

Red Blood Cell Indices and Morphology in 3rd Trimester of Pregnant Women Attending Isra University Hospital

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Abstract

Objective: To calculate RBC indices in third trimester of pregnancy among anemic and non-anemic women and to observe RBC morphology on peripheral blood smear.

Methodology: A cross-sectional comparative analysis was done at department of Obstetrics and Gynecology, Isra University Hospital Hyderabad, Sindh, from March 2019 to August 2019. All the gravid women aged 25 to 50 years old with 3rd trimester of gestation (more than 28 weeks of gestation) were included. Approximately 5 mL of blood was drawn into EDTA tubes. RBC indices, including hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were measured using an automated hematology analyzer. Peripheral blood smears were prepared from the collected blood samples and stained. The stained smears were examined under a light microscope by a qualified hematologist and the morphology of RBCs were assessed. Analysis of data was done using SPSS version 26.0.

Results: Study compared the red blood cell indices and morphology in the 3rd trimester of pregnant anemic and non-anemic women within a sample of 100 participants. Group A (non-anemic) had significantly higher mean Hb levels (11.6 ± 2.1 g/dL) compared to Group B (anemic) (7.7 ± 1.8 g/dL), with a p-value of 0.001. Mean hematocrit was significantly higher in Group A ($36.8 \pm 9.1\%$) than in Group B ($30.3 \pm 9.6\%$), and the mean RBC count (millions/ μ L) was also higher in Group A (3.49 ± 0.04) compared to Group B (2.82 ± 0.47), both with a p-value of 0.001. Further analysis showed that Group A had higher mean values for MCV (83.11 ± 11.26 fL vs. 64.56 ± 7.55 fL), mean MCH (28.28 ± 4.54 pg vs. 22.73 ± 4.28 pg), and mean MCHC ($30.57 \pm 4.33\%$ vs. $23.32 \pm 4.53\%$) compared to Group B, (p-0.0001) for all comparisons. Conversely, mean RDW was higher in Group B ($15.13 \pm 2.9\%$) than in Group A ($11.20 \pm 2.5\%$), (p-0.001).

Conclusion: The study revealed a high prevalence of anemia (65%) in the third trimester of pregnancy, characterized by significant decreases in RBC indices such as MCV and MCH. Additionally, elevated RDW levels, even with normal MCV, is an indicator of iron deficiency anemia, especially during pregnancy.

Keywords: Pregnancy, Anemia, Red blood cells, RBC indices, RDW.

Introduction

Worldwide, maternal anemia continues to pose a significant public health challenge, with limited progress in its reduction. Currently, 38% of women globally are affected by anemia during pregnancy, contributing to approximately 20% of maternal mortality cases.^{1,2} Similar to other essential nutrients, the demand for iron increases during pregnancy to accommodate the expanding hemoglobin and red blood cell mass, losses of the basal iron, fetal tissues growth and the placenta, as well as potential blood loss during childbirth.^{1,3} Physiological

adjustments unique to pregnancy are crucial for ensuring sufficient nutrition and fetal development. Throughout pregnancy, there is a significant increase in physiological iron requirements, amounting to about one gram, necessary to support the growth of the fetus and the placental development, as well as maternal adaptation and childbirth.^{4,5} While red blood cell (RBC) production increases during pregnancy, blood plasma volume increases at a greater rate, leading to hemodilution that affects both RBCs and platelets as pregnancy advances, resulting in a gradual decrease in their concentration.^{4,6} The implications of anemia are significant and encompass heightened risks of maternal mortality, and fetal, or neonatal death. Adverse pregnancy consequences including lower birth weight and the preterm births, as well as potential impacts on cognitive development, learning ability, and academic performance in children, alongside

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reduced productivity in adults, are among the observed consequences.^{7,8} Though, the specific pattern of trimester-related changes in these hematological indices, among others, has not been clearly defined. This gap has motivated researchers in the field to explore trimester-specific reference ranges for the hematological profile during typical pregnancy.^{9,10} It is advisable for all pregnant women to undergo a comprehensive blood count during the first trimester to detect anemia. Understanding which blood parameters during early pregnancy can predict anemia in the third trimester would be beneficial for both pregnant women and healthcare providers.¹¹ These measurements supply insight into the oxygen-carrying capacity of maternal blood and represent adjustments to accommodate the elevated demands on metabolism for both the pregnant woman and the developing baby.

