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Evaluation of Hematological, Biochemical and Physiological Changes During Pregnancy: A Retrospective Observational Study

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ABSTRACT

The maternal body undergoes various physiological changes across multiple organ system to upkeep the growing and development of the fetus. This study was undertaken to evaluate various changes in female body during pregnancy. A total of 100 case records were collected from a tertiary level hospital, consisting of 70 pregnant women and 30 control subjects. The data was collected using a pretested data collection form. No significant differences in the age group was noted between the pregnant and control groups. The examination of complete peripheral blood count, revealed significantly decreased rates in pregnant women. However, the neutrophil count was significantly higher in the pregnant. A significant decline in iron, ferritin, hepcidin, B12 and homocysteine levels in pregnant women cas compared to controls. In contrast, the pregnant group exhibited a notably higher total iron binding capacity. During a normal pregnancy, various physiological alterations occur, encompassing leukocytosis, neutrophilia, thrombocytopenia, lymphocytopenia and anemia. Furthermore, the

INTRODUCTION

Pregnancy encompasses the growth of one or more embryos in the uterus after fertilization[1]. It involves significant physiological alterations affecting various organs and systems in the body, facilitating a successful pregnancy, childbirth, breastfeeding and supporting the needs of the fetoplacental unit[2,3].

During pregnancy, there are changes in hematological parameters, such as WBC, RBC, hemoglobin, packed cell volume, and platelets, collectively referred to as "physiologic anemia of pregnancy"[4]. Anemia, a common nutritional deficiency in pregnant women of all ages, has been extensively researched[4]. Physiologic anemia is thought to reduce blood viscosity, thereby promoting vascular permeability and optimal perfusion of the developing uteroplacental components. As a result, the growing fetus receives sufficient oxygen as well as nutrients[5].

Optimal maternal nutrition plays a vital role in promoting fetal development throughout pregnancy[6]. Hepcidin, a hormone synthesized by the liver, serves as an important regulator of iron absorption and recycling. Its expression is increased in the presence of inflammation and high iron levels, while it is decreased during periods of increased erythropoiesis requirement and hypoxia[7]. The nutritional status of a pregnant woman has a significant impact not only on her own health but also on the well-being of her future child[8].

Deficiency of vitamin D is a widespread and prevalent metabolic problem with a substantial impact on bodily growth. Consequently, it is crucial to address the vitamin lack during pregnancy to mitigate the risk of adverse perinatal hazards. Physiological hemodilution, a normal occurrence in pregnancy, results in reduced plasma levels of certain vitamins, while levels of other vitamins remain unchanged due to increased carrier proteins[9-10].

Vitamin B12 plays a critical role in supporting proper folate metabolism, which is vital for cell proliferation during pregnancy. While strong evidence deficiency of vitamin B12 is limited, it is often associated with insufficient dietary intake and a natural decline in maternal B12 levels. The high maternal metabolism together with the active transport by the placenta contribute to this decrease. If pregnant women experience vitamin B12 deficiency, there is a possibility of their newborns also having low levels of vitamin B12[11,12].

MATERIALS AND METHODS

The study comprised 70 pregnant women from all trimesters and 30 non-pregnant women were included as the control group. Socio-demographic data, including age and sex, were collected, along with relevant clinical information. The study was conducted in accordance with ethical guidelines[13,14].

For laboratory investigations, blood samples were obtained through venipuncture and divided into two tubes. One tube contained dipotassium EDTA vaccuette for complete blood count measurement using an automated instrument. The other tube, a gel tube, was used to collect serum. After allowing the blood to coagulate at room temperature for approximately an hour, the serum was separated through centrifuge machine at 1000 rpm for 30 min. The collected serum was then stored in a deep freezer (-20°C) till further analysis.

Hepcidin levels were measured using a sandwich ELISA kit. Serum levels of Iron, Ferritin, Total Iron Binding Capacity, Vitamin B12, homocysteine and, Vitamin D were determined using the Ferrozine method and colorimetric kit.

