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Haemoglobin Distribution Width In Predicting Iron Deficiency Anaemia Among Healthy Pregnant Women In Third Trimester Of Pregnancy

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Abstract: ***Iron deficiency anaemia is one of the most common nutritional deficiency disorders in the world. In the present study, importance of haemoglobin distribution width (HDW) as predictor of iron deficiency anemia in pregnancy was determined.***

Materials and methods: Our study was carried out in 48 pregnant women in their third trimester of pregnancy. Blood collected was analyzed for complete hemogram, including haemoglobin (Hb), differential count(DC), total leucocyte count (TLC) , red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), hemoglobin distribution width (HDW), serum ferritin and serum transferrin receptors (sTfR).

Results: Out of a total 48 women, 41.7% had normal haemoglobin levels while among the remaining 58.3% anaemic women, 20.83% had mild anaemia (Hb levels between 10-11g/dl), 18.75% had moderate (Hb levels between 7-9.9g/dl) and 18.75% severe anaemia (Hb levels <7g/dl). A high HDW (>3.2g/dl) was observed in 87.5% women which was similar to a high RDW (>14.5%) observed in 87.5% of women.

It was also observed that HDW correlated positively and significantly with RDW and negatively with Hb, RBC indices and serum Ferritin. Serum ferritin correlated positively with Hb and RBC indices and negatively with RDW, HDW and sTfR. Although serum transferrin receptors did not show significant correlation with any of the parameters it correlated positively with HDW.

Conclusion: Similar to RDW, HDW may be useful in predicting iron deficiency anaemia but should be confirmed ultimately by serum ferritin estimation.

Keywords: Anaemia, haemoglobin distribution width, ferritin, iron deficiency, red cell distribution width, transferrin receptors.

I. INTRODUCTION

Anaemia is one of the most common nutritional deficiency disorders in the world. The World Health Organization (WHO) defines iron deficiency anaemia (IDA) as anaemia accompanied by depleted iron stores and compromised supply of iron to the tissues. This is a serious problem in the gestation–puerperal period as it is associated

with several adverse perinatal outcomes like prematurity, low birth weight, maternal and perinatal mortality. 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.

In India there is a high prevalence of iron deficiency anaemia and the gold standard test – serum ferritin is too expensive to be done in all cases. Other biochemical markers like serum transferrin receptors (sTfR) and zinc protoporphyrin (ZnPP) are not routinely available and are expensive too.

Various studies have shown that onset of iron deficiency anaemia can be predicted using automated blood analyzers [8] as a low haemoglobin (Hb) level along with high level of anisocytosis detectable by red cell distribution width (RDW) proves to be a good indicator of changes in blood due to depleted iron stores.

Apart from red cell indices and RDW, modern automated analyzers also provide indices like haemoglobin distribution width (HDW), percentage of mature hypochromic erythrocytes (HyPom), haemoglobin concentration (CHr) which are more sensitive indices than the Hb level.HDW is a new parameter in fully automated analyzer that may give an idea of early iron deficiency before other tests. Here the instruments determine the Hb concentration of individual red cells and hence provide the percentage of hypochromic red cells with distribution curves of Hb concentration and are able to 'flag' the presence of increased number of hypochromic or hyperchromic red cells. The percentage of hypochromic red cells depends on the concentration of Hb in individual cells rather than being a mean like MCH or MCHC. It can hence be a more sensitive marker of the availability of iron for erythropoesis because small changes in the number of red cells with inadequate Hb can be measured before there is any change in the MCHC. In the present study an attempt to understand the importance of HDW and other RBC indicators in the detection of iron deficiency anemia in pregnancy, especially in the third trimester was done.

II. MATERIALS AND METHODS

Our study was carried out in 48 pregnant women in their third trimester of pregnancy reporting to the antenatal outpatient department (OPD) of Gandhi Government Medical College and Hospital, Hyderabad, for their delivery, between the years 2013-2014.Institutional ethical committee clearance was obtained before the start of the study. All the subjects who agreed to participate in the study were explained about the study. After signing an informed consent, the women were approached to participate in the study.

