Correlation of Serum Ferritin with Red Cell Indices and Hemoglobin in Indian Women in Second and Third Trimester of Pregnancy

Rigvardhan[1], Vinod Raghav[2], Rakhi Negi[3], Punyashlok Biswal[4], Y S Rana[5]

ABSTRACT

Introduction: Iron deficiency anaemia (IDA) is the commonest cause of anaemia in pregnancy in developing countries and is associated with increased risk of low birth-weight infants. In developed countries, iron supplementation is based on serum ferritin levels. This is not feasible in India as it is not routinely available and is not cost effective. This study was undertaken to ascertain correlation of routinely available red blood cell (RBC) indices and hemoglobin (Hb) with ferritin and to document hematological profile of pregnant Indian women.

Material and Methods: We studied the correlation of ferritin concentration with RBC indices and Hb in 100 consecutive pregnant women in second and third trimester of pregnancy. Total RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and Hb were analyzed along with peripheral smear examination when needed and correlated with ferritin. The purpose was to identify the best index in pregnancy who needed iron therapy.

Results: IDA was found in 37% cases and significant correlation was found between serum ferritin and Hb, MCV and MCHC. No correlation was found between ferritin and TRBC and RDW. Serum ferritin levels <12 ng/mL was found in 29 out of 49 non-anaemic cases suggesting high prevalence of sub-clinical iron deficiency in 59.1% cases. None of the red cell indices correlated with ferritin level in this group.

Conclusion: Unlike developed countries where iron supplementation is based on serum ferritin levels, we recommend that all pregnant women in India should continue to get iron supplements as there is high prevalence of not only IDA but also subclinical iron deficiency in non anaemic pregnant women.

Keywords: Pregnancy, iron, ferritin, RBC indices

INTRODUCTION

Iron deficiency anemia (IDA) is the commonest nutritional deficiency in the world with estimated 2 billion persons worldwide being iron deficient.[1] As iron requirements during pregnancy increases they form a particularly susceptible group especially in developing countries and affects upto 52% of pregnant women.[2] IDA during pregnancy is associated with higher rates of premature and low birth weight.[3]

Centers for Disease Control and Prevention (CDC) has defined anemia in pregnancy as hemoglobin (Hb) level of less than 11 g/ dL during the Ist and IIIrd trimesters and less than 10.5 g/ dL during the IInd trimester.[4,5] The lower cut-off is due to haemodilution in pregnancy as plasma volume expands upto 30-40% as compared to 20-25% increase in red cell mass. This results in dilutional drop in Hb concentration creating a low viscosity state facilitating oxygen transport to the tissues including placenta. However, in India and most of the other developing countries the lower limit for Hb is often accepted as 10 gm per dL.[4] Also, there occurs physiological macrocytosis with average red cell size increase of 4 fL at term. Therefore, the accepted cut-off value of mean corpuscular volume (MCV) less than 78 fL to screen cases of IDA may not be true in pregnant women.[6] Giving iron supplementation during pregnancy is a norm in developing countries including India but recently there have been studies which recommend iron discontinuation due to teratogenic effects.[7] A recent Cochrane Review of 2009 also supports this observation and advocates that iron supplementation should be taken as per need.[8]

For diagnosis of IDA in pregnancy ferritin level <12 ng/ dL is considered as the gold standard.[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, serum iron can be normal or high if the pregnant female is on oral iron even with IDA.[3,4] The estimation of ferritin is expensive and cannot be routinely done in all cases especially in developing countries. Therefore, surrogate markers need to be identified in women who require iron supplementation. It is also necessary to determine the utility of red cell indices in diagnosing IDA during the course of pregnancy. The incidence of anemia during pregnancy changes with epidemiological differences in the population studied, so it is difficult to determine the true incidence.[10,11] This study was therefore done to ascertain utility of economical red cell indices to determine IDA in pregnant women in India and to compare these to the established norms so as to determine whether the norms apply or whether there was a need to establish local and population specific norms.

