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Indian Journal of Obstetrics and Gynecology Research

Journal homepage: www.ijogr.org

Original Research Article

Assessment of iron, folic acid, and vitamin B12 deficiencies and their association with red cell indices in anemic and non-anemic women

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ARTICLE INFO

Article history:
Received 08-12-2023
Accepted 29-01-2024
Available online 11-05-2024

Keywords:
Anemia
Pregnant women
Iron deficiency anemia (IDA)
Vitamin B12
Folic acid

ABSTRACT

Background: During pregnancy anemia is a common health issue with various factors influencing its occurrence. It is a major public health problem in India. It is believed that 2.36 billion individuals are affected globally, and it is also an endemic problem in India. Iron deficiency often leads to iron deficiency anemia (IDA), significantly influencing maternal and fetal health.

Objective: This study aims to investigate the complete blood count and nutritional parameters including Iron profile, vitamin B12, and Folic acid in anemic and non-anemic participants.

Materials and Methods: The study involved 458 women of which 300 were anemic women and 158 were non-anemic women, predominantly from rural areas. The severity of anemia was classified as per WHO guidelines. The CBC was estimated by a 3-part hematology analyzer Medonic M-series, while the iron profile, vitamin B12, and folic acid were measured by using an automated analyzer Abbott Architect 1000 SR.

Results: The severity of anemia among the anemic women, classified according to WHO guidelines, revealed that 38.7% had mild anemia, 32.7% had moderate anemia, and 28.6% had severe anemia. 39.3% women exhibited microcytic hypochromic cells, 38% had normocytic normochromic cells, and 22.7% exhibited macrocytic cells. 56.6% had iron deficiency, 10% had folate deficiency, 16.7% had vitamin B12 deficiency, 6.7% had iron+vitamin B12+folic acid deficiency and 10% had vitamin B12+folic acid deficiency. Based on the severity only vitamin B12 was significantly reduced in severe (145.65±58.43) than moderate (177.06±66.24) and mild group (174.43±68.43), p=0.042. Hemoglobin showed a positive correlation with iron level (r=0.523, p<0.0001), and a negative correlation with ferritin (r=-0.355, p<0.0001).

Conclusion: These findings provide valuable insights into the nutritional status of the anemic women and highlights the need for targeted interventions to address micronutrient deficiencies during pregnancy.

1. Introduction

Anemia is defined as a reduction in the aggregate amount of red blood cells (RBCs) and/or hemoglobin (Hb), leading to an impairment in the blood's ability to carry oxygen.[1,2] It is defined as having a hemoglobin level below the thresholds set for specific age groups by the World Health Organization (WHO).[3] It is a major public health problem in India. It is believed that 2.36 billion individuals are affected globally, and it is also an endemic problem in India.[4–9] It's no surprise that it's one of the most commonly encountered conditions by general practitioners.[10] According to the (National Nutrition Strategy, NITI Aayog) NFHS-5, anemia is a major health problem affecting 57% of women in

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https://doi.org/10.18231/j.ijogr.2024.036

India.[6–8]

They also documented that over 52% of pregnant women are anemic.[8] Iron deficiency, often seen during pregnancy, results in iron deficiency anemia (IDA). This condition significantly impacts maternal and fetal health and can also lead to complications such as intrauterine growth retardation and preterm birth.[11,12]

The most common cause of anemia is dietary deficiency (iron, vitamin B12, or folic acid).[13–17] Because of the unique biochemical roles of these nutrients in erythropoiesis, dietary deficiencies produce a variety of disorders. Although the association of many biochemical parameters with anemia has been explored in multiple independent research, there is a dearth of studies examining their relationships in a single study with the same group of study participants.[12–16] Hence, the present study was intended to measure the levels of nutritional parameters (iron, vitamin B12, and folic acid) in anemic and non-anemic women and their association with hemoglobin and red cell indices.

2. Materials and Methods

2.1. Participants recruitment

The research was carried out in the Department of Biochemistry with the collaboration of the Department of Pathology and Obstetrics and Gynecology at Autonomous State Medical College, Shahjahanpur. We included 300 anemic women who were diagnosed with anemia during their OPD visits.

