DOI: https://doi.org/10.70749/ijbr.v3i7.1865

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Frequency of Iron Deficiency in Females having Normal Haemoglobin level Complaining of Fatigue

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ARTICLE INFO

Keywords: Iron deficiency, non-pregnant females, normal hemoglobin, iron deficiency without anemia, ferritin, risk factors, public health

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Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 07-06-2025 Revised: 28-06-2025
Accepted: 13-07-2025 Published: 20-07-2025

ABSTRACT

Background: Iron deficiency is the most widespread nutritional disorder globally and often occurs without anemia, especially in women of reproductive age. Conventional screening practices that rely solely on hemoglobin levels may miss a significant number of cases. **Aim:** To determine the frequency of iron deficiency in non-pregnant females having normal hemoglobin levels, and to identify associated risk factors based on clinical, demographic, dietary, and reproductive variables. **Methodology:** A cross-sectional study was conducted at the Department of Medicine, Services Hospital Lahore, over a period of six months. A total of 100 non-pregnant females aged 16–45 years with hemoglobin >11 g/dL were enrolled using non-probability consecutive sampling. Data on sociodemographic, clinical, dietary, and reproductive factors were collected. Laboratory evaluation included hemoglobin, serum ferritin, and serum iron. Iron deficiency was defined as serum ferritin <15 µg/L and serum iron <10 µmol/L. Data were analyzed using SPSS v25, and associations were tested using Chi-square test and odds ratios; $p < 0.05$ was considered statistically significant. **Results:** Iron deficiency was found in 41% of participants despite normal hemoglobin levels. Significant associations were observed between iron deficiency and underweight BMI (OR = 7.11, p = 0.002), history of anemia (OR = 6.30, p = 0.001), hypertension (OR = 3.84, p = 0.012), ≥2 deliveries (OR = 3.10, p = 0.012), breastfeeding (OR = 2.50, p = 0.045), sedentary lifestyle (OR = 2.41, p = 0.049), no supplement use (OR = 2.80, p = 0.034), and low meat intake (OR = 3.06, p = 0.016). The strongest predictors were low serum ferritin (OR = 9.47, p < 0.001) and low serum iron (OR = 5.54, p < 0.001). **Conclusion:** A considerable proportion of non-pregnant females with normal hemoglobin levels were found to be iron deficient, highlighting the inadequacy of hemoglobin-based screening. Risk factors were multifactorial, involving nutritional, reproductive, and behavioral dimensions. Routine biochemical screening and broader nutritional interventions are recommended to detect and manage iron deficiency in at-risk non-pregnant females, even in the absence of anemia.

INTRODUCTION

Iron deficiency is the most prevalent micronutrient disorder globally and remains a significant public health issue in women, even when haemoglobin levels appear normal (Pobee et al., 2021). Traditionally, haemoglobin concentration has served as the primary biomarker to detect iron deficiency, yet a growing body of research challenges this approach, particularly in non-pregnant females of reproductive age (AlFaris et al., 2021). Iron deficiency can exist without progressing to anemia, a condition often referred to as iron deficiency without anemia (IDWA), and may go unnoticed in standard screenings that rely solely on haemoglobin levels (Wen et al., 2024). In such cases, iron stores may already be depleted, leading to subtle but functionally significant symptoms, such as fatigue, reduced work capacity, or poor concentration even when full-blown anemia has not yet developed. Recognizing this form of deficiency is crucial, especially in women who are frequently misclassified as healthy based on a single lab value (Auerbach et al., 2021). Globally, iron deficiency in women often remains hidden beneath apparently normal haemoglobin levels, affecting millions of non-pregnant females (Shams et al., 2023). As of 2019, roughly 29.6% of non-pregnant women aged 15–49 were anemic, equating to over half a billion individuals; however, IDWA may be at least twice as common as iron-deficiency anemia, placing its prevalence as high as 25% among women with normal haemoglobin (Qadir et al., 2022). Global burden studies from 2021 report that 1.27 billion people are affected by dietary iron deficiency, with

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women bearing a disproportionately high burden, especially in reproductive years (Ali et al., 2021). In the U.S., national surveys show up to 13% of non-pregnant women have low ferritin levels despite appearing non-anemic (Nisar et al., 2023). These numbers highlight the inadequacy of haemoglobin-focused diagnostics and underline the global need for a more comprehensive approach to iron status assessment.