Comprehensive investigation into these characteristics can aid in monitoring maternal well-being, detecting and controlling problems such as anemia and its types, and assuring the best possible outcomes for both mother and fetal health. This study measured hematological indices and identified red blood cell abnormalities to investigate potential causes of anemia in pregnant women during third trimester of pregnancy.

Methodology

This descriptive /observational study was done at department of Obstetrics and Gynecology, Isra University Hospital Hyderabad, Sindh. Study duration was six months from March 2019 to August 2019. Non-probability purposive sampling was used. All the gravid women aged 25 to 50 years old with 3rd trimester of gestation (more than 28 weeks of gestation) were included. All the identical babies and patients with systemic diseases like pulmonary tuberculosis, Systemic hypertension, diabetes mellitus, Pre-eclampsia, Eclampsia, Valvular heart disease and further systemic complications were excluded. Detailed medical history of the patients was taken accompanied by thorough clinical examination. After taking informed consent and explanation of study objectives, the blood sample was collected from each participant. Venipuncture will be performed by a trained phlebotomist, following standard procedures. Approximately 5 mL of blood was drawn into EDTA tubes. RBC indices, including concentrations of the hemoglobin, Hct, MCV, MCH, and the concentration of MCHC, were measured using an automated hematology analyzer. Subjects were separated

into two groups according to Hemoglobin percentage into: Group A = Hb more than 10gm/dl. Group B = Hb less than 10gm/dl. Peripheral blood smears were prepared from the collected blood samples and stained using Wright-Giemsa stain. The stained smears were examined under a light microscope by a qualified hematologist and the morphology of RBCs were assessed. Analysis of data was done using SPSS version 26.0. Quantitative variables were evaluated as Mean and standard deviation and frequency and percentage was obtainable for categorical. student's t-test was applied and a p-value ,0.05 was considered as significant.

Results

Group A consists of 35 patients and had a mean age of 32.8 years with a SD of 6.65 years. Group B comprises 65 patients and exhibits a mean age of 35.3 years with a standard deviation of 6.63 years. The p-value for the comparison of mean ages between the two groups is 0.08. On the comparative analysis of mean Hb level, mean hematocrit, and RBC count between two groups within a study population of 100 participants. For the Hb level (g/dL), Group A has a mean of 11.6 with a SD of 2.1, while Group B has a mean of 7.7 with an SD of 1.8. In terms of hematocrit (%), Group A shows a mean value of 36.8 with an SD of 9.1, compared to Group B, which has a mean of 30.3 with an SD of 9.6. Regarding the RBC count (millions/ μ L), Group A has a mean of 3.49 with an SD of 0.04, whereas Group B has a mean of 2.82 with an SD of 0.47. The p-value for all 0.001, indicating a statistically significant difference. Table I

| Table I: Mean Hb level, mean hematocrit, and mean RBC count of study population. (n=100) | | | |
|--|------|------|---------|
| Groups | Mean | SD | P value |
| Hb level (Gm /dl) | | | |
| A | 11.6 | 2.1 | 0.0001 |
| B | 7,7 | 1.8 | |
| Hematocrit (%) | | | |
| A | 36.8 | 9.1 | 0.001 |
| B | 30.3 | 9.6 | |
| RBC (millions/ u/l) | | | |
| A | 3.49 | 0.04 | 0.0001 |
| B | 2.82 | 0.47 | |

