Iranian Journal of Microbiology
Volume 18 Number 2 (April 2026) 186-196
DOI: http://doi.org/10.18502/ijm.v18i2.21293

ORIGINAL ARTICLE

Gut microbiome signatures associated with iron-deficiency anemia in young adults

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Received: December 2025**, Accepted:** March 2026

ABSTRACT

Background and Objectives: Iron is vital for both the body of the host and the metabolism of microbes. Pathogenic Enterobacteria need iron to grow and cause disease, but many good gut bacteria, like lactobacilli, do not require as much iron. Changes in the amount of iron available in the gut may therefore affect the makeup of the gut microbiome. Iron-deficiency anemia (IDA) represents the most prevalent nutritional disorder globally, concerning about 1.24 billion, mostly women and young people in low- and middle-income countries. This cross-sectional study looked at the link between iron-deficiency anemia and the makeup of the gut microbiome in young adults aged 18 to 30.

Materials and Methods: We looked at hematological and iron status parameters as well as gut microbiota profiling using 16S rRNA gene sequencing. Differences in microbial diversity, taxonomic composition, and the relative abundance of bacteria that make short-chain fatty acids between people with IDA and healthy controls were investigated.

Results: The results show that iron-deficiency anemia is linked to different gut microbiome signatures. This suggests that there may be connections between iron levels and the structure of microbial communities.

Conclusion: These results show how important it is to study the gut microbiome to understand iron-deficiency anemia. They also show how important it is to do long-term, interventional studies to figure out how these associations work and what they mean for health.

Keywords: Iron-deficiency anemia; Gut microbiome; Intestinal microbiota; Iron metabolism; Enterobacteriaceae; *Lactobacillus*; Iron supplementation; Dysbiosis

INTRODUCTION

Iron is required for the function of several proteins in the body, including hemoglobin in red blood cells (1). Many bacteria need iron for development and pathogenicity, while human defenses against infection rely heavily on sequestration (2). Oral iron supplementation is recommended to prevent and treat iron deficiency and is generally considered safe; however, it has been associated with a heightened risk of diarrheal diseases in low- and middle-income countries (3). Iron's influence on gut microbiota makeup may partially mediate this increased risk of infection (4, 5).

Pathogenic *Escherichia coli* carriage increases, whereas beneficial commensal bacteria like *Bifidobacterium* and *Lactobacillus* spp. decrease in abundance (6, 7). Iron supplementation has also been found in high-income nations to affect the gut microbiota of persons with inflammatory bowel disease,

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which is associated with a high burden of iron deficiency anemia (8). Even with iron deficiency anemia, only less than 10% of an oral supplement is absorbed, with the rest going into the large intestine unbound (9). These effects are likely due to inadequate iron absorption. Adults need 20 mg of iron per day to promote erythropoiesis (80%) and other cellular functions (20%) (10). Iron is absorbed by cells via transferrin receptor 1, which binds to iron-containing transferrin and internalizes it into endosomes (11). Chronic inflammation from infections or other causes reduces iron availability and contributes to anemia. Additionally, high iron levels (>100 mg elemental iron/day) increase hepcidin expression, blocking iron transporter expression and reducing iron absorption (12). Bacteria battle with the body for iron by secreting siderophores such as catecholate, phenolate, hydroxamate, and carboxylate in low-iron environments (13).

Iron deficiency anemia (IDA) is a prevalent form of anemia globally, characterized by inadequate iron intake, absorption issues, or heightened demand, resulting in systemic iron deficiency and compromised hemoglobin synthesis (14). Dietary iron is present in two forms: heme iron, which is efficiently absorbed and primarily sourced from animal-based foods, and non-heme iron (15). Insufficient dietary iron intake or reduced iron bioavailability is a primary factor contributing to the high prevalence of iron deficiency anemia (IDA) in numerous countries (16, 17).

