Trimester pattern of change and reference ranges of hematological profile among Sudanese women with normal pregnancy

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Abstract

Trimester specific reference ranges of hematological indices were described in several populations; however, comparable reports among Sudanese women with normal pregnancy are lacking. To evaluate trimester pattern of change and reference ranges of hematological profile among Sudanese women with normal pregnancy, we 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. Obstetrics and medical history was gathered using questionnaire and hematological profile was investigated using hemo-analyser. The first, second and third trimester mean (SD) [5th-95th centile] of hematological profile were as follow: RBC counts 4.30 (0.36) [3.69-4.93], 4.35 (0.36) [3.69-4.93], 4.08 (0.44) [3.44-4.78] ×106/mm3; hemoglobin concentration 10.81 (1.22) [8.92-12.74], 10.62 (0.93) [9.00-12.10], 10.83 (1.13) [8.82-12.60] g/dL; hematocrit 35.38 (3.52) [30.12-40.30], 34.43 (2.51) [30.58-38.23], 35.17 (3.18) [29.66-40.04] %; WBC counts 7.69 (1.96) [4.36-11.20], 8.45 (1.97) [5.48-12.13], 8.36 (2.11) [5.00-11.96] ×109/mm3; platelet counts 278.02 (66.93) [182.6-418.0], 251.96 (64.17) [163.8-381.8], 238.36 (57.10) [150.4-346.2] ×109/mm3. The present study is the first to establish trimester specific, reference range for hematological profile among Sudanese women with normal pregnancy. The present study aimed to evaluate trimester pattern of change of these hematological indices, and others, remained ill-defined. This fact encouraged researchers in the field to investigate for trimester specific, reference range for hematological profile during normal pregnancy. Thus, studies assessing detailed hematological profile during normal pregnancy are scarce before invention of automated hematological analyzer. Over the last decade, trimester specific reference ranges of hematological indices were reported in several western,11 eastern,1,2 and African populations.10,14 We could not find a report exploring hematological profile among Sudanese women with normal pregnancy, a part from a study exploring physiological variations of blood formed elements counts in 50 healthy pregnant Sudanese women.4 The present study aimed to evaluate trimester pattern of change and reference ranges of fifteen important hematological parameters among Sudanese women with normal pregnancy. We believed that the findings of this study would help in precise interpretation of laboratory results, correct diagnosis and appropriate management of blood disorders among pregnant Sudanese women.

Introduction

Regular assessment of hematological profile during pregnancy is an essential practice in antenatal care clinics.1 Hematological profile can reflect nutritional, immunological, hemostatic status of the pregnant women.2 Likewise, it is an important predictor of pregnancy outcomes.3 Physiologically, activation of renin-angiotensin-aldosterone system during pregnancy increases extracellular fluid4 and consequently plasma volume.5 Maternal erythropoiesis,6 neutrophil apoptosis,7 platelet activation and clearance5 are enhanced during pregnancy. The hematocrit (HCT), plateletcrit (PCT), counts of red blood corpuscles (RBC), white blood corpuscles (WBC) and platelets (PLT) are expected to change according to the degree of plasma volume expansion5,6 and the amount of blood formed elements being added6 or removed4 from the circulation. Release of young RBC and activation of platelets affect the readings of some hematological indices like mean corpuscular volume (MCV), red cell distribution width (RDW), mean platelet volume (MPV) and platelet distribution width (PDW).2,8 However, the exact pattern of trimester change of these hematological indices, and others, remained ill-defined. This fact encouraged researchers in the field to investigate for trimester specific, reference range for hematological profile during normal pregnancy.9,10 Studies assessing detailed hematological profile during normal pregnancy are scarce before invention of automated hematological analyzer. Over the last decade, trimester specific reference ranges of hematological indices were reported in several western,11 eastern,1,2 and African populations.10,14 We could not find a report exploring hematological profile among Sudanese women with normal pregnancy, a part from a study exploring physiological variations of blood formed elements counts in 50 healthy pregnant Sudanese women.4 The present study aimed to evaluate trimester pattern of change and reference ranges of fifteen important hematological parameters among Sudanese women with normal pregnancy. We believed that the findings of this study would help in precise interpretation of laboratory results, correct diagnosis and appropriate management of blood disorders among pregnant Sudanese women. With singleton pregnancy were enrolled since early gestation and followed till the third trimester. After signing an informed consent, questionnaires were used to gather the medical and obstetrics history (age, parity, gravidity, gestational age). Pregnancy and its duration were confirmed by ultrasound. Women with thyroid disease, hypertension, renal disease, diabetes, liver disease and on medication(s) were excluded from the study.

