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Evaluation of Iron Status, Haemoglobin and Protein Levels of Pregnant Women in Owerri Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ILO, JUC and EIO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CCNV, OMTBO, AMI and COA managed the analyses of the study. Authors CCA, NVA and NMA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was done to determine iron status, haemoglobin and protein levels of pregnant women in owerri metropolis. A total of 100 pregnant women were recruited for this study. The mean Hb levels in group 1, group 2, and group 3· were 12.00±1.68g/dl, 10.06±1.J4g/dl and 10.96±1.19g/dl respectively. The mean Serum ferritin level of group 1 was 67.00±88.38ng/ml, group 2, 52.48±52.47ng/ml and group 3, 51.26±48.70ng/ml. The mean Serum iron in group 1, 2 and 3 were 46.72±16.41μg/dl, 79.59±63.24μg/dl and 83.35±53.04 μg/dl respectively. In group 1, 2 and 3 the

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mean results. (μg/dl) of TIBC were 295.58 ± 109.53, 324.06 ± 178.00 and 319.88 ± 92.95 and % T.S (%) were 18.78 ± 11.77,26.59 ± 19.40 and 17.97 ± 10.87 percent respectively. The mean total protein was group 1,6.83±l1.77g/dl, group 2,6.39±0.70g/dl and group 3, 6.39 ±0.98 g/dl while the mean albumin (g/dl) in group 1, 2 and 3 were 4.84±0.47, 4.13±0.28 and 4.14±0.29 respectively. The mean values of globulin (g/dl) were 1.98 ± 0.91, 2.29 ± 0.87 and 1.89 ± 0.90 in groups 1, 2 and 3 respectively. As gestational age increased; serum ferritin, total protein, and albumin levels decreased while serum" iron and TIBC increased. The differences in the mean results between the groups were statistically significant ($p<0.05$) while % T.S and globulin levels when compared showed no significant difference ($p>0.05$). Iron status showed no statistical difference with increasing parity ($p>0.05$). However, from this study iron deficiency anaemia was most prevalent in second trimester; hence iron status estimation should be an integral part of routine antenatal care test during second trimester of each pregnancy for proper assessment and management of iron deficiency anaemia in pregnancy.

Keywords: Iron status; haemoglobin; protein; pregnant women.

1. INTRODUCTION

Pregnancy is a period between conception and delivery and has been associated with increased dietary requirements in humans including protein, iron, and vitamins etc. During their period of rapid growth, the foetus and placenta accrue proteins very rapidly [1,2]. The average requirement in the entire gestation period is approximately 4.4 mg/day [3-5]. The absorbed iron is reported to be predominantly used to expand the woman's erythrocyte mass, fulfill the foetus's iron requirements, compensate for iron losses at delivery.

Iron enters the body after absorption from the diet and it is highly conserved in humans. Absorption of iron from the small intestine and its release from macrophages is tightly controlled, as free iron has the potential to cause tissue damage through the production of reactive oxygen species. Hepcidin, a 25 amino acid peptide produced by the liver, is the principal iron-regulatory hormone providing the link between iron metabolism and innate immunity [6]. The total body iron content ranges between 2 and 4 g: approximately 50 mg/kg in men and 35 mg/kg in women.

Serum iron profile studies typically include measurement of serum ferritin, serum iron, transferrin or Total Iron Binding Capacity (TIBC), and the calculation of percentage transferrin saturation. Iron studies are usually requested to diagnose iron deficiency or iron overload, but interpretation can be difficult because of the relationship shared by iron metabolism and inflammation. Iron deficiency is the most common nutritional deficiency worldwide, with anaemia only one part of the clinical spectrum. It is now recognized that deficiency without overt anaemia is common, and can adversely affect growth, cognitive performance and behaviour in children and adolescents. It can also reduce immunity to infections, and decrease work capacity and performance in all age groups.

Some foods are high in certain amino acids, but their digestibility and the anti-nutritional factors present in these foods make them of limited value in human nutrition. Therefore, one must consider digestibility and secondary nutrition profile such as calories, cholesterol, vitamins and essential mineral density of the protein source [7]. On a worldwide basis, plant protein foods contribute over 60 percent of the per capita supply of protein, on average. Meat, products from milk, eggs, soy, and fish are sources of complete protein [8].