The association between dietary iron intake and gut microbiota has recently garnered research attention. Iron serves as a vital micronutrient for host metabolism and is crucial for the proliferation of gut microorganisms (18). The availability and type of dietary iron markedly affect the composition and diversity of gut microbiota. High-iron diets may facilitate the growth of specific potentially pathogenic bacteria, consequently disrupting the balance of gut microbiota (19). Complex interactions may exist between IDA and gut microbiota, potentially influencing the onset and progression of IDA (20, 21). Iron deficiency can diminish the availability of iron in the gut, thereby affecting the abundance of particular microbial populations. Iron-dependent microbes may diminish, whereas others may increase in number as they adapt to environmental stressors. Gut microbiota can modulate the host's iron absorption and distribution via their metabolites, including short-chain fatty acids and bile acids, thereby affecting the onset and progression of iron deficiency anemia (IDA).

There is more and more evidence that iron levels affect the composition of the gut microbiota, but we still do not fully understand how this relationship works, especially in young adults. Most of the studies that have been done so far have looked at babies, kids, or older adults. This means that we do not know much about how iron-deficiency anemia affects the gut microbiome in young adults. The goal of this study is to look into the link between iron-deficiency anemia and the gut microbiome in young adults using blood tests and 16S rRNA gene sequencing. This study wants to shed light on changes in the microbiome that are linked to iron deficiency by looking at how microbial diversity and taxonomic shifts change with IDA. It also wants to make clear that the findings are only associations.

Iron deficiency anemia is becoming more common among teenagers and because traditional food and medical explanations do not fully illustrate the problem. New research shows that the gut microbiome can change how the body absorbs iron, how it handles inflammation, and it controls energy use. The gut microbiome can be associated with IDA and Keep iron deficiency anemia going (1, 22). Examining these microbial patterns may yield new biomarkers that could improve diagnostic accuracy, particularly in cases where iron deficiency cannot be fully attributed to dietary or lifestyle factors.

Additionally, examining the microbiome in young adults is essential, as this demographic is frequently underrepresented in microbiome studies, despite their susceptibility to anemia-related academic, cognitive, and physical impairments (23). By focusing on this demographic, the study addresses a critical research gap and facilitates the development of more customized therapies. Finally, findings from this study may promote novel microbiome-informed therapy techniques for enhancing iron absorption and restoring microbial balance, making the work crucial to clinical practice and public health.

Iron insufficiency is the most common dietary deficit globally, concerning probable 5-7 billion people. ID is furthermore leading reason associated with anemia in kids and females load settings (24, 25). Two billion people, or about 30% of the world's population, and 43% of children aged 6–59 months are anemic, with the incidence of anemia five times greater in low- and middle-income countries (LMICs) than in high-income nations (26). As a result, iron defi-

ciency anemia (IDA) is the world's greatest nutritional deficiency illness and one of the top five causes of global disease burden (27). IDA is the leading cause of death globally, affecting 1.24 billion people at any given time.

Iron deficiency is linked to various conditions, including anemia and impaired organ function and development (28). ID in the deficiency of anemias has more modest signs than other micronutrient deficiencies, while being a substantial contributor to illness, premature mortality, and lost wages in impoverished nations. Even mild iron deficiency appears to impair intellectual development in young children and is lowering national intelligence quotients (29, 30).

According to the WHO, It represents one of the most significant economic burdens on healthcare systems globally, causing an estimated 4.05% of global GDP loss (31).

Many treatments, such as supplementation, food fortification, dietary diversification, and parasite infection prevention, can manage iron deficiency. Short-term methods, like supplementation or fortification, are often used by reason of their cost-efficiency and easy implementation. The WHO recommends daily iron supplement three following months for premenopausal women, young adult, and children in countries with over 40% anemia prevalence (primarily in Sub-Saharan Africa and Southeast Asia) and intermittent supplement in lower prevalence (32).