The anemia was diagnosed based on WHO guidelines, categorizing the patients into:[18]

Mild anemia (Hb- 9 to 11 g/dL, Moderate anemia (Hb- 7 to 9 g/dL), and severe anemia (Hb- <7g/dL) and 158 non-anemic women with same age group.

2.2. Sample acquisition

Blood samples were collected into plain and EDTA vials. The EDTA vials were used for complete blood counts (CBC) estimation, while the plain vials were used for assessing iron profile, vitamin B12, and folic acid levels.

2.3. Estimation of Complete blood counts (CBC) and nutritional parameters

The CBC was determined using the Medonic M series a hematology automated system, which provides a 3-part differential white blood cell along with several other parameters based on electrical impedance. Serum folic acid and vitamin B12 levels were measured by using an automated analyzer Architect 1000SR, Abott which is based on the immuno-chemiluminescence assay method. Serum iron, unsaturated iron binding capacity (UIBC), and serum ferritin were assessed by the ferrozine method using the same automated analyzer. Total iron binding capacity (TIBC) was calculated by using a formula: Iron + UIBC.

2.4. Statistical analysis

The frequency data was represented in terms of number and percentage, and the variable data was presented in mean and standard deviations. Pearson correlation coefficient analysis was used to see the correlation between two variables. The data analysis was performed by using SPSS version 21 software (IBM, Chicago, US). The p-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Baseline characteristics of the study population

The study included a total 458 women, with ages ranging from 18 to 35 years and an average age of 29.87±6.98 years. No significant differences were found between age which shows the adequate matching of both groups. The women were predominantly from rural areas (66%) compared to urban areas (34%). In terms of dietary habits, a majority of the women were vegetarians (66%), while the rest were non-vegetarians (34%). 32.7% women were illiterate, while the rest were literate. 37.3% women were from middle socio-economic status. The socio-economic status and dietary habits of the study population were significantly found which indicates that both factors influence the anemia. (Table 1)

The severity of anemia among the anemic women, classified according to WHO guidelines, revealed that 38.7% had mild anemia, 32.7% had moderate anemia, and 28.6% had severe anemia. When classifying the anemic women based on morphology, 39.3% of the participants exhibited microcytic hypochromic cells, 38% had normocytic normochromic cells, and 22.7% exhibited macrocytic cells (Figure 1).

When the anemic women were classified based on Etiology, 56.6% had iron deficiency, 10% had folate deficiency, 16.7% had vitamin B12 deficiency, 6.7% had iron + vitamin B12 + folic acid deficiency and 10% had vitamin B12+folic acid deficiency. (Figure 1)

3.2. Status of CBC parameters and nutritional parameters of the study population

The hemoglobin and red blood cell counts were significantly reduced in anemic than the non-anemic women. In red cell indices, only MCV, MCH, and RDW were significantly reduced in anemic women as compared to non-anemic women with $p<0.0001$, respectively.

As for the nutritional parameters, the average iron level was found in anemic women to be 62.99±26.92 than the non-anemic women (98.82±63.80, <0.0001). The UIBC (195.69±118.45 vs. 232.49±124.43) and TIBC

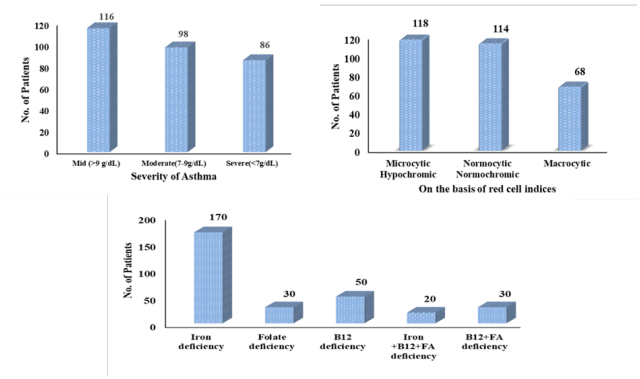

Figure 1: Distribution of anemic women: **A):** Based on Severity; **b):** Based on Red cell indices; **c):** Based on Etiology

