%)	139 (20.8%)		1,766 (20.6%)	37 (21.2%)	
Alcohol, <i>n</i> (%)	3,524 (76.9%)	2,690 (76.7%)	0.853	6,011 (76.9%)	203 (76.9%)	1.000	5,782 (77.0%)	432 (75.1%)	0.450	6,082 (77.1%)	132 (66.4%)	0.047
Diabetes, <i>n</i> (%)	773 (11.0%)	688 (12.8%)	0.029	1,386 (11.6%)	75 (21.1%)	<0.001	1,300 (11.3%)	161 (19.8%)	<0.001	1,400 (11.6%)	64 (22.5%)	0.001
Hypertension, <i>n</i> (%)	1,648 (26.9%)	1,447 (33.2%)	<0.001	2,961 (29.3%)	134 (43.5%)	0.001	2,768 (28.6%)	327 (46.8%)	<0.001	2,981 (29.2%)	114 (56.4%)	<0.001
Depression, <i>n</i> (%)	359 (6.2%)	451 (10.2%)	<0.001	744 (7.4%)	66 (26.3%)	<0.001	681 (7.2%)	129 (20.8%)	<0.001	773 (7.9%)	37 (13.1%)	0.029
Dietary iron [Mean (SD)]	15.70 (8.12)	15.37 (7.25)	0.219	15.59 (7.77)	14.46 (6.92)	0.083	15.58 (7.77)	15.05 (7.40)	0.071	15.56 (7.75)	15.06 (7.60)	0.443
Serum iron [Mean (SD)]	15.63 (6.47)	15.31 (6.23)	0.102	15.49 (6.39)	15.35 (5.62)	0.742	15.52 (6.41)	15.03 (5.66)	0.222	15.52 (6.37)	13.84 (5.96)	0.026

TABLE 2 Association of bowel leakage with iron intake and serum iron.

Item	Model 1	Model 2	Model 3
	OR (95%CI) <i>p</i> -value	OR (95%CI) <i>p</i> -value	OR (95%CI) <i>p</i> -value
Bowel leakage of gas			
Iron intake (mg/day)			
Q1 (<9.95)	Ref	Ref	Ref
Q2 (9.95–13.45)	1.08 [0.87, 1.34] 0.491	1.07 [0.86, 1.32] 0.526	1.09 [0.87, 1.37] 0.414
Q3 (13.45–18.36)	1.35 [1.06, 1.73] 0.018	1.33 [1.05, 1.68] 0.022	1.35 [1.05, 1.73] 0.021
Q4 (>18.36)	1.08 [0.87, 1.36] 0.461	1.05 [0.85, 1.31] 0.627	1.08 [0.86, 1.37] 0.476
<i>P</i> for trend	0.191	0.295	0.226
Serum iron (umol/L)			
Q1 (<11.1)	Ref	Ref	Ref
Q2 (11.1–14.5)	0.94 [0.78, 1.12] 0.443	0.91 [0.76, 1.09] 0.294	0.92 [0.76, 1.12] 0.379
Q3 (14.5–18.8)	0.98 [0.84, 1.15] 0.777	0.95 [0.81, 1.11] 0.480	0.97 [0.82, 1.13] 0.649
Q4 (>18.8)	0.90 [0.75, 1.09] 0.273	0.86 [0.71, 1.05] 0.127	0.89 [0.73, 1.08] 0.224
<i>P</i> for trend	0.331	0.147	0.262
Bowel leakage of mucus			
Iron intake (mg/day)			
Q1 (<9.95)	Ref	Ref	Ref
Q2 (9.95–13.45)	1.17 [0.70, 1.94] 0.535	1.20 [0.73, 1.96] 0.458	1.33 [0.79, 2.26] 0.260
Q3 (13.45–18.36)	1.33 [0.73, 2.42] 0.342	1.37 [0.74, 2.55] 0.296	1.59 [0.83, 3.04] 0.146
Q4 (>18.36)	0.69 [0.37, 1.29] 0.234	0.72 [0.38, 1.34] 0.284	0.83 [0.44, 1.59] 0.552
<i>P</i> for trend	0.394	0.481	0.854
Serum iron (umol/L)			
Q1 (<11.1)	Ref	Ref	Ref
Q2 (11.1–14.5)	1.58 [0.94, 2.67] 0.082	1.60 [0.95, 2.71] 0.077	1.74 [1.01, 2.99] 0.045
Q3 (14.5–18.8)	1.40 [0.86, 2.29] 0.172	1.41 [0.86, 2.32] 0.169	1.50 [0.90, 2.50] 0.111
Q4 (>18.8)	1.29 [0.78, 2.13] 0.303	1.31 [0.79, 2.15] 0.279	1.39 [0.85, 2.28] 0.178
<i>P</i> for trend	0.443	0.420	0.290
Bowel leakage of liquid			
Iron intake (mg/day)			
Q1 (<9.95)	Ref	Ref	Ref
Q2 (9.95–13.45)	1.12 [0.73, 1.72] 0.592	1.13 [0.73, 1.75] 0.566	1.23 [0.77, 1.96] 0.364
Q3 (13.45–18.36)	1.36 [0.94, 1.96] 0.097	1.36 [0.93, 1.99] 0.106	1.51 [1.00, 2.26] 0.049
Q4 (>18.36)	0.97 [0.65, 1.42] 0.854	0.96 [0.64, 1.44] 0.843	1.09 [0.71, 1.68] 0.681
<i>P</i> for trend	0.779	0.824	0.353
Serum iron (umol/L)			
Q1 (<11.1)	Ref	Ref	Ref
Q2 (11.1–14.5)	1.08 [0.77, 1.51] 0.653	1.07 [0.77, 1.51] 0.664	1.13 [0.79, 1.60] 0.473
Q3 (14.5–18.8)	1.13 [0.75, 1.71] 0.549	1.13 [0.74, 1.72] 0.563	1.22 [0.78, 1.89] 0.363
Q4 (>18.8)	0.93 [0.59, 1.47] 0.759	0.93 [0.58, 1.48] 0.747	1.03 [0.62, 1.70] 0.913
<i>P</i> for trend	0.840	0.823	0.810
Bowel leakage of solid stool			
Iron intake (mg/day)			
Q1 (<9.95)	Ref	Ref	Ref
Q2 (9.95–13.45)	0.95 [0.53, 1.72] 0.866	1.01 [0.55, 1.87] 0.961	1.07 [0.56, 2.05] 0.829

(Continued)

TABLE 2 (Continued)

Item	Model 1	Model 2	Model 3
	OR (95%CI) <i>p</i> -value	OR (95%CI) <i>p</i> -value	OR (95%CI) <i>p</i> -value
Q3 (13.45–18.36)	1.34 [0.66, 2.70] 0.407	1.44 [0.69, 3.01] 0.310	1.55 [0.73, 3.30] 0.233
Q4 (>18.36)	1.28 [0.71, 2.30] 0.398	1.40 [0.76, 2.56] 0.268	1.47 [0.79, 2.76] 0.208
<i>P</i> for trend	0.258	0.168	0.123
Serum iron (umol/L)			
Q1 (<11.1)			
Q2 (11.1–14.5)	0.66 [0.38, 1.16] 0.142	0.67 [0.38, 1.19] 0.161	0.70 [0.38, 1.29] 0.232
Q3 (14.5–18.8)	0.88 [0.58, 1.32] 0.516	0.91 [0.61, 1.36] 0.627	0.93 [0.61, 1.42] 0.703
Q4 (>18.8)	0.42 [0.20, 0.87] 0.021	0.43 [0.20, 0.91] 0.029	0.42 [0.20, 0.89] 0.027
<i>P</i> for trend	0.030	0.043	0.033

OR: Odds Ratio, CI: Confidence Interval; Bolded values indicate statistically significant associations ($P < 0.05$).

test was significant (P for trend < 0.05), suggesting that the risk of leaky stool was reduced by 58% for each quartile increase in serum.

3.3 Restricted cubic splines

Restricted cubic splines (RCS) were used to analyze the dose–response relationship between iron (dietary iron and serum iron) and leaky gut. As shown in [Figure 2a](#), the weighted RCS results showed an inverse U-shaped association between iron intake and gas FI (P for nonlinear < 0.001). The risk of gas FI increased when iron intake was between 13.68–21.55 mg/day. This parabolic downward trend suggests that high intake may have a protective effect. Conversely, serum iron concentrations were significantly negatively linearly (P for nonlinear = 0.180) correlated with the risk of solid stool leakage ([Figure 2b](#)). When the serum iron concentration exceeded 25umol/L, the comprehensive effect on solid stool leakage tended to be stable.

3.4 Subgroup analysis

3.4.1 Subgroup analysis of dietary and serum iron with FI

The association heterogeneity of iron, gas, and solid stool leakage in different sociodemographic groups was further analyzed. As shown in [Table 3](#), the association between dietary iron and bowel leakage was not significant in most subgroups. However, among non-Hispanic Black people, older women (60–74 years), and those with lower levels of education (below college), dietary iron was positively correlated with intestinal leakage risk. Moreover, the risk of solid bowel leakage was negatively correlated with serum iron levels in older adults, women, non-Hispanic White, those with low levels of education, those living in poverty, and those with a history of smoking, drinking, being overweight, or having high blood pressure. However, there was no statistically significant effect in the diabetic population.

3.4.2 Exploratory biomarker analysis

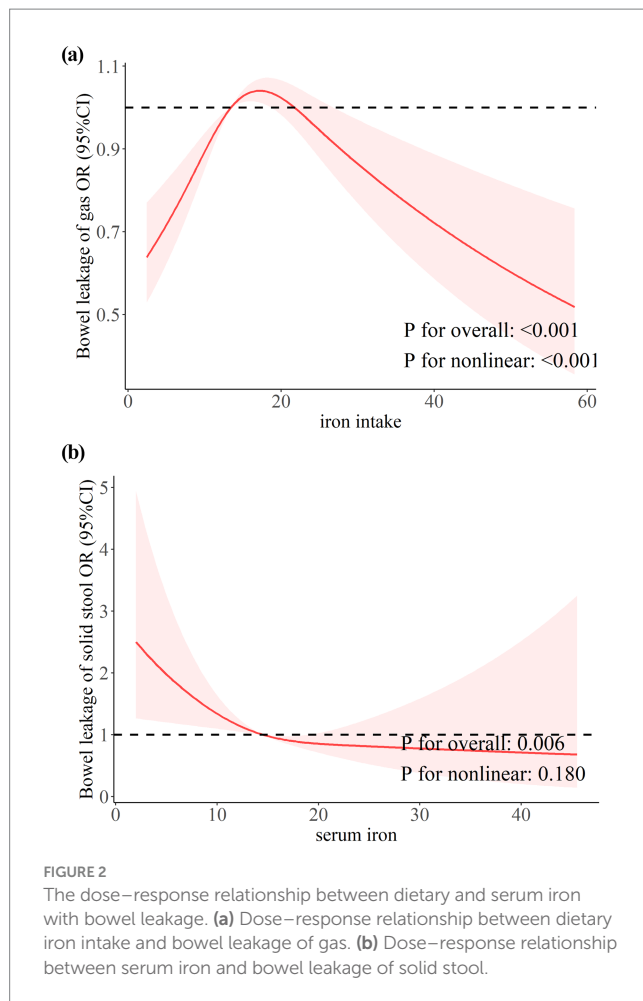
We attempted to explore the association of total iron-binding capacity and unsaturated iron-binding capacity with FI, but the NHANES 2007–2010 cycle did not include these data. Whereas,

NHANES mainly collects ferritin and transferrin receptor levels in women of childbearing age. Therefore, we performed additional analyses of serum ferritin and transferrin receptors in a subset of female participants aged 20–49 years ($n = 2,202$), as these biomarkers were only measured in participants aged 1–5 years, as well as in women aged 12–49 years, while participants who completed the bowel health questionnaire were all over 20 years of age. In this subgroup, the highest quartile of ferritin (OR = 0.30, 95% CI 0.11–0.83) was significantly associated with mucus FI after full adjustment ([Supplementary Table S2](#)). The results of the trend test (P for trend = 0.004) showed that the risk of mucus FI gradually decreased with the increase of ferritin levels.

4 Discussion

This is the first study to show that dietary iron and serum iron have a synergistic effect on FI using a nationally representative sample. Firstly, serum iron was negatively linearly associated with solid FI. Secondly, there was a nonlinear, inverted U-shaped relationship between iron intake and gas gut leakage. This association between iron and FI is most pronounced in older people, women, and groups with low levels of education. Lastly, in women of childbearing age between 20 and 49, ferritin levels were negatively correlated with mucus gut leakage. In addition, no association was found between iron and liquid gut leakage.

Our findings suggested that in the adult population, iron intake between 13.68 and 21.55 mg/day was associated with an increased risk of gas FI. At the same time, lower and higher doses did not show a statistically significant association with FI. Beneficial bacteria with low iron needs, such as *Lactobacilli* and *Bifidobacteria*, were more prevalent and could prevent pathogen colonization when iron intake is low (29–31). Thus, at lower levels, iron consumption did not raise the risk of FI. As iron intake increased, iron-loving pathogens such as *E. coli*, *Salmonella*, and *Heraldella* predominated, promoting intestinal inflammation and increasing diarrhea (32–34). Besides, diarrhea was a known risk factor for FI, and iron supplementation had also been shown to increase intestinal permeability (35, 36). Therefore, by promoting intestinal inflammation and raising intestinal permeability, higher doses of iron consumption raised the risk of FI. Higher intake of iron may have a protective effect against



FI. Several studies support our results. For example, a randomized controlled study of 53 Swedish infants aged 6 months reported a significantly higher abundance of beneficial bacteria in the intestines of infants in the high-iron group (6.6 mg Fe/day) compared with the low-iron group (1.2 mg Fe/day) (37). The iron intake of infants in both the low-iron group and the high-iron group exceeded the recommended intake (RDA) of 0.27 mg/d for infants aged 0–6 months stipulated by the National Institutes of Health (NIH) and the World Health Organization (WHO). A study of dietary nutrients in patients with FI and controls showed that iron intake was higher in the non-FI group. The iron intake in the FI group was $119 \pm 45\%$ of the RDA, while the iron intake in the control group was $128 \pm 51\%$ of the RDA (38). In addition, an animal study showed that high doses of iron can promote intestinal repair by modulating intestinal epithelial cell turnover and intestinal stem cell activity (39). According to a study by Sarah R. Bloor et al., too much iron intake damages intestinal villi and impairs the intestinal barrier, which in turn causes intestinal inflammation (40, 41). The latter, in turn, triggers the production and secretion of hepcidin, which lowers the levels of the divalent metal transporter 1 (DMT-1) and reduces iron absorption (41). Therefore, different levels of dietary iron intake may increase the risk of FI by altering the ratio of beneficial and pathogenic bacteria in the gut or altering intestinal permeability. High iron intake protected the intestines by upregulating hepcidin and promoting intestinal repair.

This appeared to challenge previous theories about the harmful effects of iron overload on the gut. In an animal experiment, for instance, mice fed a high-iron diet containing 1,000 mg per day for 70 days had a significant decrease in *Cldn8*, a marker of intestinal barrier integrity, which led to luminal bacterial leakage, a decrease in beneficial bacteria like *Tanner* and *Ekmania*, and a significant enrichment of pathogenic bacteria like *Streptococcus peptococcus* (42). In addition, the study by Tanja Jaeggi et al. reported that iron fortification (12.5 mg/day, WHO recommends 0.27 mg/day for infants) adversely affected the gut microbiome of Kenyan infants, increasing pathogen abundance and inducing intestinal inflammation (43). The contradiction between our study and previous research could be explained by the following points. A recent review of iron and gut microbiota showed that multiple studies reported different or even opposite results due to differences in the models and detection methods used – except for the *Lactobacillus* family, which always decreased during iron supplementation, the gut microbiota (*Bifidobacteria*, *Bacteroides*) of the other species showed different or even contradictory alterations (44). Therefore, the mechanism of iron's effect on the gut microbiota is complex and variable. Besides, while most of these studies were conducted through short, high-dose iron supplementation, our study was based on the daily iron intake of adults, which was much lower than the amount of iron in the experiments. In addition, different forms of iron supplementation had different effects on gut microbiota, and there were no studies on daily iron intake and gut microbiota (31).

Similarly, in this study, the majority of patients with FI were elderly and female, which was consistent with the results of previous studies (45, 46). This might be related to aging increasing colonic transit time and decreasing anal sphincter pressure (47, 48).

We found that higher serum iron levels were associated with a lower risk of solid FI. Prior research showed a negative correlation between serum iron and factors that are overexpressed in inflammatory and tumor situations, such as growth differentiation factor-15 (GDF-15) and IL-6 (49, 50). It has been reported that iron intake can increase serum iron levels, regulate goblet cell regeneration and mucin layer function, and play a positive role in the prevention of pathogenic bacteria (51). In addition, subgroup analysis showed that this association between serum iron and FI was more significant in women, older adults, poor (PIR < 2), smokers, alcohol users, and hypertension, but not in people with diabetes. These results suggested that the protective effect of serum iron might be co-regulated by aging-related metabolic remodeling, differences in the regulation of oxidative stress, and health behavior-nutrition interactions. Diabetes-related pathological mechanisms might negate its potential benefits.

Although our analysis of serum ferritin was limited to women of reproductive age, given the restrictions of NHANES data, our study demonstrated a significant inverse relationship between serum ferritin levels and mucus FI. A previous study showed that increased ferritin concentrations appeared to be associated with a reduced risk of colorectal cancer, but the results were not significant (20). In addition, systemic immune inflammation was significantly negatively correlated with serum ferritin (52). This seemed to support our results. However, some studies have pointed out that ferritin levels are negatively correlated with beneficial bacteria such as *Bifidobacteria* and lactic acid bacteria, but positively correlated with

TABLE 3 Subgroup analysis of dietary and serum iron with FI.

