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Establishing Normal Reference Ranges Of Various Hematological Parameters In Pregnant Females In A Rural Tertiary Care Hospital In North India

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Abstract

Background: There are physiologic changes in many hematological parameters in pregnancy compared to non-pregnant healthy females which are principally influenced by changes in the hormonal milieu hormonal milieu. We carried out this research to establish normal reference ranges for various hematological parameters like WBC count, RBC count, haemoglobin and platelet count in a population of pregnant females from rural setting and compare the results with studies done elsewhere.

Methods: We followed 576 apparently healthy pregnant females attending the antenatal clinic at health care facility in BPS Government Medical College, Khanpur Kalan, Sonepat from December 2020 to September 2021. Written informed consent was taken from each participant after the study got approval from Institute's Ethics Committee. A complete blood count was performed on venous blood sample using Beckman coulter LH 750 and the results were analyzed using SPSS (ver-20) software.

Result: The mean values (and 95% confidence intervals, CI) of hematological indices were as follows:
First trimester- Haemoglobin (Hb) 11.18(10.62-11.73) g/dl, TLC 6.27(5.87-6.67) 10^3/µL, DLC; Neut 61(64.33-67.36)%, Lym 34(27.39-30.45) %, Mono 3.68(3.72-4.34) %, Eos 0.9(0.91-1.30) %, Bas 0.009(0-0.10) %, RBC Count 4.41(4.25-4.56) 10^6/µL, Platelet Count 3.36(3.01-3.71) 10^5/µL
Second trimester- Haemoglobin(Hb) 11.09(10.63-11.55) g/dl, TLC 8.56(8.26-8.85) 10^3/µL, DLC; Neut 67(64.76-69.33) %, Lym 28(25.48-30.11) %, Mono 3.78(3.28-4.28) %, Eos 1,2(0.77-1.4) %, Bas 0.03(0-0.08) %, RBC Count 4.26(4.06-4.46) 10^6/µL, Platelet Count 2.79(2.56-3.02) 10^5/µL
Third trimester- Haemoglobin(Hb) 10.86(10.27-11.45) g/dl, TLC 11.22(10.75-11.69) 10^3/µL, DLC; Neut 69(66.73-71.86) %, Lym 24(22.36-26.87) %, Mono 4.72(4.13-5.30) %, Eos 1.3(0.97-1.62) %, Bas 0.03(0-0.09) %, RBC Count 4.25(4.14-4.35) 10^6/µL, Platelet Count 2.68(2.36-2.99) 10^5/µL

Discussion: The following hematological indices: TLC, DLC (Neutrophil and Lymphocyte), platelet count of women across the trimesters was highly statistical significant (p value < 0.001 in each case).

Keywords: blood count, hematology, pregnancy, reference ranges, trimester

Introduction

Hematological profile is measured all over the world to estimate general health, because it is a reliable indicator and is a simple, fast and cost-effective test.[1] It is considered to be one of the factors affecting pregnancy and its outcome. Physiological changes in pregnancy and puerperium are principally influenced by changes in the hormonal milieu and this is also reflected in the hematological profile of a pregnant female.

During pregnancy, the total blood volume increases by about 1.5 liters, mainly to supply the demands of the new vascular bed and to compensate for blood loss occurring at delivery.[2] Red Blood Cell (RBC) 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 (Hb) concentration. Thus, there is dilutional anemia.[3]

White Blood Cell (WBC) count is increased in pregnancy due to the physiologic stress and inflammatory response induced by the pregnant state.[4] This leucocytosis is mainly due to neutrophilia and immature forms like metamyelocytes and myelocytes (neutrophil left shift) may be present in the peripheral blood film along with monocytosis.[5] The neutrophilia of pregnancy is associated with a left shift to enhance phagocytosis by engaging younger band forms and there may be toxic granulations due to poor oxidative metabolism.[6] This boost in non-specific (innate) immunity is said to be a compensation for the attenuation of specific immunity (particularly cell mediated immunity) in pregnancy.[7,8] The impairment of specific immunity correlates with a reduction in lymphocyte count.[9] Monocytes help in preventing fetal allograft rejection by infiltrating the decidual tissue (7^{th} to 20^{th} week of gestation) possibly, through PGE_2 mediated immunosuppression.[10] The monocyte to lymphocyte ratio is markedly increased in pregnancy. Eosinophils and basophils also decline in number with increasing gestational age.[11]

