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The Study of Iron Related Parameters in Iron Deficiency Anaemia in Pregnancy

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ABSTRACT

Anemia is widely prevalent in India which consists of 80% of villages in India, where Majority of the people is ignorant of the hemopoietic principles of diet belong to low socioeconomic status. Pregnancy Imposes an extra demand on the the nutrients, hence anemia is rather a constant association. Factors such as food which is chiefly derived from local ingredients and secondly prevalence of parasitic infections varies and therefore the pattern of incidence, severity and type of anemia also alters. Anemia in pregnancy is a major public health problem in the developing countries. It is one of the commonest causes of the high maternal mortality rate. It is also responsible for the high incidence of premature and low birth of premature and low birth weight babies, thus increasing the perinatal mortality and morbidity. This study deals with various types of anaemia in pregnant women having Haemoglobin level less than 11gm% and study sensitivity and positive predictive value of Red cell distribution width in diagnosis of Iron deficiency anaemia.

Key words: Iron deficiency anaemia, complications, etipathogenesis, iron parameters.

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INTRODUCTION

Anemia is defined as Haemoglobin level in the blood is below the lower extreme of the normal range for the age and sex of the individual[1].The importance of anaemia as a major public health problem throughout the world is widely recognized. According to WHO, in developing countries the prevalence of anaemia among pregnant women averages 56%, ranging between 35 to 100% among different regions of the world. Various studies from different regions of the country (India) have reported the prevalence of anaemia to be between 33 and 100%.In India, anaemia is the second most common cause of maternal deaths, accounting for 20% of total maternal deaths. Anaemia affects mainly the women in child bearing age group, young children and adolescent girls[1,2]. Association of anaemia with adverse maternal outcome such as puerperal sepsis, ante-partum haemorrhage, post-partum haemorrhage and maternal mortality is no longer a debatable subject. Apart from the risk to the mother, it is also responsible for increased incidence of premature births, low birth weight babies and high perinatal mortality[2].In pregnant women WHO defined Anemia as a reduction in Haemoglobin level <11g/dl. It occurs in 40-80% of the pregnant women. Iron and Folic acid deficiencies, malaria, intestinal parasitic infections and hemoglobinopathies are the principal causes of anemia in pregnancy[3]. Haemoglobin level at or below 9 gm/dl requires detailed investigations and appropriate treatment. Anemia is responsible for 20% maternal deaths in the third world countries[4].

Anemia due to Iron deficiency is the commonest malnutrition disorder seen throughout the world and in India. The single most important cause for the widespread Iron deficiency anemia in our country is inadequate iron intake in the habitual diets compared with the poor bioavailability of dietery iron. However, risk factors such as Anemia in pregnancy can be controlled and monitored by good antenatal care and appropriate action, including referral, in accordance to the level of severity of the anemia[5,6].

MATERIALS AND METHODS

The present study was carried out in pregnant women who attended the outpatient and inpatient department of OBG from recent 2 years at KIMS Hospital and Research Centre, Bangalore. A detailed clinical history of each patient was recorded and a thorough clinical examination was performed. Venous blood was collected in all women with aseptic precautions in EDTA anticoagulant for hematological investigations. Separate blood sample was collected for biochemical investigations. Serum was separated on the same day of blood collection and stored in refrigerator between 2 to 8 °C. Biochemical study was carried out within three days of blood collection. The haematological investigations were performed on Sysmex KX-21 (Transasia Ltd) and ABX PENTRA 60 with standard calibration using fresh whole blood. As a part of CBC, red blood cell indices (MCV, MCH, MCHC), PCV, RDW, white blood cell count and platelet count were obtained by analyzer. Peripheral blood smear study was performed on each of these patients. A good peripheral smear was made and the blood film was stained by Leishman's stain. Staining characteristics and morphological abnormalities of red blood cells

were observed. Anisocytosis, poikilocytosis, elliptical cells, pessary cells, tear drop cells, white blood cell morphology distribution and platelet morphology were observed.

Inclusion Criteria

Pregnant women whose haemoglobin levels are less than 11gm%.