MATERIAL AND METHODS

100 consecutive pregnant women in the second and third trimester (12-40 wks) were enlisted for this study. All were on 60 µg elemental iron supplements per day. 25 non pregnant age matched healthy controls were also taken. Informed consent and Institutional ethical clearance was obtained.

Blood samples were collected in EDTA (2 ml) and sterile vacutainer (3 ml). The EDTA anticoagulated sample was used for estimation of Hb and the red cell indices on Sysmex KX-21 three part semiautomated hematology cell counter. Hb,

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total RBC count (TRBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width –standard deviation (RDW-SD) were tabulated. Wherever necessary, peripheral smear was seen to subtype the anemia. HbA2 estimation was done if thalassemia trait was suspected and treating obstetrician was informed to carry out genetic counseling and spouse testing. The serum was separated from the sample in sterile tube. Ferritin levels were estimated on automated chemiluminescence immune analyzer Roche (Hitachi) cobas e 411 using cobas kits. Serum iron was done on Siemens Dimension EXL 200 using Siemens kits.

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for Social Sciences, version 15.0. Scatter plots were constructed between serum ferritin and Hb, MCV, MCHC, RBC count and RDW-SD%. Receiver operator characteristic (ROC) curves were constructed to look at maximum sensitivity and specificity for Hb, MCV, TRBC, MCHC and RDW-SD % to look for statistically significant correlation with serum ferritin and also to see which one gave maximum area under curve (AUC).

RESULTS

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 and 11 g/dL in 2nd and 3rd trimester respectively for anemia and ferritin < 12 ng/mL were taken for presence of iron deficiency. It was found that 51 had anemia and 49 were non-anaemic. 37 had IDA and iron deficiency without anaemia was seen in 29/49 (59.1%) of non-anaemic ladies. Amongst the non iron deficiency anemia group, thalassemia trait was seen in two cases, five had Vitamin B12/folate deficiency and rest seven had mild anaemia due to pregnancy. Table 1 shows the distribution of these cases. The mean, median mode and SD of all Hb, red cell indices and serum ferritin of all cases were calculated. Scatter plots were drawn to look for correlation of ferritin with red cell indices (Figures. 1A, 1B, 1C). Significant correlation was seen between serum ferritin and Hb (Pearson's Corr coeff (r) = 0.3624, p = 0.0005), MCV (Pearson's Corr coeff (r) = 0.352, p = 0.0007), MCHC (Pearson's Corr coeff (r) = 0.3, p = 0.004). No significant correlation was observed between ferritin levels and RDW (Pearson's Corr coeff (r) = -0.01, p = 0.927) and TRBC (Pearson's Corr coeff (r) = -0.133, p = 0.37)(Figure 2A, 2B). When iron and ferritin levels were assessed, significant correlation was found between the two (Pearson's Corr coeff (r)

Sr No.	Status	N
1	Non-anaemic with normal ferritin (Hb >10.5 g/dL; ferritin >12 ng/mL)	20
2	Iron deficiency anemia (Hb < 10.5 g/dL; ferritin < 12 ng/mL)	37
3	Non-anaemic with iron deficiency (Hb >10.5 g/dL; ferritin < 12 ng/mL)	29
4	Anaemia not due to iron deficiency (Hb 10.5 g/dL; ferritin >12 ng/mL)	14

