



“Red Cell Parameters Variation in Third Trimester in Normal Pregnancy”

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ABSTRACT

Background: Haematological profile is considered one of the factors affecting pregnancy and its outcome. The study reported here investigated the variation in some haematological parameters during third trimester of normal pregnancy.

Subjects and Methods: The study reported here is a cross-sectional study of 171 pregnant women in their third trimester who attended a tertiary health centre in India. Full blood count was performed using a Sysmex Hematology, a six-part auto analyzer able to test all parameters.

Results: Overall, the values obtained were (mean \pm standard deviation [SD]): Red cell mass, 4.222 ± 0.9329 cells/ml; hematocrit level, $34.865 \pm 4.299\%$; hemoglobin concentration, 11.011 ± 1.6399 g/dL; Red cell distribution width, $15.801 \pm 3.004\%$; white blood cells, 10.267 ± 3.102 cells/mm³; cell volume 84.261 ± 9.514 fL, corpuscular hemoglobin, 26.717 ± 3.566 pg; and corpuscular hemoglobin concentration, 31.524 ± 1.640 g/dL; neutrophil count, $68.391 \pm 8.610\%$; lymphocyte count, $22.080 \pm 7.442\%$; monocyte count, $7.112 \pm 4.957\%$; eosinophil count, $2.547 \pm 2.636\%$. These values were compared with their non-pregnant control values and for correlation p-values were calculated. There was statistically significant association between all parameters except red cell mass and eosinophil count.

Conclusion: It can be concluded that pregnancy in women alters haematological indices such as haemoglobin, red cell mass, hematocrit, red cell distribution width, red cell indices, total leukocyte counts and differentials. The review highlights that pregnancy is a state characterized by many physiological haematological changes, which may appear to be pathological in the non-pregnant state.

Keywords: Normal Pregnancy, Third trimester, Haematology, Physiology

Introduction

The haematological profile of an individual to a large extent reflects their general health (1) and many studies have identified the haematological profile of the pregnant woman as one of the factors affecting pregnancy and its outcome (2-7). Normal pregnancy is characterized by profound changes in almost every organ and system to accommodate the demands of fetoplacental unit (8). In normal pregnancy, the physiological change in haemoglobin concentration [Hb] and platelet count during pregnancy are well known phenomena [9]. It is also one of the physiological conditions capable of causing remarkable and dramatic changes in haematological variables.

The haematological indices of an individual to a large extent reflect their general health. Many of the haematological indices are influenced by factors like sex, seasonal variation, lactation, pregnancy health, and nutritional status (10). It is also acknowledged that for comparisons between individuals and with reference data in a clinical diagnostic situation, it is necessary to consider the normal variations due to sex, age, and breed in order to increase diagnostic precision (11).

Many studies such as Osonuga et al. (12) and Shaw et al. (13) have identified the haematological indices of the pregnant woman as one of the factors affecting pregnancy. Anaemia (low haemoglobin) is a widely identified haematological abnormality (14) and it is also associated with adverse pregnancy outcome (15). Anaemia in pregnant women is variously defined with two common parameters either as haemoglobin concentration less than 11.0 g/dL or 5th percentile of the distribution of haemoglobin concentration or hematocrit in a healthy reference population (16). This assessment is possible through a series of tests measuring different variables (17). This study is of importance because systems monitored during antenatal care in an attempt to predict and/or improve pregnancy outcome are dependent on the quality and quantities of haematological indices.

Material & Methods

The study reported here is a cross-sectional study of 171 pregnant women in their third trimester who attended a tertiary health centre in India. During the study, all third trimester pregnant women who gave informed consent and satisfied the study inclusion criterion (normotensive blood pressure < 140/90 mmHg) were recruited into the study.

Pregnant women with any of the following conditions were excluded from the study: bleeding disorders, splenomegaly, connective tissue disease such as systemic lupus erythematosus, diabetes, hypertension, human immunodeficiency virus (HIV), and hepatitis B infection. In addition, women on nonsteroidal anti-inflammatory drugs such as aspirin were also excluded.

Demographic data and information on drug history were collected directly from the recruited participants, and additional data – such as HIV/hepatitis B status – were extracted from clinical notes. All study participants were on routine ferrous sulphate (200 mg three times daily), folic acid (5 mg daily), and vitamin B complex (one taken three times daily) tablets.

The research was approved by the ethics review committees of the Hospital.

A blood sample (4.5 mL) was withdrawn from each participant with minimal stasis from the antecubital

vein using a dry, sterile disposable syringe and needle. The blood was dispensed into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The specimens were labelled with the subject's age, and identification number. The EDTA samples were kept at room temperature until processing, which occurred within 4 hours of collection.

Full blood count was performed using a Sysmex Hematology, a six part auto analyzer able to test all parameters per sample including Hb concentration, PCV, RBC concentration, MCH, MCV, MCHC and WBC count. Standardization, calibration of the instrument, and processing of the samples were done according to the manufacturer's instructions.