Although iron compounds are easily accessible, they often generate non-physiological levels of reactive ionic iron. This excess can lead to detrimental effects within the circulatory system (absorbed portion) and the colon (unabsorbed portion) (33, 34). Conventional soluble iron treatments for IDA have been consistently associated with adverse outcomes in young children across developing regions (6, 35). Findings from large-scale studies show a significant correlation between these supplements and increased risks of bloody diarrhea, gut inflammation, and harmful shifts in the intestinal microbiome (36), increasing the burden from enteric infection and environmental enteropathy. Home fortification programs using multi-micronutrient powders show potential in reaching at-risk groups (37).

Despite the severity of the issue, progress has been gradual, and efforts to manage iron deficiency have not reduced the worldwide burden. Research has demonstrated that iron supplementation for young infants in Sub-Saharan Africa is ineffective and may increase infection risk (38, 39).

The Objectives of this study include:

• To evaluate the prevalence and hematological features of iron deficiency and iron-deficiency anemia in young adults.

• To examine the correlation between gut microbiome composition and iron-deficiency anemia in young adults.

• To examine potential functional and metabolic consequences derived from the relative abundance of short-chain fatty acid–producing bacteria.

• To investigate the correlation among iron-deficiency anemia, modifications in the gut microbiome, and hypothesized microbial functional pathways associated with iron metabolism.

MATERIALS AND METHODS

Research design. This study employed a cross-sectional analytical research methodology to examine the association between gut microbiota composition and iron deficiency anemia (IDA) in young individuals aged 18 to 30 years. A cross-sectional strategy was chosen as it facilitated the concurrent assessment of hematological markers, iron status indicators, and gut microbial profiles, yielding a snapshot of microbial signatures that differentiate IDA patients from healthy individuals (14).

The study encompassed clinical examination, biochemical assays, and 16S rRNA microbiome sequencing to deliver a comprehensive evaluation of biological, dietary, and microbial factors. All clinical evaluations and specimen collections took place at the Outpatient Diagnostic Clinic. In contrast, hematological analyses, iron biomarker assessments, DNA extraction, and sequencing were performed at the Clinical Nutrition and Microbiome Research Laboratory, following international laboratory standards (ISO 15189) to ensure reliability, reproducibility, and a minimized risk of contamination (4).

Participants. This study involved 84 individuals aged 18 to 30 years. After checking to see if they were eligible, 80 volunteers were chosen and split into two equal groups.

• IDA Group (n = 40) consisted of clinically diagnosed adults with iron deficiency anemia.

• The control group (n = 40) consisted of healthy adults exhibiting normal iron and hemoglobin con-

centrations as shown in Table 1.

Participants were selected through outpatient clinic referrals, public advertisements, and voluntary participation. Screening consists of complete blood counts, iron biomarkers, a review of medical history, an assessment of dietary habits, and a lifestyle evaluation to mitigate confounding variables (2). However, detailed quantitative measurements of dietary iron intake, body mass index (BMI), socioeconomic status, smoking, alcohol consumption, and physical activity were not systematically recorded.

Inclusion criteria. The IDA Group includes individuals aged 18-30 with hemoglobin levels below 12 g/dL (females) or <13 g/dL (males), serum ferritin levels below 15 ng/mL, and transferrin saturation below 20%. , No chronic inflammatory, renal, or gastrointestinal diseases, No iron supplementation during the last 2 months.

Control group. Aged 18-30, Normal hemoglobin and iron indices, No chronic disease or history of anemia, No antibiotic or probiotic usage in the previous 3 months.

Exclusion criteria. Exclusion criteria included chronic conditions impacting iron metabolism or gut microbiota, as well as gastrointestinal disorders such as IBD, celiac disease, and IBS (8). Chronic kidney disease and autoimmune diseases (2), According to Paganini et al. (5), antibiotics and probiotics not should be used within 3 months, Recent GI illnesses (6), Pregnancy and lactation (16). Tang et al. (40) reported iron supplementation in the prior two months.

Sample collection: blood sample collection. The data in Table 2 summarizes all participants provided venous blood samples following an overnight fast of 10-12 hours to guarantee precise assessment of hematological and biochemical markers. We used sterile vacutainer tubes with EDTA to get 5 mL of whole blood for complete blood counts (CBC). We also took 3 mL of serum tubes to check iron levels, such as serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation (2).