Table-1: Anaemic status of study participants

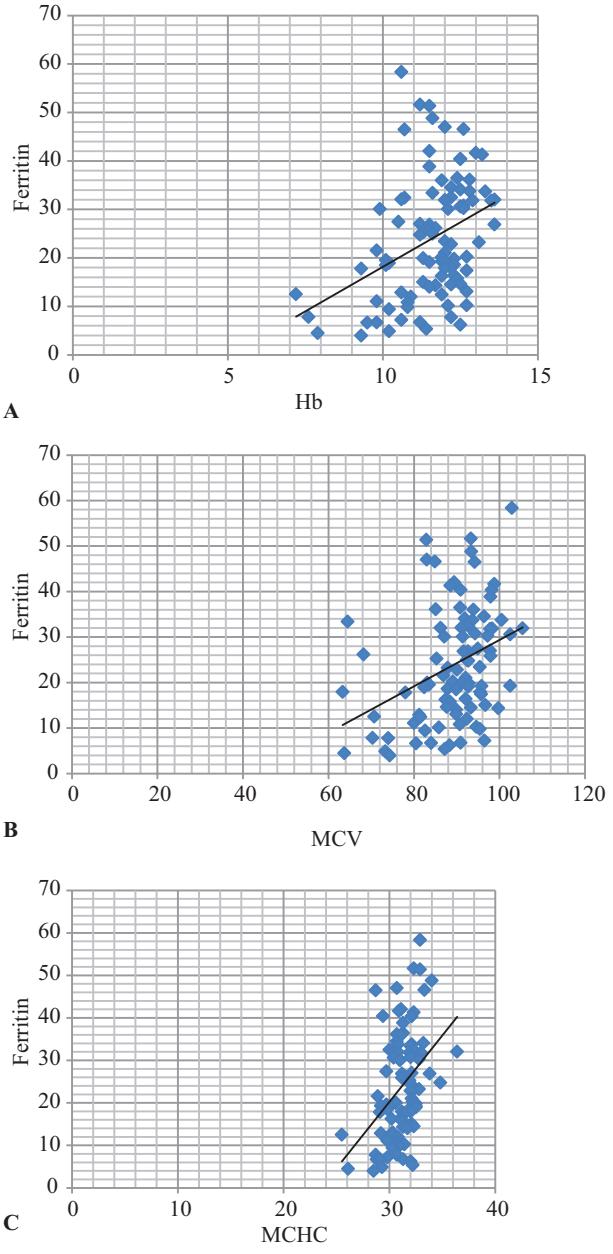

Figure-1: A: Scatter plot of Hb and serum ferritin. Pearson Correlation coefficient =0.362; p= 0.0005. B: Scatter plot of MCV and serum ferritin. Pearson Correlation coefficient =0.352; p= 0.0007. C: Scatter plot of MCHC and serum ferritin. Pearson Correlation coefficient =0.3; p= 0.004

S No	Variable	AOC	Best cut-off	Sensitivity %	
1	Hb	0.95	9.6 gm/dl	89.5	83
2	MCV	0.86	75.6 fl	85.7	80.1
3	MCHC	0.84	29.4 gm/dl	81	79.4
4	TRBC	0.7	< 4.3 million/cumm	76.8	69
5	RDW	0.7	####	72.6	67.3

Table-2: Area under ROC curve and the best cut-off value for various indices to diagnose of IDA in ANC cases

= 0.629, p = 0.000) (Figure 2C). Serum ferritin was significantly lower in the IDA cases as compared to other pregnant women and 59.1 % of non-anaemic had ferritin levels < 12 ng/dL. This difference was statistically significant (p = 0.024).

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 89.5% and specificity of 83% (Figure 2D). The 14 non-iron deficiency anaemia comprised of 2 cases of b-thalassemia trait, 5 Vitamin/folate B12 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 HbA2 estimation. Further subgroup analysis was not done due to small number. There were 49 non-anaemic cases with 29 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.

DISCUSSION

Amongst the 49 non-anaemic cases, ferritin < 12 ng/dL was found in 29 ladies, thus 59.1% cases had subclinical iron deficiency in pregnant women. The iron deficiency (anaemic and non-anaemic) was present in 66 out of 100 pregnant women. This shows that anaemia as well as IDA is very common in India as compared to the developed countries.[12] Sub-clinical iron deficiency without anaemia (serum ferritin < 12 ng/dL and Hb >10.5 g/dL) is another major health issue which was seen in 59.1 % non-anaemic pregnant women in this study.