(145.10±59.95 vs. 297.60±99.36) were also reduced in anemic participants than the non-anemic women, p<0.05 respectively. The average ferritin level was also lower in the anemic (82.36±44.82) than the non-anemic women (142.96±114.15), p<0.0001. Lastly, the vitamin B12 (116.43±66.57 vs. 250.94±177.76, p<0.0001) and folic acid (7.14±2.55 vs. 14.56±9.70, p<0.0001) levels were also significantly lower in anemic than the non-anemic women. (Table 2)

3.3. Comparison of CBC parameters and nutritional parameters based on severity

The level was hemoglobin was significantly lower in the severe group (5.62±1.21) than the moderate group (8.55±0.79) as compared to the mild group (10.79±9.94), p<0.001. In the nutritional parameters, only vitamin B12 was significantly reduced in severe (145.65±58.43) than moderate (177.06±66.24) and mild group (174.43±68.43), p=0.042. (Table 3)

3.4. Comparison of CBC parameters and nutritional parameters based on morphological classification

CBC parameters, the hemoglobin, RBCs, MCV, MCH, MCHC levels were significantly found, p<0.05. As for the nutritional parameters, the iron was lower in microcytic hypochromic (49.37±33.51) than the normocytic normochromic (62.64±28.40) as compared to macrocytic (65.65±29.91), p=0.002. The ferritin level was also reduced in the microcytic hypochromic (31.60±28.76) than the normocytic normochromic (41.78±28.10) and macrocytic (48.10±35.21), p=0.036. The vitamin B12 levels were lower in the macrocytic group (123.78±67.87) than the microcytic hypochromic (181.04±102.65) and normocytic normochromic (168.69±61.35), p<0.0001. (Table 4)

3.5. Correlation between Hb, iron, ferritin

From Figure 2, it is evident that the level of hemoglobin showed a positive correlation with iron level (r=0.523, p<0.0001), and a negative correlation with ferritin (r=-0.355, p<0.0001).

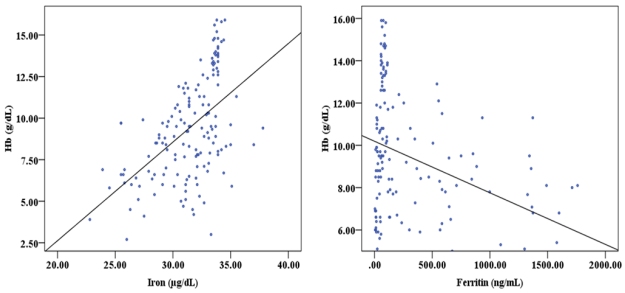

Figure 2: Correlation of hemoglobin with iron and ferritin

4. Discussion

This study delved into the status of hemoglobin concentration, iron, ferritin, vitamin B12, and folic acid levels in the serum of anemic and non-anemic participants in the Indian population. The results revealed that a significant number of anemic participants were categorized under mild anemia, with iron deficiency being the primary cause of anemia in the normal population.

The basic form of anemia treatment remains supplementation with iron, vitamin B12, and folic acid as well as a diet rich in the above-mentioned hematopoietic factors.[10] The route of administration (oral, intramuscular or intravenous) requires careful consideration of the benefits and possible side effects and assessment of the patient's clinical status.[19]

Our findings resonate with previous research in several ways. Suega et al. 2002[20] studied involving 1684 women across 42 communities in Bali. They revealed that the level of education, use of antenatal iron pills as the most common risk factors during pregnancy in anemia. Another research reported in a national survey reports that the prevalence of anemia during pregnancy was 63.5% in 1990 which decreased by 51.4% in 1995.[21]

A study conducted by Asok et al. 2003 revealed that 75% of anemic subjects have vitamin B12 deficiency and 16% of anemic subjects have folate deficiency.[22]

Our results were also similar to the findings of the study conducted by Krishna Kishor et al. 2013, which also showed deficiency of vitamin B12 levels in 50% of anemic study subjects. They concluded that 100% of subjects presenting with macrocytosis had vitamin B12 deficiency but the finding was much confounding in microcytosis and normocytosis.[23] Similarly, other study was also discussed by Gerardo et al. 2014,[7] in patients having concomitant