During each visit the blood pressure was measured using a sphygmomanometer. The weight and height measured during the first visit were used to calculate the body mass index (BMI), which was expressed as weight (kg)/height2 (m2). During each visit, 2 mL of blood was taken from every woman in an ethylene diamine tetra acetic acid, analyzed immediately for a complete hemogram using an automated hematological analyzer and following the manufacturers’ instructions as previously described.15,16 Combined tablets of iron and folic acid were prescribed to every woman.

Materials and Methods

A longitudinal study was conducted at the antenatal care clinics of Saad Abu-Alela Hospital (Khartoum, Sudan) during the period of January-December 2015. Women

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Key words: Hematological profile; pregnancy; reference range; Sudan; trimester.

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Contributions: IA designed the study; DAR MAA carried out experimental protocols; MFL analyzed data; MFL, IA, DAR, MAA, AHA participated in manuscript preparation and revision. All authors read and approved the final manuscript.

Conflict of interest: the authors declare no potential conflict of interest.

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A total sample size of 140 women was calculated using a formula for longitudinal study and the difference in the mean of the proposed variables (mainly hemoglobin, WBC and platelets) that would provide 80% power to detect a 5% difference at α = 0.05, and assumed that 10% of women will be lost during follow-up or will not respond.

Statistics

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). Proportions of the studied groups were expressed in percentages (%). Means (M) and standard deviations (SD) were used to describe the studied variables. 5th-95th centiles were used to identify reference ranges of hematological profile among Sudanese women with normal pregnancy. The repeated measure ANOVA and LSD Post hoc analyses were used to evaluate the differences in the means of the hematological indices between different trimesters. P<0.05 was considered significant.

Ethics

Ethical clearance was obtained from the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Khartoum. Written informed consent was provided by each volunteer before being enrolled in the study.

Results

Out of 175 pregnant women who were enrolled initially, 143 (81.7%) completed the follow-up till the third trimester. The rest (18.3%) were lost during follow-up due to address change. The mean age of the pregnant women enrolled in the study (N = 143) was 27.94±5.45 years (range 18-42 years). The other obstetric and socio-demographic characteristics of the studied pregnant women are summarized in Table 1.

Table 2 shows the M (SD) [5th-95th centiles] of RBC indices in the studied pregnant women. RBC counts were comparable in the first and second trimester (4.30±0.36 vs. 4.35±0.33 ×10^6/mm³, P = 0.836), but decreased significantly during the third trimester (4.08±0.44 ×10^6/mm³, P < 0.001). In comparison, hematocrit was lower in the second trimester (34.43±2.51 %) compared with the first (35.38±3.52 %), P<0.003) as well as the third trimesters (35.17±3.18 %, P<0.008). MCV increased steadily throughout pregnancy while MCH and HCHC were higher during the last two thirds of pregnancy compared with the first trimester. RDW was lower during the third trimester.

Table 1. Characteristics of the studied pregnant women (N = 143).

| Variables                  | M (SD) | N (%) |
|----------------------------|--------|-------|
| Age (years)                | 27.94 (5.45) |       |
| Gravidity                  | 2.62 (2.96) |       |
| Parity                     | 1.06 (1.24) |       |
| Inter-pregnancy interval (months) | 20.36 (25.52) |       |
| History of miscarriage     | 0.26 (0.42) |       |
| History of stillbirth      | 0.03 (0.17) |       |
| First visit BMI (kg/m²)    | 27.89 (6.08) |       |
| First visit SBP (mmHg)     | 109.30 (10.12) |       |
| First visit DBP (mmHg)     | 71.75 (3.88) |       |
| Urban residence            | 124 (86.71) |       |
| University or higher education | 124 (86.71) |       |
| Housewife (non-employee)   | 107 (4.83) |       |

Table 2. Mean (SD) [5th-95th centile] of RBC indices during pregnancy in Sudanese women.

