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ORIGINAL ARTICLE

B12 Deficiency is the Commonest Cause of Anaemia During Pregnancy in Northern India: Study from a Tertiary Care Institute

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Abstract Iron deficiency anemia is considered the leading cause of anemia during pregnancy; however, there is a lack of comprehensive studies on the etiological factors of anemia in pregnant women. The objective of this study was to systematically investigate the causes of anemia in pregnancy. Five hundred women with hemoglobin levels < 11 g/dl between 6 and 40 weeks of pregnancy underwent a complete hemogram, iron studies, serum folate, serum B12, serum copper, and serum zinc level assessments using standard methods. The median age of the patients was 26 years (range 24–29 years). The majority of patients were in the third trimester (449/500, 89.8%). Among the patients, 325 (65%) had vitamin B12 deficiency, with 159 (31.8%) having isolated B12 deficiency and 142 (28.4%) having combined B12 and iron deficiency. Isolated iron deficiency anemia was present in 74 patients (14.8%). Additionally, 28 patients (5.6%) had beta-thalassemia minor, and anemia of chronic disease was found in 17.2% (86) of the patients. Vitamin B12 deficiency was the most common cause of anemia, followed by combined B12 and iron deficiency. Further studies in diverse populations are warranted as they have broader implications for nutrient supplementation during pregnancy.

Keywords Anemia · Vitamin B12 deficiency · Iron deficiency · Epidemiology · Pregnancy

Introduction

Anemia in pregnancy is defined by the World Health Organization as hemoglobin levels below 11 g/dl [1]. It is one of the most common medical disorders encountered during pregnancy, affecting approximately one-third of the global population (over 2 billion people) [2]. Anemia in pregnancy is a significant contributing factor to maternal morbidity/mortality and neonatal morbidity [3–7]. The causes of anemia can range from nutritional deficiencies (such as iron, vitamin B12, folic acid, and other micronutrients etc.) to inherited disorders, autoimmune disorders, infections, and exposure to drugs or toxins [3–7].

While anemia can occur due to a variety of causes, iron deficiency and acute blood loss are the most common etiologies during pregnancy and the postpartum period [3]. Iron deficiency anemia is widely regarded as the leading cause of anemia in pregnancy [3], leading to the empirical use of iron supplementation as a treatment approach regardless of the underlying cause, as recommended by the Government of India. Consequently, other micronutrient deficiencies (e.g. B12 deficiency), protein malnutrition, and rare causes of

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anemia often go unnoticed and unaddressed [8]. Therefore, the aim of this study was to systematically investigate the etiology of anemia in pregnant women, encompassing a broader range of potential causes.

Methodology

This was a cross sectional study conducted in the Department of Obstetrics and Gynaecology from July 2015 to August 2016 following approval from the institutional ethical committee (NK/2209/MD/9896-97). A total of 500 eligible women were recruited after screening 2000 pregnant women. The inclusion criteria consisted of pregnant women in any trimester with hemoglobin levels below 11 g/dl who presented for antenatal check-ups to the department. Exclusion criteria included pregnant women with an obvious history of blood loss either related to pregnancy (antepartum hemorrhage) or obvious cause of bleeding (e.g. epistaxis, bleeding due to piles). Informed written consent was obtained from all recruited patients. A pre-designed proforma was used to document detailed information regarding the current pregnancy, obstetric and medical history. Each anemic woman underwent a comprehensive general physical and obstetric examination.

The socioeconomic status of patients was determined using the Kuppuswamy index [9]. Dietary habits were assessed through a 24-h recall and a two-week food frequency questionnaire. Participants were categorized as vegetarians if they followed a diet devoid of all animal flesh but included eggs and/or dairy products. Non-vegetarians were defined as individuals who consumed meat, seafood, or products containing these foods at least once a week. The following investigations were conducted for all patients to identify the cause of anemia: complete hemogram with peripheral blood film, Iron studies (serum ferritin, serum iron, total iron-binding capacity (TIBC), transferrin saturation), hemoglobin HPLC, serum copper, serum zinc, vitamin B12 and folate assays, serum urea and creatinine and liver enzymes. Further investigations (autoimmune profile, bone marrow examination, coomb test etc.) to determine the cause of unexplained anemia were conducted based on the hematologist's opinion. Patients were treated for anemia according to the cause.