Variables	<i>n</i> (%)	Dietary iron and bowel leakage of gas			Serum iron and bowel leakage of solid stool		
		OR (95%CI)	<i>p</i>	<i>P</i> for interaction	OR (95%CI)	<i>p</i>	<i>P</i> for interaction
All patients	8,612 (100.00)	1.00 (1.00~1.01)	0.212		0.97 (0.95~1.00)	0.030	
Age				0.087			0.748
<45	3,422 (39.74)	1.00 (0.99~1.01)	0.887		0.99 (0.92~1.07)	0.835	
>74	970 (11.26)	1.02 (1.00~1.04)	0.064		0.97 (0.92~1.02)	0.260	
45–59	2,143 (24.88)	1.00 (0.99~1.01)	0.581		0.98 (0.94~1.03)	0.481	
60–74	2,077 (24.12)	1.01 (1.00~1.03)	0.047		0.95 (0.91~1.00)	0.039	
Gender				0.098			0.164
Female	4,428 (51.42)	1.01 (1.00~1.02)	0.049		0.95 (0.92~0.99)	0.012	
Male	4,184 (48.58)	1.00 (0.99~1.01)	0.886		0.99 (0.96~1.03)	0.647	
Race				0.055			0.074
Non-Hispanic Black	1,518 (17.63)	1.02 (1.00~1.03)	0.028		1.00 (0.94~1.07)	0.896	
Non-Hispanic White	4,369 (50.73)	1.00 (0.99~1.01)	0.962		0.95 (0.92~0.98)	0.004	
Other	2,725 (31.64)	1.00 (0.99~1.01)	0.488		1.00 (0.95~1.05)	0.913	
Education				0.077			0.450
Below college	4,434 (51.49)	1.01 (1.00~1.02)	0.024		0.96 (0.93~0.99)	0.024	
College or above	4,178 (48.51)	1.00 (0.99~1.01)	0.637		0.99 (0.95~1.03)	0.495	
PIR				0.660			0.168
Not poor	4,905 (56.96)	1.01 (1.00~1.01)	0.157		0.99 (0.95~1.02)	0.439	
Poor	3,707 (43.04)	1.00 (0.99~1.01)	0.754		0.95 (0.92~0.99)	0.019	
BMI				0.546			0.638
Normal	2,406 (27.94)	1.00 (0.99~1.01)	0.844		0.98 (0.94~1.02)	0.262	
Obese	3,249 (37.73)	1.00 (0.99~1.01)	0.432		0.99 (0.94~1.04)	0.655	
Overweight	2,957 (34.34)	1.01 (1.00~1.02)	0.092		0.95 (0.90~1.00)	0.041	
Alcohol				0.407			0.162
No	2,398 (27.84)	1.01 (1.00 ~ 1.02)	0.133		1.00 (0.95~1.04)	0.924	
Yes	6,214 (72.16)	1.00 (1.00 ~ 1.01)	0.541		0.96 (0.93~0.99)	0.011	
Smoke				0.629			0.568
Former smoker	2,248 (26.10)	1.01 (1.00~1.02)	0.094		0.95 (0.91~1.00)	0.037	
Never smoke	4,561 (52.96)	1.00 (0.99~1.01)	0.547		0.99 (0.95~1.04)	0.783	
Recent smoker	1,803 (20.94)	1.00 (0.99~1.01)	0.804		0.95 (0.90~1.01)	0.083	
Hypertension				0.407			0.248
No	5,517 (64.06)	1.00 (1.00~1.01)	0.517		0.99 (0.95~1.02)	0.520	
Yes	3,095 (35.94)	1.01 (1.00~1.02)	0.212		0.95 (0.92~0.99)	0.013	
Diabetes				0.165			0.468
No	7,151 (83.04)	1.00 (1.00~1.01)	0.513		0.97 (0.94~1.00)	0.025	
Yes	1,461 (16.96)	1.01 (1.00~1.03)	0.085		0.98 (0.93~1.03)	0.518	
Depression				0.876			0.421
No	7,802 (90.59)	1.00 (1.00~1.01)	0.252		0.98 (0.95~1.00)	0.095	
Yes	810 (9.41)	1.01 (0.99~1.02)	0.591		0.95 (0.88~1.01)	0.114	

OR, Odds ratio; CI, Confidence interval; Bolded values indicate statistically significant associations (*P* < 0.05).

conditionally pathogenic bacteria such as *Bacteroides* and *Prevotella* (53). The association of ferritin with FI might be related to gut microbiota and inflammation, but further study is required to validate this.

A study on the effects of iron intake on mice with Crohn's disease suggested that an iron-deficient diet might suppress intestinal inflammation (54), however, several studies showed that iron overload or iron deficiency might impair gut health by affecting the gut microbiota. In our research, the risk of gas FI increased when iron intake was 13.68–21.55 mg/day. Therefore, consuming less or more iron might be a better option for FI. Previous studies showed that iron deficiency was associated with intestinal diseases such as inflammatory bowel disease. In addition, the WHO stated that the maximum safe daily intake (UL) for iron was 45 mg/day, and exceeding this dose might lead to health risks. Therefore, we recommended consuming as much iron as possible at a safe dose, i.e., 22–45 mg/day. Besides, serum iron and ferritin were found to be inversely associated with solid/mucus FI. Higher iron intake increased the levels of serum iron and ferritin, along with a reduced risk of solid FI and mucus FI. Therefore, higher iron intake in safe doses might be beneficial in reducing the risk of FI.

There are several advantages to our research. Firstly, our study uses nationally representative data from NHANES and applies the weights recommended by NHANES to improve generalization and generalization of results. Besides, our study adjusts for confounding factors such as demographics, social factors, chronic diseases, etc. In addition, previous studies have focused on the linear effects of iron deficiency or iron overload, while our study further assess the dose-effect effect of iron on FI.

There are also limitations to our study. Firstly, underreporting of FI due to stigma may underestimate the true prevalence. Secondly, the cross-sectional design prevents causal inference. A combination of anal sphincter electromyography (EMG) and iron staining techniques may be needed in the future to verify a direct association between tissue iron and loss of function, or further prospective studies may be needed to illustrate this.

5 Conclusion

Iron intake of 13.68–21.55 mg/day nonlinearly increases gas FI risk, while higher serum iron linearly reduces solid FI. Associations are strongest in women and older adults. Optimizing iron balance—avoiding moderate dietary excess and ensuring serum sufficiency—may prevent FI, particularly in high-risk groups.

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 in the article/[Supplementary material](#).

Ethics statement

The Centers for Disease Control (CDC) approved NHANES program, to which all participants consented. All experimental

protocols were approved by the NCHS Ethics Review Committee (<https://www.cdc.gov/nchs/nhanes/irba98.htm>). 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

TL: Validation, Data curation, Formal analysis, Methodology, Conceptualization, Software, Writing – original draft. JC: Writing – original draft, Formal analysis, Methodology. YZ: Writing – original draft, Visualization, Validation. QY: Writing – original draft, Visualization, Investigation, Supervision. LC: Conceptualization, Writing – review & editing, Supervision, Resources, Project administration.

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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.

Generative AI statement

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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.2025.1598172/full#supplementary-material>

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Prevalence and determinants of anemia in chronic kidney disease patients in Ethiopia: a systematic review and meta-analysis

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Background: Chronic kidney disease (CKD) is a progressive and devastating condition affecting over 10% of the global population, with more than 800 million individuals impacted worldwide. Anemia, a critical and debilitating complication of CKD, accelerates disease progression and exacerbates its overall burden. Despite numerous studies exploring the prevalence and determinants of anemia in CKD in Ethiopia, the pooled prevalence remains undefined, highlighting a critical gap in evidence.

Objectives: To determine the pooled prevalence of anemia and identify its associated factors among CKD patients in Ethiopia through a systematic review and meta-analysis.

Methods: A comprehensive search was carried out for studies with full document and written in English language through an electronic web-based search strategy from databases of PubMed, CINAHL, Cochrane Library, Embase, Google Scholar and Ethiopian University Repository online. The quality of the included studies was assessed using the Joanna Briggs Institute (JBI) checklist. Statistical analyses were performed using STATA version 17. Meta-analysis was conducted with a random-effects model. Heterogeneity among the primary studies was evaluated using Cochran's Q test and the I-squared statistic. To explore potential sources of heterogeneity, subgroup and sensitivity analyses were performed.

Results: This systematic review and meta-analysis included seven studies, involving a total of 1,714 CKD patients. The pooled prevalence of anemia among CKD patients was 60.93% (95% CI: 50.05, 71.80), with substantial heterogeneity observed across studies ($I^2 = 97.0$, $P < 0.000$). A significant association was found between anemia in CKD patients and diabetes comorbidity, with those having diabetes experiencing significantly higher odds of developing anemia (OR: 4.52, 95% CI: 1.18–7.28). Additionally, patients in stage 3 or more advanced stages of CKD had an increased risk of anemia (OR: 4.36, 95% CI: 3.15–5.57).

Conclusion and recommendations: This systematic review and meta-analysis revealed a high pooled prevalence of anemia among CKD patients, with

significant associations with diabetes mellitus comorbidity, and advanced CKD stages. Regional differences were also noted, with Oromia region showing the highest prevalence. We recommend regular anemia screening in CKD patients and the identification and management of risk factors such as diabetes and advanced CKD stages.

Systematic review registration: <https://www.crd.york.ac.uk/PROSPERO/view/CRD42024608561>, identifier CRD42024608561.

KEYWORDS

chronic kidney disease, CKD, anemia prevalence, diabetes mellitus, systematic review, meta-analysis

Background

Chronic kidney disease (CKD) is characterized by persistent abnormalities in kidney structure or function lasting at least 3 months, with significant health implications. CKD is classified according to Cause, Glomerular Filtration Rate (GFR) category (G1–G5), and Albuminuria category (A1–A3), collectively referred to as the CGA classification (1). CKD is a progressive disease that affects over 10% of the global population, impacting more than 800 million people (2). According to the Global Burden of Disease studies, CKD has become a major cause of mortality worldwide (3, 4).

According to the World Health Organization (WHO), anemia is a significant public health issue worldwide (5). It is defined as hemoglobin (Hgb) concentration of less than 12 g/dL for females and less than 13 g/dL for males (6, 7). Anemia is a significant complication of CKD, accelerating disease progression and increasing its overall health burden (8). Anemia prevalence is notably elevated among CKD patients ranging from 14% to 91% (9–13).

Anemia associated with CKD, sometimes referred to as anemia of chronic renal disease, is classified as normocytic normochromic anemia and is characterized by a reduced ability to produce red blood cells. This form of anemia is frequently observed in individuals with renal impairment and is correlated with poor health outcomes, including an increased risk of mortality among CKD patients (14). Anemia in CKD is characterized by hemoglobin levels that fall below the average range adjusted for age and sex. The primary cause of this anemia is the insufficient production of endogenous erythropoietin, which can result from either functional or absolute deficiencies of iron (15).

Various factors contribute to the development of anemia in individuals with chronic kidney disease (CKD), including diabetic nephropathy, the stage of CKD, body mass index (BMI), smoking status, white blood cell (WBC) count, and serum albumin levels (16). As CKD progresses, hemoglobin levels decline primarily due to the insufficient production of erythropoietin by impaired kidneys. Furthermore, anemia is exacerbated by hepcidin, which limits iron availability by reducing both intestinal iron absorption and

the mobilization of stored iron needed for red blood cell production (17).

Anemia is linked to reduced quality of life (QoL) and increased rates of cardiovascular disease and death (18). A study which enrolled 27,998 patients with CKD and followed them for nearly 5.5 years, showed that the prevalence rates of anemia, congestive heart failure, coronary artery disease, and type 2 diabetes mellitus were higher among patients who died compared to those who survived, despite a shorter observation period for the deceased (19).

Erythropoiesis-stimulating agents (ESAs) and iron have been integral in the management of anemia related to CKD for many years (14, 20). Recent developments have introduced innovative strategies for enhancing erythropoiesis, including novel iron formulations. One of the most promising methods involves targeting the hypoxia-inducible transcription factor (HIF) pathway. Additionally, the modulation of the activin A signaling pathway is gaining attention for its potential benefits, which include increased bone density. Furthermore, newly formulated intravenous iron products may offer the advantage of fewer required administration sessions (21).

While previous study have examined the prevalence and associated factors of anemia among CKD patients in Sub-Saharan Africa (8), there remains a critical need for an Ethiopia-specific analysis. Regional and global studies provide valuable insights but may overlook Ethiopia's unique demographic, cultural, and healthcare dynamics, which likely influence anemia prevalence and risk factors differently from other parts of globe and Sub-Saharan Africa. Given these contextual differences, synthesizing data specifically from Ethiopian studies will allow for more relevant and precise recommendations for anemia management in CKD patients within the country. This focused review will support policymakers and healthcare providers in designing targeted, culturally appropriate interventions to address the unique needs of Ethiopia's CKD population. Therefore, this study aims to address the following research questions:

1. What is the pooled prevalence of anemia among CKD patients in Ethiopia?
2. What are the factors associated with anemia among CKD patients in Ethiopia?

Objectives

The primary objective of this study is to determine the pooled prevalence of anemia among CKD patients in Ethiopia. The secondary objective is to identify the factors associated with anemia in this population.

Methods

Study design and search strategy

A comprehensive systematic review and meta-analysis were conducted to investigate the prevalence and determinants of anemia in CKD patients in Ethiopia. The methodology for this systematic review and meta-analysis was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) statement (22). The findings were reported following the PRISMA guidelines (23).

We performed a systematic and comprehensive search across multiple databases, including PubMed, CINAHL, Cochrane Library, Embase, Google Scholar, and the Ethiopian University Repository, to identify studies reporting on anemia among CKD patients in Ethiopia. To ensure no duplication of efforts, we reviewed the database available at <http://www.library.ucsf.edu> and the Cochrane Library. Additionally, we screened reference lists of relevant articles to retrieve further studies. The process involved utilizing EndNote (version X8) software for downloading, organizing, reviewing, and citing articles. A manual search for cross-references was also conducted to find any pertinent studies that may not have been captured in the initial database search.

The search utilized a comprehensive set of keywords, including “prevalence,” “anemia,” “chronic kidney disease,” “CKD,” “Ethiopian patients,” “renal disease,” and “Ethiopia.” These terms were combined using Boolean operators (AND, OR) to facilitate a thorough and systematic search across the specified databases.

Eligibility criteria

Inclusion Criteria: The following criteria were established to guide the selection of articles for this meta-analysis and systematic review:

1. **Participants:** Studies must include individuals living in Ethiopia.
2. **Type of Research:** Only community or institutional-based studies conducted in Ethiopia were eligible.
3. **Study Design:** All observational study designs, such as cross-sectional, case-control, and cohort studies, were considered for inclusion.
4. **Setting:** Studies had to be conducted in Ethiopia.
5. **Study Format:** Published works were included up until the final date of data analysis.
6. **Language:** This review only considered studies published in English.

Exclusion Criteria: Studies were excluded if they lacked full text or demonstrated poor methodological quality. Research which

scored Joanna Briggs Institute (JBI) quality index score of seven or above were categorized as low risk and below were considered as poor methodological quality. Additionally, studies that did not provide information on anemia in CKD patients or for which necessary information could not be obtained were also excluded. Three authors (E.E., B.A., M.B.) independently assessed the eligibility of the identified studies, with input from another author (E.I) to reach a consensus on potential inclusion or exclusion.

Quality assessment

Three authors (A.H and T.G) independently performed a critical appraisal of the studies included in this review, utilizing the Joanna Briggs Institute (JBI) checklist for prevalence studies to evaluate quality. This tool comprises nine criteria with options for responses: yes, no, unclear, and not applicable (24). Additionally, reviewer E.E provided input as needed during the assessment process. Studies scoring seven or higher on the Newcastle–Ottawa Quality Assessment Scale (NOS) were deemed of acceptable quality (24). Consequently, all studies were included in our review (Table 1).

The methodological quality of studies examining anemia in CKD patients and its associated factors in Ethiopia was evaluated based on the following questions:

- Was the sample frame appropriate to address the target population?
- Were study participants sampled appropriately?
- Was the sample size adequate?
- Were the study subjects and setting described in detail?
- Was data analysis conducted with sufficient coverage of the identified sample?
- Were valid methods used to identify the condition?
- Was the condition measured in a standardized, reliable way for all participants?
- Was appropriate statistical analysis used?
- Was the response rate adequate, and if not, was a low response rate managed appropriately?

Data extraction

Data was extracted and documented using Microsoft Excel, guided by the Joanna Briggs Institute (JBI) data extraction form for observational studies. Two authors (E.E., B.A) independently conducted the data extraction process. The recorded data included the first author's last name, publication year, study setting or country, region, study design, study period, sample size, response rate, population characteristics, types of management, prevalence of anemia, and associated factors. Any discrepancies in the data extraction were resolved through discussion among the extractors.

PICO components for anemia prevalence among CKD patients

The PICO statement for our review highlights the key components of the research design. The population of interest consists of patients with CKD. The intervention focuses on studies

TABLE 1 Critical appraisal results of eligible studies on prevalence of anemia and its associated factors among chronic kidney disease (CKD) patients, Ethiopia, 2024.

Name of author	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Total
F Bishaw et al.	Y	Y	Y	N	Y	Y	Y	Y	Y	8
B. Alemu et al.	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
H. Adera et al.	Y	N	Y	Y	Y	N	Y	Y	Y	7
T H Molla et al	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
A Kidanewold et al	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
M U Yusuf et al	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
S Abdi et al	Y	Y	Y	N	Y	Y	Y	N	Y	7

Y, yes; N, no; JBI critical appraisal checklist for studies reporting prevalence data: Q1 = was the sample frame appropriate to address the target population? Q2- Were study participants sampled appropriately? Q3- Was the sample size adequate? Q4- Were the study subjects and the setting described in detail? Q5- Was the data analysis conducted with sufficient coverage of the identified sample. Q6- Were the valid methods used for the identification of the condition? Q7- Was the condition measured in a standard, reliable way for all participants? Q8- Was there appropriate statistical analysis? Q9- Was the response rate adequate, and if not, was the low response rate managed appropriately?

investigating the prevalence of anemia within this population. The comparative aspect involves examining studies that report anemia prevalence among CKD patients in different regions or settings. The primary outcome of interest is the prevalence of anemia observed among CKD patients. Additionally, the secondary outcome seeks to identify the factors associated with anemia in this population.

Heterogeneity and publication bias

To assess potential publication bias and the effects of small studies, we utilized funnel plots along with Egger's test. Furthermore, we calculated the I^2 statistic to evaluate heterogeneity among the studies included in the analysis (25, 26).

Data processing and analysis

Data analysis involved importing the extracted data into STATA 14 statistical software to calculate the pooled prevalence of anemia among CKD patients in Ethiopia. A meta-analysis was performed using a random-effects model to account for variability across studies (27). Subgroup analyses were conducted to compare the prevalence of anemia and its associated factors among CKD patients across different regions of Ethiopia. The results were visually presented in a forest plot format with a 95% confidence interval. I^2 values of 0%, 25%, 50%, and 75% were interpreted as indicating no, low, medium, and high heterogeneity, respectively (28).

Results

Study selection

After conducting an initial comprehensive search on chronic kidney disease (CKD) research across multiple databases, including Medline, Cochrane Library, Web of Science, Embase, AJOL, Google Scholar, and others, a total of 748 articles were retrieved. Of these, 312 duplicate records were removed. Screening the titles

and abstracts of the remaining 436 articles led to the exclusion of an additional 294 articles that did not meet the study criteria. Subsequently, 31 articles were excluded due to unavailability of the full text. Finally, from the remaining 111 articles, seven studies, comprising a total of 1,714 CKD patients, were included in the systematic review and meta-analysis (Figure 1).

Study characteristics

All seven included studies used a cross-sectional design. Three studies were conducted in Addis Ababa, while each of the remaining four studies was conducted in a different region: one in the Southern region, one in Amhara, one in Oromia, and one in Harar. The reported prevalence of anemia among CKD patients in Ethiopia varied considerably, ranging from 44% to 85.33% (Table 2).

Pooled prevalence of anemia among CKD patients in Ethiopia

Our comprehensive meta-analysis identified a significant anemia prevalence rate of 60.93% (95% CI: 50.05, 71.80) among CKD patients in Ethiopia, with substantial heterogeneity observed across studies ($I^2 = 95.6\%$, $P < 0.000$), as shown in Figure 2. Due to this high level of variability, we applied a random-effects model, allowing for a robust adjustment to account for the significant heterogeneity among the included studies.

