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ORIGINAL RESEARCH

Correlation of serum Ferritin level with red cell indices and haemoglobin in pregnant women during second and third trimester

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Abstract:
Introduction: Iron deficiency is the most common cause of anaemia in pregnancy worldwide and is associated with increased risk of preterm births and low birth weight infants.
Aim of the study: To Correlate the serum ferritin level with red cell indices and haemoglobin in pregnant women in the second and third trimester
Material & Methods: The present study conducted among 100 consecutive pregnant women in the second and third trimester (12 to 40 wks) & 50 non pregnant age matched healthy controls were enlisted. 150 women attending to the OPD of NCR Institute of Medical Sciences, Meerut, U.P, India is included for this study. The blood sample was used for estimation of Hb and the red cell indices on haematology cell counter. Serum ferritin level was estimated on automated Minividas instrument using the Enzyme Linked Fluorescent Assay technique.
Results: The mean age of 100 consecutive pregnant women included in our study was 21.54 years (SD = 2.73). It was found that 54 had anaemia and 46 were non-anaemic. Iron deficiency was found in 36 ladies in anaemia group (36/54) and 22 ladies in non-anaemic group (22/46) in pregnant women. The red cell indices were correlated with Serum Ferritin, it showed statistically significant results using Spearmen Coefficient (r) with Hb level, MCV, MCH and HCT.
Conclusion: Thus, the international norms were found to be applicable to the pregnant Indian women and to similar populations with same stage of development as India. Also, we have concluded that we have to look for newer surrogate markers for diagnosis of sub-clinical iron deficiency in Indian women.
Keywords: Anaemia, Ferritin, Pregnant Women, Red cell Indices

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Introduction:

Anaemia in pregnancy is a major health problem in many developing countries including India, where nutritional deficiency, malaria and other parasitic infections contribute to increased maternal and perinatal mortality and morbidity.[1,2] Iron deficiency is the most common cause of anaemia in pregnancy worldwide and associated with increased risk of preterm births and low birth weight infants.[3,4]

Anaemia in pregnancy has been defined by criteria from the Centres for Disease Control and Prevention (CDC) as a haemoglobin level of less than 11 g per dL during the first and third trimesters and less than 10.5g per dL during the second trimester[5].

On the other hand, a longitudinal study[6] found that infants of mothers receiving either routine or selective iron prophylaxis during pregnancy did equally well on an average of 6.5 years of follow-up in time and number of hospitalizations. When prescribing iron supplements, we must consider compliance of patients[7] and possible adverse effects of iron supplements

Serum ferritin is considered as a better parameter to detect latent iron deficiency especially before the change of red cell morphology and red cell indices. In a developing country like India with high prevalence of sub-clinical iron deficiency, the gold standard test is serum ferritin but serum ferritinis too expensive to be done in all cases[8], we need to know whether

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western study results are applicable in our settings and what are the surrogate markers for reliably predicting iron deficiency to guide prophylaxis and therapy?

Ferritin level of <12 ng/dL is considered as the gold standard for the diagnosis of iron deficiency anaemia in pregnancy.[9] It is superior to transferrin saturation or serum iron in the diagnosis of IDA as its concentration correlates with bone marrow iron stores. Also, even with iron deficiency, serum iron can be high or normal if the pregnant female is on oral iron.[10]

The estimation of ferritin is expensive and cannot be routinely done in all, thus there is a need for surrogate markers to identify women who need iron supplementation. It is also necessary to determine the usefulness of red cell indices in diagnosing iron deficiency anaemia as well as subclinical iron deficiency longitudinally during the course of pregnancy.

Published guidelines indicate acceptable levels for Hb and red cell indices in pregnancy but they are population specific and there is no evidence confirming their applicability in Indian pregnant woman[11-13]. This study was therefore undertaken to evaluate the validity of each red cell index including Hb as a screening tool to detect early iron deficiency at an early stage of pregnancy and also to correlate the serum ferritin with red cell indices and haemoglobin in pregnant women in the second and third trimester

Material and Methods:

The present study conducted among 100 consecutive pregnant women in the second and third trimester (12 to 40 wks) & 50 non pregnant age matched healthy controls were enlisted for this study attending to the OPD of NCR Institute of Medical Sciences, Meerut, U.P, India.

The approval to conduct the study was obtained from Institutional Research Ethics Committee, before the study commenced. An informed written consent was obtained from each participant before enrolment and operation. The purpose and nature of the study was fully explained to the participants in a language they could fully understand.

Exclusion Criteria

1. Patients with diabetes, hypertension, renal failure, Cardiac/Heart Disease, thyroid, malignancy or any other serious diseases or any adverse habit.
2. Patient was on any medications

Questionnaire: A self administered structured questionnaire was developed in which sociodemographic details were taken regarding age, marital status, occupation, education and residence. Also, the history regarding any systemic disease or any adverse habit was taken. All included women were followed through second and third trimesters of pregnancy and their serum ferritin level, Hb and the red cell indices was estimated.