Group A shows higher mean values for all measured parameters compared to Group B. Specifically, Group A has a mean MCV of 83.11 fL (SD 11.26) versus 64.56 fL (SD 7.55) in Group B, with a p-value of 0.0001. The mean MCH for Group A is 28.28 pg (SD 4.54), while Group B has

a mean of 22.73 pg (SD 4.28), also with a p-value of 0.0001. For MCHC, Group A's mean is 30.57% (SD 4.33), compared to 23.32% (SD 4.53) in Group B, with a p-value of 0.0001. Lastly, Group A has a mean RDW of 11.20% (SD 2.5), whereas Group B's mean RDW is 15.13% (SD 2.9), with a p-value of 0.001. Table II

| Groups | Mean | SD | P Value |
|--|-------|-------|---------|
| Mean corpuscular volume (fl) | | | |
| A | 83.11 | 11.26 | 0.0001 |
| B | 64.56 | 7.55 | |
| Mean corpuscular hemoglobin (pg) | | | |
| A | 28.28 | 4.54 | 0.0001 |
| B | 22.73 | 4.28 | |
| Mean corpuscular hemoglobin concentration (%) | | | |
| A | 30.57 | 4.33 | 0.0001 |
| B | 23.32 | 4.53 | |
| Red cell distribution width (%) | | | |
| A | 11.20 | 2.5 | 0.001 |
| B | 15.13 | 2.9 | |

For MCV, 49 participants have low MCV (<70 fL), with 32 from Group A and 17 from Group B. Normal MCV (70-96 fL) is observed in 43 participants, including 32 from Group A and 11 from Group B. High MCV (>96 fL) is found in 8 participants, all from Group B. Regarding MCH, 54 participants have low MCH (<24 pg/dL), with 5 from Group A and 49 from Group B. Normal MCH (24-30 pg/dL) is seen in 40 participants, comprising 31 from Group A and 9 from Group B. High MCH (>30 pg/dL) is present in 6 participants, with 1 from Group A and 5 from Group. Table III

| Groups | Group A | Group B | Total |
|--|---------|---------|-------|
| Mean corpuscular volume frequency | | | |
| Low MCV (<70fl) | 32 | 17 | 49 |
| Normal MCV (70-96fl) | 02 | 11 | 43 |
| High MCV (>96fl) | 0 | 8 | 8 |
| Mean corpuscular hemoglobin frequency | | | |
| Low MCH (<24pg/dl) | 05 | 49 | 54 |
| Normal MCH (24-30 pg/dl) | 31 | 09 | 40 |
| High MCH (>pg/dl) | 01 | 05 | 06 |

Discussion

During pregnancy, hematological alterations occur to meet the needs of the growing fetus and developing

placenta, resulting in significant variations in blood volume. Inappropriate hematological profiles can impact childbirth and its outcomes.¹² This study investigated hematological indices and identified red blood cell abnormalities to explore potential causes of anemia among pregnant women in the third trimester. The mean age was 32.8 years for non-anemic women and 35.3 years for anemic women, with an anemia prevalence of 65.0%. These findings are consistent with those reported by et al¹³, where the mean age of pregnant women was 26.36 ± 7.66 years, with an overall anemia prevalence of 46.12%. In comparison, Toheed R et al¹⁴ reported a mean age of 22.59 ± 3.17 years among pregnant women, with a higher prevalence of anemia at 78.1%.

Furthermore, in this study, non-anemic women consistently exhibited higher mean hemoglobin levels (11.6 g/dL), hematocrit (36.8%), and red blood cell count (3.49 millions/ μ L) compared to anemic women (7.7 g/dL, 30.3%, and 2.82 millions/ μ L respectively), with all differences statistically significant ($p < 0.001$). In the comparison of this study, Henri E et al¹⁵ compared red blood cell indices across trimesters and found that mean hemoglobin levels were significantly higher in the first trimester as 12.1 ± 0.9 g/dL compared to the third as 11.8 ± 1.3 g/dL ($p = 0.043$). They also observed significant differences in mean hematocrit (HCT) values and RBC count between the first and second trimesters ($p = 0.001$). Low hemoglobin levels in the first trimester have been identified as a strong predictor of anemia in the third trimester, with a sensitivity of 83% for detecting anemia.¹⁶ In aligns to this study Anjum A et al¹⁷ reported that the anemia was among 75% women, and out of 100 their patient's decreased RBCs were in 91 women out of 100 and among 97 women HCT was less than 40%.