As for correlation of ferritin with red cell indices in all the 100 cases, we did not find any correlation between ferritin and RDW-CV% and RBC count. Hb, MCV, and MCHC showed correlation with ferritin levels. These findings were similar to those of a study from USA on African American population where the results showed that Hb < 9.7 g/dL and RDW > 15 (at gestational age <20 weeks) predicted iron deficiency with high specificity.[13] The present study showed that in second and third trimester pregnant women with Hb <10.5 g/dL (anaemic) the MCV and MCHC correlate with the ferritin levels and can help in the diagnosis of IDA. Our findings were different from an earlier study in India and Malaysia that found significant correlation between ferritin and TRBC and Hb only respectively.[4,14]

Our study shows that sub-clinical iron deficiency cannot be diagnosed with red cell indices given in routine hematology counters available in India as sufficient changes do not develop in them to be picked up in routine screening. Our findings were different from another study which concluded that in early iron deficiency and during recovery the count shows near normal Hb with a low MCV and a high RDW-CV%.[4] Also in pregnancy, the MCV rises by 4 fL which may have a role to play.[6] Therefore,

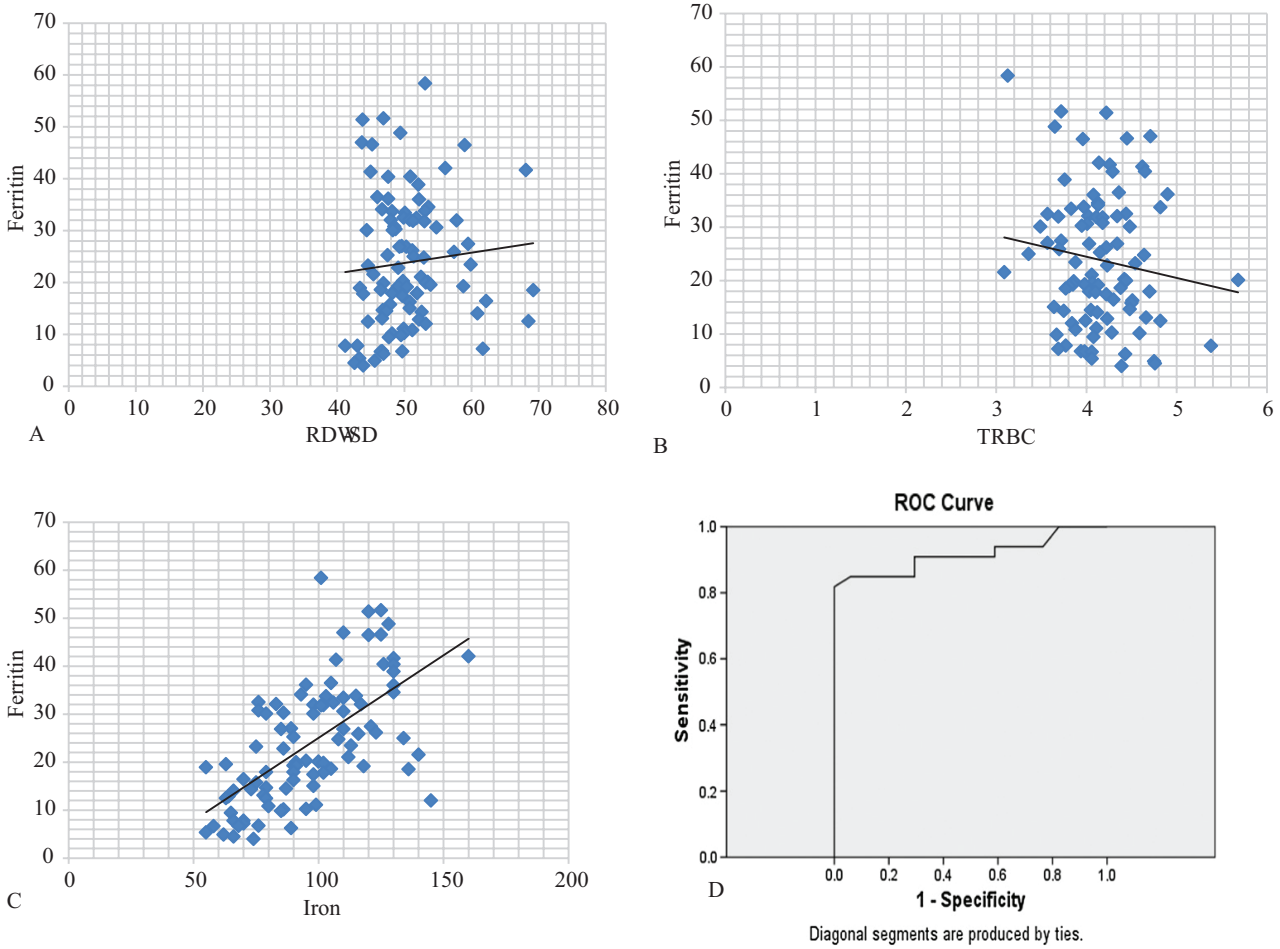

Figure-2: A: Scatter plot of RDW and serum ferritin. Pearson Correlation coefficient = -0.01; p= 0.925. B: Scatter plot of TRBC and serum ferritin. Pearson Correlation coefficient = -0.133; p= 0.37. C: Scatter plot of iron and serum ferritin. Pearson Correlation coefficient =0.629; p= 0.000. D: ROC curve for Hb. Area under curve = 0.947. The best cut-off is Hb < 9.6 gm/dL with a sensitivity of 89.5% and specificity of 83%.

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 89.5% and a specificity of 83%. This was similar to the cut-off of 9.7 g/dL given in the study among African-Americans in USA and India.[4,13] Other indices also had statistically significant correlation with ferritin. For IDA the next best index was MCV < 75.6 fl (sensitivity of 85.7% and specificity of 80.1%).