Subgroup analysis of prevalence of anemia among CKD patients in Ethiopia

To better understand the observed heterogeneity, we performed a subgroup analysis by region, which uncovered striking variations in prevalence rates. Notably, the Oromia region reported the highest prevalence of anemia among CKD patients at an alarming 85.33% (95% CI: 79.67–90.99%). In sharp contrast, the South Ethiopia region exhibited the

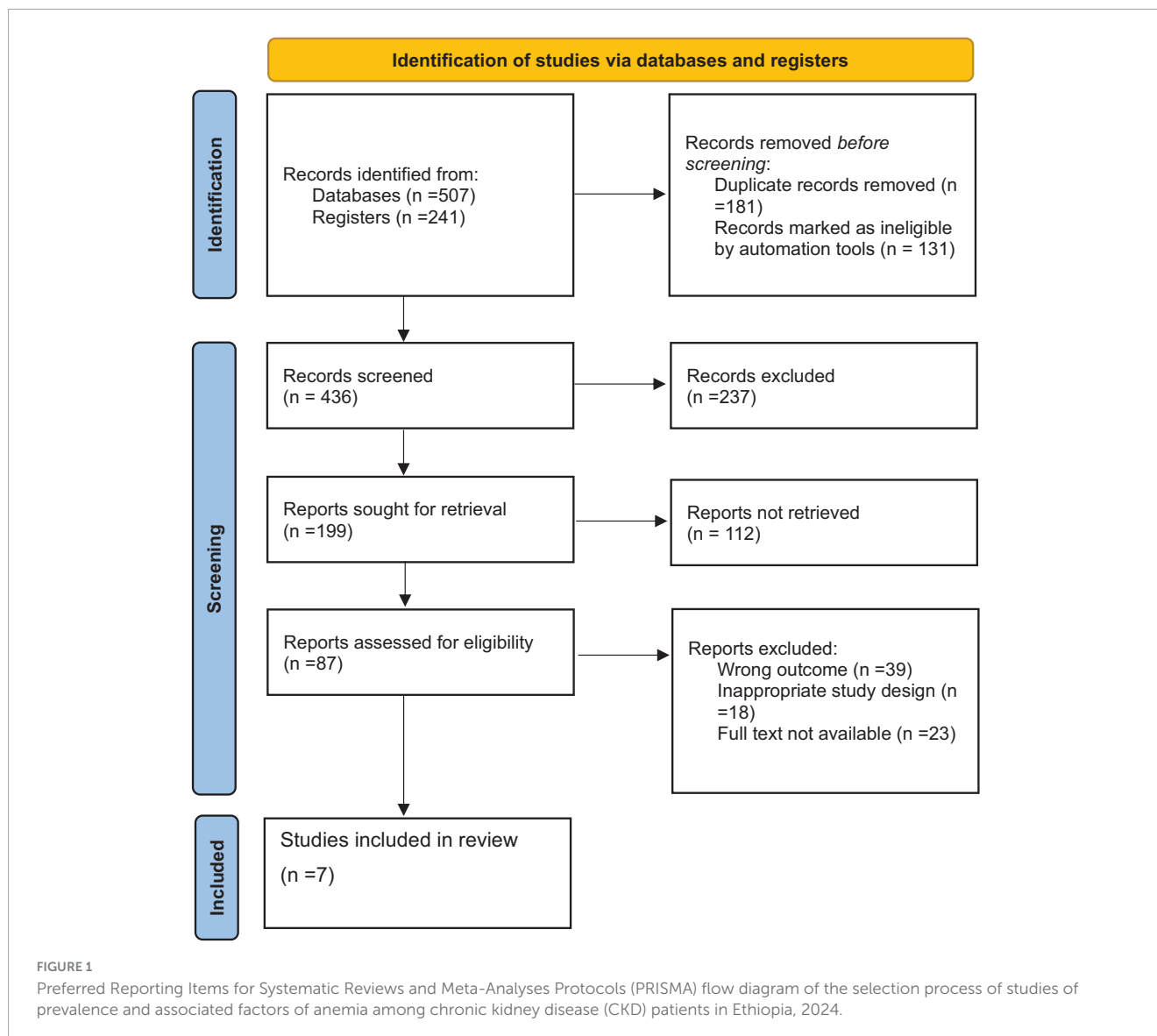


TABLE 2 Characteristics of studies included in the systematic review and meta-analysis on prevalence and associated factors of anemia among chronic kidney disease (CKD) patients in Ethiopia, 2024.

Name of author	Year	Region	Health facility name	Study design	Sample size	Anemia among CKD patients
F Bishaw et al.	2023	Oromia	Jimma university hospital	Cross-sectional	150	85.33
B. Alemu et al.	2021	Addis Ababa	Addis Ababa	Cross-sectional	387	53.5
H. Adera et al.	2019	Amhara	Gondar	Cross-sectional	251	64.5
T H Molla et al	2022	Addi Ababa	St Paul hospital	Cross-sectional	352	65.91
A Kidanewold et al	2021	South Ethiopia	south	Cross-sectional	384	44
M U Yusuf et al	2023	Eastern Ethiopia	Harar	Cross-sectional	90	64.8
S Abdi et al	2021	Addis Ababa	Black lion hospital	Cross-sectional	100	48

lowest prevalence, with only 44% (95% CI: 39.04–48.96%) of patients affected. Despite these differences, the overall heterogeneity remained significant, with $I^2 = 97$ (Figure 3), underscoring the complexity of anemia prevalence across the country.

Publication bias and heterogeneity

Our investigation into publication bias yielded insightful results. The Egger's test produced a p -value of 0.64, indicating no statistically significant evidence of publication bias in our

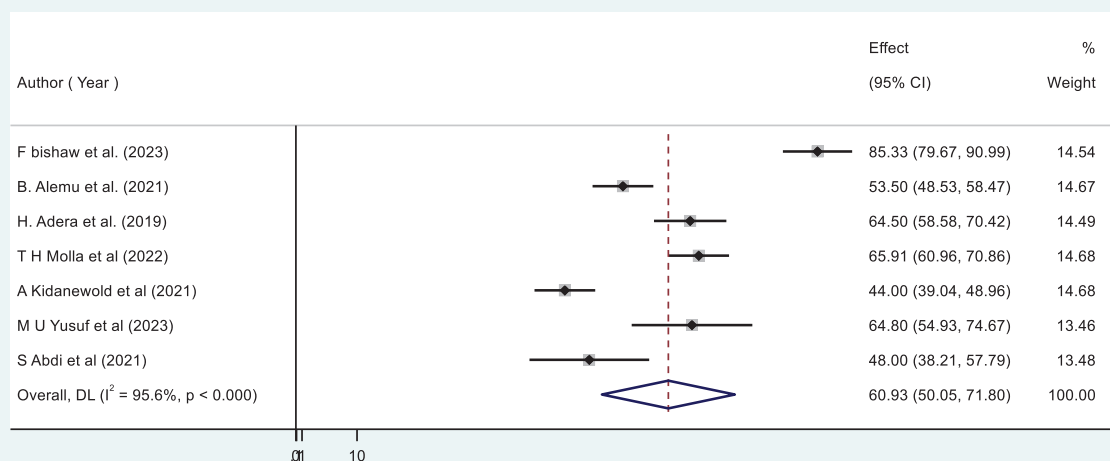


FIGURE 2

Forest plot showing the pooled proportion prevalence of anemia among chronic kidney disease (CKD) patients in Ethiopia.

meta-analysis (Table 3). Furthermore, the funnel plot (Figure 4) displayed a symmetrical distribution of the included studies, further confirming the absence of publication bias. These findings bolster the credibility of our results and underscore the robustness of the observed anemia prevalence rates among CKD patients in Ethiopia.

Additionally, to identify potential sources of heterogeneity, we conducted a meta-regression analysis that included sample size and publication year as covariates. However, our findings indicated that neither variable significantly affected the observed heterogeneity across the studies (Table 4).

Sensitivity analysis

The leave-out-one sensitivity analysis was conducted to rigorously assess the impact of individual studies on the overall pooled prevalence of anemia among chronic kidney disease (CKD) patients. In this systematic approach, each study was sequentially excluded from the analysis to evaluate its influence. The results of this thorough evaluation indicated that the exclusion of any single study did not lead to a significant or statistically meaningful change in the overall pooled estimate of anemia prevalence. These findings are illustrated in Figure 5, which demonstrates the stability and robustness of the overall pooled estimate, even with the removal of specific studies from consideration.

Factors associated with anemia among CKD patients in Ethiopia

Our meta-analysis reveals two significant factors associated with an increased risk of anemia among CKD patients in Ethiopia: diabetes mellitus (DM) comorbidity and advanced CKD stages (stage 3 and above). CKD patients with diabetes comorbidity were found to have markedly higher odds of developing anemia (OR: 4.52, CI: 1.175, 7.28; Figure 6). Similarly, those in stage 3 or more advanced stages of CKD faced an increased risk of anemia (OR: 4.36, CI: 3.15, 5.57; Figure 7).

Discussion

Anemia in chronic diseases is an emerging public health problem in low-income countries, including Ethiopia. The prevalence of anemia among CKD patients has become a significant burden in developing nations compared to developed countries. This study aimed to provide evidence on the pooled prevalence of anemia and its associated factors among CKD patients in Ethiopia.

In this systematic review and meta-analysis, we summarized the pooled prevalence of anemia among CKD patients in Ethiopia, which was 55.19% (95% CI: 42.19–68.20%). This prevalence is consistent with studies conducted in Turkey (55.9%) (29),

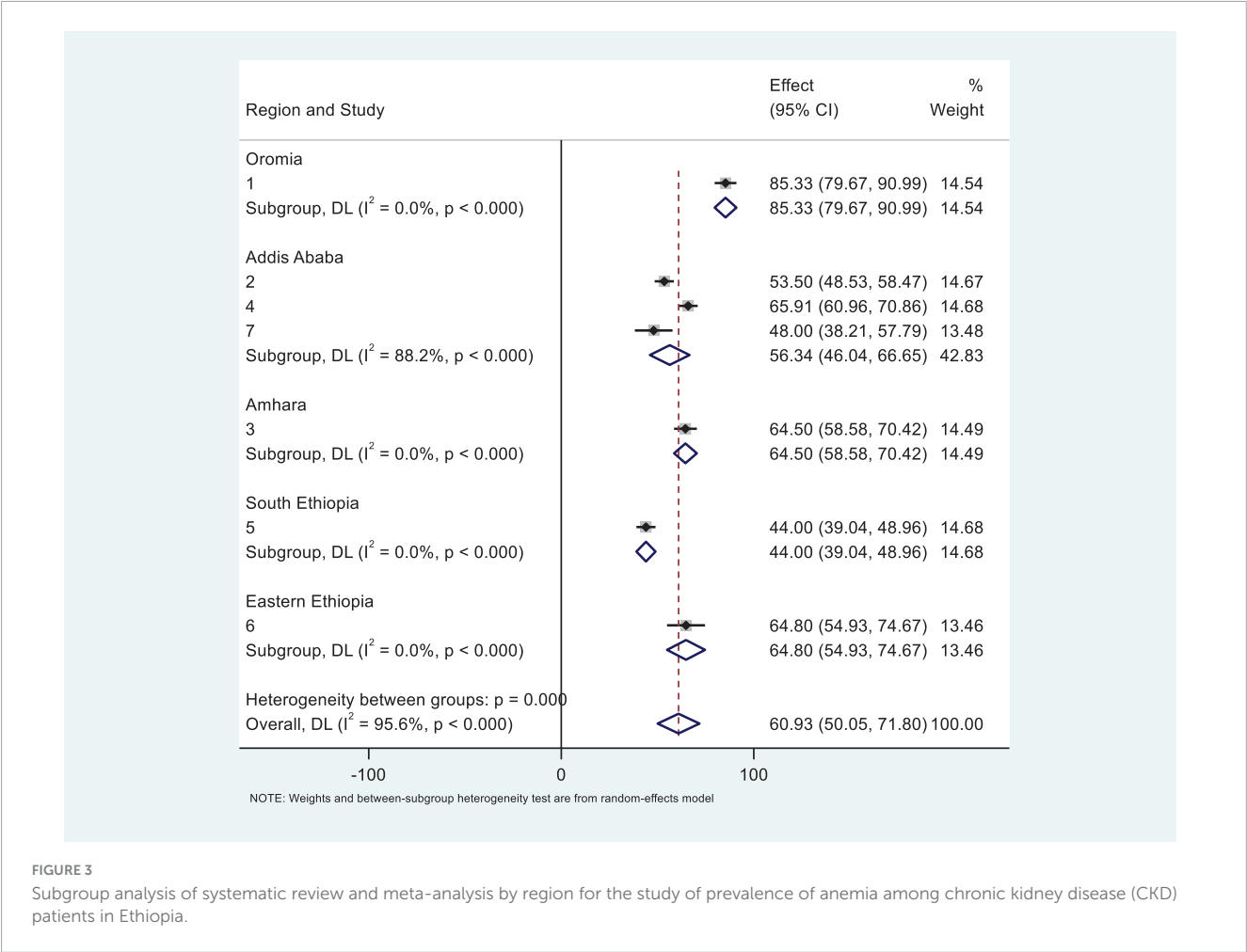


TABLE 3 Egger's test systematic review and meta-analysis of prevalence and associated factors of anemia among chronic kidney disease (CKD) patients in Ethiopia, 2024.

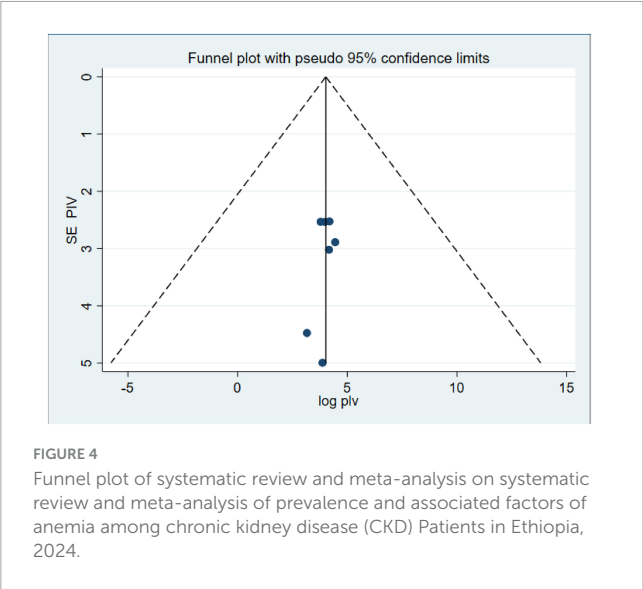
Standard effect	Coefficient	Standard error	t	P > t	95% CI
Slope	4.600698	0.5436923	8.46	0.000	5.998303
Bias	-0.1263578	0.1823594	-1.09	0.324	0.2694569

Catalonia, Spain (58.5%) (30), Nepal (53.6%) (31), and Sub-Saharan Africa (59.19%) (8). However, the results of this meta-analysis are lower than those from studies conducted in Malaysia (75.8%) (32), Brazil (86%) (33), and Pakistan (80.5%) (34). On the other hand, this finding is higher than results from studies conducted in India (39.36%) (35), Japan (32.3%) (36), and the United States (15.4%) (37). These differences may be attributed to variations in socioeconomic status, study periods, study settings, types of comorbidities and complications, methods for classifying the disease, and the quality of healthcare available to manage CKD patients.

Subgroup analysis in this meta-analysis revealed that the Oromia region reported the highest prevalence of anemia among CKD patients at 85.33% (95% CI: 79.67–90.99%), while the South region showed the lowest prevalence, with only 44% (95% CI: 39.04–48.96%) of patients affected. This inconsistency may be attributed to variations in sampling techniques, sample size, socio-demographic and behavioral characteristics, admission

diagnoses, nutritional factors, age of the participants, and clinical complications among the study subjects. Furthermore, studies from other low-income settings, such as South Asia, and Latin America, have reported varying prevalence rates of anemia among CKD patients, which may be influenced by socioeconomic and healthcare disparities (38). Regional variations in anemia prevalence are shaped by cultural, dietary, healthcare, socioeconomic, and environmental factors. Political instability further worsens anemia by disrupting food supply and healthcare. Tailored public health interventions addressing local factors are crucial for reducing anemia prevalence.

Our systematic review and meta-analysis identified significant factors associated with an increased risk of anemia among CKD patients in Ethiopia. In a random-effects model, pooled estimates indicated that diabetes mellitus (DM) comorbidity was significantly associated with anemia among CKD patients; specifically, the pooled estimates showed that the risk of developing anemia was nearly five times higher in CKD patients with DM compared to



can create a hypoxic environment in the renal interstitium, leading to impaired erythropoietin production. Additionally, erythrocyte precursor cells in the bone marrow may experience prolonged exposure to glucose toxicity, while mature red blood cells can be damaged by oxidative stress—both of which impair erythropoiesis and reduce red blood cell survival in individuals with sustained hyperglycemia. Other contributing factors to anemia in diabetic patients include chronic inflammation, elevated levels of advanced glycation end products (AGEs), erythropoietin resistance, oxidative stress, and the side effects of anti-diabetic medications (38, 41, 42).

Another important factor associated with anemia among CKD patients was advanced CKD stage (stage 3 and above), which showed a strong association with anemia. The risk of developing anemia was four times higher in CKD patients at stage 3 or beyond. This finding is consistent with previous studies conducted in Pakistan (34), Singapore, Malaysia (43), and the United States (39). These results emphasize the need for targeted interventions to manage anemia in CKD patients, particularly for those with diabetes and those in advanced stages of the disease.

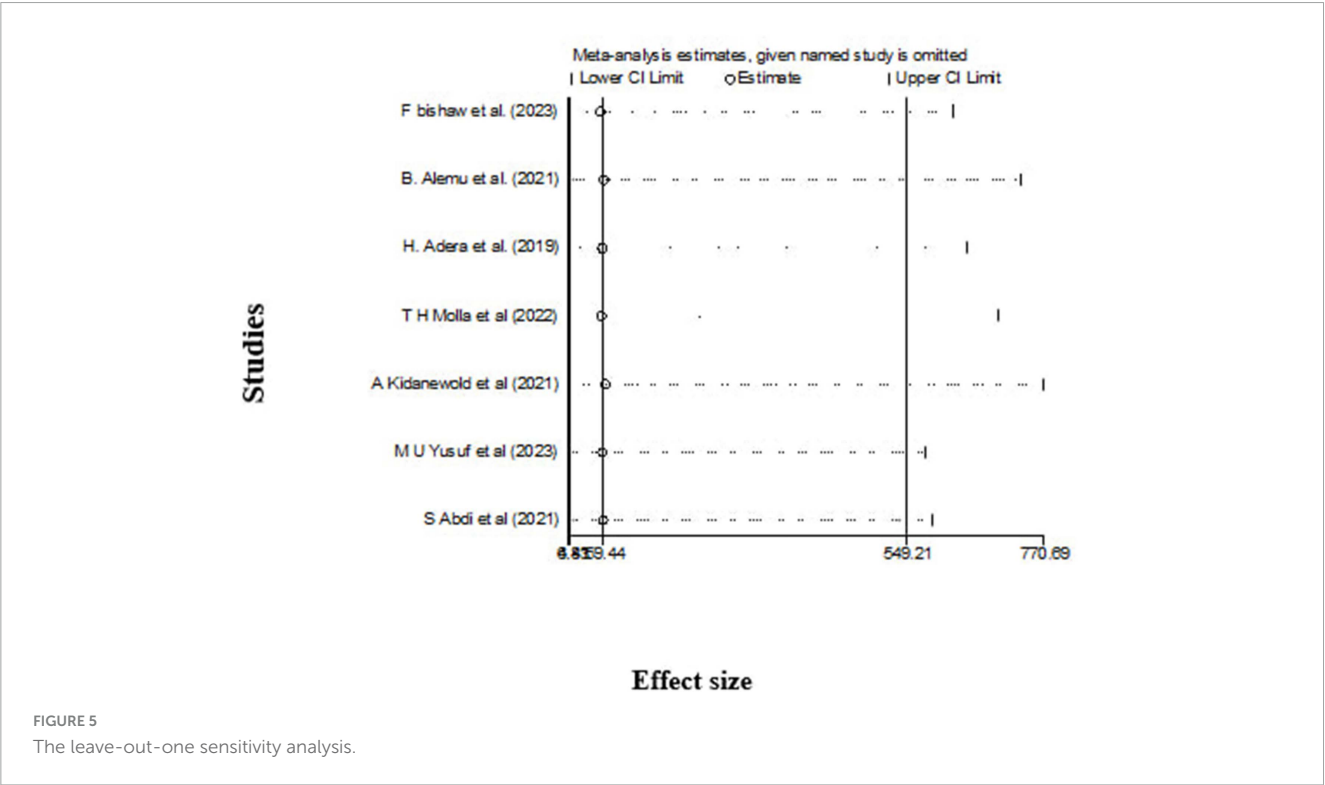
those without DM. This finding aligns with studies conducted in Sub-Saharan Africa (8), Nepal (39), and the United States (40). This association is primarily due to the effects of chronic hyperglycemia associated with diabetes. Persistent hyperglycemia

Strength and limitation of the study

This systematic review and meta-analysis present both strengths and limitations. On the positive side, it

TABLE 4 Meta-regression analysis of factors affecting between-study heterogeneity.