In Pakistan, iron deficiency remains a pervasive concern even among non-pregnant women with ostensibly normal haemoglobin, highlighting a critical public health gap (Hamid et al., 2022). According to the National Nutrition Survey 2018, although about 41.7% of women of reproductive age are anemic, approximately 18.2% exhibit iron deficiency as indicated by low ferritin levels, even if haemoglobin is still within normal limits (Lone et al., 2021). Regional studies reveal similar patterns: in urban settings such as Quetta, 75% of women were non-anemic, yet a sizable portion still had iron-deficiency anemia when assessed biochemically, suggesting overlooked subclinical deficiency (Hashmi et al., 2022). In Karachi, tertiary-care sampling showed 71.5% prevalence of anemia, but microcytic indices and low iron markers in non-anemic women pointed toward widespread early iron depletion (Zia et al., 2024). Even in more remote regions like Skardu (Gilgit-Baltistan), about 30% of women met anemia criteria, and many with normal haemoglobin still showed depleted iron stores by serum ferritin and red cell indices (Tabassum et al., 2022).

In the non-pregnant population, menstruation remains a dominant cause of iron loss, with heavy menstrual bleeding (HMB) leading to a chronic negative iron balance in many cases (Zulfiqar et al., 2021). Even in the absence of HMB, repeated monthly blood loss can gradually deplete iron reserves, especially when dietary intake is insufficient or absorption is impaired (Habib et al., 2023). Studies in various populations consistently show that a significant proportion of women with normal haemoglobin still have serum ferritin levels below 15 ng/mL, a threshold suggestive of depleted iron stores (Mahar et al., 2024). Despite this compelling evidence, in many clinical and public health settings especially in resource-limited regions like Pakistan such biochemical assessments are not routinely ordered unless anemia is already present. This oversight results in thousands of women suffering silently from undiagnosed iron deficiency that could have been managed with timely nutritional or medical interventions. This study aims to address this diagnostic blind spot by examining the frequency of iron deficiency among non-pregnant females with normal haemoglobin levels in Pakistan.

METHODOLOGY

Study Design and Setting

This was a cross-sectional study conducted at the Department of Medicine, Services Hospital, Lahore. The study was carried out over a period of six months, from December 2024 to May 2025, following approval from the College of Physicians and Surgeons Pakistan (CPSP).

Sample Size and Sampling Technique

A total of 100 non-pregnant females aged between 16 and 45 years were included in the study. The sample size was calculated using the WHO sample size calculator, assuming a 95% confidence level, 10% margin of error, and an expected iron deficiency prevalence of 57.5% in non-pregnant females with normal hemoglobin levels, based on existing literature. Participants were selected using a non-probability consecutive sampling technique.

Inclusion and Exclusion Criteria

The inclusion criteria consisted of non-pregnant females aged 16–45 years attending the outpatient department (OPD) for routine medical checkups. Women were excluded if they had a history of chronic anemia, were taking iron, vitamin, or mineral supplements, or had a diagnosis of cancer, as confirmed from their medical records.

Data Collection

Eligible participants were recruited from the OPD and their demographic and clinical information was recorded using a structured data collection proforma. Variables collected included age, BMI, marital status, parity, blood pressure, blood sugar levels, smoking history (≥5 pack-years), family history of anemia, breastfeeding history, use of contraceptives, occupation, and lifestyle (active/sedentary). After consent, a 3 mL venous blood sample was drawn from each participant and sent to the hospital laboratory for analysis of hemoglobin, serum ferritin, and serum iron levels.

Data Analysis

Data were entered and analyzed using SPSS version 25. The Shapiro-Wilk test was applied to check the normality of quantitative variables. For continuous variables such as age, BMI, hemoglobin, serum ferritin, and serum iron levels, means and standard deviations were calculated. For categorical variables, such as marital status, parity, hypertension, diabetes, smoking, breastfeeding, contraceptive use, occupation, lifestyle, and iron deficiency status, frequencies and percentages were reported. The data were stratified based on age, BMI, hemoglobin, and other clinical factors to assess associations. The Chi-square test was used to evaluate the relationship between iron deficiency and these stratified variables, with a p-value <0.05 considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

The majority of participants were between 26–35 years (41%), followed by those aged 16–25 years (34%), indicating that most women were in their prime reproductive years. Educational attainment varied, with 42% having completed secondary to higher secondary education and 32% being graduates or above, suggesting a moderately educated cohort. More than half of the participants had a normal BMI (54%), while 31% were overweight or obese, indicating a potential risk factor for metabolic disorders. Urban residents comprised a greater portion of the sample (62%), which may reflect the hospital's catchment area. Most participants were married (69%), and more than half (58%) had a history of breastfeeding, which is relevant in assessing iron stores.