As for iron deficiency in non-anaemic pregnant women, we did not find any indicator to which might help to pick up the women with ferritin <12 ng/dL. Although, haematinic deficiency in pregnancy causes raised levels of erythropoietin which in turn stimulates erythroid progenitor cells. Doing serum erythropoietin routinely is expensive and not cost effective nor is it easily available.[4] Recently newer parameters such as percentage of hypochromic red blood cells (%HYPOm), reticulocytes (%HYPOr), and reticulocyte- hemoglobin (CHr) are available in new automated cell counters like Siemens ADVIA 120 hematology system. Ervasti et al have shown that elevated % HYPOm and % HYPOr reflects iron-deficient erythropoiesis and increased erythropoietin concentration in maternal blood.[15] Thus these newer parameters can help pick up sub-clinical iron deficiency when pregnant women are screened. Although longitudinal studies during pregnancy are warranted to determine the usefulness of these cell indices in diagnosing iron deficiency, sub-clinical iron deficiency and assessing therapeutic response to hematinics. Similar studies should be done in India once such cell counters are cheaper and readily 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.[7] However, Cochrane Review on the withdrawal of iron supplementation published in 2007 recommended the continuation of supplementation.[12] In this study, we found 59.1% of non-anaemic pregnant women had serum ferritin levels <12 ng/dL in second and third trimester of pregnancy. Therefore, we recommend continuation of iron supplementation in pregnancy as has been recommended by various studies in developing world.[4,16]

CONCLUSION

In this study we found that 59.1% of non-anaemic pregnant women had iron deficiency, we recommend that iron supplementation should continue in all pregnant women in India unlike developed countries where iron supplementation is based on serum ferritin levels. Also, we conclude that newer surrogate markers like %HYPOm, %HYPOr and CHr in Indian women be studied further for diagnosis of sub-clinical iron deficiency.

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