Prevalence	Coeff.	Stad. Err.	t	<i>P</i> > t	95% conf.	Interval
Sample	−0.0859264	0.0446475	−1.92	0.127	−0.2098877	0.0380348
Year	3.5647	3.690551	0.97	0.389	−6.681913	13.81131
Const	−7118.022	7463.851	−0.95	0.394	−27840.99	13604.95



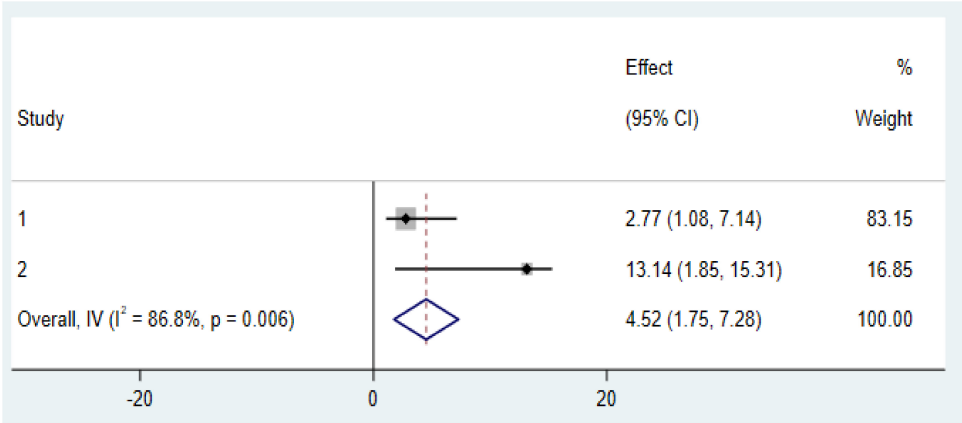


FIGURE 6
Forest plot of the association between diabetes mellitus (DM) comorbidity with prevalence of anemia among chronic kidney disease (CKD) patients in Ethiopia 2024.

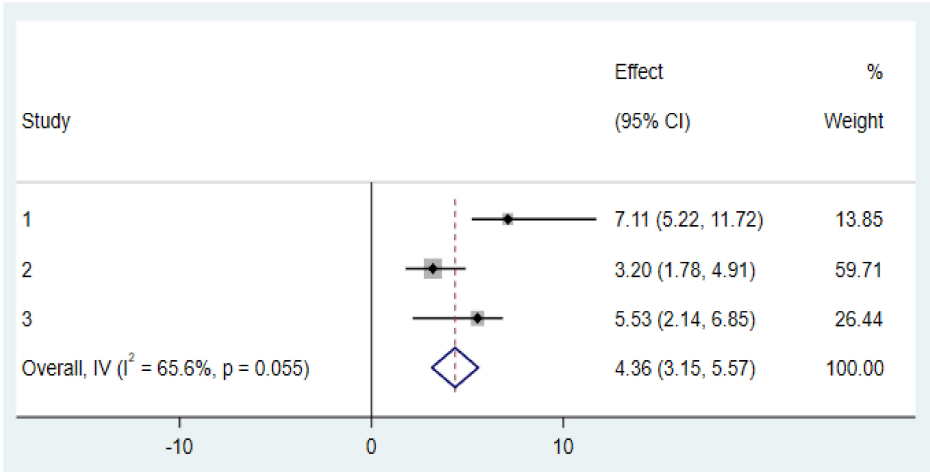


FIGURE 7
Forest plot of the association between stage 3 and above with prevalence of anemia among chronic kidney disease (CKD) patients in Ethiopia 2024.

offers an updated and comprehensive estimate of anemia prevalence and identified common factors in chronic kidney disease (CKD) patients.

Nevertheless, there are limitations to consider. Although subgroup analysis was performed, it was unable to pinpoint the sources of heterogeneity. The lack of standardized definitions for CKD and varying anemia cutoff points across the studies may have affected the accuracy of the pooled prevalence estimate. Additionally, the findings may not be fully generalizable, as the meta-analysis includes studies from a limited number of regions, which may not represent the broader CKD population. Furthermore, most of the included studies were cross-sectional, preventing the establishment of a cause-and-effect relationship between CKD and anemia. Therefore, further research with stronger study designs, considering additional causes of anemia, is necessary. Finally, the substantial heterogeneity observed among the studies, with unclear origins, makes it difficult to draw definitive conclusions from the results.

Conclusion

This study found a significantly high pooled prevalence of anemia among CKD patients. Subgroup analysis revealed the Oromia region had the highest anemia prevalence, while the Harar region had the lowest. The study also highlighted that comorbidity with diabetes mellitus and advanced CKD stages (3 and above) were significantly associated with anemia among CKD patients.

Recommendations

We recommend regular anemia screening in CKD patients, particularly in regions with high prevalence such as Oromia. It is crucial to identify and manage risk factors, including diabetes mellitus and advanced CKD stages, to reduce the incidence of anemia. Additionally, implementing targeted anemia management strategies, such as iron supplementation and erythropoiesis-stimulating agents, will help to mitigate the burden of anemia

in CKD. By adopting these recommendations, healthcare systems can improve the prevention and management of anemia in CKD, enhancing patient outcomes and quality of life.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: all datasets used in this analysis are included in the manuscript and are available from the corresponding author upon reasonable request. Requests to access these datasets should be directed to eshetuelf21@gmail.com.

Author contributions

EE: Conceptualization, Data curation, Formal Analysis, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. TG: Conceptualization, Investigation, Methodology, Writing – review and editing. EI: Visualization, Writing – review and editing. MB: Data curation, Software, Supervision, Validation, Writing – review and editing. BA: Conceptualization, Data curation, Formal Analysis, Validation, Visualization, Writing – review and editing. AM: Validation, Visualization, Writing – review and editing.

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Unveiling the hidden story of anemia among Indian Muslim women: a comprehensive analysis from 1998 to 2021

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Background: Iron deficiency among women of reproductive age, driven by factors such as inadequate dietary intake, menstrual blood loss, and heightened iron demands during pregnancy, remains a global public health concern. This research focuses on the prevalence of anemia among Muslim women in India over the last two decades, with a particular focus on disparities and trends.

Methods: Anemia among Muslim women of reproductive age (15–49 years) is studied using the four rounds of the National Family Health Survey (NFHS-2 to NFHS-5) to analyze the trends and disparities using bivariate cross-tabulations, concentration index, P/R ratios, and odds ratios across different socioeconomic factors. This study analyzes data from 212,837 Muslim women of reproductive age, collected through four rounds of the National Family Health Survey between 1998 and 2021.

Results: The findings reveal a concerning upward trend from 48.77 to 55.6% (1998–2021) in anemia prevalence among Muslim women in India. Anemia is consistently found to be highest in the age group of 15–19 across all the surveys and reported to be 59.14% in NFHS-5. Geographically, the Northeastern and Eastern regions exhibit the highest anemia prevalence rates, at 72.12 and 60.5% in 1998–99, respectively, which decreased to 41.41 and 55.95% in 2015–16, but again rose by 17.74 and 8.72% in 2019–21, respectively. The Western region increased from 37.6% in 1998–99 to 51.76% in 2019–21. Furthermore, rural areas witness a strikingly higher anemia prevalence among women, exceeding urban areas by over 8%. The Scheduled Caste/Scheduled Tribe (SC/ST) populations consistently bear the highest anemia burden. Economic disparities are evident, as wealth quintiles and education attainment display a transparent gradient, with the poorest quintile and no education consistently having the highest odds of anemia.

Conclusion: The socially disadvantaged groups, economically backward and less educated women, have constantly shown the highest prevalence of anemia for the period of the past two decades. The policies to improve public health should specifically focus on the most vulnerable sections of society. There is a need to modify existing public policies and improve population health in the context of the most vulnerable sections in developing countries.

KEYWORDS

anemia, health disparities, inequality, iron deficiency, Muslim women, women's health

1 Introduction

Anemia occurs when the blood lacks enough healthy red blood cells or hemoglobin, essential for oxygen transport. This leads to symptoms like fatigue, weakness, dizziness, and shortness of breath (1). The most common cause is iron deficiency, which hampers hemoglobin production. However, other micronutrient deficiencies, such as folate, vitamin B12, zinc, and vitamin C are also significant contributors to anemia, particularly in low-resource settings like India (2–4). Adolescents, particularly girls from low-income households, tend to consume less diverse diets and are thus more vulnerable to micronutrient deficiencies (5). These patterns call for improving dietary intake as well as nutrient bioavailability, especially for iron and folate. In this context, anemia must be understood not only as a nutritional issue but also as a socio-structural problem shaped by poverty, access, gender inequity, and other socioeconomic determinants (6, 7).

Women of reproductive age (WRA) are especially vulnerable due to factors such as heavy menstruation, frequent pregnancies, and increased nutritional requirements (8, 9). Anemia affects nearly a quarter of the global population, with women and children being the most vulnerable groups (10). Anemia's impacts extend beyond its physiological symptoms, manifesting as a multifaceted health, societal, and economic issue (11). Among children, anemia is associated with impaired cognitive and motor development, delayed academic progress, and limited social interactions, which can hinder their future economic productivity (12). Among women, anemia contributes to maternal morbidity and is linked to adverse pregnancy outcomes such as preterm birth, low birth weight, and stillbirth, as well as increased maternal mortality (9, 13). Furthermore, maternal anemia has intergenerational effects, leading to poor health outcomes in children, including stunting and impaired cognitive development (14). Nutritional and perinatal deficiencies have been found to be leading contributors to increased morbidity and mortality among women of reproductive age in India (15). Despite policy efforts that historically focused on iron and folic acid supplementation, recent studies emphasize the need to address multiple micronutrient deficiencies and structural determinants of anemia in tandem (7, 16). Studies also indicate that the relation between dietary iron intake and anemia is modulated by coexisting nutritional deficiencies and non-nutritional factors such as poverty, hemoglobinopathies, and infections (17–19). Beyond its health implications, anemia imposes significant economic burdens (20). The productivity losses associated with anemia stem from reduced physical capacity, fatigue, and impaired cognitive function. Investments in addressing anemia are therefore economically beneficial, with every US\$ 1 invested in anemia reduction among women projected to yield US\$ 12 in economic returns (21). These compounded impacts on health and productivity establish anemia as not just a medical condition but also a significant developmental and social justice challenge (20, 22).

Long-term anemia can even impact productivity and overall quality of life (12). To address anemia, strategies such as improving diets, iron and folic acid supplementation, and treating underlying causes like infections are crucial. The WHO has set a global target to reduce anemia in women of reproductive age by 50% by 2025 (1). Globally, approximately 24.8% of the population is affected by anemia (23). Among women of reproductive age, the prevalence in 2019 was 29.9%, equating to over half a billion women (24). Non-pregnant

women experienced a global anemia prevalence of 30.2%, while 41.8% of pregnant women were affected in the same period (25).

India has one of the highest burdens of anemia among women of reproductive age, with over 52.2% of pregnant women and 59.1% of women aged 15–49 affected, as per the National Family Health Survey (26). The persistent burden highlights the need for a deeper understanding of its underlying determinants. India has implemented various programs to combat anemia, such as the Anemia Mukt Bharat initiative launched in 2018, the Public Distribution System (PDS), and the Integrated Child Development Services (ICDS) program, which also focuses on providing iron and folic acid supplementation, deworming, and dietary counseling, particularly for women and adolescent girls (27, 28). However, despite these efforts, progress has been slow, primarily due to the lack of effective implementation and socioeconomic barriers, and has failed to reach the most marginalized communities, such as SCs and STs (29, 30). Evidence suggests that combining nutritional interventions with socioeconomic empowerment programs, such as skill development and education initiatives, can yield better outcomes in reducing anemia (8).

Over the past two decades, data from the National Family Health Surveys (NFHS-2, 1998–99; NFHS-3, 2005–06; NFHS-4, 2015–16; and NFHS-5, 2019–21) show persistent anemia disparities across socioeconomic groups increasing from 48.7% in NFHS-2 (1998–99) to 55.6% in NFHS-5 (31, 32). The NFHS is a nationally representative stratified survey, including 90,303 women in NFHS-2, 124,385 women in NFHS-3, 699,686 women in NFHS-4, and 724,115 women of reproductive age (16–49 years) in NFHS-5. Among these, Muslim women of reproductive age accounted for 11.93, 13.45, 13.52, and 12.52%, respectively. The high prevalence of anemia in India is deeply linked to its socioeconomic and cultural structures, where caste, class, gender, ethnicity, and religion shape access to healthcare, nutrition, and education (27). Existing research on anemia in India has primarily focused on single-axis frameworks examining gender, caste, or class independently. For instance, studies have highlighted the association of anemia with caste (33, 34), ethnicity (6, 35), and gender (27, 36, 37). Similarly, research has shown how socioeconomic factors such as low educational status (38), poor housing conditions (14), and hygiene practices (39) influence anemia prevalence. Studies show that the Northeastern and Eastern states consistently report the highest prevalence rates, with over 60% of women affected in some states like Bihar and Assam (40). Rural areas exhibit a higher burden compared to urban areas, as limited healthcare access, poor sanitation, and dietary inadequacies are more common in rural regions (24). Women from Scheduled Caste (SC) and Scheduled Tribe (ST) communities experience higher rates of anemia due to systemic marginalization, poor living conditions, and limited healthcare access (33, 41, 42). Wealth quintiles and educational attainment display a clear gradient, with the poorest and least educated women being the most vulnerable (14). However, these studies fail to account for how multiple socioeconomic dimensions interact to shape health outcomes, leaving critical gaps in understanding the lived realities of marginalized groups such as Muslim women. They experience compounded disadvantages due to socioeconomic marginalization and gender-based discrimination, as highlighted by the Sachar Committee, 2006 (43). However, their health challenges remain underrepresented in research.

Intersectionality, introduced by Crenshaw (1989), helps explain how overlapping social identities such as caste, class, gender, and

religion, shape anemia risk. A rural Muslim woman, for example, may face disadvantages from poverty, gender discrimination, and religious marginalization, intensifying her vulnerability (44). Despite its relevance, intersectionality is underutilized in Indian health research, which often adopts single-axis approaches, overlooking the interplay of multiple disadvantages (45, 46).

This study seeks to fill these gaps by adopting an intersectional framework to examine the prevalence and determinants of anemia among Muslim women in India. It explores how socioeconomic factors such as caste, class, age, place of residence, education, and geographical region interact to influence anemia prevalence among Muslim women. This research also aligns with global health goals, such as the United Nations' 2030 Agenda for Sustainable Development and the World Health Assembly's Comprehensive Implementation Plan on Maternal, Infant, and Young Child Nutrition. By focusing on Muslim women, a group often overlooked in public health policy, this study contributes to the broader effort to achieve health equity and ensure "health for all." This research addresses a critical gap in the literature by applying an intersectional lens to anemia prevalence among Muslim women in India. By shedding light on the unique challenges faced by Muslim women, this study underscores the importance of inclusive and equity-driven public health strategies and tests the effectiveness of current policies with respect to a specific section of society.

2 Materials and methods

This study utilizes data from the four rounds of the National Family Health Surveys (NFHS) conducted in India, spanning over two decades: NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16), and NFHS-5 (2019–21). The NFHS surveys, implemented by the Ministry of Health and Family Welfare (MoHFW) with technical support from the International Institute for Population Sciences (IIPS), provide nationally representative data on various health and demographic indicators. These surveys are instrumental in examining the prevalence and trends of anemia among different population groups, including Muslim women. The study focuses on women of reproductive age (15–49 years), specifically analyzing the subset of Muslim women within this demographic. The sample size for Muslim women varies across the NFHS rounds: NFHS-2 includes 10,775 ever-married Muslim women, NFHS-3 expands to 16,742 Muslim women (including both married and unmarried individuals), NFHS-4 captures 94,591 Muslim women, and NFHS-5 incorporates 90,729 Muslim women. The total pooled sample size of Muslim women across the four NFHS rounds used in this study is 212,837.

This study aims to examine the trends and disparities in the prevalence of any and severe anemia among Muslim women among Muslim women of reproductive age (15–49 years) across different socioeconomic and demographic factors such as caste (Scheduled Caste, Scheduled Tribe, Other Backward Classes, General), age groups (e.g., 15–19, 20–29, 30–39, 40–49), place of residence (rural and urban), Education levels (no education, primary, secondary, higher), wealth quintiles (poorest, poorer, middle, richer, richest), regions (e.g., North, South, East, West, Northeast). Anemia is measured using hemoglobin levels among women in India and worldwide. According to the WHO cutoffs, Anemia is typically defined as a condition where the hemoglobin

levels in a woman's blood fall below a certain threshold, which is 12 g/dL (any anemia), 10.00–11.90 g/dL (mild), 7.00–9.90 (moderate) g/dL, and <7.00 (severe) g/dL (47). In this study, "severe" (hemoglobin <7.00 g/dL) and "any" (hemoglobin <12.00 g/dL) categories are used for the analysis to make the analysis comprehensible and inclusive. To maintain consistency and transparency, the diagnosis of anemia in this study is based solely on hemoglobin concentration, following World Health Organization (WHO) thresholds. Women were categorized as anemic if their hemoglobin level was below 12.0 g/dL (for non-pregnant women) or below 11.0 g/dL (for pregnant women). Observations were excluded if they had missing hemoglobin values, biologically implausible or flagged hemoglobin readings, or incomplete pregnancy-related information that made the application of the relevant hemoglobin cutoff unfeasible. These exclusions ensure the validity and reliability of the anemia estimates used in the analysis.

The dependent variable is "anemia," which has been transformed into a binary variable coded as "1" if there is any kind of prevalence of anemia and "0" otherwise. The trends over the last two decades (1998–2021) and socioeconomic variations and heterogeneities based on socioeconomic factors have been examined along the dimensions of age, area of residence, caste, education, economic status, and different regions (which are considered as independent variables) among the Muslim women. To capture the prevalence of anemia and heterogeneities across various socioeconomic factors, the methods described below are used:

2.1 Cross-tabulation

This statistical method is used to analyze the percentage prevalence of anemia among Muslim women of reproductive age (15–49 years). Cross-tabulations allow this study to identify patterns and disparities in anemia prevalence by comparing proportions or percentages across these categories.