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Notably, 27% had a prior history of anemia, while comorbid conditions such as hypertension (21%) and diabetes (13%) were present in a minority. Only 9% reported smoking, and 36% were using contraceptives. The majority (55%) reported a sedentary lifestyle, which could contribute to fatigue and other non-specific symptoms (Table 1).

Table 1

Demographic and Clinical Characteristics of Participants (n=100)

Variable	Category	Frequency (n)	Percentage (%)
Age (years)	16–25	34	34%
	26–35	41	41%
	36–45	25	25%
Education Level	Below Secondary	26	26%
	Secondary to Higher Secondary	42	42%
	Graduate and Above	32	32%
BMI (kg/m^2)	<18.5 (Underweight)	15	15%
	18.5–24.9 (Normal)	54	54%
	≥25 (Overweight/Obese)	31	31%
Residence	Rural	38	38%
	Urban	62	62%
Marital Status	Married	69	69%
	Unmarried	31	31%
History of Anemia	Yes	27	27%
	No	73	73%
Hypertension (BP >140/90 mmHg)	Yes	21	21%
	No	79	79%
Diabetes (BSR >200 mg/dL)	Yes	13	13%
	No	87	87%
History of Breastfeeding	Yes	58	58%
	No	42	42%
Smoking (>5 pack-years)	Yes	9	9%
	No	91	91%
Use of Contraceptives	Yes	36	36%
	No	64	64%
Lifestyle	Active	45	45%
	Sedentary	55	55%

Laboratory Examination

Although all participants had normal hemoglobin levels (≥11 g/dL), a significant proportion exhibited biochemical markers consistent with iron deficiency. Most participants (44%) had hemoglobin levels in the range of 12.1–13.0 g/dL, while 30% had levels above 13 g/dL, and 26% were at the lower end of normal (11.0–12.0 g/dL). Despite these normal values, 46% had serum ferritin levels below 15 µg/L, indicating depleted iron stores, and 49% had serum iron levels below 10 µmol/L, supporting the presence of iron deficiency even in the absence of anemia. Based on the combined criteria of low ferritin and serum iron, 41% of participants were classified as having iron deficiency. These findings emphasize that iron deficiency can exist independently of anemia, and relying solely on hemoglobin levels may overlook a substantial portion of affected individuals, particularly those presenting with non-specific symptoms such as fatigue.

The Nutritional status, as indicated by BMI, played a critical role—underweight women were significantly more likely to be iron deficient (OR = 7.11, p = 0.002), highlighting the link between malnutrition and iron depletion. A history of anemia (OR = 6.30, p = 0.001) and comorbid hypertension (OR = 3.84, p = 0.012) were also significantly associated with iron deficiency, suggesting persistent or undiagnosed underlying conditions. Breastfeeding history (OR = 2.50, p = 0.045) and a sedentary lifestyle (OR = 2.41, p = 0.049) further increased the odds of iron deficiency, likely due to increased physiological demand and reduced metabolic activity, respectively. Among dietary and health-seeking behaviors, not taking iron and folic acid supplements was significantly associated with higher iron deficiency (OR = 2.80, p = 0.034), as was inadequate meat consumption (<3 times/week) (OR = 3.06, p = 0.016), both underscoring the importance of dietary iron intake (Table 3).