Table 1: Demographical characteristics of study population

Variables	Anemic women N=300 N (%)	Non-Anemic womenN=158 N (%)	p-value
Age (years)			
18-20	128(42.7)	78(49.4)	
26-30	102(34.0)	56(35.4)	0.108
31-35	70(23.3)	24(15.2)	
Non-Pregnant	114(38.0)	68(43.0)	0.294
Pregnant	186(62.0)	90(57.0)	
Place of Residence			
Urban	102(34.0)	56(35.5)	0.757
Rural	198(66.0)	102(64.5)	
Educational Status			
No education	98(32.7)	46(29.1)	
Primary	76(25.3)	39(24.7)	0.652
Secondary & Higher	126(42.0)	73(46.2)	
Socio-economical			
Lower	110(36.7)	56(35.4)	
Middle	112(37.3)	78(49.4)	0.010*
Upper	78(26.0)	24(15.2)	
Dietary Habits			
Vegetarian	198(66.0)	86(54.4)	0.015*
Non-vegetarian	102(34.0)	72(45.6)	

The Chi-square test was used to calculate the p-value. *p-value <0.05 was considered as statistically significant

Table 2: Comparison of hemoglobin, red cell indices, and nutritional variables in anemic and non-anemic women

Variables	Anemic women(N=300) Mean±SD	Non-Anemic womenN=158 Mean±SD	p-value
CBC Parameters			
Hemoglobin (g/dL)	8.25±2.29	13.05±1.48	<0.0001*
RBCs (million/mm^3)	3.10±1.01	3.54±1.21	<0.0001*
MCV (fL)	76.61±13.84	92.03±11.87	<0.0001*
MCH (pg)	26.74±12.83	29.45±11.73	0.027*
MCHC (g/dL)	30.82±12.74	32.08±11.82	0.150
RDW (%)	12.61±3.15	19.37±4.26	<0.0001*
Nutritional Parameters			
Iron (μg/dL)	62.99±26.92	98.82±63.80	<0.0001*
UIBC (μg/dL)	195.69±118.45	232.49±124.43	<0.0001
TIBC (μg/dL)	145.10±59.95	297.60±99.36	0.002*
Ferritin (ng/mL)	82.36±44.82	142.96±114.15	<0.0001*
Vitamin B12 (pg/mL)	116.43±66.57	250.94±177.76	<0.0001*
Folic Acid (ng/mL)	7.14±2.55	14.56±9.7	<0.0001*

Abbreviations: RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cells distribution width, UIBC: Unsaturated iron binding capacity, TIBC: Total iron binding capacity. The student t-test was used to calculate the p-value. *p-value was considered as statistically significant

deficiency of both ferritin and vitamin B12.

In our study, we observed that 39.3% anemic women had microcytic hypochromic, 32.7% of anemic women had normochromic normocytic pictures, and 22.7% anemic women had macrocytic pictures.

Singh et al. 2017 observed that folate, vitamin B12, ferritin, and hemoglobin levels among women of childbearing age from a rural district in south India. They also observed the lower median serum levels of biochemical parameters in their study participants. They concluded that women with folate deficiency had a two times higher prevalence of having vitamin B12 deficiency.[24]

Our study suggested that iron deficiency (56.6%) was more prominent in our study population as compared to vitamin B12 deficiency (16.7%), and folic acid (10%) deficiency. Interestingly, some participants exhibited multiple deficiencies. A combination of iron, vitamin B12, and folic acid was found in 6.7%, while a combined

Table 3: Comparison of hemoglobin, red cell indices, and nutritional variables on the basis of severity as per WHO criteria

Variables	Mild (9-11gm/dl) N=116 Mean±SD	Moderate (7-9gm/dl) N=98 Mean±SD	Severe (<7gm/dl) N=86 Mean±SD	p-value
CBC Parameters				
Hemoglobin (g/dL)	10.79±0.94	8.55±0.79	5.62±1.21	<0.0001*
RBCs (million/mm3)	3.25±1.12	3.12±1.04	2.02±1.01	0.062
MCV (fL)	85.54±10.69	86.42±18.33	79.81±11.19	0.123
MCH (pg)	27.30±4.56	27.18±4.72	25.74±7.61	0.109
MCHC (g/dL)	31.78±1.83	31.34±2.34	29.39±3.23	<0.0001*
RDW (%)	17.16±3.16	18.80±4.06	21.99±3.98	<0.0001*
Nutritional Parameters				
Iron (μg/dL)	57.23±27.74	64.46±30.05	46.54±30.21	0.075
UIBC (μg/dL)	124.60±118.92	123.99±114.10	122.55±104.45	0.500
TIBC (μg/dL)	174.54±123.54	185.67±111.98	162.54±109.76	0.738
Ferritin (ng/mL)	51.60±38.86	41.32±29.73	40.35±28.39	0.032
Vitamin B12 (pg/mL)	174.43±68.45	177.06±66.24	145.65±58.43	0.042*
Folic Acid (ng/mL)	7.50±6.52	7.87±1.50	5.84±2.54	0.358