Large cross-sectional studies done in pregnancy of healthy women (excluding hypertensive states) have shown that the platelet count decreases as the pregnancy advances. This is termed as 'gestational thrombocytopenia.' It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance as they pass over scarred and damaged surface of placenta.[12] Although most cases of thrombocytopenia in pregnancy are mild and fall within the normal range with no adverse outcome for mother or baby, occasionally a low platelet count may be part of a complex disorder with significant morbidity and may cause life-threatening post partum hemorrhage.

Establishing normal reference ranges is essential to know what is abnormal for that population and that specific group and what is caused by disease. There are changes in many hematological parameters in pregnancy compared to non-pregnant healthy females. As an example, a slight leucocytosis can be a normal finding in pregnancy which may mean an infection in a non pregnant female. Many hematological changes also, occurring during this period, are physiological. Therefore, to the clinician, it is important to know the changes in pregnancy so that they are not considered as a sign of disease. Populations in different geographic regions display slightly different reference ranges owing to the socioeconomic and racial disparity. We have to know what is normal to decide upon what is abnormal.

Ideally, it is expected that every laboratory should establish its own reference ranges. Normal reference ranges of blood parameters for pregnancy have been reported in literature. However, this is the first such study conducted in a rural setting in North Western India.

Review Of Literature:

Many studies have been carried out in different parts of the world aiming to establish normal reference ranges of hematological parameters in pregnant females.

Akinbami et alcarried out a similar study in Nigeria in 2010 taking a sample size of 274 healthy pregnant females who attended the antenatal clinic in Lagos State University Teaching Hospital. [13] They employed similar methodology and generated results using an automated hematology analyzer. A statistically significant relationship was found to exist between white blood cell count with increasing gestational age (P=0.001).

Another study was carried out by Akingbola et al in north western Nigeria in 2009. However their study spanning over 8 months also included a control group of non pregnant healthy females to compare their research findings obtained in pregnant healthy females. [14] In that study, pregnancy was characterised by lowest values of haemoglobin parameters in trimester three and there were statistically significant differences between the WBC, platelet counts, RBC count of women between the three trimesters.

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Recently Rayis et al carried out a similar study in Sudan in 2015 in order to evaluate trimester pattern of change and reference ranges of hematological profile among Sudanese women with normal pregnancy. [15] They followed 143 women with singleton gestation since early pregnancy until the third trimester in Saad Abu-Alela Hospital, Khartoum, Sudan, during the period of January-December 2015. The trimester reference range of RBC, WBC and platelets and other hematological indices are mostly parallel to international records.

In India, many studies have been conducted, which have studied hematological variables during pregnancy. Shah et alstudied the hematological variations during three different trimesters of pregnancy and their compared their result with matched non pregnant controls. [16] It was a longitudinal study conducted in a cohort of 302 normal apparently healthy rural pregnant women, attending the antenatal clinic of Dhiraj General Hospital, Vadodara, Gujarat, India. Apparently healthy 94 non pregnant women matched with age and socioeconomic status was studied as control. A significant finding was increase in RBC count with increasing gestational age as opposed to the findings of many other national and international reports which found a decrease in RBC count with advancing pregnancy.

Das et al also studied hematological parameters across the three trimesters but reported no significant difference in all hematological parameters among the three trimesters. [17]

Methodology:

Study Population

The study was a cross-sectional study of 576 pregnant women who attended antenatal clinic at health care facility at Bhagat Phool Singh Government Medical College for Women (BPS GMC (W)), Sonepat, India. During the study period between December 2020 and September 2021, all pregnant women who gave informed consent and satisfied the study inclusion criterion (normotensive blood pressure < 140/90 mmHg and found healthy on the clinical examination by Obstetrics and Gynaecology Consultant) were recruited into the study. Pregnant women with any of the following conditions were excluded from the study: hypertension, urinary tract infection, human immunodeficiency virus (HIV), and hepatitis B infection. In addition, women on non-steroidal anti-inflammatory drugs such as aspirin were also excluded.

