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To find the best correlating factor from the red cell indices with Serum Ferritin levels in women with hyperglycaemia in pregnancy

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ABSTRACT
PURPOSE OF PROJECT: *Anaemia in women with childbearing age affects more than 50% women, and hyperglycaemia in pregnancy is also a rampant problem for women in the Indian subcontinent. Pregnancy alters the red cell indices and so does hyperglycaemia. There thus arises the need for determining the best correlating factor of the red cell indices to detect/ grade iron deficiency in pregnant women with hyperglycaemia.*
METHOD USED: *In women with hyperglycaemia in pregnancy, serum ferritin and RBC indices were tested and compared to find the best correlating factor for iron deficiency in these women.*
CONCLUSION: *MCV, MCH, MCHC, Hb are positively correlated with serum ferritin and RDW is negatively correlated. MCHC is most sensitive indicator if iron deficient status in women with hyperglycaemia in pregnancy with sensitivity of 64.6%.*
SIGNIFICANCE
Anaemia, iron deficiency, pregnancy and hyperglycaemia impact red cell indices and thus we resorted to check the best correlating RBC index with all these factors at play ie; pregnant women with hyperglycaemia.
KEYWORDS
Red cell indices, MCV, MCH, MCHC, RDW, iron deficiency, hyperglycaemia in pregnancy, OGCT, best correlating RBC index, sensitivity.

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I. INTRODUCTION

Over the years, our country has been grappling with anaemia and anaemia related illnesses, especially in women with reproductive age group.
This could be evidenced by the National Family and Health Survey from 2005 to 2021, wherein >50% women were found to have anaemia and this number increased to 57% in 2021(1) .
Amongst other causes , iron deficiency remains the leading cause of anaemia in this age group which can be traced back to poor dietary intake, vegetarian diet, multiple pregnancies with short interconceptional period, lactation , excessive blood loss during child birth. (2)
RBC indices are useful in elucidating the aetiology of anaemias.
MCV or mean corpuscular volume decreased in iron deficient state upto 80 fl or even less (3) But Red cells temporarily become hypertonic in hyperglycaemia compared to the isotonic diluent fluid, resulting in enlarged cells and a higher MCV. (4)
MCHC remains near normal in iron deficient state , whilst, MCH, mirrors MCV closely and thereby decreases in iron deficient state (5) this was found to be consistent in pregnancy (6)and Alamri et al (7) found that hyperglycaemia increases MCV and MCHC.
RDW increases in iron deficiency state and also during pregnancy (6)But hyperglycaemia can decrease RDW (7) as per Alamri Et al .
The RBC count is affected differently by hyperglycaemia. When compared to individuals with normal glucose homeostasis, it is seen to be high in cases of pre-diabetes and low in situations of developed diabetes mellitus.(8)
As the RBC indices are not specific, and variable, the need arose to determine other methods to detect iron deficiency, TIBC (total iron binding capacity), Serum transferrin were some such methods, but the current gold standard for detecting iron deficiency is serum ferritin. Ferritin is the storage form of iron in our body, and also helps in the controlled release of iron. Ferritin levels less than 15 μg/Lis suggestive of iron deficient status irrespective of haemoglobin levels. (9)(10). Serum ferritin values <30μg/Lare indicative of low or absent iron

stores (10) which is the cut off we have used in this study. Studies also indicate cut off for iron deficient status can be taken as <20 µg/Land <12 µg/L(10)
We have chosen cut off as 30 microgram/l as in order to women with serum ferritin values between 12 to 30 µg/L, who may reveal to be developing iron deficiency and pregnancy progresses and iron requirement surges.
Gestational diabetes mellitus has been on the rise worldwide and has recently increase 2-3 fold with current prevalence ranging over 8.9-53.4% (11)
Hyperglycaemia in pregnancy increases the red blood cells count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). Red blood cell distribution width (RDW) was negatively correlated with poor glycaemic control. (7) As per Alamri et al. in this study we have thus resolved to study the RBC indices and compare them with ferritin to determine the best correlating factor for iron deficiency in women with deranged OGCTs that is OGCT >120 mg/dl which includes women with impaired glucose tolerance, GDM and overt diabetes.