All blood samples were processed within an hour after collection to avoid deterioration. An automated hematology analyzer conducted the CBC analysis, while iron biomarkers were assessed using standard immunoassays in alignment with global clinical standards (41). To ensure data accuracy, samples exhibiting signs of hemolysis were immediately discarded. To reduce the potential confounding influence of inflammation on iron status biomarkers, participants with clinical or laboratory findings indicating acute or chronic inflammatory disorders were eliminated during the initial screening procedure.

Stool sample collection. Each participant supplied fresh fecal samples utilizing sterile, DNA-free collection containers specifically engineered for microbiota research. Participants were told not to get urine or water on the samples. Each sample was moved to the microbiological lab in 30 to 45 minutes in insulated transport boxes that kept the temperature at 4°C (5). Table 3 displays the details of the storage temperatures and purposes of stool sample aliquots.

Table 2. The blood sample types, volumes, tube types, and storage conditions

Sample Type	Volume	Tube Type	Storage Temp	Purpose
Whole blood	5 mL	EDTA	4°C	CBC analysis
Serum	3 mL	Serum tube	-20°C	Iron biomarkers

Table 3. Details of the storage temperatures and purposes of stool sample aliquots

Aliquot	Storage Temp	Purpose
A	-80°C	16S rRNA sequencing
B	-20°C	Backup/Repeat analysis

Table 1. Demographic and clinical characteristics of participants in both groups

Group	N	Age (Mean ± SD)	Gender (M/F)	Hemoglobin Range (g/dL)	Ferritin Range (ng/mL)	Notes
IDA	40	22.5 ± 3.1	18/22	<12 (F) <13 (M)	<15	Clinically diagnosed
Control	40	23.0 ± 2.8	20/20	Normal	Normal	Healthy volunteers

Upon arrival, stool samples were split into two aliquots.

1. Aliquot A (Microbiome Sequencing) is stored at -80°C for DNA extraction and 16S rRNA sequencing.

2. Backup sample (Aliquot B) is stored at -20°C for possible future analysis.

Samples containing visible blood, mucus, or contamination were excluded. This protocol ensured preservation of microbial integrity and prevented post-collection bacterial overgrowth (42).

Laboratory procedures. Laboratory procedures were executed to ensure the precise evaluation of hematological, biochemical, and microbiological parameters. All analyses were performed in accredited research institutions utilizing rigorous quality control measures (see Table 4).

Hematological analysis. All participants had complete blood counts (CBCs) done with an automated hematological analyzer (Sysmex XN-1000). We examined hemoglobin, hematocrit, red blood cell indices (MCV, MCH, MCHC), white blood cell count, and platelet count. Quality control was in place because the equipment was regularly calibrated and standard control samples were used. To make sure the data was accurate, we threw away hemolyzed or poorly stored samples.

Examination of Iron biomarkers. We measured serum iron, ferritin, total iron-binding capacity (TIBC), and transferrin saturation with enzyme-linked immunosorbent assays (ELISA). This is what happens in the clinic. We checked each sample twice and made calibration curves for all the biomarkers to make sure they would work again. We re-analyzed samples that were outside of normal reference ranges to make sure they were correct (41).

DNA extraction for microbiome sequencing. We followed the instructions that came with the Qiagen QIAamp DNA Stool Mini Kit to get genomic DNA from stool samples. Samples were homogenized, lysed, and purified in sterile conditions to keep them from getting dirty. A Nanodrop spectrophotometer and agarose gel electrophoresis were used to find out how much DNA there was and how good it was. The DNA that was taken out was kept at -80°C until the 16S rRNA sequencing (4).

Sequencing the microbiome and doing bioinformatics analysis. The microbiome workflow used standard reporting methods for studies that used 16S rRNA sequencing. Microbiome sequencing was employed to determine the structural composition of the gut bacterial community in both the IDA and control groups. Standardized procedures were employed for 16S rRNA sequencing, read filtering, and taxonomy classification to ensure the accuracy and consistency of the results, as detailed in Table 5.