Exclusion Criteria

1. Pregnant women who had already received blood transfusion.
2. Anemia's of chronic disorders and haematological malignancies and Consent refusal

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Procedure for Leishman's stains:

Air dried film was flooded with Leishman's stain for two minutes. Double the volume of buffer water was added for 10 minutes. The stain was washed off with distilled water after drying, the back of the slide was wiped clean.

Special biochemical procedures done included:

I. Measurement of Serum Ferritin concentration.
Reference range – 15-200 ng/ml.
II. Measurement of Total Iron Binding Capacity.
Reference range- 250- 400 µg/dl
III. Measurement of Percentage saturation.
Reference range – 16- 50 %.

Estimation of serum ferritin:

[Ferritin Enzyme Immunoassay Test Kit- Chemiluminescence Immunoassay (CLIA)]

Measurement of T.I.B.C:

[Kit method - by Pinnacle Pvt. Ltd]

Principle: A known amount of ferrous ions are added to serum at an alkaline pH. The ferrous ions bind with transferrin at unsaturated iron - binding sites. The additional unbound ferrous ions are measured using the ferrozine reaction. The difference between the amount of ferrous ions added and unbound ions measured is the U.I.B.C (Unsaturated iron binding capacity).

Measurement of % Transferrin Saturation:

(Serum iron/ TIBC) X 100 = % Transferrin saturation.

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Chi Square test was applied for testing the statistically significance of the results

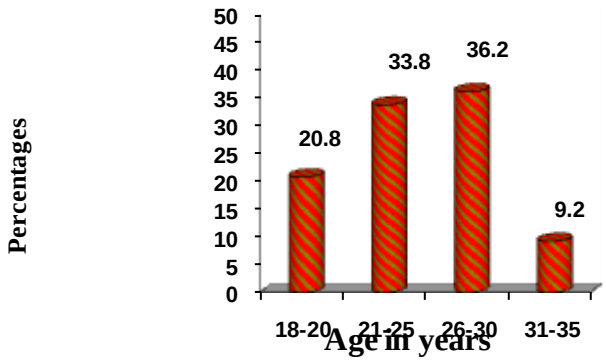
RESULTS

Study Design: Prospective clinical non –controlled study with 500 pregnant women is undertaken over a period of 2 years recently in the department of pathology, KIMS , Bangalore of pregnant women attending OPD and IPD in the department of Obstetrics and Gynecology, KIMS Hospital and Research centre, Bangalore to study the various types of anemia, and mainly to study the iron deficiency anemia in pregnant women.

Table 1: Age distribution of patients studied

Age in years	Number of patients	%
18-20	104	20.8
21-25	169	33.8
26-30	181	36.2
31-35	46	9.2
Total	500	100.0
Mean ± SD	**24.70±4.28**	

Graph 1: Age distribution of patients studied



In Age wise distribution of anemic cases in pregnancy, in the age group of 18-20 years, 104 cases(20.8%) were anemic. In the age group of 21-25 years, 169 cases(33.8%) were anemic. In the age group of 26-30 years, 181 cases(36.2%) were anemic. In the age group of 31-35 years, 46 cases(9.2%) were anemic. Maximum no: of cases were found in the age group of 21-30 years(70%).

Table 2: Gravida and Anemia

Obstetric Index	Number of patients	%
Gravida		
I	**220**	**44.0**
II	**211**	**42.2**
III	**57**	**11.4**
IV	**12**	**2.4**
Total	**500**	**100.0**

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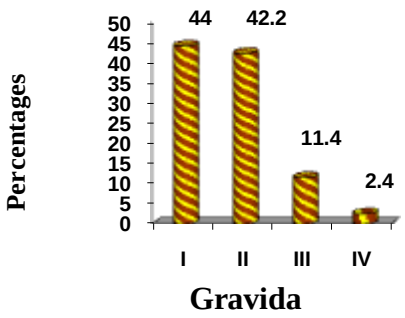
Graph 2: Gravida and Anemia

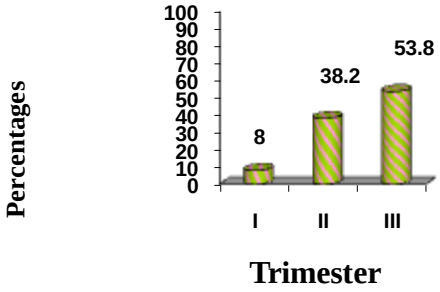


Among the 500 cases studied, no: of primigravida were 220 cases(44%). No: of Gravida II were 211 cases(42.2%). No: of Gravida III were 57cases (11.4%). No: of Gravida IV were 12 (2.4%).