Serum protein profile studies typically include measurement of total protein, albumin and globulin. Since Pregnancy involves a number of changes in anatomy, physiology and biochemistry which can challenge maternal reserves [9] a basic knowledge of these adaptations is critical for understanding normal anthropometric and laboratory measurements.

The study was done to determine the Iron status and protein profile of pregnant women in Owerri, Imo state, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Owerri, Imo State. Owerri lies within latitude 5.485°N and 7.035°E. It is the largest metropolitan area in the state and is host to many public and private establishments.

2.2 Advocacy, Mobilization and Presurvey Contacts

With the letter of introduction/identification from the Department of Medical Laboratory Science (MLS) Imo State University (IMSD), Owerri, we applied to the Chief Medical Director of Federal Medical Centre, Owerri who directed/referred me to the Ethical Committee where we obtained an approval via ethical clearance which we took to the co-ordinator antenatal unit who gave me the necessary direction and assistance from the staff to the patients (pregnant women). Informed consent was given to the participants, after explaining the purpose of the study to them. Those who gave their consent by filling the questionnaire were recruited.

2.3 Study Population

The sample size for the study was calculated using the formula below according to Araoye 2004, using a prevalence rate of 7%.

$$n = Z^2 (1 - p) P / d^2$$

Where:

n = minimum sample size
z = standard normal variance = 1.96 at 95% confidence interval
d = precision = 0.05
P = prevalence = 7%.
Using the above formula, the calculated sample size is 100.

A total of 100 pregnant subjects were recruited. These subjects were grouped into three based on their gestational age: group1 consist of 36 women in their first trimester, group 2, 32 in second trimester and group 3, 32 in third trimester.

2.3.1 Selection criteria

Personal data including age, medical history, socioeconomic status, gestational age and parity were obtained from the subjects by use of a questionnaire.

2.3.2 Inclusion criteria

Apparently healthy pregnant women between 19 to 40 years of different gestational ages attending antenatal at FMC who gave their consent.

2.3.3 Exclusion criteria

Pregnant women who did not give consent to participate in the study or who had complications of pregnancy.

2.4 Sample Collection

10ml of fresh venous blood was drawn from each subjects by a clean veinous puncture using a sterile hypodermic syringe from the antecubital vein and delivered into two different containers; 3mls was delivered into an EDTA container and the remainder was delivered into a plain container to obtain serum. The samples were then taken to Royal Image Diagnostic Services, Owerri for analysis.

2.5 Laboratory procedures

All reagents for the research were commercially purchased and manufacturer's standard operating procedures were strictly adhered to.

3. METHODOLOGY

3.1 Haemoglobin Estimation

Method: Cyanmethaemoglobin method [10].

Procedure: 1 in 201 dilution of blood were made by adding 20 μl of blood to 4 ml of diluent (Drapkin's solution) into a test tube. The test tubes were stoppered and inverted several times and were then poured into a cuvette and the absorbances were read in a colorimeter at 540 nm against the reagent blank. Haemoglobin level was read off from the calibration graph prepared.

Reference range:

Male = 13.0-18.0g/dl
Female = 12.0-15.0g/dl

3.2 Serum Ferritin Estimation

Method: Enzyme-Immunoassay method [11]. Reagent kit was purchased from BIOTEC Laboratories Ltd. Catalog No 7/352.

Procedure: Into different microplates, 20μl of the test samples and standard solution were added. An anti-ferritin monoclonal antibody conjugate of 100 μl was added into each standard and test microplate.

The microplates were sealed using plate sealer and were shaken for 30 seconds to allow proper mixing of the solution. The mixtures were in an incubator at 37°C for 30 minutes. Using microplate washer, the test and standard plates were washed with washing solution to remove unbounded antibodies. 200 μ l chromogen/substrate solution was added into each microplate and sealed with plate sealer. The solution was incubated for 10 minutes at room temperature in the dark. 1 00 μl of stop solution was added after 10 minutes to terminate the enzyme reaction and a coloured solution was obtained. The concentration of ferritin in each sample was measured using automatic microplate reader. The colour intensity is proportional to the concentration of the ferritin present in each sample.