II. MATERIAL AND METHODS

AIM:
To find the best correlating factor amongst the red cell indices with serum ferritin levels in women with hyperglycemia in pregnancy.

OBJECTIVES:

- Detect iron deficiency status in women with hyperglycemia in pregnancy with serum ferritin
- Study red cell indices ; RDW, MCV, MCH, MCHC, Hb in these women
- Establish relationship between above mentioned red cell indices and serum ferritin.

STUDY AREA
MGM Medical College and Hospital, Aurangabad, Maharashtra.

STUDY POPULATION
Pregnant women, after 28 weeks of gestation, with hyperglycemia in pregnancy, admitted (IPD) or coming to the OPD of MGM Medical College and Hospital, Aurangabad, Maharashtra, India.

INCLUSION CRITERIA

Pregnant women with gestational age above 28 weeks, and OGCT above 120mg/dl

SAMLPE SIZE
100

TYPE OF STUDY
Observational

STUDY DESIGN
Descriptive

STUDY PERIOD
2 years

SAMPLING METHOD
Convenient sampling

METHODOLOGY
Women with gestational age more than 28 weeks of gestation underwent testing for OGCT. Out of those women with OGCT 120 and above were selected randomly and then their haemogramand serum ferritin was tested.
Based on serum ferritin levels, women were divided into two groups <30µg/L (Iron deficient group) and >30 µg/L (non iron deficient group).
RBC indices *MCV, MCH. MCHC. RDW, Hb) were compared with serum ferritin reports to determine the best correlating factor of iron deficiency.

DATA ANALYSIS

Data analysis was done using SPSS software.

Pearson correlation coefficient was calculated to find the correlation between each of the RBC indices and ferritin. Sensitivity of each test was also calculated.

Unpaired T test was applied to check the presence of significant difference between both groups (iron deficient and non iron deficient group).

III. RESULTS

FIGURE 1

64% of women are in age range of 21-24 years. 19% are in the range of26-30 years, 13% are <20 yearsof age and 4% re in range of 31-35 years.

TABLE 1

31 % of women had a Gestational age of 28-32 weeks .51% had 32.1 -36 weeks and 18% had >36 weeks of Gestational age .The Group had a mean of 33.59 weeks of gestation.

TABLE 2

Of the total sample population ,55% were primigravida ,45% were Multigravida.69% of women with iron deficient status (Serum ferritin <30 µg/L) were primigravidas.

TABLE 3

Out of 55 primigravidas, 38 women (69%) were primigravidas with serum ferritin <30 µg/L, and off of these 38 women, 9 women (24%of primigravidas with iron deficiency <30 µg/L) had severe iron deficiency ie, serum ferritin <12 µg/L and

TABLE 4

The 65 pregnant women With serum ferritin <30 µg/L(HB Mean=10.15,SD=1.4) compared with 35 patients of Women with serum ferritin >30 µg/L (Mean =12.14,S.D=1.02) demonstrated significant difference in Hb in both Groups. Mean of HB in Women with serum ferritin <30 µg/L(65) is less compared with the mean of Women with serum ferritin >30 µg/L(35).t=7.410 ,p<0.0001.

The 65 pregnant women With serum ferritin <30 µg/L(PCV Mean=32.16 SD=3.6) compared with 35 patients of Women with serum ferritin >30 µg/L (Mean =37.05,S.D=3.02) demonstrated difference in PCV in both Groups. Mean of PCV in Women with serum ferritin <30 µg/L(65) is less compared with the mean of Women with serum ferritin >30 µg/L(35).t=7.410 ,p<0.0001.

Also the above datastates ,all RBC indices ie: MCV, MCH, MCHC, PCV RBC count vary significantly when compared in both groups, as in all of them , p value is <0.05.

It is also evident that the standard deviation in the group with serum ferritin value < 30 µg/L (the group with iron deficiency) is more than in the group with serum ferritin value > 30 µg/L for each RBC indices namely, Hb, MCV, MCH, MCHC, RDW, RBC count and PCV.