Collection of Sample: Blood sample was collected in the Central lab, Department of Biochemistry, NCR Institute of Medical Sciences, Meerut, U.P, India.

After informed consent, blood samples were collected 2 ml in EDTA and 3 ml in sterile vacutainer. The EDTA anticoagulated sample was used for estimation of Hb and the red cell indices on a Coulter Act 3-part automated Haematology cell counter.

The red cell indices assayed were total RBC count, mean corpuscular volume(MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width coefficient of variation (RDW-CV%). A peripheral smear was seen to subtype the anaemia.

Ferritin level was estimated on automated Minividas instrument in human serum or plasma using the Enzyme Linked Fluorescent Assay technique,

Statistical Analysis: Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA).Descriptive analysis includes the estimation of mean values and standard deviations (SD) for continuous variables. Receiver operator characteristic (ROC) curves were constructed to look at maximum sensitivity and specificity for Hb, MCV, TRBC, MCHC, HCT, TRBC and RDW-SD % to look for statistically significant correlation with serum ferritin and also to see which one gave maximum area under curve (AUC). Spearman Coefficient (r) used to correlated between red cell indices and serum ferritin and the level of significance was set at $p < 0.05$.

Results:

Table-1: Anaemic status of study participants of Pregnant Women

S. No	Status	N
1	Non-anaemic with normal ferritin (Hb >10.5 g/dL; ferritin >12 ng/mL)	24
2	Iron deficiency anemia (Hb< 10.5 g/dL; ferritin< 12 ng/mL)	36
3	Non-anaemic with iron deficiency (Hb >10.5g/dL; ferritin < 12 ng/mL)	22
4	Anaemia not due to iron deficiency (Hb<10.5 g/dL; ferritin >12 ng/mL)	18

The mean age of 100 consecutive pregnant women in second and third trimester of pregnancy was 21.54 years (SD = 2.73). As per CDC recommendation, a cut-off of Hb of 10.5 g/dL and11 g/dL in 2nd and 3rd trimester respectively for anaemia and ferritin < 12 ng/mL were taken for presence of iron deficiency. It was found that 54 had anaemia and 46 were non-anaemic. Out of 54 anaemic patients, 36 had IDA and iron deficiency without anaemia was seen in 22/46 (47.83%) of non-anaemic ladies in pregnant women. Table 1 shows the distribution of these cases.

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Table 2: Area under ROC curve and the best cut-off for various red cell indices for diagnosis of iron deficiency in ANC cases

S. No	Variables	AOC	Best cut off	Sensitivity %	Specificity %
1.	Hb	0.93	≤ 9.7 gm/dL	91.2	79.1
2.	MCV	0.82	_≤ 76.1 fL	87.9	78.2
3.	MCHC	0.81	≤ 25.05 pg	82.3	82.4
4.	TRBC	0.71	≤ 31.35 g/dL	83.8	76.5
5.	RDW- CV%	0.73	≥ 16.35%	82.4	75.8
6.	HCT	0.72	<32%	81.3	72.1

Table 2 shows the area under ROC curves for various red cell indices for diagnosis of IDA in ANC cases. The maximum area under ROC curve was for Hb and it was seen that Hb < 9.6 g/dL can predict iron deficiency anaemia in the second and third trimester with a sensitivity of 91.2% and specificity of 79.1%. The 18 non-iron deficiency anaemia comprised of 2 cases of b-thalassemia trait, 5 Vitamin B12/folate deficiency and the rest mild anaemia due to pregnancy. On the basis of red cell indices i.e. low MCV, high TRBC and low/normal RDW, b-thalassemia was suspected and confirmed with HbA2estimation. Further subgroup analysis was not done due to small number. There were 46 non-anaemic cases with 22 having serum ferritin levels < 12 ng/mL. We attempted to correlate between ferritin and red cell indices to see if any index can predict the presence of sub-clinical iron deficiency in this group but no significant correlation was found.

Table 3: Red Cell Indices and Correlation with Serum Ferritin of Pregnant Women

Variables	Mean ± SD	Spearman coefficient (r)
Hb level (g/dL)	10.6 ± 1.09	0.211*
MCV (fL)	71.8± 34.8	0.332*
MCH (pg)	26.7± 11.9	0.304*
HCT (%)	32.6± 2.81	0.199*
MCHC (g/dL)	31.4 ± 25.6	0.056
TRBC	27.8 ± 11.2	0.054

* P= 0.05 Statistically Significant

When the Red cell Indices correlated with Serum Ferritin showed statistically significant results using Spearmen Coefficient (r) with Hb level, MCV, MCH and HCT.(Table 3)

Table 4: Comparison of Red Cell Indices among Pregnant Vs Non-Pregnant Women.

