Research Article

Tracking the association of severity of anemia during pregnancy by hematological and relevant biochemical parameters

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ABSTRACT

Background: Anemia is a common ailment during pregnancy, and various factors have effect on accentuating the severity and incidence of anemia. **Objectives:** The present study was designed to determine the profile of anemia, grading the severity of anemia in pregnant women, and assess the level of various relevant biochemical parameters in the study population. **Materials and Methods:** A cross-sectional study was carried out in 530 cases of pregnant women attending antenatal clinic at M. Y. Hospital during 2015-2016. Sociodemographic information and blood samples were collected from all the participants. Parameters studied included hemoglobin (Hb), red blood cell count (RBC Count), packed cell volume (PCV), mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, iron profile and vitamin B12 in each age group, trimester, parity, and morphological types. **Results:** Results showed that parity incidence of anemia was similar in all parameters. Anemia is more common in the second and third trimesters. Anemia of all types is more prevalent in the age group of 21-30 years. **Conclusion:** It can be concluded that there were close correlations of the various hematological parameters, for example, PCV, total RBC counts, and absolute values with both the severity and the type of anemia. Iron Profile, total iron binding capacity capacities are valuable indices for diagnosing the cause of anemia and for detecting latent cases of iron deficiency anemia which may reveal them with the progression of pregnancy. All the cases of macrocytic anemia were vitamin B12 deficient.

KEY WORDS: Pregnancy; Anemia; Hematological Parameters

INTRODUCTION

A pregnancy is clout by several factors, certain of which contain environmental, cultural, socioeconomic status and access to medical care. The hematological parametric indices also have an impact on pregnancy and its outcome.[1] Anemia in pregnant women is variously defined with two common parameters either as hemoglobin (Hb) concentration <11.0 g/dl or 5th percentile of the distribution of Hb concentration or hematocrit (HCT) in a healthy reference population. This valuation is possible through a series of tests measuring different variables.[2] During pregnancy, the major change occurs in the physiology of the mother, designed to supply the fetus with nutrients necessary for growth and the mother's supplementary energy that she requires for labor (before the fetal need arises).[3] In pregnancy, plasma volume increases from 25% to 80% between the 6 and 24 weeks of gestation. However, the increase in red blood cell (RBC) mass has been found to be approximately 30% between the 25 and 36 weeks of gestation when iron and folate are supplemented.[4] In late pregnancy, plasma volume increases at a slower rate, inducing a slight rise in HCT level. These physiological changes during pregnancy make it difficult to define normal hematological reference intervals for pregnant women.[5] Anemia contributes to low birth weight and

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miscarriages and is also a primary cause of low immunity in both the mother and the child, which makes them vulnerable to several infections.[6]

Hematological parameters are very relevant in the assessment of pregnant mothers or test values that are done in the hematology laboratory with the use of specimen to assess the component of blood in health and disease, as a result of physiological conditions. Due to the physiological changes in pregnancy, certain hematological parameters could be greatly attained, especially the packed cell volume (PCV) and total white blood cell counts.[7] The PCV provides information about the percentage of erythrocytes. When there is a decrease in PVC, it implies anemia while the total white blood cell count provides information about the immunity and also detects conditions associated with acute or chronic inflammation including infection.[7] This study is of importance because systems monitored during antenatal care in an attempt to predict and/or improve pregnancy outcome are dependent on the quality and quantity of hematological indices. The objective of this study was to find the association of severity of anemia during pregnancy by hematological and relevant biochemical parameters. Keeping all these facts in view, the present study was designed to determine the variation in hematological parameters in pregnant women.

MATERIALS AND METHODS

The study was conducted by pathology department at M. Y. Hospital, Indore, from 2015 to 2016. A total of 530 anemic pregnant women, attending obstetrics and gynecology outpatient department, were studied. Prior permission from ethical committee of the hospital was obtained. In all cases, patients age, weight, clinical history, and other laboratory investigations were recorded in a pro forma. Inclusion criteria were anemic pregnant women, whose Hb level was below 11 g/dl. Complete confidentiality regarding the identity of the individual was maintained and therefore only hospital registration number is provided in the study. Written consent was obtained from every patient. The samples were collected in an EDTA vial acid and plain tube by the technician and sent along with the requisition form to the pathology laboratory. The studied parameters Hb, RBC Count PCV, MCV, MCHC, HCT, iron profile, and vitamin B12 of blood samples were estimated. Data analysis was done with appropriate statistical test.