The *inclusion criteria* were -1) All pregnant women of age 18 years and above 2) with singleton pregnancy and 3) in third trimester of pregnancy.

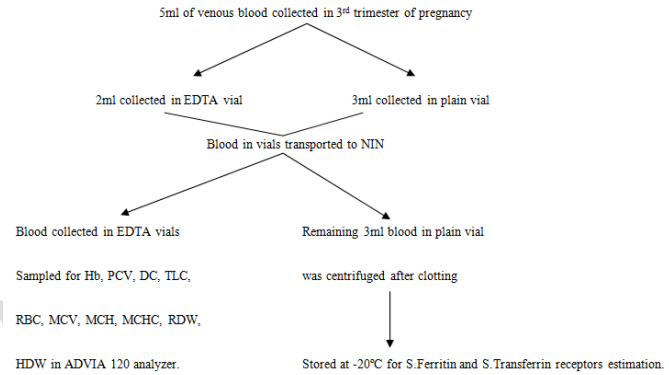
The *exclusion criteria* were – Pregnant women with 1) hemolytic anaemia 2) hypertension 3) Diabetes mellitus 4) Human immunodeficiency virus (HIV) 5) Hepatitis C Virus (HCV) 6) Hepatitis B surface antigen (HBsAg) positive mothers 7) Twin pregnancies 8) Women in 1st and 2nd trimester of pregnancy. The obstetric and medical history was obtained using pretested questionnaire.

Anaemia in pregnancy was defined as Hb concentration of <11g/dl, as defined by the World health organization (WHO).

Grades of Anaemia
- Mild anaemia (Hb 10-11g/dl)
- Moderate anaemia (Hb 7-9.9g/dl)
- Severe anaemia (Hb <7g/dl)

The following cut off values suggested by WHO and Centers for Disease Control and prevention (CDC) on pregnant women were used: RBC count<3.8 $x10^{12}$ cells/ L, hematocrit < 32%,MCV <76fl, MCHC < 26pg, MCHC <32 g/dl, RDW >14.5%, serum ferritin <12ng/ml and HDW >3.2g/dl. RDW >14.5% is considered abnormal and diagnostic for IDA.

FLOW CHART FOR BLOOD SAMPLE ANALYSIS



Serum ferritin was estimated using ferritin SA ELISA kit of Calbiotech, Inc. (CBI) which used solid phase sandwich assay method, based on streptavidin-biotin principle. sTfR was estimated using Human transferrin receptors ELISA kit of Qayee-Bio Ltd., which used double – antibody sandwich enzyme- linked immunosorbent one-step process assay (ELISA) to assay the level of transferrin receptors (TFR/CD71) in samples.

STATISTICAL ANALYSIS

The findings of the laboratory investigations were recorded in a predetermined data collection sheet. Statistical analysis was done by SPSS (Statistical package for Social Science) version 19. Descriptive statistics like mean, standard deviation (SD) and prevalence were calculated for all variables. Mean values for all variables were compared by unpaired't' test across both normal and anemia groups. Relationships between Hb, MCV, MCH, MCHC, RBC, RDW-CV%, HDW, serum ferritin and sTfR were calculated by correlation coefficients and Chi square test was done for associations. Non-parametric test was done wherever required. Sensitivity and specificity were calculated for validation of Iron variables with RBC indices. The level of significance was considered as 0.05.

III. RESULTS

During the study period a total of 50 pregnant women in their third trimester of pregnancy were enrolled of which 48 fulfilled the inclusion criteria and their data is presented in this report. The means and SDs of all the parameters were calculated and are as shown in the Table I.

Among the 48 women, 20 (41.7%) had normal Hb levels and the remaining 28 (58.3%) had anaemia of varying grades. 10 women (20.83%) among these 48 women had mild anaemia while 9 women each (18.75%) had moderate and severe anaemia.