Another observation made is that the standard deviation difference between both groups ie the one with serum ferritin > 30 µg/L vs the one the serum ferritin < 30 µg/L is much larger in MCV, MCH and RDW as compared to MCHC, PCV and RBC count.

Also applying T test (unpaired) to this data at confidence interval of 95% and degree of freedom as 98 (as in the table) , we can say that there is significant difference between the two groups (one with serum ferritin >30 µg/L and one with serum ferritin <30 µg/L) for each CBC parameter as all T values are more than 1.661.

GRAPH 1

Shows r value 0.477, and thus a positive correlation between Hb and serum ferritin.

GRAPH 2

Shows R value of -0.26, and thus a negative correlation of RDW and Serum ferritin.

GRAPH 3

Shows R value of 0.22, and thus a positive correlation of MCV and Serum ferritin.

GRAPH 4

Shows R value of 0.16, and thus a positive correlation of MCHC and Serm Ferritin:

As per graphs 1, 2, 3 and 4 In the current study, correlation between Ferritin and Haemoglobin was found to be positive correlation, i.e. when ferritin value increases, haemoglobin value also increase.

Correlation between Ferritin and RDW was found to be negative correlation, i.e. when ferritin value increases, RDW value also decrease.
Correlation between Ferritin and MCHC, MCH was found to be positive.

TABLE 5
In our study we have found MCHC to be most sensitive parameter to iron deficient status. With 64.6% sensitivity

IV. DISCUSSION

A growing baby and an increase in the volume of blood produced by the mother both contribute to an increase in the need for iron during pregnancy. In the first trimester of pregnancy, maternal changes cause a drop in Hb concentration, which continues to fall throughout the pregnancy and reaches its lowest point in the second trimester.

In our study Majority of females were in the age group 21-25 yrs with mean age 24.22 yrs .19% were in the age group of 26-30 yrs. 13% were in the age group less than 20yrs.

A reliable indicator of early iron insufficiency in pregnancy can be obtained through serum ferritin testing . Depleted iron reserves are indicated by a serum ferritin content of less than 30μg/L(12)and this has been set as the cut off for iron deficiency for our study**.**Which has given us added advantage of including those 12 women in but were found to have serum ferritin <30μg/L, but >12μg/L.The NFHS-5 has found the prevalence of anemia in women of reproductive age in India to be 57% and 52% in pregnant women(13)

In our study , we found 65 % of pregnant women with serum ferritin <30μg/L, indicating their iron deficiency status. Although not truly indicative of anemia in this population, which could be attributed to other causes than just iron deficiency and thus could have even higher prevalence in this group**.**

Importantly, the findings of this study revealed that in routine antenatal testing, assessing iron deficiency by only Hb levels , is not sufficient as even in women with serum ferritin <30μg/L , Hb was found to be 11g% or even more sporadically as can be determined by the mean and standard deviation in that group (10.15=/- 1.4).

The normal reference range for MCH, MCHC, and MCV used in this study are 26.7–31.9 pg/cell, 32.3–35.9 g/dL, and 79–93.3 fL, respectively.

MCV

In our study we have found MCV to have a positive correlation with serum ferritin (r value 0.015). This result was similar to that of Sharanya et al (14) in iron deficiency patients and Rigwardhan(15) et al in pregnant women.
Whereas Gandhi et al (16) found no correlation between MCV and serum ferritin in anemic pregnant women.

MCV showed to have a sensitivity of 23% in our study, in a study on pregnant women in 2nd and third trimester MCV to have a sensitivity of 85.7%(15) much larger than sensitivity we found in our study which is 23%. Similar results were observed by Tiwari et al(17) with sensitivity of 84.8% and a Sri lankan study where MCV was found to have sensitivity of 74.1% (18)

This disparity can be explained by the fact that due to iron deficiency, MCV is bound to decrease resulting in microcytic anemia but, due to hyperglycmeia, there can be swelling of RBCs which causes MCV to increase(7) hence in our study, since we have chosen women with hyperglycemia, and with iron deficient status, the effect of both hyperglycmeia and iron deficient has somewhat counteracted.