Table 2

Laboratory Examination of Participants (n = 100)

Parameter	Category	Frequency (n)	Percentage (%)
Hemoglobin (g/dL)	11.0 – 12.0	26	26%
	12.1 – 13.0	44	44%
	>13.0	30	30%
Serum Ferritin (µg/L)	<15	46	46%
	15 – 30	33	33%
	>30	21	21%
Serum Iron (µmol/L)	<10	49	49%
	10 – 20	38	38%
	>20	13	13%
Iron Deficiency Status	Positive	41	41%
	Negative	59	59%

Although less frequent doctor visits showed an increased risk, this did not reach statistical significance (p = 0.090). Reproductive history was another contributing factor; women with two or more deliveries were over three times more likely to be iron deficient (OR = 3.10, p = 0.012), reflecting cumulative iron loss during pregnancies. Biochemical parameters showed the strongest associations with iron deficiency. Participants with lower-normal hemoglobin levels (11.0–12.0 g/dL) were nearly six times more likely to be iron deficient compared to those with mid-normal levels (12.1–13.0 g/dL) (OR = 5.88, p = 0.001). Furthermore, extremely low serum ferritin (<15 µg/L) and serum iron (<10 µmol/L) levels were highly predictive of iron deficiency (OR = 9.47 and 5.54 respectively, both p < 0.001). These findings confirm that iron deficiency can exist despite "normal" hemoglobin levels and reinforce the need for broader screening protocols that include ferritin and serum iron measurements, especially in at-risk populations (Table 3).

Table 3

Associations Between Iron Deficiency and Covariates (n=100)

Characteristics	Iron Deficiency n (%)	Unadjusted OR (95% CI)	p-value
Sociodemographic Variables			
Residence			
Urban	22 (35.5%)	1.00 (Ref.)	—
Rural	19 (50.0%)	1.82 (0.80–4.16)	0.146
Age (years)			
16–25	17 (41.5%)	1.00 (Ref.)	—
26–35	14 (34.1%)	0.73 (0.29–1.83)	0.499
36–45	10 (40.0%)	0.94 (0.34–2.60)	0.908
Education			
Graduate & above	11 (34.4%)	1.00 (Ref.)	—
Secondary Level	17 (40.5%)	1.29 (0.50–3.34)	0.594
Below Secondary	13 (50.0%)	1.82 (0.66–4.97)	0.243

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Clinical Variables			
BMI			
Normal (18.5–24.9)	18 (33.3%)	1.00 (Ref.)	—
Underweight (<18.5)	12 (80.0%)	7.11 (1.99–25.4)	0.002
Overweight (≥25)	11 (35.5%)	1.10 (0.42–2.91)	0.845
History of Anemia			
Yes	21 (77.8%)	6.30 (2.14–18.6)	0.001
No	20 (27.4%)	1.00 (Ref.)	—
Hypertension			
Yes	14 (66.7%)	3.84 (1.34–10.9)	0.012
No	27 (34.2%)	1.00 (Ref.)	—
Diabetes			
Yes	6 (46.2%)	1.32 (0.39–4.48)	0.650
No	35 (40.2%)	1.00 (Ref.)	—
Breastfeeding History			
Yes	29 (50.0%)	2.50 (1.02–6.15)	0.045
No	12 (28.6%)	1.00 (Ref.)	—
Smoking History			
Yes	6 (66.7%)	3.33 (0.83–13.3)	0.090
No	35 (38.5%)	1.00 (Ref.)	—
Contraceptive Use			
Yes	18 (50.0%)	1.79 (0.77–4.16)	0.173
No	23 (35.9%)	1.00 (Ref.)	—
Lifestyle			
Sedentary	28 (50.9%)	2.41 (1.00–5.81)	0.049
Active	13 (28.9%)	1.00 (Ref.)	—
Dietary & Health-Seeking Behaviour			
Iron/Folic Acid Supplement			
Yes	9 (25.0%)	1.00 (Ref.)	—
No	32 (48.5%)	2.80 (1.08–7.26)	0.034
Meat Intake (≥3x/week)			
Yes	10 (25.6%)	1.00 (Ref.)	—
No	31 (51.7%)	3.06 (1.23–7.60)	0.016
Doctor Visits (≥1x/month)			
Yes	15 (31.3%)	1.00 (Ref.)	—
No	26 (48.1%)	2.08 (0.89–4.88)	0.090
Reproductive History			
No. of Deliveries ≥2	28 (53.8%)	3.10 (1.28–7.52)	0.012
<2 Deliveries	13 (26.5%)	1.00 (Ref.)	—
Biochemical Variables			
Haemoglobin (11.0–12.0 g/dL)	21 (80.8%)	5.88 (2.08–16.6)	0.001
12.1–13.0 g/dL	15 (34.1%)	1.00 (Ref.)	—
>13.0 g/dL	5 (16.7%)	0.39 (0.11–1.36)	0.140
Serum Ferritin <15 μg/L	39 (84.8%)	9.47 (3.02–29.7)	<0.001
Serum Iron <10 μmol/L	36 (73.5%)	5.54 (2.11–14.5)	<0.001