Out of the 48 women, serum ferritin estimation could be done in 32 subjects only as the blood samples collected from 16 women were found to be hemolyzed due to improper collection and transportation from the hospital and hence were unfit for processing. Serum ferritin levels of <12µg/l was observed in only 4 women (12.5%), all of whom had severe anaemia while the remaining 28 had normal serum ferritin levels of >12µg/l (87.5%).

HDW values were noted in all the 48 women where 42 (87.5%) of them had levels 3.2g/dl while only 6 cases (12.5%) had normal levels. These findings were similar to those of RDW where out of 48 women, 42 women (87.5%) had elevated levels >14.4% while only 6 women (12.5%) had normal levels**.**

In case of red cell indices, RBC count was observed to be low (<3.8 x10^{12} cells/ L) in 20 cases (41.7%) and was normal in 28 cases (58.3%). MCV was low < 76fl in 16 women (33.3%) and was normal in 32 cases (66.7%). A low MCH of <27pg was observed in 28 women (58.3%) while it was normal in 20 subjects (41.7%)**.**

Chart shows the percentage of number of subjects on the X-axis and the different RBC indices on the Y-axis. The figure in addition shows that both RDW and HDW levels were higher than the normal values in all grades of anaemia in comparison with other RBC indices like MCV, MCH and MCHC.

TABLE II SHOWS CORRELATION between the different parameters which was done to predominantly look for significant difference in the values. It was observed that significant positive correlation was seen between Hb and PCV, MCV, MCH, MCHC ($p<0.001$) while Hb was significantly negatively correlated with RDW ($p<0.001$).HDW and sTfR were negatively correlated with Hb although it was not statistically significant.

HDW (Table II): It correlated positively and significantly with RDW ($p<0.001$) and negatively but significantly with MCH and MCHC ($p<0.05$).Similar to RDW, HDW negatively correlated with Hb and serum ferritin and positively with sTfR although it was not statistically significant.

RDW (Table II): RDW positively and significantly correlated with HDW ($p<0.001$) and significantly but negatively with Hb, PCV, MCV, MCH and MCHC ($p<0.001$). Although it correlated negatively with serum ferritin and positively with sTfR, the correlation was not significant.

Serum Ferritin (Table II): Serum ferritin positively and significantly correlated with MCV, MCH and MCHC ($p<0.05$) but insignificantly and positively with Hb. It was negatively but insignificantly correlated with RDW, HDW and sTfR.

SerumTransferrin receptors (sTfR) (Table II): Although it did not show significant correlation with any of the parameters, the correlation was positive with both RDW and HDW and negative with Hb, serum ferritin, RBC, PCV, MCV, MCH, MCHC.

Comparing Serum Ferritin with HDW and RDW: Low serum ferritin of < 12µg/l with a high HDW of >3.2 g/dl was found in 4 women while 24 women (85.7%) had raised HDW of >3.2g/dl with a serum ferritin of >12 µg/l. Similar trend was also observed between RDW and serum ferritin where serum ferritin was <12 µg/l and RDW was >14.5% in 4 subjects while serum ferritin of >12 µg/l with a high RDW of >14.5% was seen in 25 subjects (89.3%).

TABLE III shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of the new parameters RDV and HDW with the standard parameters serum ferritin and hemoglobin.

HDW when compared with ferritin showed a sensitivity of 100%, a specificity of 14%. The PPV was 14% and NPV was 100%.Similarly HDW with Hb showed a sensitivity of 92% and a specificity of 20%. The PPV was 62% and NPV was 66%.

Sensitivity, specificity, PPV, NPV of RDW with Hb and S.ferritin was done. Hb showed a sensitivity of 92% and specificity of 20% with RDW while the PPV was 62% and NPV was 66%.With S.ferritin sensitivity of RDW was 100%, specificity was 10%, PPV was 10% and NPV was 100%.