RESULTS

A total of 530 cases of pregnant women were included in the present study. These cases included pregnant women from 18 to 40 years of age. Nearly 78.3% (415) of women were in the age group of 21-30 years, while only 15.5% (82) and 6.2% (33) were in the age group of <20 years and above 30 years, respectively. Out of the 82 patients who were under the age of 21 years, severe anemia was observed in 6 patients whereas moderate anemia was observed in 29 patients. In the age group of 21-30 years, severe and moderate types of anemia were observed in 33% and 134 patients respectively. There was no significant association of age groups with severity of anemia (P = 0.151). The average Hb was also same across different age groups; the severity of anemia was highest in the second trimester. About 80% of patients with severe anemia were in the second trimester. The average Hb was also lowest in the second trimester. The distribution of severity of anemia and trimester was statistically significant (P < 0.0001).

Out of the 530 patients, 238 patients were nulliparous and 195 patients were primiparous. There was no association with parity and severity of anemia (P = 0.253). The average Hb was also same across all parities. The patients were divided into four groups according to the different morphological picture of RBCs in peripheral smear. Accordingly, 195 patients were microcytic hypochromic, 34 patients were macrocytic, 37 were dimorphic and 246 were normocytic normochromic. Moderate type of anemia was most common among the microcytic hypochromic RBCs followed by mild anemia (Table 1). Macrocytic picture of RBC was more common in severe and moderate types of anemia. About 50% of patients with dimorphic picture of RBCs were normal. There was a significant association of severity of anemia with morphological RBC type (P < 0.0001).

More than half of the patients with dimorphic picture were nulliparous. A majority of patients with macrocytic picture were primiparous. Nulliparity was seem to be common in microcytic hypochromic and normocytic normochromic pictures. There was no association of type of morphology with parity. No significant association was observed between the trimester and morphological type of RBCs (P = 0.396) (Table 2).

RBC count was found to be lowest in severe anemia group (Table 3). The total RBC Count (tRBC Count) was almost similar in all the other groups. The difference in mean RBC values was statistically significant (P < 0.0001). PCV estimation was done in each case and it was lowest in severe anemic group and highest in normal group. The difference in mean PCV was statistically significant (P < 0.0001). MCV and MCH values were lowest in moderate type of anemia. The mean MCV and MCH values were also significantly different in different groups. The MCHC levels were almost near normal in all groups.

An association was also drawn between the various types of anemia on the peripheral smear and the absolute values. It was observed that the PCV was the lowest in patients with macrocytic RBCs and the highest in dimorphic RBCs. The RBC count was the lowest in patients having macrocytic RBCs. The difference in total RBC count was statistically significant. The MCV was above normal in macrocytic anemia

Table 1: Cross-tabulation of severity of anemia along with the average Hb level in each age group/trimester/parity/morphological type

Types	Hb level				
	<7 g (%)	7-9.9 g (%)	10-10.9 g (%)	≥11 g (%)	Average Hb
Age years					
≤20	6	29	13	34	10.2
21-30	33	134	100	148	10.1
>30	0	10	13	10	10.6
Trimester					
I	0	29 (16.8)	16 (12.7)	30 (15.6)	10.53
II	31 (79.5)	72 (41.6)	52 (41.3)	68 (35.4)	9.78
III	8 (20.5)	72 (41.6)	58 (46.0)	94 (49.0)	10.48
Parity					
0	13	83	55	87	10.3
1	22	58	46	69	10.1
2	4	28	20	34	10.4
3	0	4	5	2	10.3
Morphological					
Microcytic hypochromic	23	112	44	16	
Macrocytic	10	11	7	6	
Dimorphic	0	8	9	20	
Normocytic normochromic	6	42	66	150	

Hb: Hemoglobin

Table 2: Cross-tabulation between the morphological RBC types with parity and trimester of patients

Types	Dimorphic	Macrocytic	Microcytic hypochromic	Normocytic normochromic
Parity				
0	20	10	93	115
1	11	14	74	96
2	05	09	23	49
3	01	01	05	04
Trimester				
1	6	7	25	37
2	12	16	91	104
3	19	11	79	123

RBC: Red blood cell

and was the lowest in microcytic hypochromic anemia. The MCH was the lowest in microcytic hypochromic anemia and the highest in macrocytic anemia. The MCHC was the highest in macrocytic anemia and the lowest in microcytic hypochromic anemia. The RBC Count, PCV, MCV, MCH, and MCHC levels were similar across all parity groups. tRBC count was similar in all the trimester groups. PCV values were lower in the 2nd trimester group and they were highest in the 1st trimester group. The difference in mean PCV values across the groups was statistically significant ($P = 0.008$) (Table 4).

