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RETICULOCYTE ASSESSMENT AS A BIOMARKER FOR BONE MARROW ERYTHROPOIETIC FUNCTION IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN BENIN CITY, NIGERIA.

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ABSTRACT

A decrease in iron stores in pregnancy is accompanied by a significant rise in the reticulocyte count .This study was aimed at using reticulocyte production index as a biomarker for erythropoietic function in pregnant women. Packed cell volume was determined using sysmex® Automated Hematology System. Reticulocyte count was determined using the supra vital staining techniques. It was observed that there was a significant decrease in packed cell volume at stage 4(control subjects) when compared to stage 1(1^{st} trimester), 2(2^{nd} trimester) or 3(3^{rd} trimester) ($P < 0.05$) and significant increase in reticulocyte count, reticulocyte index and reticulocyte production index at stage 4 when compared to stage 1, 2 or 3 ($P < 0.05$). The significant decrease in packed cell volume coupled with an increase in Reticulocyte production index is a reflection of an increased erythropoiesis activity of the bone marrow, occasioned by the increased fetal demand for micro-nutrients which is associated with pregnancy. Proper compliance to vitamin B_{12}, folic acid and iron supplementations are advocated during pregnancy.

Keywords: Biomarkers; Reticulocyte production index; Micronutrients, Erythropoiesis.

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INTRODUCTION

Pregnancy is described as the period when a female carries a developing fetus in her uterus. The developmental stages is divided into three; first, second and third trimester; they last for 36 ± 2 weeks (1).

A balanced diet is an important aspect of a healthy pregnancy but this is not so for women in the local communities in Benin City, who often go down with anemia. As a result of these challenges, some pregnant women have poor pregnancy outcomes, including delivering premature babies, low birth weight and invariable death in some cases (2).

The federal ministry of health in Nigeria gives great attention to pregnant women in order to minimize morbidity and mortality. However, some perennial problems, including poor nutrition, position in polygamous homes, poor child spacing, non-compliance to supplementation with haematinics and women entering pregnancy with iron depleted status are frequently observed (3). In healthy subjects, up to 2 percent of erythrocytes are reticulocytes (4). In the face of decreased numbers of circulating red blood cells, there is an increased erythropoietic activity, driven by an increased erythropoietin release, which leads to the production of more reticulocytes(5). This study was aimed at using reticulocyte production index as a biomarker for erythropoietic function in pregnant women.

MATERIALS AND METHODS

Subjects aged between 18 and 49 years participated in the study. Patient consent form was obtained from 300 pregnant women on antenatal visit and 100 aged matched healthy individual on routine medical checkup. The pregnant women were divided into three groups according to the trimester. Ethical approval was obtained from Lahor Medical and Research Laboratory, Benin City, Nigeria.

COLLECTION OF SAMPLES

4 ml of venous blood was collected from the medial cubital vein using a vacutainer and needle from each of the subjects into an Ethylene diamine tetra acetic acid (EDTA) container.

Packed cell volume estimation

It was determined using the Sysmex® Automated Hematology Analyzer Kx-2IN, Sysmex Corporation, Kobe-Japan.

Reticulocyte Count Assessment

2 drops of New Methylene blue was added to 2 drops of EDTA anticoagulated blood in a test tube and mixed properly. It was then incubated at 37^{o}C for 10minutes. The mixture was then mixed and a drop of the mixture was placed on a clean glass slide to make a thin blood film and it was allowed to air dry. A drop of immersion oil was placed on the film and examined using the oil immersion objectives. The reticulocytes are recognized by their violet blue stained granules of the Ribosomal Ribonucleic acid (6).