Amplification and sequencing of the 16S rRNA gene. Using universal primers, the V3-V4 hypervariable parts of the bacterial 16S rRNA gene were made bigger. To prevent amplification bias, PCR procedures were performed in triplicate for each sample. We used magnetic bead purification to clean up the amplicons, then we used Nanodrop to measure them and mixed them together in equal amounts before sequencing. We used a 2 × 300 bp paired-end kit (Illumina, USA) on an Illumina MiSeq platform to do the sequencing. This gave us high-resolution reads for microbial profiling (43).

Quality control. We put the raw sequencing reads into QIIME2 so we could look at them. We used DADA2 to get rid of noise and chimeras and make amplicon sequence variations (ASVs). We only kept readings with Phred scores over 30 that were of good quality. To preserve statistical integrity, samples demonstrating insufficient sequencing depth (<10,000 reads) were excluded from the study. The feature table that was made was used to do diversity analysis and taxonomy profiling (44).

Table 4. Outlines the laboratory analyses, instruments/methods, and their respective functions

Analysis	Instrument/Method	Purpose
CBC	Sysmex XN-1000	Hematological markers
Serum iron/ferritin/TIBC	ELISA	Iron status indicators
DNA extraction from stool	Qiagen QIAamp DNA Stool Mini Kit	16S rRNA sequencing

Table 5. The steps for sequencing the microbiome and the tools for bioinformatics

Stage	Method/Tool	Purpose
DNA quantification	Nanodrop 2000	Assess DNA purity and concentration
16S rRNA amplification	PCR using primers V3–V4	Amplify bacterial 16S rRNA regions
Sequencing platform	Illumina MiSeq (2 × 300 bp)	Generate high-quality paired-end reads
Quality filtering	QIIME2 – DADA2	Remove noise, trim reads, denoise sequences
Taxonomic classification	SILVA Database (v138)	Assign taxonomy to ASVs
Diversity analysis	QIIME2 (Alpha/Beta metrics)	Evaluate microbial diversity across groups
Differential abundance	LEfSe	Identify taxa significantly associated with IDA

Taxonomic assignment and diversity analysis. We used the SILVA reference database (version 138) to put ASVs into groups. Profiles of relative abundance were made at the phylum, family, and genus levels. We used alpha diversity (Shannon, Chao1) and beta diversity (Bray-Curtis, UniFrac) to look at differences between groups and within samples. PERMANOVA with 999 permutations was used to find significance.

To find taxa that were more common in the IDA group than in the control group, we used Linear Discriminant Analysis Effect Size (LEfSe). LDA scores greater than 2 showed biological significance (45).

Statistical analysis. Statistical analyses were conducted to compare hematological parameters, iron biomarkers, and gut microbiota composition between the IDA and control groups. All analyses were conducted utilizing SPSS version 28, GraphPad Prism 10, and QIIME2 for microbiological statistics. All tests used a p-value of less than 0.05 to indicate significance.

Hematological and iron biomarker analysis. The Shapiro-Wilk test was used to determine if continuous variables were normal. Hemoglobin, RBC count, and MCV had normal distributions and were therefore evaluated using independent t-tests. Serum ferritin and transferrin saturation had non-normal distributions, which were assessed using Mann-Whitney U tests. Effect sizes (Cohen's d) were used to estimate the amount of changes between groups (46).

Microbiome diversity and taxonomy comparisons. The Wilcoxon rank-sum test was used to compare groups' alpha diversity indexes (Shannon, Chao1). Beta diversity measures (Bray-Curtis and weighted UniFrac) were created in QIIME2 and assessed using PERMANOVA with 999 permutations to assess overall microbial community differences (47).

LEfSe was used to perform differential abundance analysis, with significant taxa defined as $p < 0.05$ and LDA > 2 (42).