IV. DISCUSSION

In the present study, out of 48 pregnant women, 28 women (58.3%) had anaemia of different grades. This shows that there is still a high prevalence of anaemia in pregnant women in our city. This observation is similar to that by Dr Hamzullah Khan et al. Among the 28 women with anaemia in our study, 10 (20.83%) had mild anaemia and 9 women each (18.75%) had moderate and severe grades of anaemia respectively. Hence, in our study an almost equal prevalence of different grades of anaemia in the pregnant women with slight increase in number of mild anaemia cases was observed. The prevalence of severe anaemia (18.75%) observed in our study is almost similar to the report of ICMR task force study conducted in 16 districts of India by Dr Toteja.[15]

In our study serum ferritin estimation could be done in the samples of only 32 women as the remaining samples were hemolyzed due to improper collection and transport of blood samples and hence could not be used for the analysis. However, in the 32 samples only 4 samples (12.5%) showed low SFr levels of <12µg/l while the remaining 28 samples (87.5%) showed values >12µg/l.The high serum ferritin values observed in our study could be due to added inflammation/ infection which also elevate serum ferritin levels.

However, RDW which was estimated and analyzed in all the 48 women, was observed to be raised (> 14.5%) in 42 (87.5%) and <14.5% in 6 women (12.5%). A similar finding of elevated RDW with a low Hb level was reported in 84.2% subjects in a study by Sultana et al.[16]

Exactly similar to RDW, HDW, which is a new parameter obtained in the new hematology analyzers, also showed

similar values in 48 pregnant women in our study. Out of the total 48 women, 42 (87.5%) had elevated HDW values of >3.2 g/dl while only 6 (12.5%) had values <3.2 g/dl. This finding shows that values of HDW are similar to RDW in anaemia. Moreover it was observed that both RDW and HDW levels were more than the normal in a greater number of subjects and in all grades of anaemia in comparison with other RBC indices like MCV, MCH and MCHC. Both RDW and HDW correlated negatively and significantly with Hb and various RBC indices and positively but without any statistical significance with serum ferritin and sTfR. Both HDW and RDW are relatively new routine parameters which are evaluated in fully automated hematology analyzers, and are a part of complete blood count. RDW can reflect early changes in RBCs, which are accompanied by IDA. In our study HDW values were observed to be similar to RDW values and hence may be analyzed to reflect early changes in Hb levels of red cells in IDA. Therefore CBC can be used as a simple and cheap test to detect IDA through RDW[16] and also HDW estimation. Similar to RDW there is less published data on the performance of HDW during pregnancy.

HDW being a new parameter, we attempted to study its sensitivity and specificity with iron and Hb. In our study of a limited number of women, when compared with serum ferritin, HDW had a sensitivity of 100% and a specificity of 14%. The PPV was 14% and NPV was 100%.When compared with Hb, HDW showed a sensitivity of 92% and a specificity of 20% with a PPV of 62% and NPV of 66%.

Similarly sensitivity, specificity, PPV and NPV of *RDW* with Hb and ferritin was also done. Surprisingly the values were similar to those of HDW. With Hb the sensitivity of RDW in predicting anaemia was 92% and the specificity was 20% while the PPV was 62% and NPV was 66%.With ferritin the sensitivity of RDW in prediction of IDA was 100% and the specificity was 10% while PPV was 14% and NPV was 100%. In contrast, in a study among pregnant women in Sudan, the sensitivity of RDW was low (43.8%) while the specificity was moderate (73.7%).[17]Another study by Alquaiz et al [18] showed RDW value > 16.1% had a sensitivity of 59.3% and specificity of 71%. However, a high sensitivity (82.3%) and specificity of 97.4% for RDW was reported among pregnant women by Sultana et al.[16] Hence, different rates of RDW have been reported. Similar to our study, a high sensitivity of RDW (81%) and a low specificity of 53.4% was reported by Aulakh et al [19] and Van Zeben et al who found a sensitivity of RDW was 94% and a specificity of 59%.[20] A study reported that Hb level < 9.7g/dl and RDW > 15 (in a gestational age of <20weeks) predicted iron deficiency with a high specificity.[21]

Correlation between different parameters was done in our study with the primary focus on parameters like Hb, RDW, HDW, serum ferritin and sTfR.