Serum iron (SI) was observed to be lowest in microcytic hypochromic patients whereas it was highest in macrocytic patients. The difference in mean SI was statistically significant ($P < 0.0001$). Total iron-binding capacity (TIBC) was found to be highest in microcytic hypochromic patients and lowest in macrocytic patients. The difference in mean TIBC was statistically significant ($P < 0.0001$). 68 (75.6%) pregnant women were found to be iron deficient with serum ferritin <20 ng/ml. The SI and % transferrin receptor (TFR) saturation were lowest in moderate anemia group. The difference in mean SI and % TFR in different groups was statistically significant ($P < 0.0001$). The difference in mean TIBC was also statistically significant across the groups (Table 5). Serum vitamin B12 was measured in macrocytic cases to rule out vitamin B12 deficiency, we found 21 cases to be vitamin B12 deficient. Folic acid deficiency was observed in 16 cases having macrocytic RBC type.

Table 3: Severity of anemia and the average RBC count, PCV, MCV, MCH, MCHC levels

Types	Average RBC count millions/cumm	Average PCV (%)	Average MCV fl	Average MCH Pico gms	Average MCHC
Hb levels g (%)					
<7 g	2.5±0.85	18.39±3.55	79.13±20.7	27.90±115.0	30.05±6.2
7-9.9 g	4.0±0.93	29.15±5.43	75.85±14.17	23.57±6.72	30.58±3.8
10-10.9 g	4.07±0.82	33.58±3.7	83.61±12.28	26.06±5.35	31.38±3.02
≥11 g	4.11±0.94	39.76±5.6	87.98±7.81	27.03±3.3	30.68±2.28
P value	<0.0001	<0.0001	<0.0001	<0.0001	0.095

RBC: Red blood cell, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration

Table 4: Absolute counts according to morphological types/parity/trimester

Types	Average RBC count	Average PCV	Average MCV	Average MCH	Average MCHC
Morphological					
Dimorphic	4.74±0.58	38.87±4.62	82.18±2.70	23.54±1.2	28.71±1.74
Macrocytic	2.39±0.81	26.43±9.11	110.35±7.23	37.08±4.69	33.97±3.40
Microcytic hypochromic	4.44±0.92	29.94±7.14	68.32±7.08	20.9±7.21	29.77±4.05
Normocytic normochromic	4.00±0.72	35.8±7.04	89.09±5.04	28.06±2.87	31.98±2.55
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Parity					
0	4.2±0.92	33.38±8.5	80.8±12.6	25.2±7.0	30.78±3.11
1	4.0±1.00	32.7±7.7	82.71±14.1	25.97±6.61	30.77±3.91
2	4.0±0.89	33.8±6.6	85.38±13.35	26.53±5.64	30.90±3.01
3	4.3±0.88	33.35±6.4	83.75±13.7	24.69±5.41	29.41±3.5
P value	0.192	0.542	0.054	0.403	0.606
Trimester					
I	4.12±0.81	34.6±8.7	84.37±13.5	26.2±5.8	30.92±3.2
II	4.02±1.00	32.05±7.7	81.34±14.46	81.3±14.4	30.7±3.74
III	4.2±0.93	33.95±7.62	82.62±12.3	82.62±12.3	30.72±3.2
P value	0.149	0.008	0.217	0.575	0.903

RBC: Red blood cell, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration

DISCUSSION

Anemia is a common problem during pregnancy, and no reference values have been reported for hematological parameters during pregnancy. Our finding concludes that the parity incidence of anemia was similar in all parameters. Anemia is more common in the second and third trimesters. Anemia of all types are more prevalent in the age group of 21-30 years. The most common type of morphological type of anemia diagnosed on the peripheral smear is the microcytic hypochromic type, i.e., iron deficiency anemia, followed by dimorphic and normocytic normochromic anemia in that order. There is a close correlation of the various hematological parameters, for example, PCV, tRBC counts, and absolute values with both the severity and the type of anemia. The PCV and tRBC count are valuable diagnostic indices in severe anemia. Severe anemia is mainly caused due to low RBC count whereas moderate type of anemia is mainly caused by iron deficiency. SI and TIBC are valuable indices for diagnosing the cause of anemia and for detecting latent cases of iron deficiency anemia which may reveal them with the progression of pregnancy. All the cases of macrocytic anemia were vitamin B12 deficient.