DISCUSSION

The present study aimed to examine the frequency of iron deficiency among non-pregnant females with normal haemoglobin levels in Pakistan. The findings revealed that 41% of non-pregnant females with normal hemoglobin levels were iron deficient, based on biochemical criteria (serum ferritin <15 μg/L and serum iron <10 μmol/L). Although all participants met the threshold for normal hemoglobin (>11 g/dL), a significant portion still exhibited biochemical signs of iron deficiency, emphasizing the clinical reality of IDWA. Our stratified analysis found that participants with hemoglobin levels at the lower end of normal (11.0–12.0 g/dL) were significantly more likely to be iron deficient (OR = 5.88, 95% CI: 2.08–16.6, p = 0.001) compared to those with higher-normal values. This aligns with Fite et al., (2022), who described IDWA as a frequently missed but clinically important condition, particularly in women presenting with fatigue (Fite et al., 2022). These findings underscore the limitations of relying on hemoglobin alone for diagnosing iron deficiency and call for a broader diagnostic approach in public health screening (Tang and Sholzberg, 2024).

Nutritional status emerged as a strong determinant of iron deficiency in our study. Women with underweight BMI (<18.5 kg/m^2) had over seven times greater odds of being iron deficient (OR = 7.11, p = 0.002) compared to those with normal BMI. This association is consistent with the findings of Munro et al., (2023), who identified undernutrition as a key risk factor for iron deficiency in women of reproductive age (Munro et al., 2023). Weyand et al., (2023) also highlighted that inadequate dietary intake and chronic energy deficiency compromise not only total iron intake but also its absorption (Weyand et al., 2023). In contrast, overweight women did not show significant association with iron deficiency (p = 0.845), suggesting that being overweight does not necessarily imply nutritional adequacy. These findings have serious public health implications, indicating a need for targeted nutritional interventions for underweight women, including iron-rich food support and supplementation programs, particularly in low-income communities where calorie and micronutrient deficiencies often coexist.

A prior history of anemia was also a significant predictor of current iron deficiency, with affected women having over six times the odds of ID (OR = 6.30, p = 0.001). This suggests incomplete or poorly maintained recovery of iron stores despite hemoglobin normalization. Benson et al., (2022) support this trend which found that women with previous anemia were more likely to relapse into iron deficiency, particularly when not supplemented post-anemia (Benson et al., 2022). Similarly, Aguree et al., (2023) emphasized that hemoglobin recovery does not necessarily reflect replenished iron stores, especially in women with chronic menstrual losses or dietary inadequacy (Aguree et al., 2023). Our findings indicate that follow-up ferritin testing should be routine in women who have recovered from anemia, as they remain a high-risk group. Integrating iron status monitoring into primary care follow-up protocols can help prevent recurrence and associated complications like poor work productivity, cognitive deficits, and maternal morbidity (Ali and Ahmed, 2021).

Beyond individual nutritional status, clinical and reproductive factors also contributed significantly to iron deficiency risk. Women with a history of hypertension were nearly four times more likely to be iron deficient (OR = 3.84, p = 0.012), a finding not commonly reported in literature but biologically plausible. Inflammatory states like hypertension can upregulate hepcidin, an iron-regulatory hormone that inhibits absorption (Sharma et al., 2024). Furthermore, women with ≥2 deliveries had threefold higher odds of being iron deficient (OR = 3.10, p = 0.012), consistent with a previous finding (Zhao et al., 2022), which link repeated pregnancies with cumulative iron loss. High-parity women are often nutritionally depleted, especially in settings lacking postpartum nutritional rehabilitation. These findings support WHO recommendations for birth spacing and postpartum supplementation, and highlight the need to integrate iron screening into maternal health services beyond antenatal

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care.