Laboratory Analysis

Complete blood counts were performed using LH780 hematology analyzers (Beckman Coulter Inc., Florida, USA). Vitamin B12 and Folic acid levels were determined using the radioimmunoassay method on ADVIA Centaur and ADVIA Centaur XP systems. Serum iron assay was conducted based on a modification of the International Committee for Standardization in Hematology (ICSH)-recommended method, while Serum Ferritin was estimated using an indirect solid-phase ELISA (ORGenTec Diagnostika GmbH, Mainz, Germany). Serum copper and serum zinc assays were carried out using an inductively coupled plasma-mass spectrometer (ICP-MS, Make: Agilent Technologies model 7700x) in collision cell mode.

Serum Vitamin B12 levels < 150 pg/mL and Serum Folic acid levels < 2 µg/L were used as cut-off values according to reference literature [1]. Serum iron levels < 40 mg/dL, serum ferritin levels < 10 mg/L, and transferrin saturation less than 16% were used as indicators of iron deficiency anemia [1]. Cut-off values of Serum Cu < 65 µg/dL and Serum Zn < 70 µg/dL were taken from the diagnostic kit literature.

Statistical Analysis

Discrete categorical data were presented as n (%) while continuous data were reported as mean ± SD (standard deviation) with range or median and interquartile range, as appropriate. The normality of quantitative data was assessed using the Kolmogorov–Smirnov test of normality. Proportions were compared using the Chi-square test or Fisher's exact test, as applicable. Independent predictors for outcomes were identified through univariate analysis (logistic regression analysis). All statistical tests were two-sided and conducted at a significance level of $p = 0.05$. The analysis was performed using IBM SPSS Statistics (version 22.0).

Results

Demographics: The median gestational age at recruitment was 33 weeks (range 29–36 weeks). The median age of the patients was 26 years (range 24–29 years). According to the Kuppuswamy index of social classification [9], 253 out of 500 patients (50.6%) were from the upper lower class, 158 (31.6%) were from the lower middle class, and the remaining were from the lower (16%) and upper-middle (1.8%) classes (Table 1). Among the 500 anemic patients, 423 (84.6%) had been booked since the first trimester in either government or private healthcare setups and were prescribed iron and folic acid supplements. However, only 373 (74.6%) had a history of actual intake of these supplements (Table 1). Overall, 337 patients (67.4%) reported being vegetarian.

Causes of Anemia The median hemoglobin level among the 500 patients was 9.2 g/dL (range 7.8–10.1) (Table 2). Out of the 500 patients, 325 (65%) had vitamin B12 deficiency [159 (31.8%) isolated B12 deficiency, 142 (28.4%) combined B12 and iron deficiency, 20 (4%) anemia of chronic disease, and 4 (0.8%) had hemoglobinopathy]. Isolated iron deficiency anemia was present in 74 patients (14.8%) (Table 3). Among the 28 patients with beta-thalassemia minor, five had both combined B12 and iron deficiency,

Table 1 Demographic details of the study population

Age (yrs.)	Total, N = 500 (%)
≤ 20	26 (5.2)
21–25	180 (36)
26–30	208 (41.6)
31–35	68 (13.6)
≥ 36	18 (3.6)
Median (IQR)	26 (24–29)
Socio-economic status	Total, N = 500(%)
Upper	0 (0)
Upper middle	9 (1.8)
Lower middle	158 (31.6)
Upper lower	253 (50.6)
Lower	80 (16)
Booking	
Booked	423 (84.6)
Un-booked	77 (15.4)
Intake of iron and folic acid	
Yes	373(74.6)
No	127(25.4)
Diet	
Vegetarian	337(67.4)
Mixed	163(32.6)