MCH

In our study, MCH was found to have a positive correlation with serum ferritin (r value 0.22)Sharanya et al (14) too found positive correlation between MCH and ferritin in non pregnant population with iron deficiency and by Tiwari et al (17) Anwar et al (19) in pregnant women. In contrast to which, Gandhi et al did not find any correlation between MCH and serum ferritin in pregnant women with iron deficiency anemia(16).

In our study MCHwas 41% sensitive to detect iron deficient status. Tiwari et al found MCH to be 84.8% sensitive(17) in predicting iron deficiency in pregnant women in second and third trimester. Rabindrakumar et al (18)found it to be the most sensitive parameter with 88.9% sensitivity.

MCHC

We found MCHC to be positively correlated with serum ferritin and thereby with iron deficient status (r value of 0.16), same as Sharanya et al (14)in iron deficientanemicwomen (non pregnant). Rigawardhan et al (15) and Rabindrakumar et al (18) also found positive correlation between Serum ferritin and MCHC. But Tiwari et al (17)and Gandhi et al, (16) on the other hand found no correlation of MCHC with serum ferritin in their studies.

It is although interesting to note, that in the same study, Tiwari et al (17)found MCHC to be 81.8% sensitive for predicting iron deficiency. Rigawardhan et al (15) in a similar study found MCHC to have 81% sensitivityand Rabindrakumar et al (18), found 74.6% sensitivity to predict iron deficiency, this is all in congruence with our study, that has found sensitivity of 64.6%.

We also found significant difference between MCHC in iron deficient and non iron deficient women with hyperglycemia in pregnancy and the results are in accordance with a study conducted in Pakistan, Quetta,that found significant difference between MCHC of pregnant anemic and non anemic women , (19)

RDW

Our study results have indicated a negative correlation between RDW and serum ferritin. Significant correlation was established in a study conducted at Jammu and Kashmir between serum ferritin and RDW in anemic pregnant women.(16)

But, contrary to what we found in our study , RDW was found to be positively correlated with serum ferritin in non-pregnant population with iron deficiency anaemia as observed by Sharanya et al (14), as did Tiwari et al(17) in his study on pregnant women, and Rigawardhan et al found no correlation (15)of RDW and serum ferritin in a similar study.

This difference in observations could be attributed to the fact iron deficiency causes increase in RDW(20) , and that during pregnancy RDW increases between 34 weeks of gestation and the onset of labour and does not change between 16 and 34 weeks gestation as observed by Shehata et al (21) and that poor glycaemic control can increase RDW (22), and In our study we have selected women over 28 weeks of gestation , with hyperglycaemia, thereby causing the RDW to riseand establish a negative correlation. Since studies by Sharanya et al (14) was on non-pregnant women, the effect of pregnancy has been negated in her observations and in studies by Tiwari et al(17) and Rigawardhan et al (15) have not accounted for hyperglycaemia in their study population, the additive impact of hyperglycaemia on RDW has been nullified.

In our study RDW was found to be 52% sensitive indicator of iron deficiency status , this is in line with Tiwari et al's observation of 82.4%(17)sensitivity and Rigavardhan et al's observation of sensitivity of 72.6%(15) in predicting iron deficiency in pregnantwomen.

Hb

Hb in our study came out to be positively correlated to serum ferritin. Similar findings in non-pregnantanaemic population by Sharanya et al (14), and in pregnant population by Tiwari et al(17) and Rigawardhan et al (15)Rabindrakumar et al (18)and Anwar et al (19)

Hb in our study had sensitivity of 58% Rigawardhan et al(15) found sensitivity of 89.5%. and in a similar study, Tiwari et al (17)observed sensitivity of 90.9%

From all the above analysis and review of literature it is clear that none of the red cell indices can be attributed to be the best correlating factor with ferritin and thereby of iron deficiency status. More so when hyperglycaemia and pregnancy also come into play which alter these indices invariably and unpredictably.