Additionally in this study non anemic women showed significantly higher mean corpuscular volume (MCV) (83.11 fL), mean corpuscular hemoglobin (MCH) (28.28 pg), and mean corpuscular hemoglobin concentration (MCHC) (30.57%), compared to anemic women (64.56 fL, 22.73 pg, and 23.32%, respectively), all with p-values of 0.0001 for MCV, MCH, and MCHC, and 0.001 for RDW. These findings were supported by the Anjum A et al¹⁷ where MCH was decrease in 32, in 23 women MCHC was less than 30%, in nine women and MCH was less than 9 pg. Consistently Sarah B et al¹⁸ stated decrease MCV, MCH and MCHC among anemic women compared to

normal ($p < 0.001$). In this study there was a raised RDW among anemic women compared to normals ($p = 0.0001$). Consistently Sarah B et al¹⁸ reported that the mean RDW for normal and anemic subjects was $12.83 \pm 1.03\%$ and $17.32 \pm 3.42\%$, respectively ($p = 0.0001$) and among 87 anemic pregnant women, RDW was elevated in 83, with 73 of these cases indicating severe iron deficiency. Red cell distribution width (RDW) measures the variation in red blood cell size and is included in a standard complete blood count. RDW increases significantly during pregnancy, especially in the third trimester or at the onset of labor.¹⁹

Red blood cell (RBC) indices are a group of measurements that are used to assess the size, shape, and hemoglobin content of RBCs. These measures are useful in the diagnosis of anemia, a disorder characterized by a low number of red blood cells. The three major RBC indexes are: MCV represents the average volume of an RBC. MCV observed in macrocytic anemias, such as vitamin B12 and folate insufficiency. Decreased MCV is detected in microcytic anemias, such as iron deficiency anemia. MCH represents the average amount of hemoglobin in an RBC. Microcytic anemias are associated with low MCH levels. MCHC is the hemoglobin concentration in an RBC. Hypochromic anemias, especially iron deficiency anemia, are characterized by low MCHC levels. Along with to the three major RBC indices, a number of other indices can be employed to diagnose anemia.²⁰ In the 3rd trimester of gestation, RBC indices are critical in discriminating between anemic and non-anemic women. According to studies, anemia is common among women during pregnancy, with varied degrees of severity. Anemia in pregnancy is frequently linked with reduced levels of hemoglobin, hematocrit, MCV, MCH, and MCHC.²¹⁻²⁴ More specifically, anemic pregnant women have lower RBC counts, indicating a decreased ability to deliver oxygen to body tissues. Furthermore, the occurrence of microcytic anemia, which includes low MCV and elevated RDW, is a typical finding in anemic pregnant women, particularly those with iron deficiency anemia.²³ These differences in RBC indices underline the necessity of tracking these parameters to properly detect and manage anemia throughout pregnancy.

Conclusion

Study has been observed an elevated prevalence of anemia (65%) for the third trimester of gestation, accompanied by significant declines in RBC indices such as MCV and MCH. These markers are important in distinguishing between different kinds of anemia. Furthermore, elevated RDW level, in addition to normal MCV, can be used as an early indicator of iron deficiency anemia, making RDW a more accurate marker for iron insufficiency, particularly during pregnancy. Measuring these RBC parameters throughout pregnancy is critical for rapid intervention and management of mother and fetal well-being.