Statistical analysis: Data were analyzed using SPSS (v 16; IBM, Armonk, NY, USA). The descriptive data are presented herein as means \pm standard deviation (SD).

Results

TABLE 1: correlation between mean and standard deviation of test and control of various haematological parameters.

Parameters	Mean and standard Deviation of Test	Mean and Standard Deviation of Control
Hb(Haemoglobin)g/dl	11.011 \pm 1.6399	11.8 \pm 1.5
RBC(cells/ μ l)	4.222 \pm 0.9329	4.41 \pm 0.23
PCV(Packed cell volume)%	34.865 \pm 4.299	40.1 \pm 1.06
RDW (Red cell distribution width) %	15.801 \pm 3.004	14.04 \pm 1.56
MCV (Mean corpuscular volume) fl/cell	84.261 \pm 9.514	79.56 \pm 5.61
MCH (Mean corpuscular haemoglobin) pg/cell	26.717 \pm 3.566	28.74 \pm 2.18
MCHC (Mean corpuscular haemoglobin concentration) g/dl	31.524 \pm 1.640	33.01 \pm 1.45
TLC (Total leukocyte count) cells/mm ³	10.267 \pm 3.102	8.7 \pm 1.20
NEUTROPHIL (%)	68.391 \pm 8.610	63.91 \pm 7.54
LYMPHOCYTE (%)	22.080 \pm 7.442	19.15 \pm 4.65
MONOCYTE (%)	7.112 \pm 4.957	3.81 \pm 2.76
EOSINOPHIL (%)	2.547 \pm 2.636	2.57 \pm 2.52

TABLE 2: difference between the observed means in two independent samples, significance value (p-value) and 95% confidence interval (CI) of the difference.

Parameters	Difference	Standard Error	95% CI	T-Statistic	DF	Significance Level
Hb	0.789	0.208	0.3788-0.1992	3.788	258	0.0002
RBC	0.188	0.101	-0.0100- 0.3660	1.870	258	0.0626
PCV	5.235	0.463	4.322-6.147	11.301	258	0.0001
RDW	-1.761	0.340	-2.4310- -1.0910	-5.176	258	0.0001
MCV	-4.701	1.097	-6.8602- -2.518	-4.287	258	0.0001
MCH	2.023	0.413	1.2091-2.8369	4.894	258	0.0001
MCHC	1.486	0.206	1.0799-1.8921	7.206	258	0.0001

Parameters	Difference	Standard Error	95% CI	T-Statistic	DF	Significance Level
TLC	-1.567	0.342	-2.2397- -0.8943	-4.587	258	0.0001
NEUTROPHIL	-4.481	1.080	-6.6072- -2.3548	-4.150	258	0.0001
LYMPHOCYTE	-2.930	0.866	-4.6347- -1.2253	-3.385	258	0.0008
MONOCYTE	-3.302	0.567	-4.4177- -2.1863	-5.828	258	0.0001
EOSINOPHIL	0.023	0.339	-0.6454- 0.6914	0.068	258	0.9460

Discussion

Pregnancy is a state characterized by many physiological haematological changes, which may appear to be pathological in the non-pregnant state. This review highlights most of these changes along with the scientific basis for the same, as per the current knowledge, with a special reference to the red blood, white blood cells and red cell indices.

During pregnancy, the total blood volume increases by about 1.5 litres, mainly to supply the demands of the new vascular bed and to compensate for blood loss occurring at delivery (18). Of this, around one litre of blood is contained within the uterus and maternal blood spaces of the placenta. Increase in blood volume is, therefore, more marked in multiple pregnancies and in iron deficient states. Expansion of plasma volume occurs by 10–15 % in 6–12 weeks of gestation (19,20). During pregnancy, plasma renin activity tends to increase and atrial natriuretic peptide levels tend to reduce, though slightly. This suggests that, in pregnant state, the elevation in plasma volume is response to an under filled vascular system resulting from systemic vasodilatation and increase in vascular capacitance, rather than actual blood volume expansion, which would produce the opposite hormonal profile instead (i.e. low plasma renin and elevated atrial natriuretic peptide levels) (21,22). Red cell mass (driven by an increase in maternal erythropoietin production) also increases, but relatively less, compared with the increase in plasma volume, the net result being a dip in haemoglobin concentration. This was identical to our case and RBC mean value was statistically not significant with a p-value of >0.05.

It was discovered that there was significant difference (p-value <0.0001) in the PCV of the test group ($34.865 \pm 4.299\%$) when compared to the control ($40.1 \pm 1.06\%$). This finding is in line with those of James et al. (23). The decrease in PCV may be due to increase in plasma volume during pregnancy which causes haemodilution, and increased rate of infection especially malaria, hormonal

changes, and conditions that promote fluid retention and iron deficiency.