2.2 Concentration index

The Concentration Index is a widely used tool in health economics to measure inequality in health outcomes across different socioeconomic groups. It ranges from -1 to $+1$, where a negative value means the health condition (in this case, anemia) is more concentrated among the poorer segments of the population, and a positive value indicates a higher prevalence among the wealthier segments (48). A value of 0 implies perfect equality, that is, anemia is evenly distributed across all economic groups. This index is derived from a concentration curve, which plots the cumulative percentage of the health variable (e.g., anemia) against the cumulative percentage of the population, ranked by income or wealth. The farther the curve is from the diagonal line of equality, the greater the inequality (49). In this study, the CI is used to track how anemia prevalence among Muslim women varies with economic status across different NFHS rounds. It offers a summary statistic that not only captures the existence of disparity but also reveals its direction and magnitude, which is especially useful for policy targeting and equity monitoring (50).

2.3 Poor-rich (P/R) ratios

The P/R ratio is used to measure the disparity in anemia prevalence between women belonging to the poorest and richest wealth quintiles. It is defined as the ratio of the prevalence percentage of anemia in the poorest quintile to the prevalence percentage in the richest quintile.

A P/R ratio of 1 indicates no economic disparity, meaning anemia prevalence is equally distributed across the poorest and richest groups. A P/R ratio greater than 1 suggests higher anemia prevalence among women in the poorest wealth quintile compared to those in the richest quintile (50). This ratio is a simple and widely used measure to assess socioeconomic disparities in health and nutrition outcomes. It has been adopted in prior literature (e.g., Pathak and Singh, 2011) to summarize economic inequality, particularly in the context of undernutrition among children (48). The P/R ratio helps to highlight the disproportionate burden of anemia on economically disadvantaged populations.

2.4 Logistic regression model

The logistic regression model has been used by transforming the linear combination of predictor variables and coefficients into probabilities and odds; the relationship between socioeconomic factors, such as caste, age, place of residence, education, wealth quintiles, and region, and the probability of women experiencing anemia has been discerned. A logistic regression model was employed to assess the association between anemia prevalence and various socioeconomic and demographic factors, including caste, age, place of residence, education, wealth quintiles, and region. Odds ratios were estimated to determine the likelihood of anemia among Muslim women in each category, compared to relevant reference groups (51). In the context of this study, the odds ratio (OR) derived from the logistic regression model is used to determine the likelihood (or odds) of anemia among Muslim women of reproductive age in India based on various socioeconomic and demographic factors.

2.5 Spatial analysis

Across different states, the geographical variance using ArcGIS software is used to assess anemia prevalence among Muslim women, both at the state and regional levels. This approach highlights the importance of considering state and regional economic disparities to identify localized health challenges and tailor public health interventions to address the specific needs of different regions effectively.

3 Results and preliminary findings

3.1 Distribution of socioeconomic characteristics

Figure 1 provides a percentage breakdown of Indian women aged 15–49 years, categorized by various socioeconomic factors. These include religion (such as Muslim, Hindu, Christian, Sikh,

Buddhist, Jain, and others), age (8, 15–48), caste (grouped as SC/ST, OBC, and Others), and place of residence (urban or rural). It also captures levels of education, ranging from no formal education to completed primary (5 years), secondary (10 years), and higher secondary (12 years). Additionally, the table organizes the data into wealth quintiles and different geographic regions, offering insight into the socioeconomic landscape of this population. This detailed categorization helps highlight the diverse socioeconomic background of women of reproductive age, which could be critical for examining health outcomes like anemia prevalence across different segments of the population. The data reveal a clear trend toward urbanization and increased education among women in India over the two decades, which likely contributes to better access to healthcare and nutritional resources. As more women move to urban areas and attain higher levels of education, awareness about health and nutrition improves, which could help reduce the prevalence of anemia. However, significant economic disparities persist, particularly between the wealthiest and poorest groups, which can limit access to essential resources like nutritious food and healthcare, especially for women in lower-income households. Regional differences also play a role, with certain areas experiencing population shifts that may influence healthcare distribution. Additionally, while the inclusion of more SC/ST and OBC women in surveys shows improved representation, these groups still face systemic challenges that could make them more vulnerable to anemia and other health issues. Overall, while some progress has been made, socioeconomic and regional inequalities continue to present significant challenges in addressing anemia among women in India (Table 1).

3.2 Trends in anemia among Muslim women in India

We find evolving trends in anemia prevalence among Muslim women over time, as shown in Table 2. Anemia prevalence declined across most socioeconomic groups between NFHS-3 and NFHS-4, but rose again in NFHS-5. Regional disparities persist, with higher rates in the East and Northeast, lower in the West and Central regions, and the lowest in the South, reflecting potential regional influences (19). These findings highlight the shifting nature of anemia prevalence among Muslim women and underscore the need for continuous monitoring and targeted interventions, particularly in high-prevalence regions.

Studies show that Muslims consistently have higher anemia rates than Hindus, Christians, and other religious groups (52). Contributing factors include poverty, inadequate access to clean water, healthcare, and sanitation (39, 53). The Sachar Committee Report identifies additional socioeconomic disadvantages, including limited education, restricted social security and job access, and discrimination, particularly affecting Muslim women (54). Class and gender further shape anemia prevalence in India. Among Muslim women, anemia rose from 48.77% in NFHS-2 to 55.56% in NFHS-5, with a sharp increase between NFHS-4 and NFHS-5. Hindu women also experienced a rise, but the sharper trend among Muslims suggests a need for focused interventions. Education and wealth significantly influence anemia prevalence, with the highest rates among the uneducated and poorest women. These findings stress the importance of policies addressing economic and educational disparities to reduce anemia rates effectively.



FIGURE 1

Trends in economic disparities with respect to anemia among Muslim women using P/R ratio across (A) caste, (B) age groups (in years), (C) residence, (D) educational groups, and (E) regions for NFHS-2 (1998-99), NFHS-3 (2005-06), NFHS-4 (2015-16) and NFHS-5 (2019-21).

TABLE 1 Percentage distribution of socioeconomic characteristics of women in India (aged 15–49), based on NFHS (1998–2021).

Socioeconomic characteristics	NFHS-2	NFHS-3	NFHS-4	NFHS-5
Religion				
Hindu	81.75	80.52	80.57	81.36
Muslim	12.53	13.62	13.79	13.48
Christian	2.53	2.45	2.38	2.35
Sikh	1.59	1.79	1.66	1.57
Buddhist	0.76	0.81	0.92	0.63
Jain	0.37	0.33	0.18	0.23
Others	0.46	0.49	0.5	0.38
Age				
15–19	9.39	20.34	17.70	17.27
20–29	39.18	35.41	34.66	33.35
30–39	29.96	25.51	25.43	25.83
40–49	21.47	18.74	22.21	23.55
Caste				
SC/ST	27	26.73	29.55	31.18
OBC	32.91	39.30	43.42	42.92
Others	40.09	33.98	27.02	25.91
Place of residence				
Urban	26.18	32.82	34.62	32.49
Rural	73.82	67.18	65.38	67.51
Education				
No education	53.44	40.59	27.46	22.43
Primary	16.9	14.7	12.47	11.73
Secondary	21.77	37.41	47.31	50.18
Higher	7.89	7.3	12.76	15.65
Wealth quintiles				
Poorest	19.66	17.46	17.73	18.5
Poorer	19.99	18.99	19.57	20.00
Middle	20.15	20.17	20.55	20.52
Richer	20.01	20.99	21.15	20.81
Richest	20.18	22.4	21	20.17
Region				
North	12.87	13.28	13.59	14.11
Central	22.42	23.26	23.65	24.89
East	21.99	22.44	22.11	22.76
West	14.64	14.83	14.37	14.09
South	24.59	22.24	22.76	20.45
North East	3.49	3.95	3.52	3.69

SC/ST, Scheduled castes and scheduled tribes; OBC, Other backward castes. Author's calculation based on NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16) and NFHS-5 (2019–21) data.

3.3 Trends and differentials in anemia among Muslim women using the concentration index

Across all socioeconomic factors (Caste, Age, Residence, Education, and Region), the Concentration Index (CI) values are

negative across all NFHS rounds (NFHS-2 to NFHS-5). Negative CI values indicate that anemia is more concentrated among poorer Muslim women across all survey rounds. SC/ST women consistently exhibit the highest negative CI values, meaning they suffer from the most significant economic disparity in anemia prevalence. In NFHS-2 (−0.157) vs. NFHS-5 (−0.057), the CI value

TABLE 2 Percentage prevalence of anemia among Muslim women in India (aged 15–49), by socioeconomic characteristics, based on NFHS (1998–2021).

Socioeconomic characteristics	NFHS-2		NFHS-3		NFHS-4		NFHS-5	
	Any	Severe	Any	Severe	Any	Severe	Any	Severe
All-India	48.77	1.32	54.66	1.15	50.60	0.90	55.60	0.77
Caste								
SC/ST	54.25	1.71	53.90	2.26	51.87	0.81	56.49	1.16
OBC	46.98	1.93	52.69	1.16	50.49	1.02	52.05	0.72
Others	49.12	1.11	55.73	1.10	50.60	0.80	58.62	0.78
Age								
15–19	46.97	0.49	55.20	1.07	51.82	0.70	58.33	0.77
20–29	49.02	1.41	55.42	1.27	50.33	0.87	55.14	0.62
20–39	49.01	1.12	53.14	1.01	50.95	0.93	54.09	0.85
40–49	48.61	1.81	54.54	1.14	50.05	1.12	55.69	0.95
Place of residence								
Urban	42.41	1.33	50.37	1.11	48.86	0.87	50.82	0.73
Rural	52.14	1.32	57.27	1.18	52.05	0.93	58.83	0.80
Education								
No education	54.48	1.50	58.46	1.34	53.77	1.28	58.32	0.87
Primary	48.33	1.42	54.59	1.39	51.65	0.92	55.79	1.08
Secondary	37.87	1.02	50.42	0.80	48.86	0.69	54.97	0.67
Higher	32.67	0.00	41.71	0.74	45.66	0.61	51.15	0.60
Wealth quintiles								
Poorest	64.35	1.90	63.81	1.01	55.63	1.08	62.36	0.89
Poorer	56.42	1.55	60.12	1.14	52.41	0.88	60.30	0.80
Middle	49.21	1.48	55.03	1.42	51.47	1.04	56.31	0.93
Richer	41.67	1.39	50.98	1.31	48.69	0.90	52.26	0.76
Richest	37.94	0.44	44.76	0.79	45.92	0.63	46.65	0.48
Region								
North	47.51	1.45	52.51	2.41	50.11	1.27	57.79	1.10
Central	47.83	1.23	50.77	1.05	51.82	1.24	50.51	0.68
East	60.50	1.66	63.52	0.65	55.95	0.57	64.67	0.58
West	37.60	0.69	47.71	0.98	48.95	0.71	51.76	1.09
South	34.60	1.46	49.26	1.29	45.20	0.99	44.77	0.82
North East	72.12	0.40	57.58	1.58	41.41	0.40	59.15	0.69

SC/ST, scheduled castes and scheduled tribes; OBC, other backward castes. Author's calculation based NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16) and NFHS-5 (2019–21) data.

has decreased significantly across castes, indicating a reduction in economic inequality over time. Younger women (15–19 years) have the least negative CI values, implying they experience the least socioeconomic inequality in anemia prevalence. Whereas women aged 20–39 and 40–49 consistently have higher negative CI values, indicating that older women in lower economic groups are more affected by anemia. The difference between urban (−0.045) and rural (−0.035) CI in NFHS-5 suggests that rural poor Muslim women remain more vulnerable to anemia. Women with no education have higher absolute CI values (−0.081 in NFHS-2 to −0.035 in NFHS-5), showing persistent inequality. The negative CI value for higher education (−0.054 in NFHS-5) suggests that even well-educated but economically disadvantaged women still face

anemia-related inequalities, though less severe than before. The South region initially showed high inequality in NFHS-3 (−0.067) but later improved in NFHS-4 (−0.032) before slightly worsening in NFHS-5 (−0.046), indicating fluctuating progress. The West region displayed a steady reduction in inequality, reaching NFHS-5 (−0.006), while the East region experienced moderate fluctuations, with inequality rising slightly in NFHS-4 (−0.037) before significantly improving in NFHS-5 (−0.011). Central India showed an improving trend until NFHS-4 (−0.011) but saw a reversal in NFHS-5 (−0.030), suggesting a loss of earlier progress. The North region followed a similar pattern, with inequality improving in NFHS-4 (−0.017) but worsening again in NFHS-5 (−0.054). The North-East region maintained relatively low disparities overall,

though inequality peaked in NFHS-3 (−0.036) before improving in NFHS-5 (−0.007) (Table 3).

3.4 Trends and differentials in anemia among Muslim women using the P/R ratio

The P/R ratio declined from 2.43 (NFHS-2) to 1.37 (NFHS-3), indicating reduced caste-based inequality, but rose slightly to 1.41 in NFHS-5, signaling widening disparities. Women aged 40–49 years had the highest P/R ratio in NFHS-5 (1.42), while adolescents (15–19 years) consistently had the lowest, declining from 1.38 (NFHS-2) to 1.22 (NFHS-5), suggesting lower disparities among young women. Urban areas showed higher P/R ratios than rural areas in all rounds except NFHS-5. Urban disparities declined from 1.63 (NFHS-2) to 1.29 (NFHS-4) but slightly increased to 1.32 (NFHS-5). Rural disparities followed a similar pattern, dropping from 1.65 (NFHS-2) to 1.17 (NFHS-4) before rising to 1.30 (NFHS-5). Women with no education had the highest disparities in NFHS-2 (1.67) and NFHS-3 (1.32), which declined to 1.12 (NFHS-4) but rose again to 1.25 (NFHS-5). Highly educated women had the lowest ratio in

NFHS-3 (0.71), but it increased to 1.36 in NFHS-5, indicating growing inequality among them. Primary and secondary education groups also saw rising disparities, reaching 1.37 and 1.34 in NFHS-5. Regional trends were mixed: the East region saw a peak in NFHS-4 (1.37) before dropping to 1.11 (NFHS-5), reflecting reduced inequality, while the North showed worsening disparities, rising from 1.02 (NFHS-4) to 1.37 (NFHS-5). Overall, the decline in inequality from NFHS-2 to NFHS-4 suggests improved equity in anemia prevalence, but NFHS-5 shows a reversal, with rising disparities across most categories. SC/ST women, older women (40–49 years), rural residents, uneducated women, and those in the South, Central, and North regions face consistently higher disparities, underscoring the need for targeted interventions to address anemia among Muslim women in India.

3.5 Trends and differentials in anemia among Muslim women using odds ratios

Table 4 presents Odds Ratios (OR) for anemia prevalence among Muslim women across socioeconomic and demographic factors from NFHS-2 to NFHS-5, offering insights into the likelihood of anemia

TABLE 3 Trends in economic disparities in the prevalence of any anemia among Muslim women, based on the Concentration Index from NFHS (1998–2021).

Socioeconomic characteristics	NFHS-2		NFHS-3		NFHS-4		NFHS-5	
	CI	SE	CI	SE	CI	SE	CI	SE
Caste	−0.107***	0.006	−0.067***	0.004	−0.035***	0.002	−0.056***	0.002
SC/ST	−0.157***	0.03	−0.032	0.021	−0.046***	0.006	−0.057***	0.006
OBC	−0.106***	0.013	−0.065***	0.008	−0.037***	0.003	−0.052***	0.003
Others	−0.104***	0.007	−0.068***	0.005	−0.032***	0.003	−0.049***	0.002
Age	−0.109***	0.006	−0.067***	0.004	−0.035***	0.002	−0.055***	0.002
15–19	−0.073***	0.021	−0.048***	0.009	−0.023***	0.004	−0.034***	0.004
20–29	−0.112***	0.009	−0.084***	0.007	−0.036***	0.003	−0.057***	0.003
20–39	−0.117***	0.011	−0.056***	0.009	−0.035***	0.004	−0.057***	0.004
40–49	−0.111***	0.014	−0.072***	0.011	−0.044***	0.004	−0.068***	0.004
Place of residence	−0.107***	0.006	−0.067***	0.004	−0.035***	0.002	−0.056***	0.002
Urban	−0.067***	0.01	−0.054***	0.006	−0.035***	0.003	−0.045***	0.003
Rural	−0.099***	0.007	−0.057***	0.006	−0.024***	0.002	−0.035***	0.002
Education	−0.107***	0.006	−0.067***	0.004	−0.035***	0.002	−0.056***	0.002
No education	−0.081***	0.007	−0.048***	0.006	−0.021***	0.003	−0.035***	0.003
Primary	−0.081***	0.014	−0.051***	0.011	−0.026***	0.005	−0.058***	0.005
Secondary	−0.031*	0.016	−0.054***	0.007	−0.028***	0.003	−0.059***	0.002
Higher	−0.014	0.03	−0.04**	0.018	−0.022***	0.007	−0.054***	0.006
Region	−0.107***	0.006	−0.067***	0.004	−0.035***	0.002	−0.056***	0.002
North	−0.061***	0.012	−0.044***	0.009	−0.017***	0.004	−0.054***	0.003
Central	−0.04**	0.018	−0.037***	0.011	−0.011***	0.003	−0.03***	0.004
East	−0.051***	0.011	−0.032***	0.008	−0.037***	0.004	−0.011***	0.003
West	−0.04*	0.022	−0.035**	0.014	−0.013*	0.008	−0.006	0.006
South	−0.05***	0.018	−0.067***	0.01	−0.032***	0.006	−0.046***	0.006
North East	−0.002	0.011	−0.036***	0.011	−0.027***	0.006	−0.007*	0.004

SC/ST, Scheduled castes and Scheduled Tribes; OBC, Other Backward Classes; CI refers to the Concentration Index, which measures the socioeconomic inequality in the prevalence of anemia among women; SE, standard error. * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Author's calculation based on NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16) and NFHS-5 (2019–21) data using STATA.

TABLE 4 Trends and differentials in the prevalence of any anemia among Muslim women, based on odds ratios from NFHS (1998–2021).