Table 3: Trimester and Anemia

Trimester	Number of patients	%
I	40	8.0
II	191	38.2
III	269	53.8
Total	500	100.0

Graph 3: Trimester and Anemia



In trimester wise distribution of anemia, 40 cases were seen in first trimester(8%), 191 cases were seen in second trimester(38.2%),269 cases were seen in 269 cases (53.8%).

Table 4: Peripheral smear Examination

Peripheral smear Examination	Number of patients	%
Dimorphic anemia	55	11
Microcytic hypochromic Anemia	334	66.8
Macrocytic Anemia	111	22.2
Total	500	100.0

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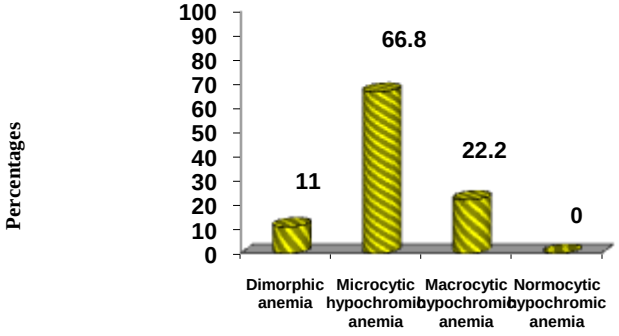
Graph 4: Peripheral smear Examination

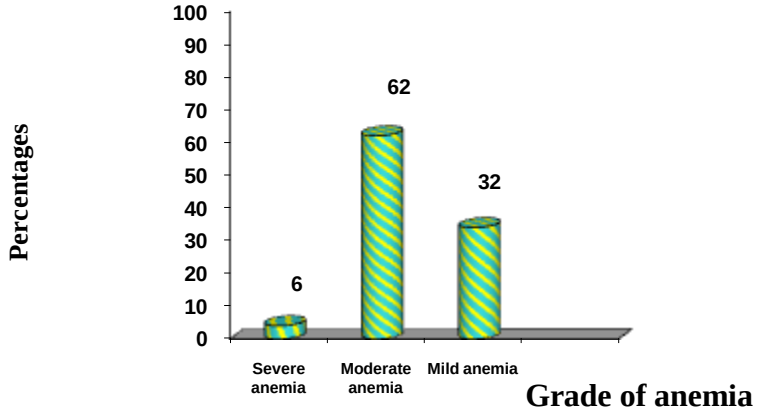


In the study of 500 cases of anemia in pregnancy, on Peripheral smear examination following types of anemia were found. Microcytic Hypochromic anemia was 334 cases(66.8%), Macrocytic anemia was 111 cases(22.2%), and Dimorphic anemia was 55 cases (11%).

Table 5: Grade of anemia

Grade of anemia	Number of patients	%	95%CI
Severe anemia	20	6	2.45-6.85
Moderate anemia	310	62.0	57.67-66.15
Mild anemia	170	32.0	28.06-36.21
Total	500	100.0	-

Graph 5: Grade of anemia



Grade of anemia as studied in 500 cases were as follows. Mild anemia was seen in 170 cases(32%), Moderate anemia was seen in 310 cases(62%) and Severe anemia was seen in 20 cases(6%).