An overall prevalence rate of anemia in pregnant women in our study was 63.77%. This level is substantially high as compared to other countries such as 41.3% in Saudi Arabia.[8] The prevalence of anemia in Malaysia was 35% which is much lower.[9] Other countries did show a lower prevalence of anemia. However, the prevalence of anemia in developing countries such as Nigeria was higher, 76.5%.[10] The rate of anemia for pregnant women in this study is approaching to the WHO estimation for developing countries and far behind from economically developed countries such as in Malaysia, where their prevalence was 25.1%.[9] This high figure is surprising, considering the routine practice at all primary health-care centers to provide pregnant women with prophylactic elemental iron. This reflects a poor nutritional health of antenatal mothers in this country. Hb levels varied from a low of 4.1 g% to a high of 14.0 g%. These levels

Table 5: Iron profile in different morphological subtypes and severity of anemia

Types	SI	TIBC	% TFRs
Morphological			
Dimorphic	41.37±15.99	356.15±137.7	12.07±3.02
Macrocytic	88.62±31.98	295.7±87.6	32.72±12.5
Microcytic	53.04±61.6	520.04±103.92	10.0±10.28
Normocytic	67.62±18.75	297.78±79.07	22.95±3.68
Hb levels g (%)			
<7 g	54.05±26.54	413.38±143.66	16.32±11.94
7-9.9 g	49.15±25.6	449.50±137.31	12.82±8.6
10-10.9 g	75.56±70.59	389.94±145.83	20.16±13.01
≥11 g	65.41±24.5	312.59±104.37	21.74±8.67

SI: Serum iron, TIBC: Total iron-binding capacity, TFRs: Transferrin receptors, Hb: Hemoglobin

were classified into three groups as mild (23.2%), moderate (32.6%), and severe (7.3%) anemia. The above cases were classified as mild, moderate, and severe anemia as those with Hb levels <7.0 g% being classified as with severe anemia, those with Hb levels 7.0-9.9 g% being classified as with moderate anemia, and those with Hb levels from 10.0 to 10.9 g% being classified as with mild anemia. Out of 66 pregnant women, 40.92% were mild anemic, 54.54% were moderate anemic, while 4.54% were severe anemic. The severity of anemia is higher in our study compared to other studies.[11] According to Shah et al.,[12] out of 51 pregnant anemic women, 9 (18%) were mildly anemic, 30 (58%) were moderately anemic, while 9 (18%) were severely anemic and 3 (6%) were very severely anemic. The severity of anemia is higher in this study as compared to our study. Anemic status of mothers may predispose themselves as well as their offsprings to several unwanted health outcomes such as preterm deliveries, low birth weight, and prenatal mortality conditions.[12] These health implications result from the impairment of oxygen delivery to placenta and fetus.[13] Apart from that, low Hb level of the mothers has been proved to cause an increase in maternal mortality rate.