Variables	Pregnant Women Mean ± SD	Non pregnant Mean ± SD	p value
Hb level (g/dL)	10.6 ± 1.09	11.08±0.87	0.001*
MCV (fL)	71.8± 34.8	78.35± 26.6	0.49
MCH (pg)	26.7± 11.9	31.5± 14.9	0.03*
HCT (%)	32.6± 2.81	35.67±2.14	0.001*
MCHC (g/dL)	31.4 ± 25.6	33.90± 28.6	0.58
TRBC	27.8 ± 11.2	29.8 ± 16.3	0.37

* P= 0.05 Statistically Significant

Table 4 shows the comparison of red cell Indices among Pregnant Vs Non-Pregnant Women using t test. Non pregnant women are comparable higher red cell indices then Pregnant Women. Hb, MCH, HCT shows statistically significant results when compared with each other.

Discussion: Ferritin is a major iron storage protein, mainly located in the spleen, liver, and bone marrow. It is also found inthe mucosal cells of the small intestine, the placenta, kidneys, testes, skeletal muscles, and circulating plasma[14]. The most important function of ferritin is to provide iron for the synthesis of iron-containing proteins, including haemoglobin and myoglobin.

During pregnancy, the total increase in plasma volume is about 42%, whereas the total red cell volume is less than one-third of the increase in plasma volume[15].This leads to haemodilution, and it was suggested that estimation of haemoglobin alone might not be a good indicator of iron status. On the other hand, Letsky[16] has claimed that mean corpuscular volume is a more sensitive indicator of iron deficiency.

It was found that 54 had anaemia and 46 were non-anaemic. 36 had IDA and iron deficiency without anaemia was seen in 22/46 (47.82%) of non-anaemic ladies. The total number with iron deficiency (anaemic and non-anaemic) was 58 out of 100. The present study shows that anaemia as well as iron deficiency anaemia is very common in India as compared to the developed countries.[2] The other major problem in our country is sub-clinical iron

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deficiency without anaemia (serum ferritin ≤12 ng/dL and Hb >10.5 g/dL.

Also in pregnancy, the MCV rises by 4 fL which may have a role to play. Therefore using MCV <78 fL as cut-off to screen IDA may not hold true in pregnancy. By constructing ROC curves, we found that the maximum area under curve for Hb and a cut-off < 9.6 g/ dL had a sensitivity of 91.2% and a specificity of 79.1%. This was similar to the cut-off of 9.7 g/dL given in the study among African-Americans in USA and India[12,17]. Other indices also had statistically significant correlation with ferritin. For IDA the next best index was MCV < 75.6 fl (sensitivity of 87.9% and specificity of 78.2 %).

The present study showed that in second and third trimester pregnant women with Hb <10.5 g/dL (anaemic) the MCV, MCH, MCHC, RBC count and RDW-CV% correlate with the ferritin levels and can help in the diagnosis of iron deficiency anaemia. Our findings were different from that of a study from Malaysia which found correlation only between Hb and ferritin levels[18].

The results of this study indicate that haemoglobin level and red cell indices mean corpuscular volume, mean cell haemoglobin, and haematocrit all showed significant correlation with serum ferritin concentration. However, the area under the ROC curve constructed from haemoglobin level is larger than all the others from mean corpuscular volume, mean cell haemoglobin and haematocrit, showing that haemoglobin level is superior to the other three red cell indices in predicting the serum ferritin concentration, thus the iron status of the pregnant women, despite the problem of haemodilution. Our findings also disputed the suggestion that the earliest effect of iron deficiency on the erythrocyte is a reduction in cell size and that the mean corpuscular volume is the most sensitive indicator of underlying iron deficiency[16].

As earlier studies also did not help a recent study found that haematinic deficiency in pregnancy results in increased stimulation of erythroid progenitor cells by erythropoietin[19]. However, as this is not an easily available test and not cost effective, workers from Europe have worked on newer indices which are available onthe new automated cell counters like ADVIA 120 haematology system and found that percentage of hypochromic red blood cells (%HYPOm) and reticulocytes (%HYPO r), and cellular haemoglobin in reticulocytes (CHr) were in good agreement with the results based on the usage of a combination of three commonly used tests (Hb, MCV, ferritin)[20]. The same group in another study published in 2008 has shown that elevated %HYPOm and %HYPO r reflecting iron-deficient erythropoiesis are associated with an increase in EPO concentration in maternal blood and can help pick up sub-clinical iron deficiency as it is accompanied by a compensatory EPO response.

Further studies are needed to determine the usefulness of the cell indices in diagnosing iron deficiency longitudinally during the course of pregnancy and may be similar studies should be done in India once such cell counters are cheaper and available in India.

Although, iron and folic acid supplementation is recommended for all antenatal cases in India and developing world, adverse effects of iron supplementation has been highlighted[21]. However, Cochrane Review on the withdrawal of iron supplementation published in 2007 recommended the continuation of supplementation[2].

Conclusion: Thus, the international norms were found to be applicable to the pregnant Indian women and to similar populations with same stage of development as India. Also, we have concluded that we have to look for newer surrogate markers for diagnosis of sub-clinical iron deficiency in Indian women. In our study 47.82% of non-anaemic pregnant women had iron deficiency, so we recommend that all pregnant women in India should get iron supplements unlike what is recommended in the developed countries where iron supplementation is based on serum ferritin levels.

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