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Table 6: Distribution of Serum Ferritin, TIBC, Transferring Saturation, MCV and RDW

Investigation	Criteria	Number of patients	%	95%CI
S.Ferritin µg/L (n=389)	<15	374	96.14	92.56-99.95
	15-200	15	3.85	2.16-5.95
	>200	0	0.0	-
TIBC (n=389)	<250	0	0.0	-
	250-400	15	3.85	2.16-5.95
	>400	374	96.14	92.56-99.95
Transferring saturation (%) (n=389)	<16	374	96.14	92.56-99.95
	16-50	15	3.85	2.16-5.95
	>50	0	0	-
MCV fl/dt	<78	326	65.2	60.92-69.24
	78-100	60	12.0	9.44-15.14
	>100	114	22.8	19.34-26.68
RDW	<11.5	1	0.2	0.04-1.12
	11.5-15.0	70	14.0	11.23-17.32
	>15.0	429	85.8	82.47-88.59

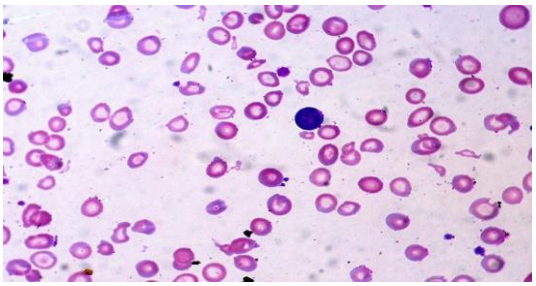

Fig 1: Microcytic Hypochromic Anemia showing microcytes and a small lymphocyte (Black arrow). [Leishman's stain,oil immersion(x100)].

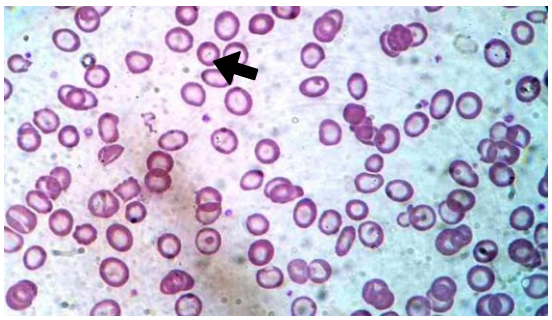

Fig 2: Microcytic Hypochromic Anemia showing many target cells (Black arrow). [Leishman's stain, oil immersion(x100)].

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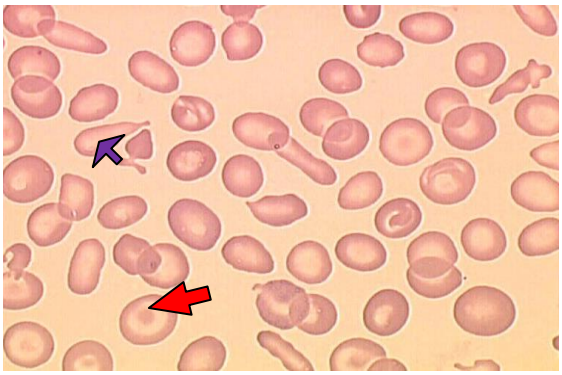

Fig 3: Microcytic Hypochromic Anemia exhibiting marked anisopoikilocytosis. seen are tear drop cells(Purple arrow) and target cells(Red arrow).[Leishman's stain, oil immersion(x100)].

DISCUSSION

The present study is a prospective study over a period of 2 years recently in the department of pathology, Kempegowda Institute Of Medical Sciences, Bangalore. Total no: of 500 cases of pregnant women attending OPD and IPD in the department of Obstetrics and Gynecology, KIMS Hospital and Research centre, Bangalore were included in the study.

Anemia in pregnancy constitutes a major public health problem in developing countries. The prevalence of anemia in pregnancy in developing countries, is still high. Nearly half the pregnant women in the world are estimated to be anemic,52% compared to 23% in industrialized countries[7].During the last 2 decades, automated blood cell counters have undergone a formidable technological evolution owing to the introduction of new physical principles for cellular analysis. For some consolidated parameters, such as WBC and RBC counts, hemoglobin concentration, or mean corpuscular volume (MCV), analytic performance is generally excellent.

From the RBC volume distribution histogram, modern analyzers calculate an index of heterogeneity known as the RDW, almost always expressed as a percentage coefficient of variation and, less frequently, as the SD. The usefulness of the anisocytosis obtained from the measurement of RBC size (diameter) has been recognized[8].