Correlation analysis. Spearman correlation coefficients were used to analyze the relationship between major bacterial taxa and iron-related indicators such as hemoglobin, ferritin, and transferrin saturation. To account for multiple testing, the false discovery rate (FDR) was corrected (Benjamini and Hochberg, 1995).

Ethical considerations. This study met international ethical standards for human research and followed the principles described in the Declaration of Helsinki (World Medical Association, 2013). The Clinical Nutrition and Microbiome Research Center's Institutional Review Board provided ethical clearance for the research methodology, participant recruitment, sample collection, and laboratory operations.

All participants received comprehensive verbal and written explanations of the study's objectives, methodologies, potential risks, and their rights as research subjects. Each participant provided written informed consent prior to recruitment, in compliance with international clinical research standards (23). Confidentiality was maintained by providing unique identifying IDs to participant data, and all biological samples were treated anonymously.

Participants were informed that they could exit the study at any time without consequence. There was no financial incentive, and no invasive or high-risk procedures were employed, as the collection of blood samples and feces is regarded as low-risk research activities (41).

To minimize the risks associated with handling human biological materials, all collected samples were

stored, managed, and disposed of in accordance with biosafety regulations and institutional laboratory safety protocols.

RESULTS

Participants' baseline characteristics. The final analysis included 80 subjects, with 40 having IDA and 40 being controls. The age and gender distribution between the two groups did not differ significantly ($p > 0.05$), indicating that the baseline demographic data were adequately aligned.

Participants in the IDA group exhibited significantly lower baseline hemoglobin and ferritin levels compared to controls, thereby validating the clinical classification of the groups. Table 6 shows the subjects' basic demographic and clinical information.

Hematological and Iron status. There were significant variations in hematological and iron status markers between the IDA and control groups. Individuals with iron-deficiency anemia had significantly lower levels of hemoglobin, serum ferritin, and transferrin saturation than healthy controls ($p < 0.001$). Red blood cell indices (MCV, MCH) were similarly significantly decreased in the IDA group, indicating microcytic hypochromic anemia (Table 7).

These data corroborate the clinical diagnosis of iron deficiency anemia in the IDA group and verify the study's inclusion criteria.

Analyzing alpha diversity. We utilized Shannon and Chao1 alpha diversity indices to assess the richness and evenness of microbes in the IDA and control groups. The IDA group showed a much lower alpha diversity than the controls ($p < 0.05$), which means that the gut microbial population was less diverse.

The Shannon diversity values were consistently lower in the IDA group, indicating reduced species evenness, while the Chao1 values indicated a lower estimated species richness. These data indicate that iron deficiency anemia correlates with a significant decrease in overall gut microbial diversity. This decrease in alpha diversity corresponds with previous research indicating that iron deficiency alters the stability and richness of gut microbiota due to insufficient nutrient availability and increased intestinal stress.

Study of beta diversity. We employed beta diversity analysis to examine the overall structure of the microbial communities within the IDA and control groups. Principal Coordinate Analysis (PCoA) plots utilizing Bray-Curtis dissimilarity and weighted UniFrac distances revealed a significant difference between the two groups, suggesting distinct microbial community compositions.

The PERMANOVA test (999 permutations) revealed significant differences in microbial structure for both Bray-Curtis ($p < 0.01$) and weighted UniFrac metrics ($p < 0.01$).

These findings indicate that individuals with iron-deficiency anemia have gut microbiomes that signifi-

Table 6. Demographic and clinical information of study groups

Variable Age	**IDA Group (n=40)**	**Control Group (n=40)**	**p-value**
(years)	22.5 ± 3.1	23.0 ± 2.8	0.52
Gender (M/F)	18 / 22	20 / 20	0.64
Serum Ferritin (ng/mL)	9.2 ± 3.5	45.6 ± 12.4	<0.001
Hemoglobin (g/dL)	10.4 ± 0.9	13.8 ± 1.1	<0.001
Transferrin Saturation (%)	14.1 ± 3.2	29.7 ± 4.8	<0.001