References

1. WHO. The global prevalence of anaemia in 2011. World Health Organization; Geneva, Switzerland: 2015.
2. Agbozo F, Abubakari A, Der J, Jahn A. Maternal dietary intakes, red blood cell indices and risk for anemia in the first, second and third trimesters of pregnancy and at predelivery. *Nutrients*. 2020 Mar 15;12(3):777. <https://doi.org/10.3390/nu12030777>
3. Scholl TO. Maternal iron status: Relation to fetal growth, length of gestation, and iron endowment of the neonate. *Nutr Rev*. 2011;69 <https://doi.org/10.1111/j.1753-4887.2011.00429.x>
4. Figueroa-Mujica R, Ccahuantico LA, Ccorahua-Rios MS, Sanchez-Huaman JJ, Vásquez-Velasquez C, Ponce-Huarancca JM, Rozas-Gamarra RE, Gonzales GF. A critical analysis of the automated hematology assessment in pregnant women at low and at high altitude: association between red blood cells, platelet parameters, and iron status. *Life*. 2022 May 13;12(5):727. <https://doi.org/10.3390/life12050727>
5. Fisher AL, Nemeth E. Iron homeostasis during pregnancy. *Am J Clin Nutr*. 2017 Dec 1;106:1567S-74S. <https://doi.org/10.3945/ajcn.117.155812>
6. Getrajdman C, Sison M, Lin HM, Katz D. The effects of hemodilution on coagulation in term parturients: an in vitro study utilizing rotational thromboelastometry. *J Matern Fetal Neonatal Med*. 2022 May 19;35(10):1969-77. <https://doi.org/10.1080/14767058.2020.1776250>
7. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. *Am J Clin Nutr*. 2000 May 1;71(5):1280S-4S. <https://doi.org/10.1093/ajcn/71.5.1280s>
8. Gebreweld A, Bekele D, Tsegaye A. Hematological profile of pregnant women at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia. *BMC Hematol*. 2018;18:1-7. <https://doi.org/10.1186/s12878-018-0111-6>
9. Mohamed AO, Hamza KM, Babker AM. Physiological changes in some hematological and coagulation profile among Sudanese healthy pregnant women. *Int J Med Sci Public Health*. 2016;5:525-8. <https://doi.org/10.5455/ijmsph.2016.30092015149>

10. Rayis DA, Ahmed MA, Abdel-Moneim H, Adam I, Lutfi MF. Trimester pattern of change and reference ranges of hematological profile among Sudanese women with normal pregnancy. *Clin Pract*. 2017 Jan 11;7(1):888.
<https://doi.org/10.4081/cp.2017.888>
11. Zeng Y, He G. Association of blood parameters in early pregnancy with anemia during late pregnancy: a multicenter cohort study in China. *J Matern Fetal Neonatal Med*. 2024 Jan 2;37(1):2299110.
<https://doi.org/10.1080/14767058.2023.2299110>
12. Gebreweld A, Bekele D, Tsegaye A. Hematological profile of pregnant women at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia. *BMC Hematol*. 2018 Dec;18:1-7.
<https://doi.org/10.1186/s12878-018-0111-6>
13. Ashraf B. Frequency of anemia and associated risk factors among pregnant women; a study from the remote outskirts of Quetta, Balochistan. *J Soc Obstet Gynaecol Pak*. 2023;13(2):82-6.
14. Toheed R, Ayub TB, Ali HS, Mumtaz S, Haneef A. Prevalence of anemia and its main determinants among primigravidae in antenatal population of a tertiary care hospital of Lahore. *PJMHS*. 2015 Jul 1;9(3):907.
15. Henri E, Valere MK, Lucas EE, Calixte PI, Ngalame CM, Grâce TT, Ekobo AS, Moukoko CE. Hematological profile and risk factors of anemia in pregnant women: A cross-sectional descriptive and analytical study in Douala, Cameroon. *Open J Obstet Gynecol*. 2019 Jul 18;9(7):968.
<https://doi.org/10.4236/ojog.2019.97094>
16. Noshiro K, Umazume T, Hattori R, Kataoka S, Yamada T, Watari H. Hemoglobin concentration during early pregnancy as an accurate predictor of anemia during late pregnancy. *Nutrients*. 2022 Feb 17;14(4):839.
<https://doi.org/10.3390/nu14040839>
17. Anjum A, Manzoor M, Manzoor N, Shakir HA. Prevalence of anemia during pregnancy in district Faisalabad, Pakistan. *Punjab Univ J Zool*. 2015;30(1):15-20.
18. Sarah B, Sheikh K, Shah T. Red cell distribution width is an early marker for detection of iron deficiency anemia during pregnancy. *J Liaquat Univ Med Health Sci*. 2018;17(3):165-9.
<https://doi.org/10.22442/jlumhs.181730571>
19. Purohit G, Shah T, Harsoda JM. Hematological profile of normal pregnant women in Western India. *Sch J Appl Med Sci*. 2015;3(6A):2195-9