Table 7: Showing Age Distribution of Anemic Cases in comparison with other studies

Authors	18-20 Yrs	21-25 Yrs	26-30 Yrs	31-35 Yrs
Ahmad N [9]	45.8%	30.9%	20.9%%	2.36%
Pai PM [10]	25%	48%	14%	13%
Haniff J et al [7]	4.29%	53.6%	37.9%	4.2%
Present Study	20.8%	33.8%	36.2%	9.2%

In our present study we included 500 cases of pregnant women whose hemoglobin was less than 11 gm%. Anemia is one of the most common medical condition met during pregnancy. This was noted in most of the women attending the antenatal clinic. Iron

deficiency anemia accounted for 72.4%, and dimorphic anemia 17%, and megaloblastic anemia accounts for 25.2% in our present study.

In the present study, the age wise distribution of anemia, maximum no: of cases were observed between 21-30 years accounting for 70%. The present study correlates closely to observations by Ahmad N et al [9] (51.8%), Pai PM et al [10](62%) , Haniff J et al[7] (91.5%).

Table 8: Showing Trimester Wise distribution of anemic cases in comparison with other studies

Authors	First trimester	Second trimester	Third trimester
Belgnaoui S [11]	19.7%	46.7%	33.6%
Rasheed P [12]	27.7%	37.3%	50.2%
Present study	8%	38.2%	53.8%

In the present study, 8% were in first trimester, 38.2% were in second and 53.8% were in third trimester. Our present study correlates with Rasheed P. et al [12] found in his series 27.7% in first trimester, 37.3% in second trimester and 50.2% in third trimester. Belgnaou S [11]found in his series 19.7% in first trimester, 46.7% in second trimester and 33.6% in third trimester. Comparing various authors studies, it is seen that majority of cases are in third trimester.

Table 9: Showing Gravida wise distribution of anemic cases in comparison with other studies

Authors	G1	G2 to G3	G4 to Grand Multi
Haniff J et al[7]	27.42%	62.77%	9.79%
Satyanarayana M [13]	16%	74%	10%
Sidhu GS [14]	15.15%	57%	27%
Present Study	44%	53.6%	2.4%

In our present study we found 44% primigravida, 53.6% G2 to G3 and 2.4% of G4 to grand multi gravida. Our present study correlates with Haniff J et al[7] found 27.42% of primigravida, 62.77% of G2 to G3 and 9.79% of G4 to grand multi gravid. Satyanarayana M [13]found 16% of primigravida, 74% of G2 to G3 and 10% of G4 to grand multi gravida. Sidhu GS[14] found 15.15% of primigravida, 57% of G2 to G3, 27% of G4 to grand multigravida.

Table 10: Showing Percentage distribution of Degree of Anemia in comparison with other studies

Authors	Mild Anemia	Moderate Anemia	Severe Anemia
Ratnam R[15]	30.2%	35.8%	3.3%
Ahmad N[9]	30.17%	50.9%	18.9%
Present Study	32%	62%	6%

In the present study keeping haemoglobin standard as 11 gm% we found 32% patients as mild anemia, 62% were moderate and 6% were severe. Our present study correlates with Ratnam R[15] keeping standard as 11gm%, found 30.2% as mild anemia,

35.8% as moderate and 3.3% as severe anemia. Ahmad N [9]. keeping standard as 10 gm%, found 20% as mild, 30% as moderate anemia and 42% as severe anemia.

Table 11: Showing Percentage distribution of Type of Anemia in comparison with other studies.

Authors	Iron Deficiency	Dimorphic	Megaloblastic
Ratnam R [15]	84%	9%	6%
Present study	72.4%	2.4%	25.2%

Our present study shows 72.4% as Iron deficient, 2.4% as Dimorphic and 25.2% as Megaloblastic anemia. Our present study correlates with Ratnam R[15] found 84% Iron deficiency, 9% as Dimorphic and 6% as Megaloblastic anemia in his study.

Table 12: Showing percentage distribution of Serum Ferritin in Iron deficiency anemia in comparison with other studies.