Socioeconomic characteristics	NFHS-2		NFHS-3		NFHS-4		NFHS-5	
	Odds ratio	[95% CI]	Odds ratio	[95% CI]	Odds ratio	[95% CI]	Odds ratio	[95% CI]
Caste (SC/ST®)								
OBC	0.934	[0.718, 1.213]	1.316***	[1.105, 1.568]	−0.031	[−0.084, 0.022]	0.914***	[0.865, 0.965]
Others	0.874	[0.681, 1.122]	1.361***	[1.151, 1.609]	−0.019	[−0.071, 0.034]	1.043	[0.987, 1.101]
Age (15–19)®								
20–29	1.138	[0.971, 1.334]	0.98	[0.897, 1.069]	−0.037**	[−0.074, 0]	0.852***	[0.818, 0.887]
20–39	1.123	[0.952, 1.324]	0.948	[0.859, 1.046]	−0.057***	[−0.099, −0.016]	0.812***	[0.776, 0.849]
40–49	1.084	[0.91, 1.292]	0.922	[0.824, 1.033]	−0.091***	[−0.137, −0.045]	0.858***	[0.817, 0.902]
Residence (Urban®)								
Rural	0.903*	[0.808, 1.009]	0.956	[0.884, 1.034]	0.078***	[0.046, 0.109]	1.199***	[1.158, 1.242]
Education (No education®)								
Primary	0.956	[0.848, 1.078]	0.897**	[0.81, 0.994]	−0.077***	[−0.12, −0.033]	0.933***	[0.888, 0.979]
Secondary	0.8***	[0.702, 0.913]	0.907**	[0.828, 0.993]	−0.109***	[−0.145, −0.073]	0.968	[0.93, 1.007]
Higher	0.716***	[0.565, 0.907]	0.794**	[0.667, 0.946]	−0.167***	[−0.227, −0.107]	1.015	[0.956, 1.077]
Wealth (Poorest®)								
Poorer	0.737***	[0.624, 0.871]	0.863**	[0.752, 0.99]	−0.039*	[−0.084, 0.006]	0.946**	[0.904, 0.99]
Middle	0.719***	[0.608, 0.849]	0.697***	[0.61, 0.797]	−0.062**	[−0.11, −0.015]	0.893***	[0.85, 0.937]
Richer	0.594***	[0.5, 0.706]	0.62***	[0.539, 0.713]	−0.072***	[−0.123, −0.021]	0.847***	[0.804, 0.893]
Richest	0.499***	[0.407, 0.611]	0.526***	[0.45, 0.614]	−0.131***	[−0.189, −0.073]	0.704***	[0.663, 0.747]
Regions (North®)								
Central	1.01	[0.87, 1.172]	0.85***	[0.762, 0.948]	−0.002	[−0.04, 0.036]	0.571***	[0.547, 0.597]
East	1.54***	[1.335, 1.776]	1.244***	[1.113, 1.39]	0.106***	[0.062, 0.149]	0.823***	[0.786, 0.863]
West	0.857*	[0.727, 1.01]	1.055	[0.929, 1.199]	−0.115***	[−0.177, −0.053]	0.656***	[0.62, 0.695]
South	0.67***	[0.584, 0.769]	1.017	[0.914, 1.132]	−0.232***	[−0.281, −0.183]	0.478***	[0.455, 0.502]
North East	2.119***	[1.794, 2.503]	0.746***	[0.66, 0.845]	−0.513***	[−0.562, −0.463]	0.623***	[0.593, 0.656]
_cons	1.506**	[1.074, 2.111]	1.327**	[1.066, 1.65]	0.243***	[0.168, 0.318]	2.272***	[2.095, 2.463]

SC/ST, Scheduled castes and Scheduled Tribes; OBC, Other Backward Classes; 95% CI, confidence interval at 95% level of significance. Author's calculation based on NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16) and NFHS-5 (2019–21) data using STATA; cons represents the intercept, i.e., the baseline odds of anemia for the reference categories of all covariates. $p < 0.1 = *$, $< 0.05 = **$, $< 0.01 = ***$.

based on different characteristics. OR values above 1 indicate an increased likelihood of anemia compared to the reference group, while values below 1 suggest reduced odds.

Analyzing by caste, OBC Muslim women had higher odds of anemia in NFHS-3 (OR = 1.316, $p < 0.001$), but these odds dropped in subsequent rounds, reaching 0.944 in NFHS-5, suggesting diminishing caste-based disparities. Similarly, Other caste Muslim women showed elevated odds in NFHS-3 (OR = 1.361, $p < 0.001$), but these differences also became non-significant over time, indicating a reduction in caste-related anemia disparity. Regarding age, women in the 20–29 and 30–39 age groups had slightly increased odds in early rounds but lower odds in NFHS-4 and NFHS-5. For instance, in NFHS-5, the 20–29 group had an OR of 0.852 ($p < 0.001$), suggesting younger women (15–19) are more at risk. The 40–49 group shows a similar decrease in odds over time, with anemia risks diminishing as women age, particularly in recent rounds.

Residence impacts anemia likelihood, with rural women showing lower but non-significant odds in NFHS-2 (OR = 0.903). However, by NFHS-5, rural Muslim women exhibited higher odds of anemia (OR = 1.199, $p < 0.001$), marking an increasing rural–urban disparity, where rural women now face higher anemia risks. Education consistently emerges as a protective factor. Women with higher education showed reduced odds across rounds, although the effect was not statistically significant in NFHS-5 (OR = 1.015). Primary and secondary education also lowered anemia odds in NFHS-4 and NFHS-5, with secondary education yielding significantly reduced odds in NFHS-5 (OR = 0.933, $p < 0.001$), confirming the protective influence of educational attainment.

In terms of wealth, there is a clear gradient in anemia odds across quintiles, with wealthier Muslim women consistently displaying lower odds. In NFHS-5, those in the richest quintile had an OR of 0.841 ($p < 0.001$), indicating significantly reduced anemia likelihood compared to the poorest. Even those in intermediate wealth quintiles showed reduced odds in later rounds; for example, the second quintile in NFHS-5 had an OR of 0.946 ($p < 0.01$), underscoring the beneficial impact of economic stability. Regional disparities are also pronounced, as women in the East and Northeast consistently showed higher anemia odds compared to the North. In NFHS-5, the East region had an OR of 1.214 ($p < 0.01$), while the Northeast showed lower odds (OR = 0.623, $p < 0.01$). Women in the South and West generally exhibited lower anemia odds, with the South achieving significantly lower odds in NFHS-5 (OR = 0.442, $p < 0.001$), potentially reflecting improved health and nutritional resources in these regions.

Across all three NFHS rounds, there is a significant difference in the odds of anemia based on caste. SC/ST populations consistently have the highest odds of anemia, followed by OBC and Others. Notably, in NFHS-5, there is a significant decrease in the odds of anemia among all caste groups compared to NFHS-3 and NFHS-4. Urban areas consistently show lower odds of anemia compared to rural areas in all three NFHS rounds. While urban areas showed a decrease in the odds of anemia from NFHS-3 to NFHS-4, it increased slightly in NFHS-5. Women with higher levels of education tend to have lower odds of anemia. The gap in anemia prevalence between education levels is most pronounced in NFHS-3 and narrower in NFHS-4 and NFHS-5. Even in NFHS-5, women with no education or primary education still exhibit higher odds of anemia compared to those with secondary or higher education. There is a clear gradient in anemia prevalence across wealth quintiles, with the poorest quintile

consistently having the highest odds of anemia. In all NFHS rounds, the richest quintile has the lowest odds of anemia. Notably, there is a significant decrease in anemia prevalence among the poorest quintile in NFHS-5 compared to NFHS-3 and NFHS-4. Significant regional disparities exist in the odds of anemia across all three NFHS rounds. In NFHS-3, the North and East regions had the highest anemia prevalence, while the South region had the lowest. In NFHS-4, the Central region had the highest anemia prevalence, and the Northeast region had the lowest. In NFHS-5, the North and Central regions again show higher anemia prevalence, while the South and Northeast regions exhibit lower prevalence. Notably, in NFHS-5, there is a significant decrease in anemia prevalence in the North region compared to NFHS-4. These findings highlight the persistence of socioeconomic disparities in anemia prevalence among Muslim women in India, with caste, education, wealth, place of residence, and region all playing significant roles. While there have been improvements in certain areas (e.g., decreased anemia prevalence among the poorest quintile in NFHS-5), ongoing efforts are needed to address and further reduce anemia rates, especially among vulnerable populations.

4 Discussion

While the overall analysis shows that anemia prevalence among women in India has increased over the two decades, a closer look through the socioeconomic lens tells the tale of disparities. The present study highlights the significant burden of anemia among Muslim women in India, emphasizing the influence of socioeconomic, demographic, and geographical factors. Using data from NFHS-2 to NFHS-5, the study demonstrates the unequal prevalence of anemia among Muslim women across different classes, age groups, caste affiliations, and wealth quintiles. The North and Central regions consistently exhibit higher anemia prevalence, while the South and Northeast regions generally display lower rates. The East region emerges as an exception, demonstrating the lowest prevalence of anemia in most categories. [Figure 1](#) presents a state-wise choropleth map of anemia prevalence among all women across NFHS rounds, highlighting consistently high burden in several eastern and central states. [Figure 2](#) focuses specifically on Muslim women, revealing similar spatial patterns, though with sharper disparities in states like Bihar, Assam, and Uttar Pradesh. These maps visually reinforce the statistical findings on regional heterogeneity and emphasize the need for geographically targeted policy responses. The regional variations underscore the influence of geographical factors on anemia prevalence and emphasize the need for tailored interventions addressing region-specific challenges. Recent regional studies across Indian states further validate the disparities identified in our national-level analysis and enhance the external validity of our findings. For instance, Mog and Ghosh conducted a spatial–temporal assessment in Maharashtra and highlighted persistent anemia among women of reproductive age, especially in under-resourced districts (40). In Bihar, Chauhan et al. found a high prevalence of anemia among adolescents, attributing it to limited dietary diversity and poor reproductive health education (55). Similarly, Banerjee and Duflo demonstrated that anemia among women in rural Bihar could be significantly reduced through iron-fortified salt interventions, underscoring the importance of targeted nutrition strategies (56). Biradar documented anemia prevalence

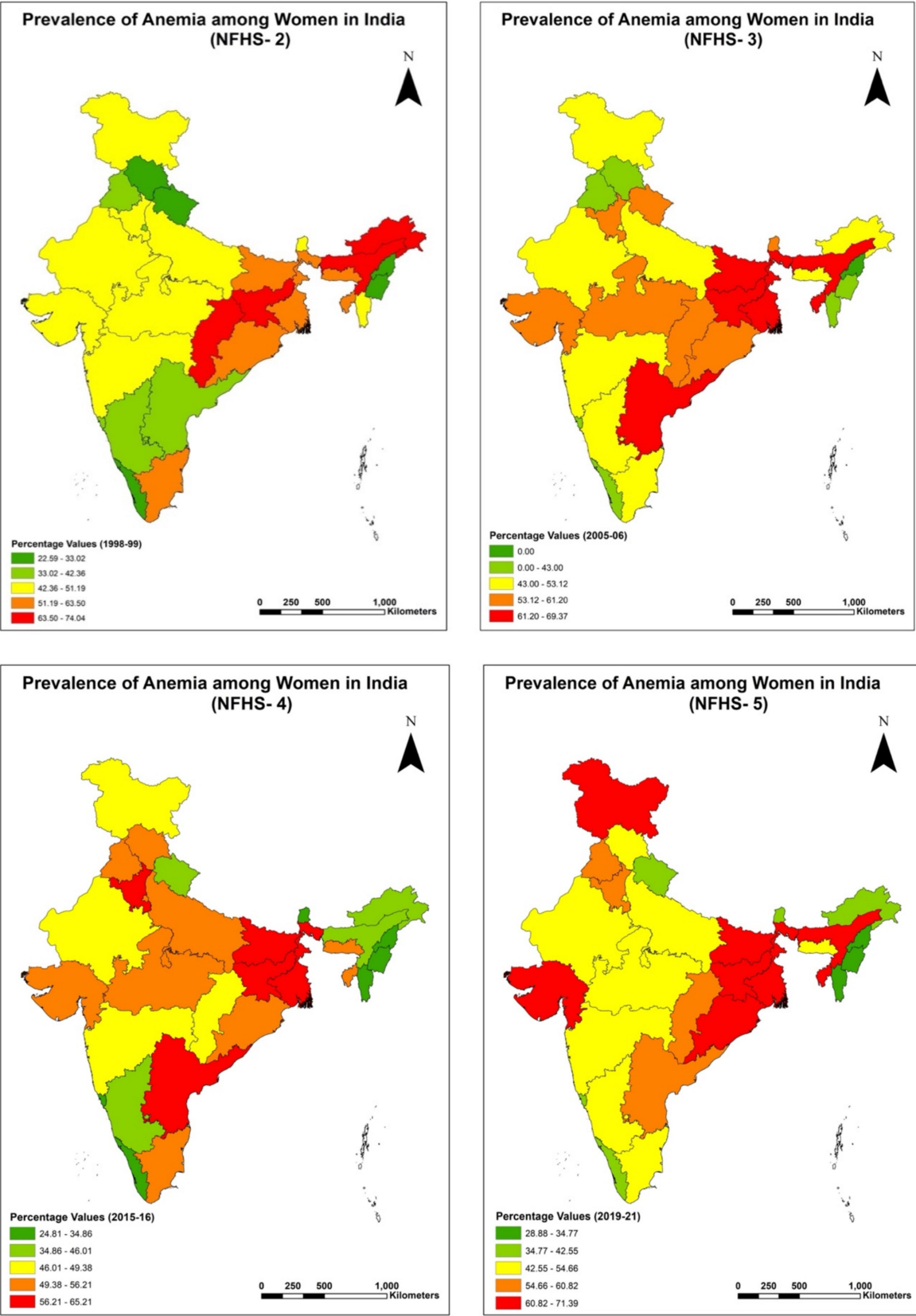


FIGURE 2
Regional analysis of percentage prevalence of any anemia among overall women (15–49 years) in India for NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16) and NFHS-5 (2019–21).

among preconception young married women in India, with regional clusters of vulnerability across northern states (18). In West Bengal, Rao et al. found micronutrient deficiencies to be a leading cause of anemia among rural children, which likely extends into adolescence and adulthood (3). Bharati et al. analyzed anemia trends among reproductive-aged women and found widening rural–urban and state-level disparities over time (57). These findings align with our results and further illustrate the role of regional health infrastructure, social determinants, and nutrition behavior in shaping anemia outcomes. Additionally, Gupta et al. highlighted that women's empowerment and intra-household decision-making power are closely linked to iron status, reinforcing the importance of gender-sensitive strategies across regions (8).

Furthermore, this research highlights the enduring impact of socioeconomic characteristics on anemia prevalence among Muslim women in India. Caste, education, wealth status, and place of residence all demonstrate significant associations with anemia risk. Scheduled Castes and Scheduled Tribes (SC/ST) consistently show higher anemia rates, necessitating targeted interventions to address disparities within these communities. Educational attainment and wealth status exhibit a clear gradient, with higher levels of education and economic prosperity associated with lower anemia prevalence. Urban areas generally fare better than rural areas, though a slight increase in anemia prevalence in urban areas in the most recent NFHS-5 round warrants attention. The findings also reveal that marginalized groups, particularly Scheduled Tribes (ST) and Scheduled Castes (SC), face a disproportionately higher risk of anemia compared to other social groups. This trend is consistent with prior research indicating the socioeconomic disadvantages faced by these communities (3, 14, 41). ST and SC Muslim women, especially those from economically disadvantaged households, exhibit the highest prevalence of anemia (24). These groups often face challenges such as poverty, poor access to healthcare, limited educational opportunities, and inadequate nutrition, which exacerbate their vulnerability to anemia (24). Previous studies have also observed a similar trend, with ST and SC populations experiencing higher rates of malnutrition, morbidity, and anemia due to restricted access to food, healthcare, and education (16, 40, 58). Additionally, the lack of political representation and slower poverty alleviation efforts have further marginalized the ST population compared to SC groups, leading to poorer health outcomes (8). The findings also highlight that rural Muslim women, particularly those from ST and SC communities, are more susceptible to anemia. Rural areas often lack basic infrastructure, including healthcare services, clean water, and sanitation facilities, which increases the prevalence of anemia (59). Studies by Biradar and Sharif et al. align with these results, showing that women in rural areas, especially those belonging to disadvantaged groups, have consistently exhibited higher anemia prevalence across 1998–2021 (18, 24). Economic status and education emerge as critical determinants of anemia prevalence among Muslim women. Women from poorer households are more likely to suffer from anemia, as economic deprivation limits their access to nutritious food and healthcare. Mog et al. found similar associations between economic class and anemia prevalence, emphasizing the role of household income in determining health outcomes (60). Education also plays a protective role, as higher levels of education are associated with better health awareness, improved dietary diversity, and greater access to healthcare services (16). Studies suggest that women's empowerment within households is associated with better health

outcomes, including reduced anemia prevalence (61). Autonomy in fertility decisions and delayed marriages further contribute to improved maternal health, as women in such households are less likely to experience frequent pregnancies or low birth weights (35).

The findings of this study align with the theory of intersectionality, which explains how multiple social identities, such as caste, class, gender, and religion, interact to create unique experiences of privilege and disadvantage (62). Muslim women in India face intersecting forms of discrimination that amplify their risk of anemia (63). For instance, a rural Muslim woman belonging to an ST or SC community is more likely to experience poverty, inadequate healthcare access, and limited educational opportunities, all of which contribute to higher anemia prevalence (42, 44). The Sachar Committee Report revealed that Muslim communities face systemic socioeconomic disadvantages, including lower levels of education, limited access to social security, and employment discrimination (43). These factors disproportionately affect Muslim women, further marginalizing their position in society and increasing their susceptibility to anemia (54). This intersection of caste, class, religion, and gender shapes the health outcomes of Muslim women, highlighting the need for targeted interventions that address these overlapping inequities. The results of this study are consistent with prior research on anemia in India. Studies have shown that caste identity significantly influences anemia prevalence, even after controlling for demographic factors such as education and household wealth (33, 64). Similarly, other studies have observed higher anemia rates among socio-economically disadvantaged groups, particularly in rural areas (24, 34). These findings reinforce the idea that structural inequalities, manifested through caste, class, and gender, play a central role in shaping health outcomes in India.

Despite the implementation of major national initiatives such as Anemia Mukt Bharat, Poshan Abhiyaan, and the Iron and Folic Acid Supplementation Programme, our findings suggest that these efforts have not adequately reached all vulnerable groups. The persistently high anemia prevalence among Muslim women, particularly those in rural areas, SC/ST communities, and economically disadvantaged households, along with the recent increase in anemia among urban women in NFHS-5, indicates limited impact or uneven coverage of current interventions. These trends suggest that structural barriers, such as poor healthcare access, sociocultural exclusion, and low awareness, may be undermining the effectiveness of national programs. Ahmad et al. highlighted several bottlenecks in the public health supply chain for iron and folic acid supplementation in India, reinforcing the need to address logistical and systemic constraints under the Anemia Mukt Bharat strategy (65). As such, these “negative” findings are not just limitations, but they are crucial signals for policy reassessment and provide evidence-based justification for strengthening program implementation, ensuring intersectional targeting, and improving monitoring and accountability within existing health and nutrition frameworks.