The Hb cut-off value for pregnant women is 11g/dl (24). During pregnancy there is increase in iron requirement due to growing foetus and increase in maternal blood volume. The drop in haemoglobin is typically by 1–2 g/dL by the late second trimester and stabilize thereafter in the third trimester, when there is a reduction in maternal plasma volume (owing to an increase in levels atrial natriuretic peptide). Women who take iron supplements have less pronounced changes in haemoglobin, they increase their red cell mass in a more proportionate manner than those not on hematinic supplements. Maternal change leads to decrease in Hb concentration in first trimester which further decline and reaches its lowest level during second trimester. During second trimester Hb concentration diminish approximately 5 g/L. It rises again during third trimester (24). Currently, there are no WHO recommendations on the use of different Hb cut-off points for anaemia by trimester (24). In our study, we observed Hb level above cut-off value of 11g/dL during the third trimesters, as recommended by the WHO. As established, we did not observe drop in Hb level during the third trimester. In our study, result of the blood haemoglobin showed a statistically significant difference (p-value <0.0002) between the test and the control group.

The red blood cell indices change little in pregnancy. However, there is a small increase in mean corpuscular volume (MCV), of an average of 4 fl in an iron-replete woman, which reaches a maximum at 30–35 weeks' gestation and does not suggest any deficiency of vitamins B1 and folate. Increased production of RBCs to meet the demands of pregnancy, reasonably explains why there is an increased MCV (due to a higher proportion of young RBC which are larger in size). However, MCV does not change significantly during pregnancy, in our study there was a statistically significant difference in between the test (mean MCV was 84.261 ± 9.514) and the control groups (mean was 79.56 ± 5.61).

Similarly, Our MCH and MCHC of test was statistically significant with a p-value of <0.0001 as compared to the controls. Casanova BF, Sammel MD et al explained this phenomenon as a possible emergence of young red blood cells of different sizes and degrees of hemoglobinization, recruited by the high erythropoietic demand of pregnancy which co corroborated with our present study (25).

Red cell distribution width (RDW) is a measure variation of red blood cell width that is reported as part of a standard complete blood count. RDW increased significantly during pregnancy, mainly in III trimester or at onset of labour. The unexpected rise in RDW during last few weeks suggest increased bone marrow activity. Studies reported it as a useful indicator of impending parturition (26). In our case also, we observe a significant difference between the test and the control groups.

White blood cell count is increased in pregnancy with the lower limit of the reference range being typically 6,000/cumm. Leucocytosis, occurring during pregnancy is due to the physiologic stress induced by the pregnant state (27). Neutrophils are the major type of leucocytes on differential counts (28,29). This is likely due to impaired neutrophilic apoptosis in pregnancy (28). The neutrophil cytoplasm shows toxic granulation. Neutrophil chemotaxis and phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female (30). There is also evidence of increased oxidative metabolism in neutrophils during pregnancy. Immature forms as myelocytes and metamyelocytes may be found in the peripheral blood film of healthy women during pregnancy and do not have any pathological significance (31). They simply indicate adequate bone marrow response to an increased drive for erythropoiesis occurring during pregnancy.

There is an absolute monocytosis during pregnancy, especially in the first trimester, but decreases as gestation advances. Monocytes help in preventing foetal allograft rejection by infiltrating the decidual tissue (7th– 20th week of gestation) possibly, through PGE2 mediated immunosuppression (32). The monocyte to lymphocyte ratio is markedly increased in pregnancy. Eosinophil and basophil counts, however, do not change significantly during pregnancy (33).

The stress of delivery may itself lead to brisk leucocytosis. Few hours after delivery, healthy women have been documented as having a WBC count varying from 9,000 to 25,000/cumm.

According to our study, Total leucocyte count (TLC), Neutrophils, lymphocytes and monocytes also showed

significant differences (p-value <0.0001) although the eosinophil count showed no significant difference, there was an increased level compared to the control; thus the observation of a no significant difference in the total WBC count is in variance with the studies of Osonuga et al. (34). who observed a significant variation in the total WBC count of test groups compared to control. The observation of the various significant variations between the TLC, granulocytes and lymphocytes has also been observed in previous studies by Wahed et al (35) and previous work by Luppi (36).

This may be as a result of the body building the immunity of the fetus and it is achieved by a state of selective immune tolerance, immunosuppression, and immunomodulation in the presence of a strong antimicrobial immunity. There is also downregulation of potentially dangerous T-cell-mediated immune responses, while activating certain components of the innate immune system, such as neutrophils. is unique dysregulation between different components of the immune system plays a central role in the maternal adaptation to pregnancy.

In our study, mean WBC count was 10.267 ± 3.102 (Control values = 8.7 ± 1.20). In our differential count, Neutrophil, lymphocyte, monocyte had statistically significant difference with a p-value of <0.0001 and eosinophil had p-value of 0.9460 which was non-significant.

According to the study of Edlestam G, Lowbeer C, Kral G et al eosinophil counts do not show any change which was in concordance to our study.

Conclusion

The objective of the study was to evaluate the values of some major haematological indices among normal pregnant women. Thus this study focuses on the diagnostic evaluation of various conditions specifically the role of variation in white blood cell (WBC) count, haemoglobin, packed cell volume (PCV), granulocytes, lymphocyte, and RDW in the diagnosis of complications or challenges during pregnancy.

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