3.3 Serum Iron and TIBC Estimation

Method: spectophotometric [11].

The reagent kit was obtained from Teco diagnostic Industry, USA.

Procedure

Serum Iron: The tubes were labeled blank, standard, control and test accordingly. Into each tube, 2.5ml Iron buffer reagent was added. 0.5ml (500μl) sample was added to each tubes, while 500μl iron-free water was added to blank. The spectrophotometer was zero with the blank and read at 560 nm wavelength. The absorbance of all tubes (A_1 reading) were read and recorded. After recording A_1 reading, 0.05ml (50μl) of Iron colour reagent was added to all tubes. The solution was mixed and placed in water bath at 37°C for 10 minutes. The spectrophotometer was zeroed Lusing the blank at 560 nm wavelength. The absorbance of all tubes were read (A_2 reading) and recorded.

Calculation:

A = Absorbance
Std= Standard

$\frac{A_2 \text{ Test} - A_1 \text{ Test}}{A_2 \text{ Std} - A_1 \text{ Std}} \times$ Cone. Of std = Total Iron (μg/dl)

UIBC (Unsaturated Iron-binding Capacity): The tubes were labeled, blank, standard, control and test accordingly. Into each tube, 2.0 ml UIBC buffer reagent was added. 0.5ml (500 μ 1) iron-free water and 0.5ml (500μ1) of standard was added. to the tube labeled "standard", while 0.5ml (500 μ 1) sample plus 0.5ml (500 μ 1) Iron Standard was added to the tube labeled "Test". 1.0ml iron-free water was added to blank. The spectrophotometer was zero with the blank and read at 560 nm wavelength. The absorbance of all tubes (A_1 reading) were read and recorded. 0.05ml (500μ1) of Iron colour reagent was added to all tubes. The solution was mixed and placed in water bath at 37°C for 10 minutes. The spectrophotometer was zeroed using the blank at 560nm wavelength. The absorbance of all tubes were read (A_2 reading) and recorded.

UIBC Calculation:

$$\text{Cone, Of Std} - \frac{[A_2 \text{ Test} - A_1 \text{ Test}]}{[A_2 \text{ Std} - A_1 \text{ Std}]} \times \text{Cone. Of std} = \text{UIBC } (\mu\text{/dl})$$

TIBC (Total Iron-binding Capacity) Calculation:

Iron Level + UIBC = TIBC (μg/dl)

Transferrin Percentage Saturation Calculation:

$\frac{\text{Serum Iron}}{\text{TIBC}} \times 100 = \%$ Transferrin Saturation

Reference range:

Serum Iron = 60-150μg/dl
TIBC = 250-400 μg/dl
Transferrin Saturation = 20-55%

3.4 Total Protein Estimation

Method: biuret (cheesebrough, 2005).

The reagent kit was obtained from Teco diagnostic Industry, USA.

Procedure

The tubes were labeled, Blank, Standard, Control, and test accordingly. 3.0 ml of reagent was added into each tube 50μl of standard was added to the tube labeled "standard", while 50 μ l sample was added to the tube labeled "Test". The solution was mixed by inversion and was allowed to stand

at room temperature (15-30^{0}C) for 10 minutes. The spectrophotometer was zeroed using the blank at 540 nm wavelength. The absorbance of all tubes were read and recorded.

Calculations:

Abs. of Unknown X Cone, Of Standard
Abs. of Standard

3.5 Albumin Estiamation

Method: Bromocresol Green (BCG) [12].

Procedure: Three test tubes were labeled test, standard and blank. 10microlitre of distilled water was added to the blank test tube, 10micolitre of standard was also added to standard test tube and 10.

3.6 Statistical Analysis

The data was expressed as mean, standard deviation, and presented in tables. The test of significance was carried out using the Chi - square.