The collected data were analyzed using Mean±standard deviation and statistical significance was determined at a $p \leq 0.05$. The statistical analysis was conducted utilizing the Epi Info software.

RESULTS

No significant differences were observed in the mean age of pregnant (26.15±5.81) and control group (27.1±5.67). Table 1 shows the trimester wise distribution of study participants. Majority of women belonged to third trimester.

The complete peripheral blood count examination indicated a significant decrease in these parameters among the pregnant group compared to controls. It is noteworthy to mention that the pregnant group exhibited a significantly higher neutrophil count when compared to the control group. The results are summarized in Table 2.

The parameters examined included. The pregnant group demonstrated a significant decrease in the levels of biochemical markers, including Iron, Ferritin, Hepcidin, B12 and Homocysteine, in comparison to the control group. Conversely, the Total Iron Binding Capacity was seen to be significantly increased in the pregnant women compared to the controls. The findings are presented in detail in Table 3.

DISCUSSIONS

During the course of pregnancy, women experience numerous physiological changes affecting various systems within the body. Consequently, there are notable alterations observed in the mean count of WBC and neutrophils in pregnant women, as compared

Table 1: Trimester wise distribution of study participants

Trimester	No. of patients	Percentage
First	11	15.71
Second	24	34.29
Third	35	50.00
Total	70	100.00

Table 2: Changes in hematological parameters in study population

Hematological parameters	Non-pregnant (control) (N = 30)	Pregnant (N = 60)	p-value
WBC count (10^3 mm^{-3})	8.047±1.361	11.980±1.8	<0.05
Neutrophil count (10^3 mm^{-3})	4.015±1.075	9.052±2.617	<0.05
Lymphocytes count (10^3 mm^{-3})	1.876±0.505	3.225±1.874	<0.05
Erythrocytes count (10^3 mm^{-3})	4.315±0.584	3.105±0.667	<0.05
Hemoglobin (g dL^{-})	12.18±1.39	7.815±1.256	<0.05
PCV (%)	36.69±4.36	24.340±4.185	<0.05
Thrombocytes count (10^3 mm^{-3})	250.9±53.85	128.50±16.88	<0.05

Table 3: Biochemical changes in pregnancy

Biochemical parameter	Controls (N = 30)	Pregnant (N = 670)	p-value
Serum iron (µg dL^{-1})	90.12±34.72	27.8±4.21	<0.05
Serum hepcidin (ng mL^{-1})	14.22±7.542	0.8291±0.198	<0.05
Serum vitamin D3 (ng mL^{-1})	37.92±4.493	10.1±2.952	<0.05
Serum ferritin (ng mL^{-1})	44.65±25.11	7.5±1.987	<0.05
Total Iron binding capacity (µg dL^{-1})	404.7±26.87	588.4±74.29	<0.05
Serum vitamin B12 (pg mL^{-1})	452.3±138.6	127.1±28.3	<0.05
Serum homocysteine (µmol L^{-1})	8.112±1.133	2.11±0.4875	<0.05

to the controls[14]. Notably, the research conducted by Pughikumo et al. demonstrates that these values exhibit variations with the progression of gestational age. This observation is consistent with the previous investigations carried out by Luppi, who also reported the occurrence of leukocytosis during gestation as a consequence of physiological strain induced by the pregnancy state[15,16]. The aforementioned leukocytosis, attributed to the gestational state, likely serves as a homeostatic response to alterations in the expression of neutrophils during pregnancy, possibly due to apoptosis[17]. Moreover, it is worth noting that the increase in leukocytes primarily corresponds to a rise in the number of neutrophils[18].