In this study, the highest rate of anemia was at the second trimester, the difference was statistically significant. A study done in Saudi Arabia show a significantly high prevalence of anemia during the third trimester.[8] The average Hb level in the second and third trimester of pregnancy was 9.78 and 10.48 g%, respectively. Low Hb level at the second trimester is in fact due to high physiological expansion of maternal plasma volume in the second trimester. In our study, we did not find any association of parity with the severity of anemia. A survey among pregnant women in Nigeria found that the primigravida has higher prevalence of anemia (69.7%) ($P < 0.05$).[13] However, this study did not support that finding. The reason would be that we took the first antenatal clinic visit at our hospital in the study and therefore it did not represent the true prevalence in this population. It was observed that the maximum prevalence of anemia was in the age group of 21-30 years. The average Hb level in the age group of 21-30 years was 10.1 g%. However, age was not found to be a risk factor for anemia in the study done in Saudi Arabia in which teenage pregnant females were not at a higher risk of anemia than older women if good prenatal care was provided.[14] In our study, the peripheral blood smear examination for red cell morphology was done in every case. Typing of anemia in each case was done, and it was found that 36.8% of cases were of the microcytic hypochromic type, 7.0% cases had a dimorphic picture, while (4-5% seen in higher socioeconomic status) 6.4% cases had macrocytosis. Normocytic normochromic type was present in 49.8% of cases. Our study was significant as compared to the other study done by Verma et al.[15] who reported 55.4% of microcytic hypochromic type, 37.5% of normocytic normochromic type, and 1.7% of dimorphic type of anemia whereas Kumari et al.[16] reported microcytic hypochromic (53.3%), normocytic normochromic (35.8%), and macrocytic types (10.8%). Peripheral blood examination showed microcytic morphology in 30% of patients, hypochromic in 34% of patients, normocytic in 6% of patients, and normochromic in 2% of patients. Polychromasia was found in 15% of patients, and target cell was detected in 12% of patients. 4-5% were also seen in higher socioeconomic status.

In our study, a correlation was also drawn between the various types of anemia on the peripheral smear and the absolute values. It was observed that the PCV was the lowest in macrocytic morphology and the highest in dimorphic morphology. The tRBC count was the lowest in macrocytic morphology and near normal in all the other subtypes. The MCV was above normal in macrocytic morphology and was the lowest in microcytic hypochromic morphology. The MCH was the lowest in microcytic hypochromic morphology and the highest in macrocytic morphology. The MCHC was the highest in macrocytic morphology and the lowest in dimorphic morphology. In another study, mean RBC count was 3.52, 3.78, and 3.56; mean MCV was 77.79, 79.49, and 76.23; MCH was 21.17, 24.47, and 23.03; and MCHC was 27.42, 27.67, and 28.04 in the in the age groups of <20, 20-25, and >25 years, respectively. Mean HCT was 29.43, 31.24, and 32.20 in the age group of <20, 20-25, and >25 years, respectively.[17,18] Overall mean RBC count (106/ml), PCV (%), Hb (g/dl), MCV (fl), MCH (Pg), and MCHC (g/dl) were 4.00, 34.49, 9.69, 90.23, 25.63, and 28.80 in pregnant women in the age group of <25, 26-32, and >33 years, respectively. In our study, the prevalence of maternal anemia was 60.53%. In the study done by Okeke,[19] the prevalence of maternal anemia was 38.8%. The mean of Hb in the anemic population was 10.1 g/dl, HCT was 35%, MCV was 87.5 fl, and RDW-CV was 13.5%. In another study by Sunitha et al.,[20] RBC level in anemic pregnant women was observed as 3.70, 3.69, and 3.56 in the age group of 15-24, 25-34, and >35 years, respectively, while in our study, it was 3.74, 3.76, and 3.44 in the age groups of ≤20, 21-30,

and ≥30 years where RBC count level was 3.52, 3.78, and 3.56 in the age group of ≤20, 21-30 and ≥30 years. In the study of Sunitha et al., Hb level of pregnant women was 8.27, 8.63, and 9.08 in the second trimester. MCV level in their study was 80.76, 80.35, and 80.86, in the pregnant women in 15-24, 25-34, and >35 years age group comparable to the present study that was 73.65, 73.69, and 76.87. MCH level in their study was 22.56, 23.60, and 24.41, while in the present study, it was in the age group ≤20, 21-30 and ≥30 years. In the present study, the level of MCHC was comparable with the study of Sunitha et al.[20]

CONCLUSION

To conclude, it can be said that the hematological parameters are easily performed when properly interpreted along with their cutoff values, as suggested in this study. There were close correlations of the various hematological parameters such as Hb concentration, PCV, MCV, MCH, MCHC, tRBC count and absolute values with both the severity and the type of anemia. The PCV and tRBC count are valuable diagnostic indices in severe anemia. Severe anemia is mainly caused due to low RBC count whereas moderate type of anemia is mainly caused by iron deficiency. SI and TIBC are valuable indices for diagnosing the cause of anemia and for detecting latent cases of iron deficiency anemia which may reveal them with the progression of pregnancy. All the cases of macrocytic anemia were vitamin B12 deficient. This study can aid in the early recognition of type of anemia during pregnancy. In essence, while individual parameters have their limitation, a combination of different parameters certainly improves their usefulness.

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