Demographic data and information on drug history were collected directly from the study participants and additional data – such as HIV/Hepatitis B status – were extracted from clinical notes. All study participants were on routine iron and folic acid supplementation.

Ethics

The research was approved by the Institute's Ethics committee.

Sample Collection

A blood sample (3 mL) was withdrawn from each participant with minimal stasis from the antecubital vein using a dry, sterile disposable syringe and needle under strict aseptic conditions. The blood was dispensed into vacutainers containing the anticoagulant potassium ethylene diamine tetra acetic acid (EDTA). The specimens were labeled with the subject's age, and identification number. The EDTA samples were kept at room temperature until processing in the Haematology laboratory at BPS GMC (W), which occurred within 2-3 hours of collection.

Laboratory Analysis

Complete blood count was performed using Beckman Coulter LH 750 Hematology Analyzer, a five part auto analyzer able to test various hematological parameters per sample including Hb concentration, Packed Cell Volume (PCV), RBC concentration, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), WBC count, Differential Leucocyte Count (DLC) and platelet count. Standardization, calibration of the instrument, and processing of the samples were done according to the manufacturer's instructions.

Procedure

Results of the analysis were displayed after about 30 seconds and white cell counts, platelet count and haemoglobin levels were obtained from the computer print-outs from the machine. Slides for the differential leukocyte count were prepared from the

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same blood sample and examined manually under the light microscope. Hundred cells were counted.

Statistical Analysis

The data generated was analyzed using the Microsoft Excel 2007 and SPSS (ver-20) software. The medians were calculated and reference ranges were determined at 95% confidence interval. Mean and standard deviations were computed for each of the hematological parameters of the study subjects. Differences between the means in the groups were assessed using the student's t-test. The p-values < 0.05 were considered as statistically significant and p-values < 0.01 were considered as statistically highly significant.

RESULT:

The mean age of the pregnant women was 25.81 years (S.D. 4.72) and ranged between 19 and 38 years. 32% of the women were in their first trimester, 36 % in their second trimester while 32% were in their third trimester at booking.

Hemoglobin Concentration:

Hb concentration decreased progressively with gestational age. First trimester; 11.18 ± 1.6g/dl, second trimester; 11.09 ± 1.4g/dl, third trimester; 10.86 ± 1.7g/dl. The differences between the means were not statistically significant from the first to the second trimester (p = 0.65); from the second to the third trimester (p = 0.5).

WBC Count:

WBC Count increased progressively with gestational age. First trimester; 6.27 ± 1.14 x 103/µL, second trimester; 8.56 ± 0.9 x 103/µL and third trimester, 11.22 ± 1.34 x 103/µL. The differences between the means were statistically significant from the first to the second trimester (p = 0.01); from the second to the third trimester (p = 0.01). The difference in mean WBC count between the first and the third trimester was highly significant (p = 0.024).

DLC:

Neutrophils increased progressively from 61 ± 6% in first trimester to 67 ± 7% in second trimester and 69 ± 7% in third trimester. However lymphocytes continued to decline with the advancement of the pregnancy from 34 ± 6% in first trimester to 28 ± 7% in second trimester and 25 ± 6% in the third trimester. Rise in monocytes was not marked.

RBC Count:

RBC Count decreased progressively with gestational age. First trimester; 4.41 ± 0.46 x 10^6 /µL, second trimester; 4.26 ± 0.60 x 10^6/µL and third trimester, 4.25 ± 0.31 x 106/µL. The differences between the means were not statistically significant from the first to the second trimester (p=0.24); from the second to the third trimester (p=0.6).

Platelet Count:

Platelet count decreased progressively with gestational age. First trimester; 3.36 ± 1.01 x 10^5/µL, second trimester; 2.79 ± 0.7 x 10^5/µL and third trimester, 2.68 ± 0.9 x 10^5/µL. The differences between the means were statistically significant from the first to the second trimester (p=0.01); insignificant from the second to the third trimester (p=0.6). The difference in mean Platelet count between the first and the third trimester was highly significant (p=0.007).