4. RESULTS

Table 1 shows the mean ±S.D values for haemoglobin, serum ferritin level, serum iron, TIBC, Iron saturation, total protein, albumin and globulin levels of the three groups of study. In first trimester the mean ±S.D values of haemoglobin, serum ferritin, serum iron, TIBC, total protein were l2.0±1.68, 67±88.38, 46.72±16.41, 295.58±109.53, and 6.83±l.0 respectively. In second trimester, the mean ±S.D of serum ferritin, serum iron, TIBC, and % transferrin Saturation were 10.6±1.34, 52.48±52.47, 79.59±63.24, 324.06±178.230, and 6.39±0.7 respectively. The mean ±S.D of serum ferritin, serum iron, TIBC, and % transferrin Saturation of third trimester were 10.96±1.19, 51.26±48.71, 83.35±53.04, 319.88±92.95, and 6.04±0.98 respectively.

Table 2 shows the relationship between iron status and gestational age in pregnant women studied. With advancing gestation age, Haemoglobin and serum ferritin decreased significantly (P<0.05) with statistical significance of x^2=20.854 and 9.061 respectively. Serum Iron increased significantly (P>0.05) with a statistical significance of 12.429 from first to third trimester while TIBC and % transferrin saturation also increased with statistical significance of 4.513 and 2.083 respectively (P>0.05).

Table 3 shows the relationship between protein profile and gestational age in pregnant women studied. With advancing gestation age, total protein and albumin decreased significantly (P<0.05) with statistical significance of x^2= 11.859 and 17.754 respectively. Globulin values was not significantly different (P>0.05) with increase gestational age.

Table 4 shows the relationship between iron status and gestational age in pregnant women studied. The iron status declined but not significantly (P>0.05) with increasing parity.

Table 1. mean ± S.D values of iron status and protein profile of the pregnant women under study

Parameters	First Trimester	Second Trimester	Third Trimester
Haemoglobin (g/dl)	12.0±1.68	l0.6±1.34	10.96±1.19
S.Ferritin (ng/ml)	67±88.38	52.48±52.47	51.26±48.71
Serum Iron (□g/dl)	46.72±1-6.41	79.59±63.24	83.35±53.04
TIBC (□g/dl)	295.58±l09.53	324.06±17.08	319.88±92.95
T. Saturation (%)	18.78±l1.77	26.59±19.4	17.97±10.87
Total protein (g/dl)	6.83±1.0	6.39±0.7	6.04±0.98
Albumin (g/dl)	4.84±0.47	4.13±0.28	4.l4±0.29
Globulin (g/dl)	1.98±0.91	2.29±0.87	1.89±0.9

Table 2. Relationship between gestational age and iron status

Parameter	From 1st to 3rd trim.	Test	Df X^2	P-value
Haemoglobin	decreased	20.854	2	P<0.05
Serum ferritin	decreased	9.061	2	P<0.05
Serum iron	increased	12.429	2	P<0.05
TIBC	increased	4.513	2	P>0.05
0/0 Trans. Saturation	increased	2.083	2	P>0.05

Table 3. Relationship between Gestational Age and Protein Profile

Parameter	From 1st to 3rd trim	Test X^2	Df	P- value
Total protein	decreased	11.859	2	P<0.05
Albumin	decreased	17.754	2	P<0.05
Globulin	was stable	1.1949	2	P>0.05

Table 4. Relationship between parity and Iron Status

Parameter parity	Increasing Test	X2	Df	P-value
Haemoglobin	decreased	0.21	2	P>0.05
Serum ferritin	decreased	4.225	2	P>0.05
Serum iron	increased	5.812	2	P>0.05
TIBC	increased	0.794	2	P>0.05
$^0/_0$ Trans. Saturation	increased	0.318	2	P>0.05

5. DISCUSSION

From the result obtained in this study, there was a significant decrease ($p<0.05$) in serum ferritin level (ng/ml) as the gestational age advances. The highest level of serum ferritin was seen in pregnant women in their first trimester (67.00 ± 88.38). In the second trimester, the level decreased (52.48 ± 52.47) and further in third trimester (51.26 ± 48.71). This finding is in line with the work of Kubik et al. [13] and okwara et al. [14] on serum ferritin levels in pregnant women. The progressive decrease in serum ferritin may be due to the increased mineral transfer from mother to fetus as pregnancy advances. The high iron need in pregnancy necessitates the mobilization from its stores which consequently with further decrease may result in complete iron store depletion, According to Lewis et al. [15] , as iron stores are depleted and exhausted, haemoglobin concentration decreases leading to iron deficiency anaemia.