Among the total 48 pregnant women of our study a statistically significantly (p <0.05) positive correlation was observed between Hb and RBC, PCV, MCV, MCHC and it was statistically significantly negatively correlated with RDW. Both HDW and sTfR were negatively correlated with Hb albit it was not statistically significant. sTfR is known to be negatively correlated with Hb as sTfR levels are found to increase with falling Hb levels.

RDW was positively and significantly correlated with HDW while negatively and significantly correlated with Hb, PCV, MCV, MCH and MCHC. It was negatively correlated with serum ferritin and positively correlated with sTfR although not significantly.

Thus, HDW being a new parameter significantly correlated with RDW and significantly but negatively with MCH and MCHC. Moreover although HDW correlated negatively with serum ferritin and Hb and positively with sTfR, the correlation was not significant. Based on these features, HDW if estimated in more number of subjects, like RDW, may predict early onset of decrease in the concentration of Hb in cells in anemic pregnant women.

In our study, serum ferritin statistically significantly correlated with MCV, MCH and MCHC. Although there was positive correlation with Hb, it was insignificant. Similarly, it was negatively but insignificantly correlated with RDW, HDW and sTfR. In contrast in a study by Lt. Manu Tiwari et al, [22] they did not find any correlation between ferritin and Hb, MCV, MCH, MCHC respectively. In contrast in their study only RDW and RBC count correlated with the ferritin levels.

Researchers from Europe have worked on newer indices which are available on the new automated cell counters like ADVIA 120 and they found that the percentage of hypochromic red cells (% HYPOm) and reticulocytes (% HYPOr) and cellular Hb in reticulocytes (CGr) correlated with the results based on the usage of the combination of three commonly used tests(Hb, MCV, Ferritin).Hence, further studies are needed to determine the usefulness of the red cell indices in diagnosing iron deficiency during the course of pregnancy and such tests will be very useful in our country once such cell counters are cheaply available in India.

Our country being a developing country with many women finding it difficult to afford expensive investigations and anaemia also being a very common disorder in both pregnant and non-pregnant women, tests like RDW and HDW are very simple and can be conducted to predict anaemia in pregnancy. Hence, anaemia, especially iron deficiency anaemia can be predicted in pregnancy using lower cost tests, which could be incredibly useful tool in areas with limited resources and a high prevalence of the disease.

Our study however has few limitations. Firstly, the number of subjects is very small in order to conclude from the results. Secondly, markers of inflammation like C-reactive protein and Interleukin-6 should have been done to exclude inflammation. Thirdly, serum ferritin and sTfR could be estimated in a lesser number of samples as the samples were hemolyzed.

V. CONCLUSION

Our study confirmed that anaemia is still prevalent in our city with mild anaemia being prevalent in most cases. Iron deficiency as well as iron deficiency anaemia can be screened out early by increased levels of RDW and HDW even when RBC indices are normal. Based on this study, similar to RDW, HDW may also be useful in predicting iron deficiency but the finding needs to be confirmed in a study with larger sample size.

COMPLIANCE WITH ETHICAL STANDARDS

✓ Disclosure Of Potential Conflicts Of Interest

All the authors declare that they have no conflict of interest related to this study.

✓ Ethical Approval

All procedures performed in the study were in accordance with the ethical standards of our institute and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

✓ Informed Consent

Informed consent was obtained from all individual participants included in the study.