| Variables                  | T1 (10.92 (3.37) Weeks) | T2 (20.64 (3.94) Weeks) | T3 (31.12 (3.19) Weeks) | P          |
|----------------------------|--------------------------|--------------------------|--------------------------|------------|
| RBC (x10^6/mm³)            | 4.30 (0.36) [3.69 4.93]  | 4.35 (0.36) [3.69 4.93]  | 4.08 (0.44) [3.44 4.78]  | Inter-Trimesters < 0.001 |
| HB (g/dl)                  | 10.81 (1.22) [8.92 12.74]| 10.62 (0.93) [9.90 12.10]| 10.83 (1.13) [8.82 12.60]| Inter-Trimesters = 0.0047 |
| HCT (%)                    | 35.38 (3.52) [30.12 40.30]| 34.45 (2.51) [30.58 38.23]| 35.17 (3.18) [29.66 40.04]| Inter-Trimesters = 0.001 |
| MCV (fL)                   | 82.88 (7.64) [65.50 93.02]| 85.16 (6.99) [71.35 94.70]| 86.40 (6.51) [73.40 95.68]| Inter-Trimesters < 0.001 |
| MCH (pg)                   | 25.16 (2.78) [19.40 28.74]| 26.68 (5.84) [20.83 30.15]| 26.57 (2.56) [21.34 30.18]| Inter-Trimesters < 0.001 |
| MCHC (g/dL)                | 30.45 (1.19) [28.54 32.30]| 30.80 (1.16) [28.78 32.40]| 30.73 (1.13) [28.82 32.50]| Inter-Trimesters < 0.001 |
| RDW (%)                    | 13.99 (1.76) [12.00 17.94]| 14.33 (2.31) [12.40 18.81]| 13.82 (1.72) [12.11 17.50]| Inter-Trimesters = 0.001 |

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trimester (13.82±1.72%) compared with the first (13.99±1.76%) and the second (14.33±2.31 %) trimesters.

Table 3 shows the M (SD) [5th-95th centiles] of the total and differential WBC counts during pregnancy in Sudanese women. The total WBCs counts were lower during first trimester (7.69±1.96×10^6/mm^3) compared with the last two thirds of pregnancy (8.45±1.97×10^6/mm^3, P<0.001; 8.36±2.11×10^6/mm^3, P = 0.002). Lymphocyte count was least during the third trimester; however, the count of other types of leukocytes remained comparable throughout pregnancy. Table 4 shows the M (SD) [5th-95th centiles] of platelets indices in the studied pregnant women. The platelets count and PCT decreased steadily while MPV remained with no significant change as pregnancy progress. PDW decreased significantly in the second compared with the first trimester (15.57±0.37 vs 15.68±0.36 %, P=0.002), but remained with no significant change thereafter.

**Discussion**

It is evident from the current results that maximum decrease in RBCs count was in the third trimester. In comparison, lowest HB and HCT as well as highest MCV, MCH, MCHC and RDW readings were achieved in the second trimester. Gradual decrement in RBCs count with progression of pregnancy was reported in a Sudanese study\(^9\) and several previous reports\(^10-12\) but not others.\(^13\) Similar to the present results, lowest means of HB and HCT were noted in the second trimester in at two separate Jamaican\(^11\) and Chinese\(^12\) studies.

![Table 3](image1.png)

![Table 4](image2.png)
Physiological, increased vascular capacity secondary to systemic vasodilatation stimulates renin-angiotensin-aldosterone system, which explains blood volume expansion during pregnancy. Based on Evans blue dye dilution methods, at least 10-15% increment in plasma volume occurs at the start of the second trimester. Maternal erythropoiesis is also enhanced during pregnancy; however, the increase RBC count (18-25%) is usually less compared with the ultimate expansion of plasma volume (50%). The resulted dilutional anemia causes significant drop in RBCs count, HB and HCT during the second trimester, which remains unchanged thereafter in the third trimester. Absence of further drop over the third trimester is probably due to high levels of atrial natriuretic peptide and consequently contraction in maternal plasma volume. Enhanced maternal erythropoiesis and HCT reported by Akingbola et al., among Nigerian pregnant women were markedly lower compared with our results as well as international records. Likewise, the drop in HB concentration and HCT in the first trimester [11.24 (10.41) g/dL and 29.36 (4.22) respectively] compared to the third trimester [9.81 (1.32) g/dL and 29.36 (4.22) % respectively] were more than expected. Such significant drop in HB concentration and HCT between first and third trimesters is difficult to explain on physiological basis and suggest an etiology other than the normal hematological response to pregnancy.