Table1: Hematological indices across the three trimesters in pregnant female

Indices	Parameters	Overall	First Trimester	Second Trimester	Third Trimester
Hb(g/dl)	Mean±S.D.	11.05±1.6	11.18±1.6	11.09±1.4	10.86±1.7
	Ref Range	10.7-11.36	10.62-11.73	10.63-11.55	10.27-11.45

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TLC (10^3/µL)	Mean±S.D.	8.68±2.3	6.27±1.14	8.56±0.9	11.22±1.34
	Ref Range	8.23-9.13	5.87-6.67	8.26-8.85	10.75-11.69
DLC: Neut(%)	Mean±S.D.	65.8±7.7	61.03±6.5	67.05±7	69.3±7.4
	Ref Range	64.33-67.36	58.77-63.28	64.76-69.33	66.73-71.86
Lym(%)	Mean±S.D.	28.9±7.8	34.4±6.5	27.8±7.1	24.62±6.5
	Ref Range	27.39-30.45	32.14-36.65	25.48-30.11	22.36-26.87
Mono(%)	Mean±S.D.	4.03±1.6	3.63±1.4	3.78±1.53	4.72±1.7
	Ref Range	3.72-4.34	3.14-4.11	3.28-4.28	4.13-5.30
Eos(%)	Mean±S.D.	1.11±1	0.91±1.02	1.11±1.03	1.3±0.93
	Ref Range	0.91-1.30	0.55-1.2	0.77-1.4	0.97-1.62
Baso(%)	Mean±S.D.	0.05±0.26	0.09±0.46	0.03±0.16	0.03±0.17
	Ref Range	0-0.10	0-0.22	0-0.08	0-0.09
RBC Count(10^6/µL)	Mean±S.D.	4.30±0.48	4.41±0.46	4.26±0.6	4.25±0.31
	Ref Range	4.21-4.39	4.25-4.56	4.06-4.46	4.14-4.35
Platelet Count (10^5/µl)	Mean±S.D.	2.9±0.93	3.36±1.01	2.79±0.7	2.68±0.9
	Ref Range	2.75-3.12	3.01-3.71	2.56-3.02	2.36-2.99

Table 2: Comparison of trimester specific mean (SD) of hematological parameters between our study and previous international reports

Parameters	Trimester	Our Study	West India[16]	China[1]	Jamaica[23]	Sudan[15] (N. Africa)	Morocco[24]
Hb(g/dl)	T1	11.18(1.6)	10.48(0.89)	12.20(0.92)	12.73(1.14)	10.81(1.22)	12.23(0.93)

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	T2	11.09(1.4)	10.06(1.04)	11.30(0.89)	11.41(1.16)	10.62(0.93)	11.68(0.96)
	T3	10.86(1.7)	10.02(1.26)	11.50(0.99)	11.67(1.18)	10.83(1.13)	11.22(1.06)
TLC (10^3/µL)	T1	6.27(1.14)	7.85(1.41)	8.30(2.02)	8.27(2.60)	7.69(1.96)	7.52(1.78)
	T2	8.56(0.9)	9.70(2.43)	9.50(2.22)	9.66(2.84)	8.45(1.97)	8.03(2.02)
	T3	11.22(1.34)	10.17(1.14)	9.10(2.17)	8.79(2.50)	8.36(2.11)	9.53(2.39)
DLC(10^3/µL)	T1	3.82(0.78)	-	-	-	6.54(10.14)	4.68(1.59)
	T2	5.77(1.03)	-	-	-	7.07(8.95)	5.18(1.8)
	T3	7.83(1.58)	-	-	-	5.89(1.87)	6.56(2.15)
Lymph	T1	2.16(0.58)	-	-	-	2.20(1.32)	2.16(0.56)
	T2	2.35(0.49)	-	-	-	1.98(0.45)	2.15(0.6)
	T3	2.72(0.62)	-	-	-	1.87(0.48)	2.20(0.66)
RBC Count(10^6/µL)	T1	4.41(0.46)	4.00(0.42)	4.05(0.36)	4.33(0.40)	4.30(0.36)	4.21(0.36)
	T2	4.26(0.6)	4.07(0.24)	3.66(0.33)	3.80(0.33)	4.35(0.36)	4.02(0.39)
	T3	4.25(0.31)	4.16(1.83)	3.79(0.36)	3.94(0.36)	4.08(0.44)	3.92(0.41)
Platelet Count(10^3/µL)	T1	336.0(101.2)	333.0(63.0)	164.0(50.77)	280.55(64.40)	278.0(66.9)	235.85(57.6)
	T2	279.0(73.7)	312.0(39.9)	155.0(46.94)	250.32(67.95)	252.0(64.2)	229.9(58.44)
	T3	268.0(93.2)	254.0(43.0)	150.0(45.15)	234.15(67.67)	238.4(57.1)	240.3(67.02)