These findings have significant implications for public health policies aimed at reducing anemia prevalence in India. Addressing anemia among Muslim women requires a comprehensive approach that considers the intersections of caste, class, gender, and place of residence. Targeted interventions, such as providing iron and folic acid supplements, promoting dietary diversity, and improving access to healthcare services, are essential for reducing anemia

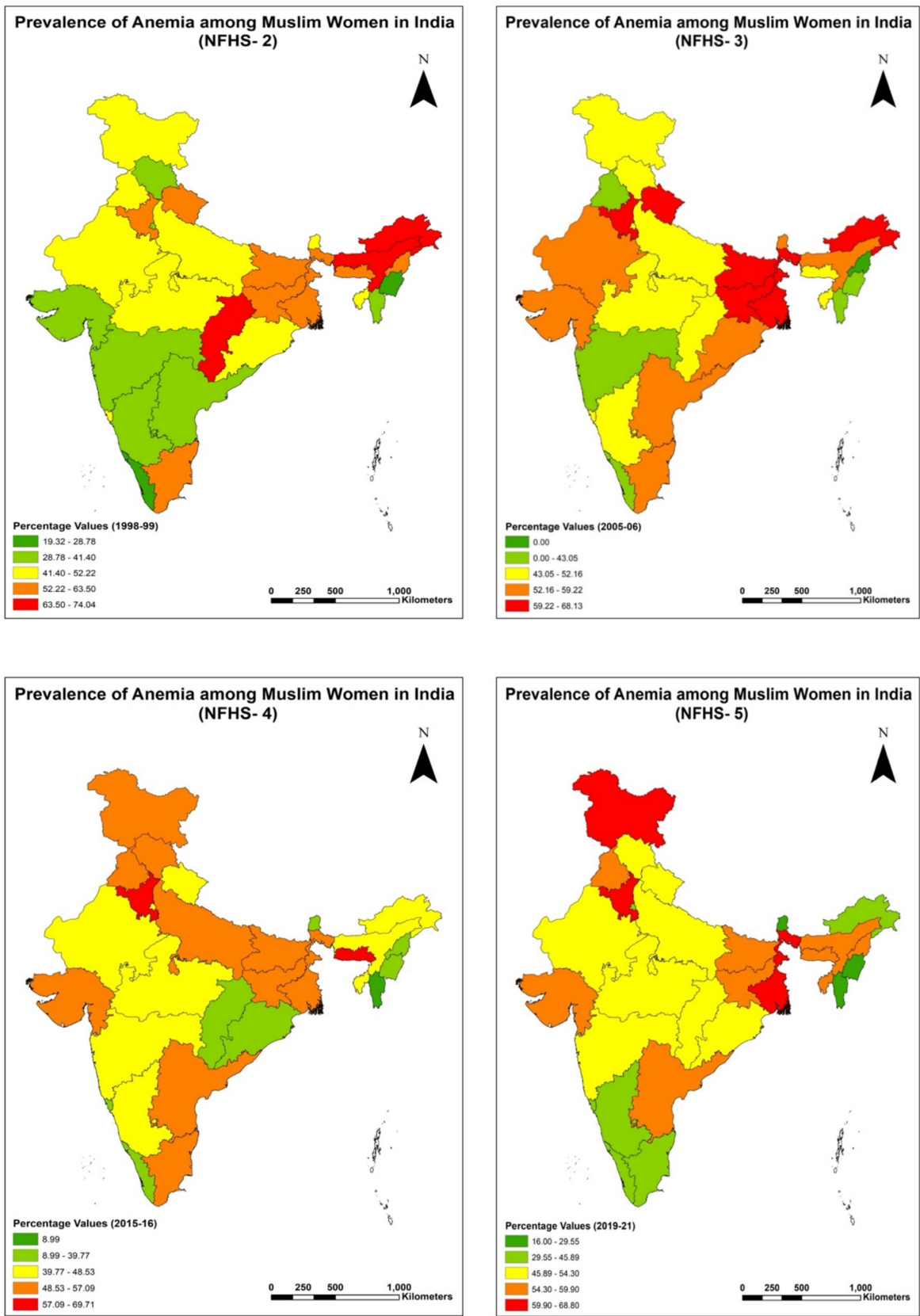


FIGURE 3 Regional analysis of Percentage prevalence of any anemia among Muslim women (15–49 years) in India for NFHS-2 (1998–99), NFHS-3 (2005–06), NFHS-4 (2015–16) and NFHS-5 (2019–21).

prevalence among marginalized groups. Policymakers must also address the structural barriers that perpetuate health inequities. This includes improving access to education and employment opportunities for Muslim women, addressing discriminatory practices in healthcare delivery, and ensuring the availability of basic amenities such as clean water and sanitation. Strengthening the healthcare infrastructure in rural areas and prioritizing the needs of ST and SC populations can further reduce the disparities in the prevalence of anemia among women of reproductive age in India (Figure 3).

5 Limitations and future research

This study has certain limitations that should be considered when interpreting the findings. Being a cross-sectional study, it cannot establish cause-and-effect relationships between socioeconomic factors and anemia prevalence. The analysis relies on secondary data from the NFHS, which may not fully capture the contextual factors influencing anemia, such as cultural practices or dietary habits. Future research could explore these intersections among diverse population groups. Additionally, longitudinal studies are recommended to assess the long-term impacts of socioeconomic and demographic factors on anemia prevalence, providing a more comprehensive understanding of its determinants.

6 Conclusion

India has progressed rapidly in terms of economic growth in the past three decades, but still suffers from substantial socioeconomic (including religion and gender-based) inequalities in economic and non-economic (such as health) outcomes. Within health, hunger and malnutrition have been chronic problems and challenges in Indian society, thereby causing more serious problems like anemia. Although progress has been made in reducing anemia prevalence in some demographic groups, women from rural areas, Scheduled Castes (SC), and Scheduled Tribes (ST), within Muslim households, consistently exhibit disproportionately higher rates compared to their urban and higher-caste counterparts (40, 58). These disparities reveal the structural barriers to achieving health equity in India.

Our findings indicate a modest but meaningful decline in economic disparities in anemia prevalence among Muslim women in India across the NFHS rounds. This trend is reflected in the consistently negative Concentration Index (CI) values and the gradual decrease in their absolute magnitude over time, suggesting a pro-poor distribution of anemia and some progress toward reducing inequality. However, this progress has not been uniform. Persistent regional disparities point to the need for localized and context-specific public health strategies. These patterns have important implications for policymakers and health practitioners, reinforcing the urgency of sustained and targeted efforts to reduce anemia, particularly among socially and economically disadvantaged populations. Drawing on four rounds of NFHS data spanning over two decades, this study offers timely evidence on both the progress made and the enduring gaps in anemia outcomes among Muslim women of reproductive age in India. Nonetheless,

the persistence of regional disparities in anemia prevalence remains a concern.

In light of these findings, it is imperative for policymakers and healthcare providers to prioritize sustained efforts aimed at promoting health equity and reducing the prevalence of anemia among economically disadvantaged populations, particularly among Muslim women. Strategies should encompass region-specific initiatives to address regional disparities effectively. Overall, this research contributes to the broader discourse on public health and underscores the importance of continued vigilance and targeted interventions in the pursuit of improved health outcomes and reduced socioeconomic disparities among women of reproductive age in India.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://www.dhsprogram.com/data/dataset_admin.

Author contributions

ZH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. AS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Value for health: how fortified infant cereals provide cost-effective solutions to iron deficiency anaemia in Egypt

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Background: Anaemia prevalence among Egyptian children under 5 years of age increased from 27.2% in 2014 to 43% in 2024, primarily attributed to iron deficiency anaemia (IDA). The World Health Organization and the United Nations International Children's Emergency Fund recommend iron-fortified foods and supplements to combat IDA. In the absence of longitudinal data among Egyptian children with anaemia, a microsimulation and cost-effectiveness analysis was conducted to evaluate the economic and health impacts of consuming iron-fortified cereals (IFC) in reducing IDA prevalence among Egyptian children under 2 years of age.

Methods: Data of 1707 children under 2 years of age from Egyptian Family Health Survey 2021 (EFHS) were used to create a virtual cohort of 100,000 through Monte Carlo simulations, stratified by age, gender, wealth index, and anaemia severity. A Markov model projected transitions in anaemia severity over 10 years for IFC and non-IFC consumers. Costs for IFC and home-based foods were derived from market research and existing literature, with cost-effectiveness evaluated using the incremental cost-effectiveness ratio (ICER), indicating the additional cost required to gain one additional unit of effectiveness (in our case the disability-adjusted life years [DALYs]) when two approaches are compared.

Results: The per-day cost of home-based food was 0.37 United States Dollar (USD) per child, with an additional 0.17 USD for IFC consumers. Based on 5% IFC consumption (EFHS 2021) anaemia prevalence was projected to reduce to 32% over 10 years. DALYs averted among IFC consumers were 0.006 DALY/day and 22 DALYs over a period of 10 years. The obtained ICER of −4.14 suggests that an IFC intervention can be more effective and less costly than no intervention.

Conclusion: IFC interventions among Egyptian children under 2 years of age are crucial for reducing IDA. IFC consumption lowers DALYs and offers significant cost savings over 10 years, making it an effective health and economic strategy. With 4.058 million children under 2 years of age in Egypt, IFC interventions could save 7.79 million USD for 1 day of disability averted. This study provides evidence-based policy insight, urging prioritisation of IFC recommendation in public health strategies to combat IDA in children and reduce economic burdens.

KEYWORDS

iron deficiency anaemia, dietary supplements, fortified infant cereals, health economics, cost-effectiveness analysis

1 Introduction

Micronutrient deficiencies during early childhood (before 2 years of age) can significantly hinder a child's physical growth and neurodevelopment, leading to short- and long-term health issues with social and economic repercussions later in life (1, 2). This is particularly concerning for iron deficiency anaemia (IDA), which affects 40%–50% of the global population and 41.4% of children under 5 years of age (3, 4). Anaemia can impair cognitive and physical development, increase infant mortality and morbidity, and reduce adult work productivity, resulting in sociocultural and economic impacts (2). The prevalence of anaemia among children in the Eastern Mediterranean region ranges between 23% and 60% in various population subgroups (5). In Egypt, the prevalence of anaemia among children increased from 27.2% in 2014 to 43% in 2024 (6, 7), regardless of the free paediatric iron supplementation programme supported by the local government. The 2021 Egyptian Family Health Survey (EFHS) indicated that 20.9% of children under 5 years of age were affected by mild anaemia, 20.8% by moderate anaemia, and 1.4% by severe anaemia (8).

To ensure appropriate dietary intake and prevent deficiencies in children, the World Health Organization (WHO) and the United Nations International Children's Emergency Fund recommend initiating complementary feeding after 6 months of age, incorporating micronutrient-fortified foods to reduce the risk of micronutrient deficiencies, including IDA (9). To prevent IDA, the WHO advises dietary fortification of 2 mg/kg body weight and daily iron supplementation for children under 5 years of age (9, 10). However, adherence to daily iron supplements is frequently suboptimal among children due to factors such as taste, palatability, forgetfulness, and cultural considerations (9, 11). Universal access to iron-rich foods in the child's early years is a crucial public health intervention to support children's growth and development, building a more productive society (4). This study was conducted to evaluate the economic and health impact of consuming iron-fortified cereals (IFC) in reducing IDA prevalence among Egyptian children under 2 years of age.

2 Methods

2.1 Data source and study population

The base data for this study were sourced from the recent EFHS 2021 survey, a nationally representative survey, in which data from 1707 children aged 6 months to 2 years were analysed to assess haemoglobin (Hb) levels and IFC consumption (8). The EFHS 2021 survey was selected as it provides comprehensive information on dietary practises, including consumption of IFC, across various regions of Egypt. Although the virtual cohort used in the modelling study is based on a subset of 1,707 children from this survey (as base data), the broader EFHS 2021 sample was

designed to reflect Egypt's national-level demographic diversity, including both urban and rural populations. Key variables included age, gender, home-based infant feeding practises (types of food, breastfeeding frequency, formula feeding, and complementary foods), access to IFC (availability and ability of caregivers of infants and young children to obtain and utilise IFC), maternal dietary practises, and relevant social and cultural factors. A multivariate analysis was conducted to adjust for any confounding factors. To determine the significant difference in Hb levels among all the key variables, a *post hoc* analysis with Bonferroni correction was conducted.

Anaemia was defined as an Hb concentration below 11 g/dL and was further categorised into mild anaemia (Hb 10–10.9 g/dL), moderate anaemia (Hb 7–9.9 g/dL), and severe anaemia (Hb <7 g/dL) (8). Additionally, children were stratified into socioeconomic groups based on a wealth index, categorising households as poorest, poor, middle, upper-middle, and wealthiest. Home-based foods were grouped into a single category hence dietary variations were not considered in the microsimulation model.

2.2 Microsimulation study

In the absence of longitudinal population data among Egyptian children with anaemia, a microsimulation analysis followed by a cost-effectiveness analysis (CEA) was conducted to evaluate the impact of consuming IFC during early childhood (6 months to 2 years of age) in reducing the prevalence of IDA and to assess its long-term benefits (estimated for a duration of 10 years). Monte Carlo simulations were used to create a virtual cohort of 100,000 children derived from the initial 1,707 children in the base data. From this, two subgroups were created: Group 1 comprised of children who consumed iron-fortified age-adapted foods as per the base data at least once a day until 2 years of age, with an average iron content of 5.5 g per serving (IFC group) and Group 2 consisted of children not consuming IFC (non-IFC group) (12). Transition probabilities were derived from a combination of empirical data and extrapolation. The extrapolation of data was performed using existing literature. A review of relevant literature was conducted to identify the odds ratio and relative risk of developing anaemia among children in Egypt (2, 13–18). Empirical derivation was based on the rates at which individuals transitioned between health states (19). Equations for transition probabilities for stages of anaemia are presented in [Supplementary Table S1](#) (Equations 1–4). To best account for the transitions between various anaemia states, probabilities for state transitions up to 10 years (6-month intervals) were developed using the Markov model ([Figure 1](#)) (19). Each transition probability represented the likelihood of shifting between no anaemia and mild, moderate, or severe anaemia and vice versa at 6-month intervals. The IFC group, consisting of children who received IFC during early childhood (up to 2 years of age), accounting for ~5.6% of boys and 4.3% of girls in the base data for that group range, was compared with children not consuming IFC. Home-based foods, as mentioned in the EFHS 2021 data, were consumed by the children in both groups.

Abbreviations: IFC, Iron-fortified cereals; CEA, Cost-effectiveness analysis; WTP, Willingness-to-pay.

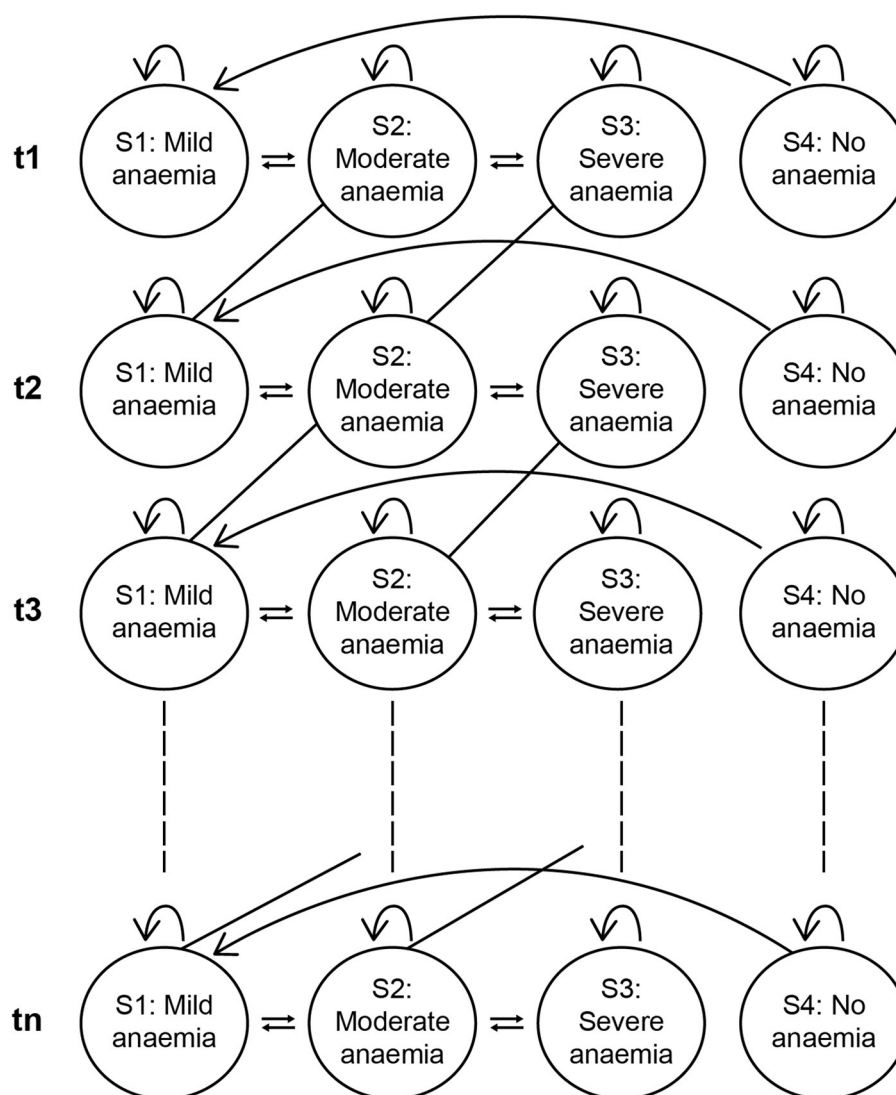


FIGURE 1
Conceptual model using transition probabilities Markov model.

2.3 Estimating disability-adjusted life years (DALYs)

DALYs were calculated for 10 years using years of life lost and years lived with disability, incorporating discount rates of 3% (applied after 1 year) to account for the time preference of health benefits (since being healthy now is valued more than being healthy in the future, a discount rate is applied to reduce the value of future health outcomes and reflect the preference for current health) (20). Disability weights for anaemia among children were taken from the Global Burden of Disease 2019 (4).

2.4 Estimating incremental cost-effectiveness ratio

The EFHS 2021 survey provided detailed data on feeding practises for children under 2 years of age. To evaluate the

cost-effectiveness of IFC in reducing IDA among Egyptian children, the ICER value was calculated, which is a way to compare the costs and benefits of different approaches—IFC consumers vs. non-consumers. The ICER is the incremental change in costs to have IFC divided by the incremental change in health outcome [cost per DALY saved {cost (intervention)-cost (comparator)/DALY averted (intervention)-DALY averted (comparator)}] (21).

The analysis involved comparing the costs and health benefits (Hb levels and anaemia reduction only) over 10 years between children consuming IFC and those not consuming IFC in the first 2 years of their life. [Supplementary Table S2](#) presents the monthly consumption proportions of various home-based foods as reported in the EFHS 2021 (8, 22, 23). The costs of these foods were gathered from multiple sources (22, 23). The costs of semi-solid and solid foods, dairy products (such as yoghurt and cheese), fruits, and vegetables, as well as meat, fish, and poultry products were sourced from the International Food Policy Research Institute (22). [Supplementary Table S3](#) represents the disability weights for anaemia among children aged 6–24 months as per the Global

Burden of Disease 2019 (4). Market research data provided the cost estimates for fortified baby foods and infant formulas (23). The duration for IFC consumption by the children was capped at 2 years. Thus, considering 0–6 months as exclusively breastfeeding, IFC consumption starting between 6 and 12 months (considering one timepoint corresponded to a duration of 6 months) will have three timepoints (first consumption timepoint between 6 and 12 months, second consumption timepoint between 12 and 18 months, and third consumption timepoint between 18 and 24 months). Similarly, those between 12 and 18 months will consume IFC at two timepoints (first consumption timepoint between 12 and 18 months, second consumption timepoint between 18 and 24 months) and children between 18 and 24 months will consume IFC at a single timepoint (consumption timepoint between 18 and 24 months).

2.5 Sensitivity analysis

To evaluate how uncertainties in the Markov microsimulation model influence ICER per capita per day, both one-way and probabilistic sensitivity analyses were conducted (24). In the one-way sensitivity analysis, every parameter was adjusted individually within a specified range to determine the impact of each parameter on the model outputs in isolation (25). Upper and lower bounds were derived from the minimum and maximum values available

in databases. This approach allowed us to observe the impact of each parameter on the model outcomes, enabling the identification of key drivers of cost-effectiveness (26). Additionally, the results of the system model were enunciated through sensitivity analysis, which highlights the significant impacts of uncertainties on system behaviour and performance. For probabilistic sensitivity analysis, model inputs were sampled multiple times from predefined probabilistic distributions. We applied gamma distributions for cost parameters and beta distributions for all other variables. The 95% confidence interval of a specific parameter represents the plausible range of values used in the sensitivity analysis. The model was executed 1,000 times, simulating a cohort of 100,000 children. We then determined how each strategy changed the ICER per capita under various scenarios, stratified for gender and wealth index groups. Median ICER values were reported along with the interquartile range to capture variability.

3 Results

The study included 1,707 infants (872 [51.08%] boys and 835 [48.9%] girls) aged 6 months to <2 years from the EFHS 2021. There was no statistically significant difference in age group distribution between the boys and girls ($p = 0.430$). Among these children, anaemia (Hb levels <11 g/dL) was observed in 58.6% of children ($n = 1002$), with 24% ($n = 410$) having mild

TABLE 1 Description of base data (8).