Table 2 Haematological profile of the study population

Parameter	Median	Range
Hb (g/dl)	9.2	7.8–10.1
TLC (/microliter)	9400	7200–1080
Platelet (lacs/microliter)	252,600	193,434–328,000
RBC (million/ microliter)	2.08	1.6–3.2
Reticulocyte count (N, %)	N	(%)
< 1%	13	2.6
1–2.7%	224	45.5
> 2.7%	255	51.8
MCV (fl)	N	(%)
< 80	240	48
80–100	241	48.2
> 100	19	3.8

Table 3 Vitamin and trace element profile among the pregnant women

Analyte	N (%)	Median	IQR
Se iron (µg/dl)			
< 40	217(43.4)	46	41–67
40–75	271(54.2)		
> 75	12(2.4)		
Se ferritin (ng/ml)			
≤ 10	221(44.2)	13	5–32
> 10	279(55.8)		
Folate level (µg/ml)			
< 2	9 (1.9)	8.9	6.8–13
2–20	467(93.4)		
> 20	24(4.8)		
Vitamin B12 (pg./ml)			
< 150	325(65)	136	112–171
150–911	148(29.6)		
> 911	27 (5.4)		
Serum copper (µg/dl)			
< 80	4(0.8)	220	182–260
80–160	401(80.2)		
> 160	95(19)		
S zinc (µg/dl)			
< 73	34(6.8)	211	172–245
73–140	318(63.6)		
> 140	148(29.6)		

Table 4 Aetiological profile of anemia in pregnant women (n = 500)

Aetiology	Total (%)
Combined deficiency	142(28.4)
IDA	74(14.8)
Vit B12 deficiency	159(31.8)
Anemia of chronic disease	86(17.2)
Hemoglobinopathy	28(5.6)
Aplastic anemia	2(0.4)
Others (haemolytic)	9(1.8)

and eight had isolated vitamin B12 deficiency. Two patients with anemia also had a decrease in other cell lines (pancytopenia). After obtaining a hematologist's opinion, both underwent bone marrow examination and were diagnosed with aplastic anemia. Nine patients were diagnosed with hemolytic anemia based on their medical history, clinical examination, high reticulocyte counts, and positive Coombs test (Table 4). Patients with iron deficiency were further investigated for worm infestations (by stool examination for ova, cysts); however, no endoscopies were performed to look for occult blood loss from the gastrointestinal tract. Thirty-four (6.8%) pregnant patients had low zinc levels, while 4 (0.8%) patients had copper deficiency. However, it is difficult to attribute anemia to these factors alone.

The etiology of anemia based on peripheral blood film examination is shown in Table 5. The most common aetiology among patients with normocytic normochromic anemia was anemia of chronic disease (51.6%). The most common aetiology of microcytic hypochromic was combined deficiency of iron and vitamin B12. (34.7%) (Table 5).

Out of 500 patients, 75 had associated comorbidities. These were seven patients with proven chronic liver disease,

Table 5 Causes of anemia according to peripheral blood film findings in pregnant women

	Normocytic normochromic N (%)	Microcytic hypochromic N (%)	Dimorphic N (%)	Macrocytic N (%)
Iron deficiency anemia	37(30.3)	30(17.9)	5(3.3)	2(3.2)
Vitamin B12 deficiency	9(7.3)	51(30.5)	59(39.5)	40(64.5)
Beta thalassemia minor	12(9.8)	5(2.9)	9(6)	2(3.2)
Combined deficiency	1(0.8)	58(34.7)	73(48.9)	10(16.2)
Anemia of chronic disease	63(51.6)	23(13.7)	3(2.0)	8(12.9)

eight patients with chronic kidney disease, six patients had heart disease, ten patients had seizure disorder, three had autoimmune disorders, three had a history of essential hypertension, four had a history of diabetes, three had a history of treated tubercular infection, and the rest 32 patients had a history of multiple comorbidities. These patients were also followed up with their disease specialty simultaneously.

