Original Research Article

Study of hematological parameters in anemia in pregnancy

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Abstract

Background: To evaluate the major hematological parameters and morphological typing in anemic pregnant females and correlation of the above findings with the trimester and gravid status of the patient. **Material and Methods:** This study was conducted on 250 pregnant women attending antenatal OPD having hemoglobin level less than 11 gm/dl in 1st and 3rd trimesters and less than 10.5 gm/dl in the 2nd trimester. Age, gravida, trimester, educational status of each pregnant subject was noted as well as grading and morphological typing of anemia was done. **Results:** Maximum number of cases were found in age group of 21-30 years i.e. 79.2% (198 cases out of 250 cases). Maximum number of cases of anemic pregnant subjects were primigravidae i.e. 42%. Highest percentage of anemia was in third trimester i.e. 52.4% (131 out of 250 cases). Among all morphological types microcytic hypochromic anemia was most predominant i.e. 89.2%. Moderate anemia was the predominant grade of anemia i.e. 67.6% (169 out of 250). On correlating age group with grade of anemia, moderate anemia was found to be predominant among all age groups. Out of 169 cases of moderate anemia, 74 cases (43.8%) were in 21-25 age group. **Conclusion:** All the four factors i.e. age, gravida, trimester and educational status were correlated with grade and morphological type of anemia. Microcytic hypochromic anemia was the predominant morphological type of anemia in all age ranges, all gravidae and all trimesters. There was statistically significant association between age and morphological type of anemia.

Keywords: Hematological Parameters, Morphological, Trimester, Gravid Status

Introduction

Anemia is a major public health problem. It is now one of the most frequently observed nutritional diseases in the world. It is especially prevalent in women of reproductive age, particularly during pregnancy. Pregnancy is one of the unique periods of women's life cycle. It is a condition of great stress because many anabolic activities take place and fetal growth is accomplished by extensive changes in maternal body composition and metabolism. Maternal nutrition is the most important determinant that influences the development of fetus. Poor nutritional status during pregnancy is associated with inadequate weight gain, anemia, retarded fetal growth low birth weight, still births, preterm delivery, intrauterine growth retardation, morbidity and mortality rates [1].

The Importance and significance of the knowledge of hematological parameters in advanced pregnancy stems from the fact that anemia, especially iron deficiency anemia is the most common ailment affecting pregnant women of India. Most of the data available about the hematological parameters of pregnant women are the result of studies carried out in western countries which does not apply for Indian women. The cultural and socio-economic differences between the western countries and India have contributed to the differences in basic physiological values. For instance, Hemoglobin level of 11 gm/dl in an adult Indian woman is considered as average whereas a similar value in the West may be a cause for concern. Because of these differences it becomes essential to carry out a study on the hematological parameters in pregnancy in India [2].

Anemia is the most common hematological disorder during pregnancy. The main causes of anemia in developing countries include: inadequate intake and poor absorption of iron, malaria, hookworm infestation, diarrhea, HIV/AIDS and other infections, genetic disorders (e.g., sickle cell and thalassemia), blood loss during labor and delivery, heavy menstrual blood flow and closely spaced pregnancies. Iron deficiency and

Manuscript received: 30th August 2019
Reviewed: 10th September 2019
Author Corrected: 17th September 2019
Accepted for Publication: 23rd September 2019

anemia during pregnancy are associated with low birth weight, preterm delivery, increased perinatal and neonatal mortality, inadequate iron stores for the newborn, increased risk of maternal morbidity and mortality, and lowered physical activity, mental concentration, and productivity [3].

Several studies on intra-household food allocation show that women get less food than men relative to their nutritional needs. Unequal access to food, heavy work demands, nutritional deficiencies including iron, makes Indian women susceptible to many ailments and anemia. Low intake of ascorbic acid and meat, due to low income reduces the absorption of iron. While malnutrition is prevailing among all segments of the population, poor nutrition among women begins in early years and continues during their lifetime. Usually, female members in a family are the last to eat. Consequently, if there is not enough food, they are the ones to suffer mainly [2-5].

In 1993, the World Health Organization instituted its Safe Motherhood Initiative with a goal of reducing the number of maternal deaths by half before the year 2000. A key component was to eradicate anemia in pregnancy, focusing on the greater risk in younger women. Little progress has been made in reducing iron-deficiency anemia among women in developing countries, in spite of the introduction of iron supplementation programs in many of them. WHO has estimated that prevalence of anemia in pregnant women was found 14% in developed, 51% in developing countries and 65-75% in India. Prevalence of anemia in all the groups is higher in India as compared to other countries. WHO recommends that haemoglobin ideally should be maintained at or above 11.0 gm/dl and should not be allowed to fall below 10.5gm/dl in the second trimester. Anaemia contributes to low birth weight and miscarriages and is also a primary cause of low immunity of both mother and child, that makes them vulnerable to several infestations [6].