Child's age in months	All children (1,707) [<i>n</i> (%)]	Boys (872) [<i>n</i> (%)]	Girls (835) [<i>n</i> (%)]	<i>p</i> -value
6 to <12	27.48 (469)	28.78 (251)	26.11 (218)	0.430
12 to <18	34.86 (595)	33.83 (295)	35.93 (300)	
18 to <24	31.75 (643)	31.9 (326)	31.62 (317)	
Anaemia levels				
0 = Not anaemic	41.30 (705)	41.06 (358)	41.56 (347)	0.604
1 = Mild	24.02 (410)	22.94 (200)	25.15 (210)	
2 = Moderate	33.63 (574)	34.86 (304)	32.34 (270)	
3 = Severe	1.05 (18)	1.15 (10)	0.96 (8)	
Haemoglobin level (g/dL) (mean ± SD)	10.56 ± 1.60	10.51 ± 1.63	10.61 ± 1.57	0.134
Wealth index quintile				
1 = Lowest quintile (poorest)	17.51 (299)	17.78 (155)	17.25 (144)	0.504
2 = Second quintile (poor)	18.92 (323)	20.18 (176)	17.60 (147)	
3 = Third quintile (middle)	21.56 (368)	21.22 (185)	21.92 (183)	
4 = Fourth quintile (upper middle)	23.61 (403)	23.74 (207)	23.47 (196)	
5 = Highest quintile (wealthiest)	18.39 (314)	17.09 (149)	19.76 (165)	
Feeding practises				
Children consuming fortified infant cereals (baby foods)	85 (5.0%)	49 (5.6%)	36 (4.3%)	0.211
Receiving subsidies for infant formula (<i>n</i> = 248)	100 (5.86%)	51 (5.85%)	49 (5.87%)	0.960
Availability of infant formula at local PHC clinic (<i>n</i> = 226)	103 (6.03%)	55 (6.3%)	48 (5.75%)	0.569
Children eating grains and cereal-based foods	1,258 (73.7%)	633 (72.6%)	625 (74.9%)	0.308

PHC, Primary healthcare centre; SD, Standard deviation.

TABLE 2 Cost-effectiveness analysis for IFC among IFC consumers vs. non-consumers.

Indicators for cost effectiveness	Boys	Girls
DALY with IFC per day (in 10 years)	0.021 (77)	0.021 (77)
DALY without IFC per day (in 10 years)	0.027 (99)	0.027 (99)
Cost of food per person/day with IFC (for 18 months)	0.540 (291) USD	0.540 (291) USD
Cost of food per person/day without IFC (for 18 months)	0.370 (199.8) USD	0.370 (199.8) USD
ICER 10 years	−4.14.	−4.14

DALY, Disability-adjusted life years; ICER, Incremental cost-effectiveness ratio; IFC, Iron-fortified cereals; USD, United States Dollar.

anaemia, 33.6% ($n = 574$) having moderate anaemia, and 1.05% ($n = 18$) having severe anaemia. The difference in anaemia levels between boys and girls was not statistically significant ($p = 0.604$). The mean Hb level was 10.6 ± 1.6 g/dL with no statistically significant difference among boys (10.51 ± 1.63) and girls (10.61 ± 1.57) ($p = 0.134$). Similarly, there was no statistically significant difference among boys and girls when categorised according to the wealth index quintile ($p = 0.504$; [Table 1](#)). The results of meta-regression analysis showed non-significant difference among geographic variations (rural/urban); hence, this was not considered as an input parameter for the microsimulation model. The results of *post-hoc* analysis are presented in [Supplementary Table S4](#).

3.1 Cost of the intervention

Based on the dietary practises highlighted under the EFHS, the average cost of home-based foods per child per day (below 2 years) was estimated as 0.37 United States Dollar (USD) and the average cost of IFC per child per day was identified as 0.17 USD. Thus, the cost of consuming IFC along with home-based foods for each child was around 0.54 USD ([Table 2](#)). This means that the total cost of the intervention is equal to a maximum of USD 291 for children who will start the consumption of IFC at 6 months and will finish at 2 years old and equal to USD 199.8 for those who will not consume IFC.

3.2 Effects of iron fortification on anaemia

Based on the transition between mild, moderate, and severe anaemia, changes are anticipated in the prevalence of anaemia in the next 10 years ([Supplementary Tables S5, S6](#); [Figure 2](#)). At baseline (timepoint t1), extrapolated from the EFHS data, the anaemia prevalence in the population consuming IFC along with home-based foods was almost similar in both groups (with no gender disparities): around 59% among IFC consumers vs. 57% among non-IFC consumers. It is important to note that the EFHS lacks information on the consumption pattern of IFC. By the end of the simulated period (timepoint t20), anaemia prevalence decreased to 27% in the IFC group, while it remained higher at 36% in the non-IFC group. Across all time points, the IFC

group consistently exhibited a lower anaemia prevalence than the non-IFC group ([Supplementary Tables S5, S6](#)).

3.3 Effects of anaemia on DALYs

Both boys and girls who consumed IFC along with home-based foods in their initial years of life (<2 years of life) experienced a lower number of days lost due to disability (for example, fatigue, weakness, and shortness of breath, making activities of daily living difficult) arising from IDA compared to the girls and boys who did not consume IFC ([4](#)). The days lost to disability due to IDA in a child consuming IFC were 77 days (0.021 DALY), while the days lost in a child not consuming IFC were 99 days (0.027 DALY). DALYs averted among IFC consumers compared with non-IFC consumers were 0.006 DALY/day. Thus, IFC consumers will save around 22 days of disability arising from IDA in 10 years, indicating that IFC contribute to improved health outcomes by preventing or reducing the severity of anaemia.

3.4 Cost-effectiveness analysis of iron fortification

This study highlighted that an 18-month intervention with IFC leads to an ICER equal to −4.14 USD over a 10-year period ([Table 2](#)). When using DALYs, a negative ICER can occur if either the numerator (incremental cost) or the denominator (DALYs averted) is negative. Negative cost (numerator) implies that the new intervention is less costly than the reference intervention. However, this is not the case in the present model: fortified cereals come with an additional cost. In the present model, we have incremental costs (positive numerator) and negative DALYs (denominator). This means that the new intervention leads to fewer DALYs (more health gains) than the reference intervention (no consumption of fortified cereals). Among children consuming IFC, the total days saved from disability were 2.19 days per annum. The cost of saving 1 day of disability was 1.92 USD.

3.5 Evaluating model robustness

One-way sensitivity analysis demonstrated that variations in the cost of IFC had the most significant impact on ICER ([Supplementary Figures S1A, B](#)). A 10% reduction in the cost of IFC shifted the median ICER from a cost-saving value of −29.8 USD per capita per day to a positive value of 5.09 USD, with a wide interquartile range, indicating substantial uncertainty. In contrast, a 20% reduction in cost yielded a median ICER of 5.63 USD with a narrower range, suggesting more stable but still mixed outcomes. Subgroup analyses revealed that children from the poorest wealth index had the most favourable ICERs, indicating consistent cost savings. Conversely, the wealthiest subgroup displayed a wider range, with potential for both cost savings and modest cost increases. Gender differences had a minimal influence on the ICER, closely aligning with the base case. These findings strongly underscore that cost parameters and socioeconomic

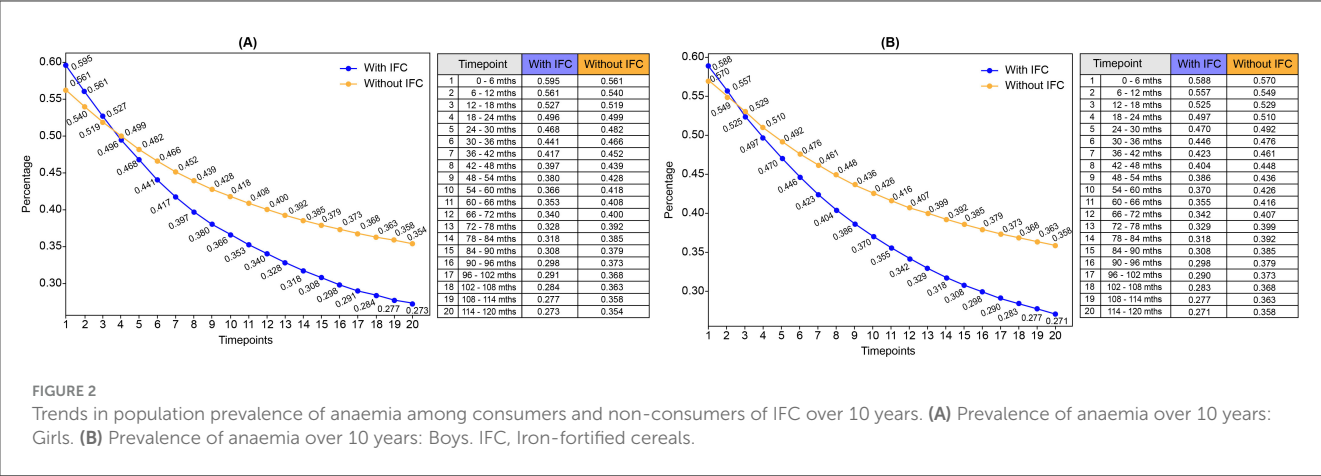


FIGURE 2 Trends in population prevalence of anaemia among consumers and non-consumers of IFC over 10 years: (A) Prevalence of anaemia over 10 years: Girls. (B) Prevalence of anaemia over 10 years: Boys. IFC, Iron-fortified cereals.

strata are crucial determinants of the model's outcomes and should be key considerations in policy implementation. The cost-effectiveness acceptability curve shows an 80% probability that IFC are a cost-effective intervention at an additional cost of 0.17/day USD, increasing with cost reductions (10%–20% cost reduction) but dropping to 40% with no funding support. This demonstrates that affordability and willingness-to-pay (WTP) thresholds critically determine the intervention's cost-effectiveness (Supplementary Figure S1B).

4 Discussion

The study findings suggested a significant reduction in the prevalence of anaemia from 59% to 27% over 10 years among both boys and girls who consumed IFC at least once a day until 2 years of age. IFC intervention was considered for children under 2 years of age because the first 1,000 days (from conception to 24 months) is the most critical window for physical and cognitive development. The majority of the linear growth deficits that make up the under-5 stunting burden are accumulated during this time, reflecting nutritional deficiencies (27, 28). These findings emphasise the critical role of iron food fortification, specifically IFC, in early childhood in mitigating IDA and the related long-term consequences.

The current study is consistent with previous research. Harrison et al. (18) reported a significant reduction in the prevalence of IDA among infants in Ghana who consumed micronutrient-fortified cereals, providing 3.75 mg of iron as ferrous fumarate per 50 g of cereal. Daily consumption varied by age: 50 g/day for infants aged 6–8 months, 75 g/day for those aged 9–11 months, and 100 g/day for those aged 12–18 months. In the fortified cereal group, the prevalence of IDA decreased from 81.1% to 42.8%, compared to a reduction from 89.1% to 62.8% in the non-fortified group ($p = 0.006$) (18). Similarly, Moumin et al. (29) conducted a study in Australia involving infants aged 6–12 months who were given 18 g (300 kJ) of iron-fortified infant cereal per serving. This intervention significantly reduced the prevalence of iron inadequacy from 75% to 5%. Furthermore, Ekoe et al. (17) reported a significantly lower prevalence of IDA (14.6%)

among children aged 18–59 months receiving micronutrient-fortified cereals compared to 53.4% in those who did not receive the fortified cereals ($p < 0.001$). Collectively, these studies reinforce the positive impact of iron fortification in reducing anaemia prevalence and improving iron status in young children, highlighting the efficacy of such interventions across different populations and settings.

Many studies show that iron supplementation results in a 58%–72% reduction in anaemia prevalence, compared to a 23%–62.8% reduction in children consuming IFC (14, 15, 18). Although the reduction in anaemia prevalence is higher among children consuming iron supplementation, iron supplementation often encounters issues such as poor compliance (9). Moreover, supplementing iron in iron-replete infants or those with unknown iron status can negatively impact growth and weight gain (9). IFC have better compliance and offer a preventive, long-term, sustainable approach. This strategy, when combined with home-based foods, can effectively meet nutritional needs during the critical period of 6–23 months of age (17). IFC were also associated with enhancements in neurodevelopmental outcomes. Specifically, children consuming IFC showed significantly higher scores on the Bayley Scales of Infant and Toddler Development, Third Edition, in areas such as language ($p = 0.003$), motor development ($p = 0.018$), and social-emotional ($p = 0.004$) and adaptive behaviour ($p < 0.001$), though not in cognitive development ($p = 0.980$), compared to those not consuming IFC (15). While individual studies have reported these positive health outcomes, extrapolation of these data over a 10-year period is not feasible due to the overlapping nature of micronutrient deficiencies and health outcomes. Aggregating the burden of micronutrient deficiencies from individual studies may lead to overestimation (Supplementary Table S7) (2). For all these reasons, these additional health outcomes were excluded from the current analysis.

Although this study considered IFC intervention up to 2 years of age, long-lasting effects in improving health status are expected. These findings indicate that regular consumption of IFC containing 5.5 g of iron per serving at least once daily during the first 2 years of life along with home-based foods results in a substantial reduction in anaemia prevalence over time and is an efficacious public health strategy in reducing anaemia prevalence. While IFC

incur a small increase per-day household food cost, adding 0.17 USD to the baseline 0.37 USD for home-made foods, this reflects only the direct cost of food and does not capture the broader economic implications of anaemia-related health outcomes. In the current CEA, where the cost of the intervention is higher than the comparator but the DALYs of the intervention are lower than the comparator, it means that the intervention is more expensive, but it also results in better health outcomes (fewer DALYs). In other words, the DALYs gained (health benefits) come at an additional cost. Thus, in such cases, the negative ICER (−4.14 USD) cannot be directly interpreted. Hence to calculate the cost-effectiveness of the current intervention, WTP threshold is considered. This means comparing the cost per DALY averted (4.14 USD in the current study) independently from its sign (negative in this case) to the country's gross domestic product (GDP) per capita or policy norms. If the intervention's additional cost is <1 GDP per capita, it can be considered cost-effective. If the cost per DALY averted is below the WTP threshold, the intervention is cost-effective despite its higher cost. If the cost per DALY averted exceeds the WTP threshold, the intervention is not cost-effective (30). In this study, given the Egyptian daily GDP per capita equal to 6.6 USD (31, 32), the intervention can be considered cost-effective. As per WHO-CHOosing Interventions that are Cost-Effective (CHOICE), the current IFC intervention can be considered as highly cost-effective as the ICER is <1 × GDP per capita per DALY averted (33, 34).

For the total population of Egyptian children under 2 years of age, which is 4.058 million (35), the IFC intervention is expected to be cost saving, with an estimated savings of ~7.79 million USD ($1.92 \times 4,058,000$) for each day of disability averted. This suggests that the intervention is not only effective and less costly over a 10-year period but also holds significant policy implications. By demonstrating economic benefit, it highlights the importance of incorporating such interventions into comprehensive public health strategies to enhance outcomes for young children while ensuring efficient resource utilisation. A common method to assess cost-effectiveness is to compare the ICER to a WTP threshold. Since the ICER for the IFC intervention is negative, it is lower than the standard cost-effectiveness threshold, including Egypt's per capita daily income of 6.6 USD (31, 32). This suggests that IFC is a cost-effective intervention, providing health benefits at a lower cost than what is typically considered affordable or sustainable based on the country's average income. Although the intervention involves a higher upfront cost for food, it leads to net savings by alleviating the health burden and reducing the broader economic impact of anaemia. The analysis accounts for total costs, encompassing healthcare expenses, productivity losses, and the long-term economic consequences of anaemia. By effectively reducing anaemia prevalence, IFC is associated with fewer healthcare visits, reduced treatment costs, and improved productivity. These savings have the potential to offset or exceed the additional daily cost. Furthermore, the analysis spans over 10 years, allowing the cumulative benefits of reduced anaemia prevalence to outweigh the modest increase in daily food costs.

However, several barriers to the consumption of IFC persist. These include inadequate caregiver awareness and nutritional

education on the benefits and proper use of IFC along with cultural beliefs favouring traditional unfortified dietary practises. Furthermore, accessibility issues in rural or underserved areas, gaps in healthcare provider support, potential inadequacies in government policies to implement national-level strategies for micronutrient intervention such as IFC consumption, lack of subsidy programs, weak monitoring and evaluation systems, under-resourced health information infrastructure, and supply chain inefficiencies contribute to these challenges (27). Addressing these barriers is essential for optimising IFC utilisation and improving child health outcomes. To scale up the distribution of IFC, a collaborative approach involving strengthening the health system and multisectoral collaboration among government, public health agencies, and healthcare providers can be implemented. The distribution of IFC can be integrated into existing maternal and child health programs thereby utilising their infrastructure to reach a larger population (28). Public-private partnerships with food manufacturers will help increase the production, affordability, and availability of fortified cereals. Additionally, awareness campaigns can be implemented, with training for health workers and community leaders to educate families on the benefits of iron fortification. The Global Alliance for Improved Nutrition has also recognised the advantages of fortified micronutrient foods in addressing deficiencies. However, it has been reported that, on average, only 50% of fortified foods meet national standards in low- and middle-income countries like Egypt (36). To maximise their effectiveness and be available to a wider population, IFC awareness and accessibility programmes need to be enhanced.

Implementing IFC intervention in children is crucial from a societal perspective. IFC benefits both families and the healthcare system by improving child health outcomes and reducing hospital-related costs. This will lead to lower healthcare resource utilisation and economic savings for families and society as a whole. Additionally, it is also essential from a policy perspective, as the study provides evidence for policymakers to prioritise IFC interventions and guide cost-effective public health strategies.

This microsimulation study has some limitations. For instance, the analysis did not fully account for the influence of confounding factors, which may affect the association between early childhood iron deficiency and long-term health and economic outcomes. Additionally, the model's estimates may be affected by the presence of other micronutrient deficiencies and multiple diseases that can contribute to IDA, introducing additional complexity and uncertainty to the findings of this model. The assumptions in the Markov modelling may not accurately reflect real-world variations. An unobserved heterogeneity can potentially impact the outcomes of the Markov modelling. Additionally, the absence of direct, longitudinal observational data from Egyptian children and relying solely on data collected from the EFHS 2021 restricts the generalisability of the findings. Furthermore, cost estimates based on market research may introduce bias, potentially impacting the accuracy of the economic outcomes. Despite these limitations, this health economic model strategy serves as a valuable tool for assisting public health professionals in evaluating both the effectiveness and cost-efficiency of various interventions.

5 Conclusion

This study highlights that regular consumption of IFC during early childhood is a cost-effective solution to mitigate IDA which is associated with long-term personal and societal negative implications. Despite the benefits demonstrated, barriers such as insufficient awareness, cultural preferences, and accessibility issues remain. Addressing these challenges is imperative for reducing the prevalence of iron deficiency and its extensive impact on child health while mitigating the associated health and economic burdens. Government bodies should enhance the availability and accessibility of fortified age-adapted foods and ensure compliance with quality standards. Paediatric associations should integrate iron-fortified foods into clinical and complementary feeding guidelines, and healthcare providers must educate caregivers on the short- and long-term benefits of fortified foods in early childhood.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

SE: Writing – review & editing, Conceptualization, Formal analysis, Methodology. SM: Writing – review & editing, Data curation, Formal analysis. LD: Writing – review & editing, Conceptualization, Data curation, Formal analysis, Methodology. HS: Writing – review & editing, Data curation, Formal analysis. HG: Writing – review & editing, Data curation, Formal analysis.

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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.2025.1570683/full#supplementary-material>

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