The above physiological reasons explained why the mean value of haemoglobin concentration (g/dl) decreased with increase in gestational age in this study. The decrease was statistically significant ($p<0.05$) when compared among the three groups. This finding is in accordance with the previous work done by Okwara et al. [14]; Scholl et al. [16]; Netal and Ayub [17] and Lee [18] on haemoglobin level and iron status in pregnant women. A prevalence of 32% (4%, 12% and 8% in first, second third trimesters respectively) anaemia and 20% (4% in first and12% in third trimester) iron deficiency was recorded from this study. This is similar to 30.2% anaemia by Lee [18] at Korea, 24.5% anaemia by Gwarzo and Ugwa [19]. In spite of the differences in findings, which could be due to short pregnancy intervals and low educational status of their study population; these researchers found anaemia and iron depletion prevalent in second trimester and more in third trimester hence it's in keeping with the present findings of this study.

Serum iron level (ug/dl) progressively increased from first to third trimesters in this study. The increase was statically significant ($P<0.05$). First trimester had the lowest value (46.72 ± 16.41) while the second and third trimester were (79.59 ± 63.24) and (83.54 ± 53.04) respectively. Similarly, the mean ± S.D of TIBC and percentage transferrin saturation from this study increased progressively as the gestational age increases but this was not statistically significant ($P>0.05$) when compared among the three groups. In accordance with this finding, Kubik et al. [13] recorded an increase in percentage transferrin saturation and the other iron status parameters as pregnancy advances. Moreso, in the work of Okwara et al. [14] TIBC and serum iron increases across the trimester. These findings are in contrast with the work done by Netal and Ayub [17], who observed decrease in transferrin saturation, TIBC, and serum iron as gestational age advances. They attributed the differences to high parity, and low socioeconomic status of their studied area, which consequently gave rise to high prevalence of iron deficiency found in the study.

From second trimester, iron requirement begin to increase and continue to do so throughout the remaining stages of pregnancy. During this period, expansion of red cell mass and increased iron transport and transfer to both the growing fetus and placental are expected to decline the maternal iron stores leading to latent iron deficiency [20,5]. With regard to parity, this study recorded no significant

difference (P>0.05) between increasing parity and iron status, though there was a slight decline in the status. Netal and Ayub [17] observed iron depletion with increased parity which they attributed to the short pregnancy interval in their study area. The reasons for this comparatively difference may due to good family planning as shown by average child spacing and averagely low parity owing to high educational and socioeconomic status of the women in this study area, though multiparity especially when the pregnancies have occurred in quick succession drains the maternal reserve increasing chances of development of iron deficiency anaemia in pregnancy.

The mean ± S.D level of total protein (g/dl) and albumin (g/dl) level in this study showed a progressive decrease as the gestational age advances and was statistically significantly ($p<0.05$). The mean ± S.D of globulin level (g/dl) were 1.98 ± 0.91, 2.29 ± 0.87 and 1.89 ± 0.90 in first, second and third trimesters respectively. Globulin level when compared among the groups of study was not statically significant ($p>0.05$) with advancing gestation. The variations in the protein profile are associated with the physiological increase in plasma volume during pregnancy. The low total protein concentration is due to dilution [21] which subsequently alters the albumin level. This negative feedback is usually balanced by globulins [22]. The finding from this study is in agreement with the work by Adedeji et al.[23] that detected and thus stated that serum proteins concentrations decreased significantly and progressively as gestation. The results obtained from this study revealed that the mean concentration of serum proteins exhibited variations with advancing gestational age. Hence, the relationship between protein profile and gestational age from this finding is statistically significant ($p<0.05$).

6. CONCLUSION

The study showed changes in iron status and serum proteins during normal pregnancy. The study recorded 32% and 20% prevalence of anemia and iron deficiency respectively.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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