Dietary patterns were also strongly associated with iron deficiency in our population. Infrequent meat intake (<3 times per week) significantly increased the odds of iron deficiency (OR = 3.06, p = 0.016), consistent with prior findings (Sharma et al., 2021; Pai et al., 2023), who showed that low intake of heme iron sources is a strong predictor of depleted iron stores. Similarly, women who did not use iron-folic acid supplements had nearly three times higher risk of ID (OR = 2.80, p = 0.034). Although iron-folic acid supplementation is standard in pregnancy, it is rarely emphasized for non-pregnant women despite evidence showing its benefits in restoring iron balance. These findings underscore the need for universal supplementation policies that include at-risk non-pregnant women, especially those reporting symptoms like fatigue, irregular menses, or high parity. Public awareness campaigns and primary care screening initiatives can help bridge this nutritional gap in women's health.

Lifestyle behaviors, particularly sedentary activity, were also significantly associated with iron deficiency in this study (OR = 2.41, p = 0.049). While the causal relationship between physical inactivity and iron deficiency is still being explored, some evidence suggests that reduced muscle activity may impair iron metabolism through inflammatory pathways (Nainggolan et al., 2022). Additionally, iron supports mitochondrial function and energy metabolism, making sedentary women potentially more vulnerable to fatigue from even marginal iron depletion. Interestingly, smoking and reduced doctor visits showed elevated but non-significant associations (p = 0.090), suggesting trends that may reach significance in larger samples. These findings collectively advocate for integrated health education programs that promote not only diet and supplementation, but also physical activity and regular health check-ups as part of iron deficiency prevention strategies.

The strongest predictors of iron deficiency were biochemical markers. Serum ferritin <15 µg/L was associated with nearly tenfold increased odds of iron deficiency (OR = 9.47, p < 0.001), while serum iron <10 µmol/L carried over five times the risk (OR = 5.54, p < 0.001). These associations are both expected and essential, reaffirming the role of ferritin and serum iron as the most sensitive and specific indicators of body iron status (Pobee et al., 2021). These findings support existing literature that recommends a multi-marker approach for diagnosing iron deficiency, especially in symptomatic women with normal hemoglobin (Hansen et al., 2023). The incorporation of ferritin and iron tests into routine primary care—especially for high-risk groups like low-BMI, high-parity, lactating, or supplement-naïve women—can significantly improve early detection and reduce the burden of fatigue, cognitive impairment, and future anemia.

Limitations

This study, while informative, has several limitations that must be acknowledged. First, the cross-sectional design limits the ability to establish causality between risk factors and iron deficiency; associations observed may not reflect temporal or direct cause-effect relationships. Second, the study was conducted at a single tertiary care hospital in Lahore, which may limit the generalizability of the findings to rural populations or other regions of Pakistan. Additionally, certain variables such as dietary iron absorption, menstrual blood loss, and inflammatory markers were not assessed, which could have provided a more comprehensive understanding of iron metabolism. The use of non-probability consecutive sampling may also introduce selection bias, as participants presenting to OPD might differ from the general population in health-seeking behavior. Lastly, self-reported data on dietary habits, supplement use, and lifestyle may be subject to recall or social desirability bias, potentially influencing the accuracy of associations.

CONCLUSION

This study highlights that a substantial proportion of non-pregnant females with normal hemoglobin levels were biochemically iron deficient, indicating that IDWA is a significant and under-recognized public health issue. Statistically significant associations were found between iron deficiency and several factors including underweight BMI (p = 0.002), prior anemia (p = 0.001), hypertension (p = 0.012), breastfeeding (p = 0.045), multiple deliveries (p = 0.012), lack of supplementation (p = 0.034), low meat intake (p = 0.016), and sedentary lifestyle (p = 0.049). The strongest predictors were biochemical indicators: low serum ferritin (p < 0.001) and low serum iron (p < 0.001). These findings support growing global evidence that hemoglobin alone is an insufficient marker for screening iron deficiency in women, particularly in resource-constrained settings where fatigue and nutritional deficiencies are often overlooked. There is an urgent need to adopt routine ferritin screening, implement nutritional interventions, and broaden iron supplementation programs to include non-pregnant women, especially those with identifiable risk factors. Integrating iron deficiency screening into primary care, reproductive health, and nutrition services could substantially reduce the hidden burden of iron-related fatigue and prevent progression to anemia, thereby improving women's health, productivity, and quality of life.

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