In the present study, a noteworthy finding was the significantly lower platelet count observed in pregnant individuals when compared to the control group. This condition, known as thrombocytopenia, is accompanied by an increased responsiveness of platelets to aggregating biochemicals, which is linked with increased production of thromboxane A2. Thrombocytopenia in pregnancy can be attributed, at least in part, to factors such as hemodilution, heightened thrombocyte activation and increased thrombocyte clearance[19]. Furthermore, as pregnancy progresses, there is an evidence indicating a significant and consistent increase in the width of the platelet volume distribution, supporting the previously mentioned factors. Consequently, the mean platelet volume becomes an indifferent indicator of platelet size as the pregnancy advances[20].

The assessment of hemoglobin levels has traditionally been employed as an indicator for determining the severity of iron deficiency. However, relying solely on hemoglobin levels lacks both specificity and sensitivity and should not be considered the only indicator of iron levels[21]. In previous studies conducted in industrialized nations, some researchers have proposed a hematocrit level below 30% as the threshold value for diagnosing anemia in pregnant women. This cutoff value has been suggested for clinically practical application purposes.The lower PCV level observed during pregnancy is primarily attributable to haemodilution resulting from increased blood volume and enhanced glomerular filtration rate. Additionally, the fetus may consume a proportion of the mother's PCV during the gestational period[22].

This study indicates that serum iron, serum ferritin, hemoglobin, hepcidin and TIBC serve as indispensable indicators for assessing anemia in pregnancy[23]. Pregnancy, as a physiological state, frequently exerts minimal impact on the general health of expectant women. However, it does give rise to hormonal, hemodynamic, hematological and certain biological changes[24,25]. Iron plays a crucial role during gestation, including: (1) Supporting the development of the placenta and fetal development, (2) Contributing to the rise in maternal RBC mass, (3) Compensation blood losses during childbirth and (4) Reinstating iron reserves[26].

In the present study, iron deficiency was observed among pregnant women at various stages. The occurrence of iron deficiency was notably significant, especially when considering the preventability of this condition through appropriate dietary guidance and the utilization of iron supplements during pregnancy[27]. Furthermore, females with adequate iron stores exhibited a smaller rise in plasma volume compared to those with iron deficiency. This phenomenon of limited expansion of volume of plasma may mask the decline in hemoglobin in some iron-deficient females[28].

The concentration of ferritin in the bloodstream serves as a reliable indicator of iron reserves and is particularly useful in conjunction with other iron status tests to assess depleted iron storage. Hemoglobin, iron, ferritin, total iron binding capacity (TIBC) and Iron/TIBC ratio are valuable criteria for defining iron inadequacy in pregnancy and utilizing numerous combinations of these measurements can enhance the specificity of frequency estimates and help define different stages of the iron deficiency[29].

Traditionally, many criteria had been utilized for diagnosing iron deficiency anemia, including hemoglobin values, iron measurements and bone marrow investigation. However, each of the methods has limitations. Recently, a decreased Iron/TIBC has emerged as a dependable method for identifying iron deficiency anemia[30]. Throughout pregnancy, the TIBC progressively increases[31]. The significantly decreased blood iron levels and increased TIBC observed in pregnant females are partly attributed to dietary deficient iron intake. Hence, iron treatment during pregnancy is useful for preservingiron and TIBC level closer to those of non-pregnant females[32].

The rise in TIBCin second trimester of pregnancy coincides with the period of greatest iron requirement for the fetus. The current findings establish a strong association between the decline in serum iron concentrations and the increase in iron-binding capacity. If the observed alterations were solely attributable to fluctuations in plasma volume, the deviations would consistently manifest in the same direction[32]. As iron reserves diminish, there is an augmented availability of transferrin for iron binding. As anticipated during pregnancy, total iron-binding capacity (TIBC) exhibits a substantial increase with gestational age[33].

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CONCLUSION

The period of pregnancy is associated with notable hematological changes, including physiological anemia, increased leukocyte and neutrophil counts, decreased lymphocyte count and thrombocytopenia. Furthermore, the iron status plays a crucial role in diagnosing anemia in pregnant women. Enhancing the utilization of screening process for anemia during pregnancy can lead to enhanced patient management and a reduction in potential complications related to anemia.

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