Discussion

Anemia is a significant problem that particularly affects pregnant women, especially in developing countries [1, 10]. It exerts detrimental effects on both the mother and the fetus. Although iron deficiency anemia has been extensively studied, there is a dearth of research on other types of anemia. Therefore, the objective of our study was to investigate the etiology of anemia in pregnant women. Our findings revealed that the most prevalent type of anemia was associated with vitamin B12 deficiency, accounting for 65% of cases overall. Of these cases, 31.8% were isolated B12 deficiency, while 28.4% were associated with iron deficiency. While our study did not delve into detailed investigations to ascertain the causes of B12 and iron deficiency, the majority of cases were attributed to nutritional factors, as most patients belonged to a low socioeconomic status and exhibited poor dietary habits [8]. Additionally, the relatively lower prevalence of iron deficiency in our study may be attributed to the fact that the majority of patients were recruited in the third trimester and 84% were already receiving iron and folic acid supplements, potentially rectifying iron and folic acid deficiency-related anemia within our study group. Several studies conducted in India have demonstrated that nutritional factors constitute the primary causes of iron, folic acid, and B12 deficiencies in the general population, and this finding holds true for pregnant women as well [8, 10–12].

The conventional approach to treating anemia in pregnancy has always involved iron and folic acid supplementation [3]. The Government of India has implemented various programs to address anemia, including the National Nutritional Anemia Prophylaxis program in 1970, the National Anemia Control program in 1990, the Weekly Iron and Folic Acid Supplementation program in 2012, the National Iron Plus Initiative in 2013, the Poshan Abhiyaan in 2018, and the Intensified National Iron Plus Initiative in 2019 (Anemia Mukt Bharat). However, despite these initiatives, the desired improvements in anemia rates have not been achieved [13, 14]. Various studies [4, 7, 11, 13, 15–18] conducted in different parts of India have reported a prevalence of B12 deficiency ranging from 41 to 60% among pregnant women and childbearing-age women. However, most of these studies had a small sample size, with fewer than 200 participants, except for the study focused on the childbearing age group. The findings from our study, combined with the results of these previous studies, indicate a high prevalence of B12 deficiency among pregnant women. Therefore, considering the suboptimal outcomes of various national programs, it is strongly recommended to include B12 supplementation, along with iron and folic acid, for pregnant women. Table 5 in our study also highlights the fact that peripheral blood film analysis cannot be relied upon to diagnose iron or B12 deficiency, as many patients exhibit deficiencies in multiple micronutrients, and accurate diagnosis requires assessment of blood levels.

One of the strengths of our study is its large sample size compared to other studies in the literature. We assessed serum levels of iron, ferritin, vitamin B12, folic acid, copper, and zinc in all patients. However, it is important to consider several limitations. Firstly, our study only included women who visited our hospital and were aware of hospital delivery, which may limit the generalizability of our results to the broader population. Additionally, the majority of our patients were recruited in the third trimester and were already receiving iron and folic acid supplements, which might have influenced the incidence of iron deficiency in our study group. We did not separately analyze the subtypes of anemia due to their low incidence. Moreover, we did not measure vitamin B12 levels in non-anemic women, which could have provided further insights into the nutritional status of the population.

Conclusion

Our study reveals a high prevalence of B12 deficiency among pregnant women, underscoring the importance of further research in peripheral hospitals to confirm its significance as a contributor to anemia. If confirmed, it would be crucial to reconsider the current approach to antenatal care supplementation by including vitamin B12 alongside iron and folic acid. This comprehensive approach holds promise for effectively addressing and managing anemia in this vulnerable population.

Author contributions Dr. AB and Dr. VS conceived the present idea and designed the study. Dr. PM planned the test, contributed to the design, and helped in the analysis. Dr. NV and Dr. SVA carried all the biochemical tests. Dr. PS and Dr. SSS supervised the research findings. Dr. AG took the lead in writing the manuscript and analysis. All the authors provided critical feedback and helped shape the research, analysis, and manuscript.

Declarations

Conflict of interest None.

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