The present study was a prospective study over a period of 24 months in the department of pathology in a tertiary care institute in an urban setting. This study was conducted on 250 pregnant women attending antenatal OPD having hemoglobin level less than 11 gm/dl in 1st and 3rd trimesters and less than 10.5 gm/dl in the 2nd trimester. Age, gravida, trimester, educational status of each pregnant subject was noted as well as grading and morphological typing of anemia was done. All the four factors i.e. age, gravida, trimester and educational status were correlated with grade and morphological type of anemia.

Materials and Methods

Study design/Type of study

The study entitled 'Study of hematological parameters in anemia in pregnancy' was conducted in Department of Pathology in collaboration with department of Obstetrics and gynecology at Dr. D. Y. Patil Hospital and Research Center, Navi Mumbai.

Study period: The duration of the study period was 24 months conducted between September 2015 to August 2017.

Sample size: Total 250 cases of pregnant females attending antenatal OPD having Hemoglobin less than 11gm/dl in first and third trimester of pregnancy. Hemoglobin less than 10.5gm/dl in second trimester of pregnancy

Inclusion criteria

All pregnant females of age more than18 years attending ANC clinic with

1. Hemoglobin less than 11gm/dl in first and third trimester of pregnancy
2. Hemoglobin less than 10.5 gm/dl in second trimester of pregnancy

Exclusion criteria

1. All pregnant females of age less than 18 years
2. Pregnant females with Hemoglobin more than 11gm/dl in first and third trimester of pregnancy and Hemoglobin more than 10.5gm/dl in second trimester of pregnancy
3. Pregnant females with other comorbid conditions which can contribute to anemia

Data collection procedure: History and other clinical details of each patient as per case papers available was recorded in a format of Case record form. Informed written consent was taken from each patient. For preliminary hematological investigations, venous blood was collected with aseptic precautions with the help of vaccutainer which contained optimum concentration of EDTA for the volume of blood collected in.

Hematological investigations were performed immediately on Automated hematology analyzer with standard calibration used. Following parameters were obtained by automated hematology analyzer.

1. Hemoglobin

2. CBC (TLC, DLC, RBC & Platelets)

3. RBC indices (MCV, MCH, MCHC & HCT)

Data analysis: Peripheral blood smear was made from blood samples showing Hemoglobin less than 11 gm/dl. A good peripheral blood smear was made and stained with Fields's stain.

Step 1 - First blood smear was fixed with 10% methanol in a coplin jar.

Step 2 - Dip fixed smear in Field's Stain B (Red stain) for 5 to 6 seconds than wash.

Step 3 - Dip smear in Field's Stain B (Blue stain) for 10 to 30 seconds, then smears were allowed to dry and then observed under oil immersion under light microscope.

Staining characteristics and morphological abnormalities of RBCs were observed. Anisocytosis, poikilocytosis, elliptical cells, tear drop cells, white blood cell morphology, differential WBC count as well as platelet count on smear and platelet morphology was assessed.

Follow-up: Morphological typing of anemia was done using following criteria (Table 1):

1) **Microcytic Hypochromic Anemia:** RBCs with a diameter less than 7.0 µm and MCV less than 80 fl and central pallor more than 1/3rd of the RBC

2) **Macrocytic Anemia:** RBCs larger than normal RBCs having a diameter more than 8.0 µm and MCV more than 100 fl

3) **Dimorphic Anemia:** Smear showing both microcytic and macrocytic forms, normocytic and microcytic forms, normocytic and macrocytic forms of RBC's.

Table-1: Peripheral Smear Examination (N=250)

Peripheral smear	Number of cases	Percentage
Microcytic hypochromic anemia	223	89.2
Macrocytic anemia	22	8.8
Dimorphic anemia	5	2
Total	**250**	**100**

Grading of anemia: Mild - 10 to 11gm/dl; Moderate - 7 to 9.9 gm/dl; Severe - less than 7 gm/dl. A master chart was prepared showing following parameters: Registration no, age, educational status, gravida, trimester, TLC, RBC, HB, MCV, MCH, MCHC, PCV, PLT, RDW, Peripheral smear, grade of anemia.