Discussion:

The aim of this study was to establish normal reference ranges for hematological indices in pregnancy and trimester-specific mean values for blood parameters in pregnant females. We saw a decline in Hb concentration from first to second trimester but marked drop in third trimester. This is

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consistent with the findings of a similar study carried out by Akingbola et al in south western Nigeria which also saw a marked drop in Hb conc. from second to third trimester as shown in Table 2.[13] The progressive fall in Hb concentration from the second to third trimester may be due to an increased demand for iron as pregnancy progresses. The foetus continues to grow and the oxygen demand increases considerably which explains the increased requirement of iron for hemoglobin synthesis.

Although there is increase in RBC production by the bone marrow in order to meet the increased oxygen demand of the metabolically active cells in female and growing foetal tissue, there is progressive decline in RBC count with advancement of pregnancy. The total Red Cell Mass increases but the RBC count decreases. This can be attributed to the additional progesterone and estrogen secreted by the placenta during pregnancy which causes release of renin from the kidneys. Renin stimulates the renin-angiotensin-aldosterone system, leading to sodium retention, thereby increasing plasma volume. The increase in plasma volume is relatively greater than the increase in red cell mass, which results in a fall in maternal Hb, hence accounting for the physiological anemia that occurs in pregnancy.

The increase observed in WBC count from the first to third trimester in the study is consistent with the findings of Pughikumo et al and Onwukeme and Uguru.[18,19] The increase is primarily due to an increase in neutrophils and may represent a response to stress due to redistribution of the WBCs between the marginal and circulating pools and increased bone marrow synthesis causing immature band forms to spill into circulation. The trimester distribution of leucocyte counts in this study shows a progressive increase with gestational age statistically significant across all the three trimesters, (p=0.001), it is a pointer to the concept of a boost in leucocyte function in pregnancy. To the clinician, especially the Obstetrician, it is noteworthy that a mild to moderate leucocytosis and even a slowly rising leucocytosis is not a good indicator of an infection in pregnancy. The peripheral blood film may show a mild to moderate left shift and even toxic granulations in a normal pregnant woman without any pathological significance.[18]

The study also reported a gradual reduction in Platelet count as pregnancy advanced, which is consistent with a similar study carried out by Shen et al in China in 2010.[1] The decline was more marked between first and second trimester and was statistically significant across the trimesters(p=0.007). Due to hemodilution secondary to expansion of plasma volume, the Platelet count in normal pregnancies may decrease by approximately 10%, with most of this decrease occurring during the third trimester, although the absolute Platelet count tends to remain within the normal reference range in most patients.[20,21] After anemia, thrombocytopenia is the second most common hematologic abnormality that occurs during pregnancy. The overall incidence of thrombocytopenia in pregnancy is 8%, but when patients with obstetric or medical conditions are excluded, the incidence drops to 5.1%.[22]

A limitation of this study could be lack of a control group of unsupplemented women. However, such a group may not have gained ethical approval, keeping in view the high prevalence of anemia in a rural setting like ours. Another important limitation of this study was reliability of the information provided by participants and data obtained from clinical notes.

Conclusion:

It is important to consider normal reference ranges specific to pregnancy when interpreting some laboratory results that may be altered by the normal changes of pregnancy. The result of our study compares well with results of other studies and suggests that there are minor, yet significant variations in hematological parameters on a regional and inter country basis. The findings of this study reinforce the need for supplementation and generation of additional information on hematological reference values in a normal pregnancy in rural settings in India.

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