In our study however we have found significant difference between CBC indices in pregnant women with hyperglycaemia (OGCT >120mg/dl) with and without iron deficiency, determined by serum ferritin (cut off being 30 µg/l). all of the indices (Hb, MCV, MCH, MCHC, RBC count, PCV. RDW have p value <0.05). r values (Pearson correlation coefficient shows positive correlation with Hb, MCV, MCH, MCHC but negative correlation with RDW. It is important to highlight here that none of the r values are >0.7 or -0.07, thus we can say that although there is significant difference in values of CBC indices in pregnant women with and without iron deficiency status with hyperglycaemia, the correlation is not strong enough to establish perfect linear or even near linear relationship.

Hence, we can say that to determine iron deficiency in this demographic, serum ferritin remains the best indicator.

LIMITATIONS

1. Ours is a descriptive study, a case control model would have helped for better analysis.
2. A larger sample size would have helped to frame better results.

CONCLUSIONS

1. In our study, with sensitivity of 64.6% , MCHC has been found as the best correlating RBC index with serum ferritin
2. Serum Ferritin is the best indicator of iron deficient status in pregnant women with hyperglycemia in pregnancy.
3. MCV, MCH, MCHC, Hb are positively correlated with serum ferritin and RDW is negatively correlated.

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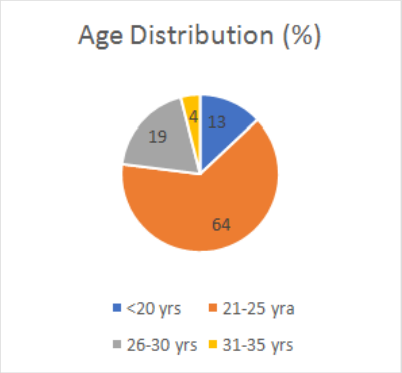
IMAGES AND TABLES

FIGURE 1: Age Distribution

n= 100



Range	19-32 yrs
Mean	24.22
SD	3.65

TABLE1: GESTATIONAL AGE

n=100

Age group	Cases
28-32 Weeks	31
32.1-36 weeks	51
>36 weeks	18
Range	28-40 weeks

Mean	33.59 weeks
S.D	3.65 weeks

TABLE 2: Obstetric Score

n= 100

Parity	Number	Serum ferritin <30 µg/L	Serum ferritin >30 µg/L
Primi	55	38 (69%)	17 (31%)
Multi	45	27 (60%)	18 (40%)
Total	100	65	35

TABLE 3 : Women with Serum ferritin between 12-30 µg/L

Parity	Serum ferritin <30 µg/L	Serum ferritin <12 µg/L	Serum ferritin 12-30 µg/L
	65	12(18%)	53(82%)
Primi	38	9(24%)	29(76%)
Multi	27	3(11%)	24(89%)

TABLE 4: Distribution of red cell indices according to iron status of pregnant women(n=100)

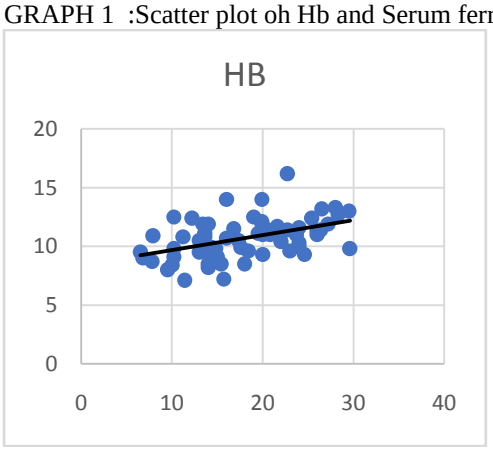
Red cell Indices	Women with serum ferritin <30 µg/L(65)	Women with serum ferritin >30 µg/L(35)	P Value	T statistic	DF
Hb	10.15 ±1.4	12.14 ±1.02	< .00001	7.410	98
MCV	82.26 ±9.35	88.80 ±6.13	0.003*	3.725	98
MCH	26.00 ±3.4	28.75 ±2.42	0.001*	4.238	98
MCHC	31.41 ±1.8	32.37 ±1.3	0.006*	2.785	98
RDW	16.21 ±3.0	14.19 ±1.04	0.002*	-3.85	98
RBC	3.9 ±0.51	4.24 ±0.43	0.001*	3.352	98
PCV	32.16 ±3.6	37.05 ±3.02	< 0.0001*	6.840	98