Table 5. Comparison of trimester specific mean (SD) of hematological profile between the present study and previous international reports.

|          | Sudan T1 | Nigeria T1 | India T1 | Jamaica T1 | China T1 |
|----------|----------|------------|----------|------------|----------|
| RBC x10³/mm³ | 4.30 (0.36) | 3.99 (0.56) | 4.00 (0.42) | 4.33 (0.40) | 4.05 (0.36) |
|          | 4.35 (0.36) | 3.42 (0.61) | 4.07 (0.24) | 3.80 (0.33) | 3.66 (0.33) |
|          | 4.08 (0.44) | 3.31 (0.59) | 4.16 (1.83) | 3.94 (0.36) | 3.79 (0.36) |
| HB g/dL   | 10.81 (1.22) | 11.24 (1.04) | 10.48 (0.89) | 12.73 (1.14) | 12.20 (0.92) |
|          | 10.62 (0.93) | 10.03 (1.41) | 10.06 (1.04) | 11.41 (1.16) | 11.30 (0.89) |
|          | 10.83 (1.13) | 9.81 (1.32) | 10.02 (1.26) | 11.67 (1.18) | 11.50 (0.99) |
| HCT (%)   | 35.38 (3.52) | 35.20 (3.74) | 37.51 (2.60) | 37.05 (2.96) | 36.0 (2.55) |
|          | 34.43 (2.51) | 29.34 (4.42) | 32.88 (2.96) | 33.12 (3.00) | 34.0 (2.30) |
|          | 35.17 (3.18) | 29.36 (4.22) | 33.7 (3.27) | 34.03 (2.97) | 35.0 (2.81) |
| MCV (fL)  | 30.17 (0.19) | 34.20 (1.16) | 31.49 (2.87) | 34.35 (1.05) | 33.80 (0.82) |
|          | 26.57 (2.78) | 26.15 (2.86) | 29.53 (2.92) | 30.15 (3.01) | 31.20 (1.81) |
|          | 26.68 (5.84) | 26.96 (3.31) | 30.15 (3.01) | 31.20 (1.81) | 31.00 (2.04) |
| RBC x10³/mm³ | 3.05 (0.19) | 34.20 (1.16) | 31.49 (2.87) | 34.35 (1.05) | 33.80 (0.82) |
|          | 30.80 (1.16) | 35.10 (2.10) | 32.99 (1.97) | 34.42 (1.30) | 33.30 (1.02) |
|          | 30.73 (1.13) | 35.30 (2.50) | 30.47 (6.65) | 34.33 (1.43) | 33.00 (1.07) |
| RDW (%)   | 13.39 (1.76) | 12.74 (1.93) | 14.07 (1.01) | - | - |
|          | 14.33 (2.31) | 12.56 (1.23) | 15.07 (2.43) | - | - |
|          | 13.82 (1.72) | 12.82 (1.32) | 18.9 (3.85) | - | - |
| WBC x10³/mm³ | 7.65 (1.96) | 5.49 (1.75) | 7.85 (1.41) | 8.27 (2.60) | 8.30 (2.02) |
|          | 8.45 (1.97) | 6.61 (2.13) | 9.70 (2.43) | 9.66 (2.84) | 9.50 (2.22) |
|          | 8.36 (2.11) | 6.80 (2.27) | 10.17 (1.14) | 8.79 (2.50) | 9.10 (2.17) |
| GRAN x10³/mm³ | 6.54 (10.14) | 3.71 (1.74) | - | - | - |
|          | 7.07 (8.05) | 4.19 (1.78) | - | - | - |
|          | 5.89 (1.87) | 4.39 (1.74) | - | - | - |
| LYM x10³/mm³ | 2.20 (1.32) | 1.93 (0.70) | - | - | - |
|          | 1.98 (0.45) | 1.57 (0.61) | - | - | - |
|          | 1.87 (0.48) | 1.95 (0.67) | - | - | - |
| MID x10³/mm³ | 0.58 (0.21) | 0.44 (0.20) | - | - | - |
|          | 0.65 (0.65) | 0.49 (0.24) | - | - | - |
|          | 0.63 (0.33) | 0.48 (0.31) | - | - | - |
| Platelet x10³/mm³ | 278.9 (66.9) | 227.5 (81.11) | 333.0 (63.0) | 280.55 (64.40) | 164.0 (50.77) |
|          | 252.9 (64.2) | 229.5 (88.34) | 312.9 (39.9) | 250.32 (67.35) | 155.0 (46.94) |
|          | 238.4 (75.1) | 186.5 (62.22) | 254.0 (42.0) | 234.15 (67.67) | 150.0 (45.15) |
| MPV (fL)  | 8.13 (0.84) | 8.09 (0.88) | - | - | - |
|          | 8.10 (0.87) | 7.90 (0.88) | - | - | - |
|          | 8.19 (0.87) | 7.88 (0.87) | - | - | - |
| PDW (%)   | 15.57 (0.37) | 14.08 (2.60) | - | - | - |
|          | 15.68 (0.36) | 16.08 (2.19) | - | - | - |
|          | 16.90 (12.40) | 16.12 (1.96) | - | - | - |
| PCT (%)   | 0.24 (0.24) | 0.18 (0.06) | - | - | - |
|          | 0.20 (0.05) | 0.18 (0.07) | - | - | - |
|          | 0.20 (0.04) | 0.15 (0.05) | - | - | - |
poiesis during pregnancy results in release of more young erythrocytes to the circulation, which are usually larger in size compared with the mature RBCs. High young erythrocytes count explains the steady increment of MCV throughout pregnancy. A longitudinal study among women with normal pregnancy demonstrated no variations in RDW between 16 and 34 weeks but significant increase afterward, up to the onset of labor.\textsuperscript{9,10} The unpredicted increase in the RDW over the 4-6 weeks before the onset of labor points to enhanced bone marrow activity; however, stimulus for this remained unexplained. Noteworthy, the physiological changes in RBCs count, HB, HCT, MCV and RDW during pregnancy are further modified by nutritional,\textsuperscript{20} medical and obstetric complications,\textsuperscript{21} which explains trimester variations of hematological profile described in previous studies.\textsuperscript{6,14}