Table 7. Hematological and iron parameters of study group

Parameter	**IDA Group (Mean ± SD)**	**Control Group (Mean ± SD)**	**p-value**
Hemoglobin (g/dL)	10.4 ± 0.9	13.8 ± 1.1	<0.001
MCV (fL)	71.3 ± 5.8	86.5 ± 4.2	<0.001
MCH (pg)	22.1 ± 2.1	28.9 ± 1.9	<0.001
Transferrin Saturation (%)	14.1 ± 3.2	29.7 ± 4.8	<0.001
Serum Ferritin (ng/mL)	9.2 ± 3.5	45.6 ± 12.4	<0.001

cantly differ from healthy controls, both in species richness and phylogenetic composition. This variation in beta diversity may result from alterations in microbial growth, metabolic processes, and competition within the gut influenced by iron.

Profiles of relative abundance. A comparison of the relative abundances of various bacterial phyla indicated that the IDA and control groups exhibited significantly distinct compositions.

The gut microbiota of healthy individuals primarily consists of Firmicutes and Bacteroidetes, representing the predominant microbial population. The IDA group, on the other hand, saw a significant drop in Firmicutes and a corresponding rise in Proteobacteria, which is a phylum that is often linked to gut dysbiosis and inflammation.

Beneficial short-chain fatty acid-producing taxa, such as *Faecalibacterium* and *Roseburia*, were dramatically reduced in the IDA group. Individuals with iron-deficiency anemia, on the other hand, had more potentially harmful and iron-dependent genera, like *Escherichia/Shigella.*

These changes in the types of microbes in the gut suggest that a lack of iron changes the environment in the gut, making it easier for opportunistic bacteria to grow and harder for beneficial commensals to grow. These changes could make the gut barrier less effective, make microbial metabolites less available, and make intestinal inflammation worse, all of which make anemia-related stress worse.

Taxa with different levels of abundance. Differential abundance analysis identified several bacterial taxa exhibiting statistically significant differences between the IDA and control groups. Employing non-parametric Wilcoxon rank-sum testing with false discovery rate (FDR) correction, certain genera were identified as either more or less prevalent in individuals with iron-deficiency anemia. *Faecalibacterium*, *Roseburia*, and *Blautia*, advantageous bacteria that synthesize butyrate were markedly less common in the IDA cohort ($p < 0.01$). These genera are essential for preserving intestinal barrier integrity and providing anti-inflammatory effects; their deficiency may linked to impaired gut function in individuals with anemia.

The IDA group exhibited significantly elevated levels of iron-dependent and potentially detrimental taxa, including *Escherichia/Shigella*, Enterobacter, and Clostridium sensu stricto ($p < 0.01$). The prevalence of these species corresponds with metabolic alterations occurring in low-iron environments, possibly indicating compensatory microbial adaptations to modified iron availability.

The observed pattern suggests that IDA is associated with a microbial imbalance characterized by the depletion of beneficial commensals and the expansion of opportunistic, pro-inflammatory bacteria. Microbes can cause these changes, which may make inflammation in the intestines worse and make it harder for the body to absorb nutrients. This could make anemia worse.

Relationship between gut microbiome and indicators of Iron status. Correlation studies were performed to examine the associations between significant bacterial genera and hematological iron parameters, including hemoglobin, serum ferritin, and transferrin saturation. Spearman correlation coefficients indicated several significant associations, corroborating the observed microbial alterations in individuals with iron deficiency anemia. Helpful SCFA-producing species, like Faecalibacterium and Roseburia, showed strong positive links with serum ferritin and hemoglobin levels ($\rho = 0.45–0.58$, $p < 0.01$). These data indicate that a higher prevalence of these taxa correlates with improved iron status and intestinal function.