Abbreviations: RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cells distribution width, UIBC: Unsaturated iron binding capacity, TIBC: Total iron binding capacity. The ANOVA test was used to compare the groups. *p-value <0.05 is considered as statistically significant

Table 4: Comparison of hemoglobin, red cell indices, and nutritional variables on the basis of morphological classification as per Mean cell volume

Variables	Microcytic Hypochromic(<80 fL) N=118 Mean±SD	Normocytic Normochromic(80-100 fL) N=114 Mean±SD	Macrocytic(>100 fL) N=68 Mean±SD	p-value
CBC Parameters				
Hemoglobin (g/dL)	7.99±2.10	8.71±2.25	7.13±2.43	0.043*
RBCs (million/mm^3)	2.28±0.98	2.87±1.03	3.02±1.12	0.045*
MCV (fL)	72.37±6.80	89.12±5.61	110.38±8.29	<0.0001*
MCH (pg)	21.21±3.27	28.03±2.98	34.73±6.44	<0.0001*
MCHC (g/dL)	21.21±3.27	28.03±2.98	34.73±6.44	0.022*
RDW (%)	19.58±4.79	18.36±4.01	20.35±2.64	0.134
Nutritional Parameters				
Iron (μg/dL)	49.37±33.51	62.64±28.40	65.65±29.91	0.002*
UIBC (μg/dL)	123.06±113.54	119.70±76.56	131.68±87.76	0.076
TIBC (μg/dL)	179.34±98.87	182.23±98.23	198.76±112.12	0.055
Ferritin (ng/mL)	31.60±28.76	41.78±28.10	48.10±35.21	0.036*
Vitamin B12 (pg/mL)	181.04±102.65	168.69±61.35	123.78±67.87	<0.0001*
Folic Acid (ng/mL)	6.51±5.39	7.74±2.59	8.09±6.32	0.068

Abbreviations: RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cells distribution width, UIBC: Unsaturated iron binding capacity, TIBC: Total iron binding capacity. The ANOVA test was used to compare the groups. *p-value <0.05 is considered as statistically significant

deficiency of vitamin B12 and folic acid deficiency was observed in 10% of the anemic women.

Dhanuka et al. 2019 studied the profile of nutritional anemia and its correlation with iron, vitamin B12, and folic acid level among the tribal population of the northern districts of West Bengal, India. They revealed iron deficiency (37.5%) was the most common cause of anemia in their population. They also observed combined deficiency of iron and folate.[25]

The strength of our study is that this is the first study of the rural and urban population of Shahjahanpur, Uttar Pradesh. Further, it is a comprehensive study with a battery of nutritional and hematological parameters assessed in all the study participants.

There are some limitations to this study that the study group was relatively small due to low sample size and also can be addressed in future research such as ensuring the sample size is large enough to be representative of the population, and ensuring that time constraints do not limit the duration of the study. These potential limitations should be considered when interpreting and conclusions of this study.

5. Conclusion

This study provides valuable insights into the prevalence of anemia in the Indian population and micronutrient deficiencies among pregnant women. The findings reveal that mild anemia is most common with iron deficiency being the predominant cause. The study also highlights the influence of factors during pregnancy such as level of education, and dietary habits on anemia. Furthermore, the study underscores the need for preventive strategies for pregnant women such as food fortification, and health education. Despite some limitations, these findings contribute significantly to our understanding of anemia and pave the way for future research in this area.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Cite this article: Sharma M, Frayez M, Rahman A, Kumar A. Assessment of iron, folic acid, and vitamin B12 deficiencies and their association with red cell indices in anemic and non-anemic women. *Indian J Obstet Gynecol Res* 2024;11(2):172-177.