The current results showed significant rise in the total WBC count during the last two thirds of pregnancy compared with first trimester. Lymphocytes counts followed reverse pattern of change, being comparable during the first two trimesters but dropped significantly over the last one. In contrast, the counts of GRAN and MID were comparable throughout the three trimesters. The relatively increased total WBC count demonstrated in the current results is supported by several previous reports.\textsuperscript{10-13} Total WBC count is hardly less than 7000/mm\textsuperscript{3} during pregnancy (Table 4). The exact cause of relative leukocytosis during gestation is yet to be explored by further researches; however, physical and emotional stress associated with pregnancy\textsuperscript{22} and depressed neutrophil apoptosis\textsuperscript{23} could be possible causes. Similar to the present findings, Kühnert \textit{et al.}, demonstrated slight elevation of lymphocytes count at the start pregnancy but gradual decrease over the following trimesters.\textsuperscript{23} In a longitudinal study, Pitkin \textit{et al.} attributed the rise in total WBC count to synonymous increase in the number of neutrophils.\textsuperscript{24} Regarding other types of leukocytes, the study showed increased monocyte and decreased lymphocytes, eosinophils, and basophils as pregnancy advanced.\textsuperscript{24} Another report confirmed monocytosis during pregnancy, but denied significant rise in eosinophil and basophil counts,\textsuperscript{6} which disagreed with Pitkin \textit{et al.} implications.

The present data showed steady decrease of platelets count and PCT, increase of PDW and unchanged MPV as pregnancy progress. Decreased platelets count and PCT were repeatedly demonstrated during normal pregnancy.\textsuperscript{10,12,25} Thrombocytopenia during pregnancy was attributed to plasma volume expansion, enhanced platelet activation and clearance.\textsuperscript{8} Theoretically, hemodilution and platelet activation associated with pregnancy can induce platelets swelling, pseudopodia formation and hence increase MPV and PDW. According to one study, PDW displayed substantial increase from the first to the third trimester of pregnancy; however, MPV failed to show the same trend.\textsuperscript{26} Noteworthy, comparable trimester changes of MPV and PDW were reproduced by the present study and others.\textsuperscript{10} The preferential rise PDW, but not MPV, as pregnancy advances suggests that variations in platelets size during gestation are principally due to platelet activation rather than hemodilution.\textsuperscript{26}

Noteworthy, this study enrolled pregnant women with regular antenatal follow-up. Studied women received proper medical advice, regular checkup investigations and hematologic. The hematological reference range derived from the present study would be helpful in evaluating pregnant women with healthcare, socioeconomic and racial background comparable to our participants. However, a potential limitation remained because our results could not uncover pattern of trimester change in hematological profile of pregnant women with inadequate antenatal care. Another limitation of the study is the lack of controls for hematological profile readings (preconception or 6-weeks postpartum).

Conclusions

The present study is the first to establish detailed, trimester specific, reference range for hematological profile among Sudanese women with normal pregnancy. The reference range of RBC, WBC and platelets indices are mostly parallel to international records. The trimester pattern of change of hematological profile among Sudanese is comparable with previous reports in this regard. The plasma volume expansion, physiological stress associated with pregnancy and preferential increased hematopoiesis of some blood formed elements, but not others, seemed to be the main grounds for hematological profile changes during pregnancy.

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