Calculation

$$\text{Reticulocyte count} = \frac{\text{Number of Reticulocyte counted in 10 high power field} \times 100}{\text{Total number of cells counted}}$$

$$\text{Reticulocyte index} = \frac{\text{Reticulocyte count} \times \text{packed cell volume} \times 100}{45}$$

Where: 45 = Correction factor for packed cell volume.

$$\text{Reticulocyte production index} = \text{Reticulocyte index} \times 0.5$$

Where: 0.5 is the reticulocyte maturation index (6)

Statistical Analysis

All results were presented as Mean ± Standard Deviation(SD) and analyzed using one way analysis of variance (ANOVA) and Turkey – Kramer Multiple comparison test using SPSS – 18.0 statistical program. P values < 0.05 were considered significant.

Results

Table 1 shows the Mean ± Standard Deviation of packed cell volume (PCV), Reticulocyte count (RC), Reticulocyte Index (RI) and Reticulocyte Production index (RPI) of pregnant women at different trimester with the control subjects. It was observed that there was a significant decrease in the PCV at stage 4 when compared to stage 1, 2 and 3, ($P < 0.05$) and a significant increase in RC, RI and RPI at stage 4 when compared to stage 1, 2 and 3 ($P < 0.05$).

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Table1: The mean ± standard deviation of PCV, RC, RI and RPI of pregnant women at different trimester with control subjects.

Parameter	Stage 1 N = 100	(Stage 2) N = 100	(Stage 3) N = 100	(stage 4) N = 100
Packed cell volume (%)	28± 0.01^{A}	25± 0.02^{B}	24± 0.03^{C}	38± 0.04
Reticulocyte Count (%)	6± 0.02^{A}	8 ± 0.04^{B}	10± 0.01^{C}	2.3± 0.01
Reticulocyte Index (%)	3.7± 0.01^{A}	4.4 ± 0.02^{B}	5.3 ± 0.01^{C}	2.0± 0.01
Reticulocyte Production Index (%)	1.87± 0.02^{A}	2.2± 0.01^{B}	2.7 ± 0.02^{C}	1.02 ± 0.01

Keys

A = Significant (P < 0.05) comparison between stage 1 and 4

B = Significant (P < 0.05) comparison between stage 2 and 4

C = Significant (P < 0.05) Comparison between stage 3 and 4

Stage 1 = 1st Trimester

Stage 2 = 2nd Trimester

State 3 = 3rd Trimester

State 4 = Control Subject

N = Number of Subjects

DISCUSSION

The significant decrease in PCV at stage 4 when compared to stage 1, 2 or 3 coupled with a significant increase in RC, RT and RPI at stage 4 when compared with stage 1, 2 or 3 could be attributed to the increased physiological demand occasioned by the growing baby for micronutrients, which are consistent with an increase in erythropoietic activity associated with pregnancy. These are in accordance with these findings. Pregnancy is one of the major causes of an increased Reticulocyte count; it is attributed to subsistence poverty and malnutrition occasioned by poor compliance to vitamin B12, folic acid and iron supplementation (2, 3, 7). .An Increased physiological demand occasioned by the growing baby for micronutrients are responsible for higher bone marrow activity reflected by higher reticulocytosis (8). Anaemia is the most common hematological problem in pregnancy (9). What is referred to as physiologic anaemia of pregnancy is a dilution process secondary to an increase in plasma volume, The demands for micronutrients, especially iron and folate is increased and maternal body stores and dietary intake may be insufficient for adequate erythropoiesis (10–12) .Severe anaemia increases prenatal morbidity and mortality by causing intrauterine growth retardation and pre-term delivery (13). The level of Reticulocytosis in pregnancy may reflect the level of erythropoiesis. (8) A decrease in iron stores in pregnancy is accompanied by a significant rise in the reticulocyte count, which is consistent with an increase in erythropoietic activity (14) .An elevated reticulocyte count reflects an increase in erythropoiesis process particularly in hypoxia and gestation. (5, 15)

CONCLUSION

The significant decrease in packed cell volume, coupled with an increase in reticulocyte production index, is a reflection of an increased erythropoiesis activity of the bone marrow, occasioned by the increase in fetal demand for micro-nutrients which is associated with pregnancy. Proper compliance to vitamin B12, folic acid and iron supplementation are advocated during pregnancy.

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