PARAMETER	MINIMUM VALUE	MAXIMUM VALUE	MEAN ± SD
Hb in g/dl	3.6	13.9	10.01 ± 2.6
RBC s X10^6 cells/µL	2.0	5.7	3.9 ± 0.82
PCV/HCT in %	13.8	47.1	32.5 ± 7.1
MCV in fl	65.4	113.8	83.3 ± 11.3
MCH in pg	16.1	33.4	25.6 ± 4.8
MCHC in g/dl	20.2	35.4	30.3 ± 3.3
RDW in %	13.1	28.1	19.1 ± 4.1
HDW in g/dl	2.5	9.0	4.2 ± 1.0
S.Ferritin in µg/l	8.1	82.5	29.8 ± 19.8
Transferrin Receptors ng/ml	206.6	765.9	286.2 ± 94.1

Table 1: showing Minimum and maximum values of different parameters along with mean values and standard deviation

Hb- Haemoglobin, RBC- Red blood cell, PCV- Packed cell volume, HCT- hematocrit, MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC- Mean corpuscular hemoglobin concentration, RDW- Red cell distribution width, HDW- Haemoglobin distribution width, S.Ferritin- Serum ferritin, sTfR- serum transferrin receptors, g/dl- gram per deciliters, µL- microlitres, fl- femtolitres, pg- picograms, %- percentage, µg/l – microgram per litre, ng/ml- nanogram per milliliter, SD- standard deviation.

	Hb	RBC	PCV	MCV	MCH	MCHC	RDW	HDW	S.Ferritinin	sTfR
Hb	1	0.735**	0.950**	0.416**	0.663**	0.657**	-0.598**	-0.147	0.321	-0.153
RBC	0.735**	1	0.835**	-0.145	0.031	0.211	-0.154	0.181	0.048	-0.029
PCV	0.950**	0.835**	1	0.362*	0.500**	0.441*	-0.423*	0.007	0.295	-0.172
MCV	0.416**	-0.145	0.362*	1	0.874**	0.352*	-0.350*	-0.190	0.475**	-0.215
MCH	0.663**	0.031	0.500**	0.874**	1	0.707**	-0.640**	-.338*	0.487**	-0.197
MCHC	0.657**	0.211	0.441**	0.352*	0.707**	1	-0.670**	-0.374*	0.368*	-0.017
RDW	-0.598**	-0.154	-0.423**	-0.350*	-0.640**	-0.670**	1	0.491**	-0.344	0.084
HDW	-0.147	0.181	0.007	-0.190	-0.338*	-0.374*	0.491**	1	-0.075	0.037
S.Ferritinin	0.321	0.048	0.295	0.475**	0.487**	0.368*	-0.344	-0.075	1	-0.094
sTfR	-0.153	-0.029	-0.172	-0.215	-0.197	-0.017	0.084	0.037	-0.094	1

** Correlation is significant at the 0.001 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 2: showing Correlations Between Different Parameters

Hb- Hemoglobin, RBC- Red blood cell, PCV- Packed cell volume, MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC- Mean corpuscular hemoglobin concentration, RDW- Red cell distribution width, HDW- Hemoglobin distribution width, S.Ferritin- Serum ferritin, sTfR- serum transferrin receptors.

Parameter	Sensitivity in %	Specificity in %	PPV in %	NPV in %
Between HDW and Ferritin	100	14	14	100
Between RDW and Ferritin	100	10	10	100
Between HDW and Hb	92	20	62	66
Between RDW and Hb	92	20	62	66

PPV- Positive predictive value
NPV- Negative predictive value
HDW- Hemoglobin distribution width
RDW- Red cell distribution width
Hb- Hemoglobin

Table 3: showing Sensitivity, specificity, positive and negative predictive values between selected parameters like HDW, RDW, haemoglobin and ferritin

LEGENDS

✓ Table I: Minimum and maximum values of different parameters along with mean values and standard deviation.
✓ Table II: Pearson's correlations between different parameters.
✓ Table III: Sensitivity, specificity, positive and negative predictive values between selected parameters like HDW, RDW, haemoglobin and ferritin.

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