Ethical approval: Taken

Statistical analysis: After getting the required information, the collected data were coded, tabulated and analyzed. The various statistical techniques i.e. the mean, standard deviation and test of significance (t-test and chi-square-test) were used for drawing valid conclusions. Statistical analysis done using student t-test. SPSS 13.0 software was used to calculate p value. $P<0.05$ was taken as statistically A descriptive analysis was done on all variables to obtain a frequency distribution. The mean + SD and ranges were calculated for quantitative variables. Continuous variables were compared by the Student t test. Proportions were analyzed with the chi-square test**.**

Results

Maximum number of cases were found in age group of 21-30 years (Table 2). Maximum number of cases of anemic pregnant subjects were primigravidae i.e. 42%. Highest percentage of anemia was in third trimester i.e. 52.4%. Among all morphological types microcytic hypochromic anemia was most predominant i.e. 89.2%. Moderate anemia was the predominant grade of anemia i.e. 67.6% (169 out of 250).

Table-2: Correlation of age with grade of anemia (n=250).

Age in years	Severe anaemia	Moderate anemia	Mild anemia	P value
18-20	5 (12.8%)	25 (14.8%)	6 (14.3%)	0.4
21-25	18 (46.2%)	74 (43.8%)	20 (47.6%)	
26-30	14 (35.9%)	61 (36.1%)	11 (26.2%)	
31-35	2 (5.1%)	9 (5.3%)	5 (11.9%)	
Total	39 (100%)	169 (100%)	42 (100%)	

On correlating age group with grade of anemia, moderate anemia was found to be predominant among all age groups. Out of 169 cases of moderate anemia, 74 cases (43.8%) were in 21-25 age group. After correlating age with type of anemia, Microcytic hypochromic anemia was predominant across all age groups and all three types of anemia were most commonly found in the age range of 21-30 years (Table 3). The above association of age and type of anemia was statistically significant. Considering trimester and grade of anemia, severe and moderate grades of anemia were most commonly seen in third trimester (61.5 % of severe anemia and 52.1% of moderate anemia found in third trimester) (Table 4).

Table-3: Correlation of age with morphological type.

Age	Microcytic hypochromic anemia	Macrocytic anemia	Dimorphic anemia	P value
18-20	32 (14.3%)	3 (13.6%)	1 (20%)	0.001
21-25	107 (48%)	4 (18.2%)	1 (20%)	
26-30	72 (32.3%)	11 (50%)	3 (60%)	
31-35	12 (5.4%)	4 (18.2%)	Nil	
Total	**223 (100%)**	**22 (100%)**	**5**	

Table-4: Relation of trimester with grade of anemia.

Trimester	Severe	Moderate	Mild	P value
I	3 (7.7%)	17 (10%)	3 (7.2%)	0.32
II	12 (30.8%)	64 (37.9%)	20 (47.6%)	
III	24 (61.5%)	88 (52.1%)	19 (45.2%)	
Total	39 (100%)	169 (100%)	42 (100%)	

However above association was not statistically significant. On correlating gravida with grade of anemia, moderate anemia was predominant in all gravidae. On correlating gravida with type of anemia, microcytic hypochromic anemia was predominant in all gravidae (Table 5). Highest percentage of microcytic hypochromic anemia was in primigravidae i.e. 44% and maximum cases of macrocytic anemia were in gravida II i.e. 50%. Considering trimester and type of anemia, Microcytic hypochromic anemia was most common morphological type of anemia in all trimesters.

Table-5: Relation of gravida with type of anemia.

Gravida	Microcytic hypochromic anemia	Macrocytic anemia	Dimorphic anemia	P value
1	98 (44%)	6 (27.3%)	1 (20%)	0.14
2	85 (38.1%)	11 (50%)	1 (20%)	
3	36 (16.1%)	4 (18.2%)	2 (40%)	
4	4 (1.8%)	1 (4.5%)	1(20%)	
Total	**223 (100%)**	**22 (100%)**	**5 (100%)**	

Maximum cases of Microcytic hypochromic anemia were in third trimester i.e. 52.4% (Table 6). Correlating grade of anemia with type of anemia it was observed that maximum cases were of moderate and microcytic hypochromic anemia i.e. 68.6% (Table 7). There was high prevalence of anemia among pregnant females with lower educational status i.e.56%-59% among illiterate and women educated till primary school as compared to 31% to 44% among females educated till college.

Table-6: Relation of trimester with type of anemia.