1. *N* number of pregnant women, *SD* standard deviation, *Hb* haemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration, *RDW* red blood cell distribution width and *RBC* red blood cell.

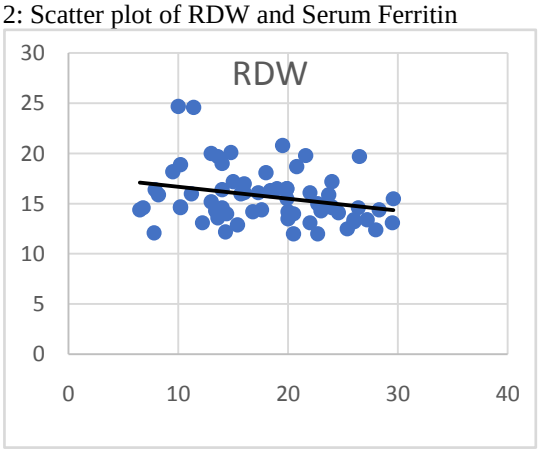
TABLE 5: Sensitivity of different RBC indices

	Iron deficient group(S. ferritin <30µg/L)	Non iron deficient group (S. ferritin >30 µg/L)	Sensitivity of index in predicting iron deficient status
RDW>15	34	5	52%
RDW <15	31	30	
MCV<79	15	3	23%
MCV >79	50	32	
MCH<26.7	27	5	41%
MCH>26.7	38	30	
MCHC <32.3	42	16	64.6%
MCHC >32.3	23	19	
Hb <11	38	3	58%
Hb>11	27	32	

GRAPH 1 :Scatter plot oh Hb and Serum ferritin

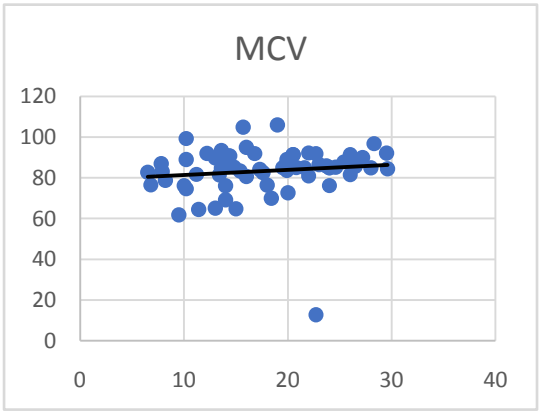


GRAPH 2: Scatter plot of RDW and Serum Ferritin

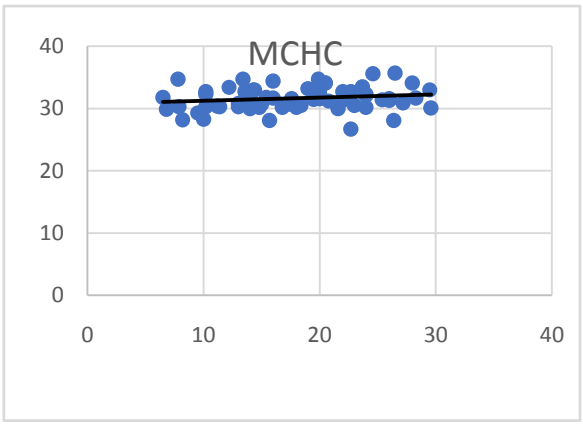


R value: -0.26R value: 0.477

GRAPH 3 : Scatter plot of MCV and serum ferritin



GRAPH 4: Scatter plot of MCHC and Serum Ferritin



R value: 0.015R value: 0.16