Authors	Serum Ferritin(<15µg/l)
Thoradeniya T [16]	74.2%
Zeben VD[17]	90%
Mast AE[18]	73%
Alper BS [19]	54%
Present study	96.14%

In our present study serum Ferritin levels were less than 15 µg/l in 96.14% cases. So present study correlates with Zeben VD[17] (90%), Thoradeniya T [16](74.2%), Mast AE[18](73%), Alper BS [19](54%). The above studies done by various authors, have considered serum Ferritin levels <30 µg/l to be indicative of iron deficiency anemia. Other studies also showed that serum Ferritin levels are useful in determining iron deficiency cases.

Table 13: Showing TIBC and Percentage saturation in Iron deficiency anemia in comparison with other studies.

Mean	Present Study	Tater ML[20]
TIBC (µg/dl)	555.6	416.7
Percentage saturation (%)	9.5	12.2

The findings in our study of TIBC 555.6 (Mean) and Percentage saturation 9.5%(Mean) are thus in accordance with those of Tater ML[20] who had TIBC Mean as 416.7 and Percentage saturation Mean as 12.2.

The % saturation of transferrin provided a clear separation between iron deficiency anemia and non iron deficiency anemia.

If % saturation was below 16% it indicates iron deficiency anemia.

If % saturation was above 16% it does not indicate iron deficiency anemia.

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Table 14: Showing Sensitivity and Positive predictive value of RDW in comparison with other studies

Name of authors	Sensitivity	Positive predictive value
Bessman JD[27]	100%	96%
Mc Clure S [21]	100%	66%
Flynn MM [22]	94%	51%
Thompson G [23]	71%	54%
Simel DL [24]	77.1%	54%
Present study	98.55%	73.70%

In our present study we have found that RDW sensitivity 98.55% and positive predictive value to be 96%. Our present study correlates with the above authors. Bessman JD [27] found RDW sensitivity 100% and Positive predictive value to be 96%. Mc Clure S[21] found RDW sensitivity 100% and Positive predictive value 66%. Flynn MM[22] found RDW sensitivity 94% and Positive Predictive value 51%. Thompson G [23] found RDW sensitivity 71% and Positive predictive value 54%. Simel DL[45] found RDW sensitivity 77.1% and positive predictive value 54%.

Varying selection criteria for cases to be included in the study was probably the contributing factor to the variability in sensitivity of RDW.

Beutler E [25], in his series of 80 patients with iron deficiency anemia, found out that in most of his patients red cell indices indicated the presence of hypochromasia and microcytosis. Among the less severely anemic patients normal red cell indices were common and examination of stained smears was not superior to determination of red cell indices. The examination of stained smears was important but could not exclude the diagnosis of iron deficiency anemia on the basis of normal appearance of red cells on smear examination alone. This was particularly true in mildly anemic patients.

In our study examination of stained peripheral smears revealed a microcytic hypochromic anemia in 334 women (66.8%).
Thus our study is in accordance with the study done by Beutler E[25] . Jen P [26] , in their study concluded that blood smear examination performed no better than RBC indices in detecting probable iron deficiency anemia.

Our study is in accordance with the above findings and it was found that red cell indices were more sensitive than peripheral smear findings. This was true specifically in cases with mild anemia.

According to our present study objective we categorized anemia under improved classification of anemia based on MCV and RDW[27] . So we have categorized our cases according to the above classification.

1.) MCV Low, RDW Normal (Microcytic homogenous) : NIL
2.) MCV Low, RDW High (Microcytic heterogeneous) : Iron deficiency anemia(362 cases).

3.) MCV Normal, RDW Normal (Normocytic homogenous) : NIL
4.) MCV Normal, RDW High (Normocytic heterogeneous) : Dimorphic anemia (12 cases).

CONCLUSION

- The incidence of anemia in pregnancy is high. Moderate to severe anemia is common in third trimester.
- In 500 cases studied, 362 cases(72.4%) belonged to Iron deficiency anemia.
- Anemia is common in the age group between 21 to 30 years due to repeated and short interval of pregnancies.
- RDW has been useful but of limited value in diagnosing Iron deficiency anemia. So further studies are indicated.
- Automated hematology analyzer was simple, economical, cheap, reliable instrument used during this study period.

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