Trimester	Microcytic hypochromic anemia	Macrocytic anemia	Dimorphic anemia	P value
I	20 (9%)	2 (9.1%)	1(20%)	0.69
II	86 (38.6%)	7 (31.8%)	3 (60%)	
III	117 (52.4%)	13 (59.1%)	1 (20%)	
Total	223 (100%)	22 (100%)	5 (100%)	

Table-7: Relation of grade with type of anemia.

Grade	Microcytic hypochromic anemia	Macrocytic anemia	Dimorphic anemia	P Value
Mild	34 (15.2%)	7 (31.8%)	1 (20%)	**0.14**
Moderate	153 (68.6%)	12 (54.6%)	4 (80%)	
Severe	36 (16.2%)	3 (13.6%)	0 (0%)	
Total	223 (100%)	22 (100%)	5(100%)	

Table-8: Mean values of parameters in trimesters.

Trimester	HB (gm/dl)	RBC	MCV (fl)	MCH (pg)	MCHC (%)	PCV
I	8.8	3.7	77.3	25.6	32	27.3
II	8.7	3.7	75.5	24.7	31.9	27.1
III	8.5	3.5	76	24.8	31.8	26.1

Discussion

Fleming AF et al studied the aetiology of severe anaemia (haemoglobin<7.0 g dl^{-1}) in 37 pregnant Zambians. Aetiology was usually multiple; 31 (84%) had Plasmodium falciparum malaria., 23 (62%) were folate deficient, 13 (35%) were iron deficient, one had sickle-cell anaemia and one had the acquired immunodeficiency syndrome (AIDS). Folate deficiency was most often secondary to malarial haemolysis: iron deficiency was nutritional, but hookworm was contributory in about one-third of patients.

The anaemia of malaria and folate deficiency was both more common and more severe than anaemia due to iron deficiency: it was seen in younger women although primigravidae were not over-represented, it occurred earlier in pregnancy, and was associated with low birth weight. Because of the risk of transmission of human immunodeficiency virus, it is more important than ever to prevent anaemia and malaria in pregnancy, and to give blood transfusion only as a life-saving treatment [1].

Salhan S et al did evaluation of hematological parameters in partial exchange and packed cell transfusion in treatment of severe anemia in pregnancy. This study was conducted to compare the improvements in hematological parameters of patients receiving partial exchange blood transfusion and transfusion of packed cells without exchange. One hundred and twenty-five severely anemic antenatal mothers were admitted from outpatient service. Partial exchange transfusion was given to sixty-six patients while fifty-nine received transfusion of packed cells with frusemide cover.

The two groups were comparable in terms of age, height, weight, religion, diet, education, occupation of self and husband, and income. The study produced an equally significant improvement in hematological parameters in partial exchange and packed cell transfusion. Platelet counts were significantly less in partial exchange as compared with packed cell transfusion [2]. Elgari M. et al did evaluation of hematological parameters of Sudanese pregnant women

attending at Omdurman Al Saudi maternity hospital. There are subtle and substantial changes in hematological parameters during pregnancy and the puerperium total blood volume increases by about 1.5 liter mainly to supply the needs of the new vascular bed. This a case control study in which 100 pregnant women were enrolled as study group and 50 non pregnant healthy women as control subjects.

The study revealed that there were significant decreased in RBCs count, hemoglobin (Hb) and packed cell volume (PCV) of pregnant women compared to non-pregnant women (P value <0.05) and significant decreased in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of pregnant women (P value <0.05). The authors found the most common type of anemia among Sudanese pregnant women is microcytic hypochromic type and likely to be of iron deficiency second class is normocytic normochromic type, and less of occurrence type is dimorphic picture types with increased reticulocyte production index results from prophylaxes iron response [3].

Study of hematological parameters in pregnancy was done by Sifakis S et al and Sarkar S et al. Osonuga et al studied hematological profile of pregnant women in southwest of Nigeria at different trimesters of pregnancy. The research involved 33 healthy pregnant women as the study group and 11 non-pregnant women as control. The age range of these women was 20-40 years. The blood was properly mixed and analyzed for packed cell volume (PCV), total white cell count, differential counts and erythrocyte sedimentation rate (ESR). Hematology was done according to standard methods.

The result showed that study group exhibited statistically significant lower values of PCV, monocyte and lymphocyte while WBC, eosinophil and ESR were not significantly changed. There was no significant difference in all hematological parameters among the three trimesters. Healthy pregnancy may have effect on hematological parameters. Therefore, there is a need to monitor these parameters during pregnancy. We also find that stages of pregnancy have no influence on hematological parameters [4, 5].