In contrast, opportunistic and iron-responsive taxa, particularly *Escherichia/Shigella* and *Enterobacter*, demonstrated significant negative correlations with ferritin, transferrin saturation, and hemoglobin ($\rho = -0.41$ to -0.53, $p < 0.01$). This indicates that an overabundance of pro-inflammatory taxa may be correlated with diminished iron absorption and protracted hematological recovery. These association patterns demonstrate a bidirectional relationship between iron metabolism and gut microbiome composition, suggesting that microbial dysbiosis may contribute mechanistically to the onset and severity of iron deficiency anemia.

DISCUSSION

The results of this study demonstrate that iron-deficiency anemia (IDA) in young adults is significantly correlated with pronounced modifications in the gut microbiome, marked by diminished microbial diversity and atypical compositional alterations. The reduction in alpha diversity within the IDA group in-

dicates a less stable and functionally limited microbial community, supporting prior studies that demonstrate iron deficiency hinders the growth of beneficial taxa and disturbs the gut's ecological equilibrium (6). The beta diversity data also showed that the groups were very different from each other, which backs up this idea. This suggests that IDA is linked to substantial changes in the structure of the microbial community.

The study found that the short fatty acid-producing taxa went down in the IDA group. The short-chain fatty acid-producing taxa include *Faecalibacterium* and *Roseburia*. *Faecalibacterium* and *Roseburia* help keep the gut barrier strong and send anti-inflammatory signals. When *Faecalibacterium* and *Roseburia* are missing, the gut lining can become weak. The body may not absorb nutrients well. The study also shows that lower butyrate synthesis hurts colonocyte function. Lower butyrate synthesis also raises the chance of inflammation (49). Conversely, the heightened levels of *Escherichia/Shigella* and other opportunistic Proteobacteria in IDA patients promote the development of microbial dysbiosis, marked by inflammation-induced bacterial proliferation. These bacteria possess robust iron-scavenging mechanisms that enable their survival in environments with limited iron availability, even at the cost of beneficial commensals (50).

The connections between the microbiome's composition and iron levels point to a connection between microbial dysbiosis and iron metabolism. Positive correlations between beneficial genera and serum ferritin/hemoglobin levels indicate that a more advantageous microbial composition may enhance iron absorption and homeostasis. In contrast, the adverse correlations between opportunistic taxa and iron indicators imply that dysbiosis may impede iron absorption directly or indirectly through either inflammatory pathways, oxidative stress, or competition for iron within the gut lumen (51). These data collectively indicate a bidirectional relationship wherein iron deficiency modifies the gut microbiota, potentially exacerbating anemia through resultant symbiosis.

The results of this study align with an expanding corpus of literature indicating that the gut microbiota is integral to iron homeostasis. The reduced diversity in the IDA group, the elimination of beneficial SCFA-producing bacteria, and the proliferation of opportunistic taxa collectively support the hypothesis that microbiome-targeted strategies may function as an adjunct to traditional iron supplementation. Prebiotics, probiotics, and dietary modifications may help restore microbial balance, improve iron absorption, and reduce inflammation in individuals with iron deficiency anemia (IDA). Associated correlations cannot be established due to the study's cross-sectional design. Longitudinal and interventional research are needed to evaluate whether the observed gut microbiota changes are the cause or result of iron-deficient anemia.

CONCLUSION

This research found that IDA significantly affects both the gut microbiota and the intestinal barrier. This study demonstrated that iron deficiency anemia in young adults is associated with significant changes in gut microbiota diversity and composition. Individuals with IDA exhibited diminished microbial diversity, anomalous clustering patterns, an absence of critical butyrate-producing bacteria, and an excess of opportunistic and iron-dependent taxa. These microbial alterations were significantly associated with hematological iron indicators, suggesting a bidirectional interaction whereby iron deficiency modifies the gut microbiome, and the resulting dysbiosis may hinder iron absorption and intestinal health.

Overall, the results show that when treating iron-deficiency anemia, it's important to think about the balance of gut microbiota. They also show that therapies that target the microbiome could be useful in addition to taking iron supplements regularly.

The study's findings offer recommendations to stimulate further research and improve the clinical management of iron deficiency anemia.

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