In populations where chronic inflammation and iron deficiency anaemia coexist, the criteria to accurately define iron status are not always clear. Similarly, in pregnancy, with marked physiological changes, cut-off points for biochemical parameters need to be re-examined. In this study Den Broek V et al examined the diagnostic accuracy of iron parameters including mean cellular volume (MCV), serum iron, transferrin, total iron binding capacity (TIBC) and its saturation, zinc protoporphyrin (ZPP), ferritin and serum transferrin receptor (TfR) for the assessment of iron status in a population of anaemic pregnant women in Malawi. Stained bone marrow aspirates were used as the standard for comparison.µg/l is used.

A number of other commonly used parameters of iron status were shown to have limited diagnostic accuracy. Logistic regression was used to obtain mathematical models for the prediction of bone marrow iron status using a combination of available parameters.

Results show that for the purpose of screening, serum ferritin is the best single indicator of storage iron provided a cut-off point of 30 [6, 7].

Idowu OA et al did a survey of anaemia in pregnant women in Abeokuta, Nigeria. This study was carried out to determine the prevalence of anaemia among pregnant women receiving antenatal care in two hospitals and a traditional birth home in order to obtain a broader prevalence data. Pregnant women were enrolled in the study at their first antenatal visit and were monitored through pregnancy for anaemia.

Packed cell volume (PCV) was used to assess level of anaemia; Questionnaires were also administered to obtain demographic information. In all the antenatal centers more women were anaemic in the 2nd trimester of pregnancy. Forty-seven (9.8%) of the enrolled women booked for antenatal care in the first trimester, while 303(63.5%) booked in the second trimester and 127 (26.6%) in the 3rd trimester of their pregnancies. 62.5% of these women were already anaemic at the time of antenatal booking, with a higher prevalence among the primigravidae (69.7%) (P< 0.05).

Absence of symptoms of ill health was the major reason for late antenatal booking. Anaemia was higher among unemployed women and those with sickle cell traits. The authors concluded that educating women on early antenatal booking and including those in TBHs in health interventions is necessary to reduce the problem of anaemia in pregnancy in Nigeria [8].

Lokare PO et al did a study of prevalence of anemia and sociodemographic factors associated with anemia among pregnant women in Aurangabad city. Anemia in pregnancy accounts for one fifth of maternal deaths worldwide and is a major factor responsible for low birth weight. In India, 16% of maternal deaths are

attributed to anemia. The association between anemia and adverse pregnancy outcome, higher incidence of preterm and low-birth weight deliveries has been demonstrated. However, high prevalence of anemia among pregnant women persists in India despite the availability of effective, low-cost interventions for prevention and treatment.

A knowledge of the sociodemographic factors associated with anemia will help to formulate multipronged strategies to attack this important public health problem in pregnancy [9].

Many similar studies were done showing association of anaemia and associated factors in pregnant women Gautam VP et al studied prevalence of anemia amongst pregnant women and its sociodemographic associates in a rural area of Delhi [10]. Ahmad N et al did work on the prevalence of anaemia and associated factors in pregnant women in a rural Indian community [11].

Khan H et al Sultana F et al evaluated Knowledge about Anemia among Pregnant Women in Tertiary Hospital [12-13]. Kurhade GA et al also did similar work on serum level of iron and transferrin in pregnancy and postpartum period [14].

Limitation of our study

1. Small sample size
2. Chances of bias
3. Single center trial

Conclusion

Microcytic hypochromic anemia was the predominant morphological type of anemia in all age ranges, all gravidae and all trimesters.

There was statistically significant association between age and morphological type of anemia. As lower educational attainment was associated with high prevalence of anemia, educational status played important role in awareness about anemia and nutrition.

What this study adds to existing knowledge?

Prevalence of anemia was high in pregnancy i.e. 39.5% Highest percentage of anemia was seen in third trimester. Anemia was predominantly seen in primigravidae. 21-30 years was the most commonly affected age group. Moderate anemia was the predominant grade of anemia in all age groups, all gravidae and all trimesters.

Original Research Article

Contribution by different authors

Dr. Anita Sharan: Concept and Data collection

Dr. Sagar Shah: Data Analysis and Discussion

Funding: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

Ethical approval: Taken

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How to cite this article?

Sharan A. Shah S. Study of hematological parameters in anemia in pregnancy. Trop J Path Micro 2019;5(10): 762-769.doi:10. 17511/jopm.2019.i10.03.

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