Original Research Article

Iron Status of Pregnant Women at Nsukka, Enugu State, South East, Nigeria.

Abstract:

Background

Anaemia is a serious health issue, particularly in remote areas such as Nsukka, Enugu State , South East geopolitical zone of Nigeria as a result of poor awareness. A comprehensive study on the iron status of pregnant women attending antenatal clinic in Nsukka, is rare.

Objectives

The study aimed to evaluate the iron status of pregnant women living at Nsukka in Enugu State, South, East Nigeriavia monitoring prevalence and severity of anaemia in pregnant women in first, second and third trimester using haemoglobin and ferritin concentration as bio- indicators of anaemia among the subjects, effect of age, gestational age and intake of routine drugs on haemoglobin and ferritin concentration of the subjects.

Methods

A cross-sectional study was conducted involving 127 pregnant women, within the ages of 20-49years , grouped based on the stages of pregnancy namely first trimester 30 pregnant women (23.6%) , second trimester 40 pregnant women 31.5% , and third trimester 57 pregnant women (44.9%) , attending antenatal clinic in Nsukka Divisional Health Centre were recruited for the study using simple random technique.All data and statistical analyses were conducted using Statistical Package for Social Sciences software, version 27. Descriptive statistics such as means and percentages were calculated and reported by groups. Test of statistical significance was carried out using One-Way Analysis of Variance (ANOVA). Pearson test was performed to assess the correlation between haemoglobin , ferritin concentrations with other stated variables.

Results

The age distributions of the 127 pregnant women were 20 – 24 years (26.8%), 25 – 29 years (37%), 30 – 34 years (20.4%), 35 – 39 years (12.6%), 40 -44 years (2.4%) and 45 – 49 years (0.8%). Study revealed that 39.4% of the pregnant women were anaemic because their haemoglobin concentration was less than 11g/dl. Exactly 27.6% of women were anaemicbased on ferritin concentration below 15 µg/l . None of the subjects were anaemic in first trimester, 26% of the subjects were anaemic in second trimester while 11.3% of the subjects were anaemic in third trimester. Based on ferritin level alone, 66.6% of the subjects were anaemic in first trimester, 46% in second trimester while 53.5% of the subjects in third trimester were anaemic. Conversely, 16.7% of the subjects in first trimester, 22% in second trimester and 23.9% in third trimester were anaemic based on low haemoglobin and ferritin level respectively.Haemoglobin concentration significantly ($p < 0.05$) showed negative correlation with age effect and positive correlation with ferritin concentration although not significant ($p > 0.05$). Gestational age correlated with haemoglobin and ferritin concentration showed negative correlation which was not significant ($p > 0.05$). Intake of routine drug correlated with haemoglobin and ferritin respectively showed negative and positive correlation respectively, although not significantly different ($p > 0.05$).

Conclusion and Recommendations

Maternal iron status is heavily affected by pregnancy even in a high-resource, generally iron-supplemented population. Our study revealed that anaemia during pregnancy was highly prevalence in Nsukka, Enugu State because more than halve of the subjects screened were anaemic in both first, second and third trimester. Early screening of pregnant women for iron status using serum or plasma ferritin standard measure of iron deficiency according to the world Health Organization is highly encouraged . There is a need to educate and implement strategies that will improve iron status of pregnant women for the safety of the mother and the unborn child.

Key words: Anaemia, Ferritin, haemoglobin, Pregnant women, Nsukka

Introduction

Iron is an essential heavy metal that forms the central core of haemoglobin .It plays the role of oxygen transportation in erythrocytes, production of deoxyribonucleic acid and metabolism in muscle (Pantopoulos et al ., 2012 ; Yiannikourides and Latunde-Dada, 2019). The most common nutritional deficiency in the globe is iron deficiency and it is the leading cause of anaemia (Churchill et al ., 2022) . A recent estimate based on World Health Organization (WHO) criteria indicate that around 600-700 million people worldwide have a marked iron deficiency anaemia. The important and commonly used assay for iron status is serum or plasma ferritin as it is a standard measure for iron deficiency (WHO, 2020). Mei et al., (2015) recommended universal prenatal iron-folic acid and other micronutrients as measure to prevent anaemia during pregnancy. Iron deficiency anaemia in pregnancy has been defined by the National Academic of Science Panel on Nutrition as ferritin level lower than 15 µg/l while the World HealthOrganization defined anaemia in pregnancy as haemoglobinlevel less than 11g/dl (Elzahrani, 2012). For non – pregnant women, the World Health Organization (WHO, 2015) defined anaemia as haemoglobin concentration less than 12g/dl and iron deficiency anaemia as serum ferritin concentration less than 15 µg/l.

Anaemia (due to iron deficiency) in pregnancy is a problem of public health concern around the globe (Obianeli et al , 2024). The World Health Organization estimates that 40% of pregnant women worldwide are anaemic and the three major causes include iron deficiency, haemoglobinopathies and malaria (WHO, 2020). Physiologically, there is an increase in the level of iron needed in pregnancy because there is transfer of iron to the growing fetus and placenta. The erythrocytes also expand in mass. There is variation of iron needed in different trimesters. The level of iron needed during the first trimester is very low because loss of blood by menstruation has stopped. However , there is steady rise in iron level in the second trimester , through to the third trimester (Bothwell, 2000). When iron is deficient in pregnancy, the woman will have problem in breathing, suffer fainting, palpitation, sleep difficulties, pre-eclampsia and bleeding (Abu-Ouf and Jan, 2015). During second trimester of pregnancy, anaemia has been linked to low birthweight, prematurity, fetal death, cognitive domain and neuro-behavioural development are impaired (Abu-Ouf and Jan, 2015). There is a current concern that irrespective of worldwide campaign against anaemia in pregnancy, women of reproductive age in developing nations like Nigeria still enter into pregnancy without proper iron stores. These pregnant women could give birth to children with inadequate iron deposit. Anaemia associated with iron deficiency in pregnant women in South East Geopolitical zone of Nigeria is a serious medical issue particularly in remote areas due to poor awareness. Therefore, there is urgent need to carry out public health campaign so as to sensitize pregnant women in need of early antenatal booking and iron supplementation during pregnancy. The scientific information from this research will enhance this sensitization. The aim of this research was to Investigate the iron status of pregnant women at the first, second and third trimester in Nsukka, Enugu State, South East, Nigeria.

Materials and Methods

Subjects

A cross-sectional study was conducted involving 127 pregnant women, within the ages of 20-49years , grouped based on the stages of pregnancy namely first trimester 30 pregnant women (23.6%) , second trimester 40 pregnant women 31.5% , and third trimester 57 pregnant women (44.9%), attending the antenatal clinic in Nsukka Divisional Health Centre were recruited for the study using simple random technique.All experimental protocols were carried out in accordance with the World Medical Association Declaration of Helsinki and all subjects provided written consent. Ethical approval (UNN/FBMS/EC/1017) was obtained from the Ethics and Biosafety Committee of Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. The objectives and benefit of this study was carefully explained to the subject before they accepted to take part in the study.

Study Area

Pregnant women attending the antenatal clinic in Nsukka Divisional Health Centre were recruited for the study. This is the major public maternity unit within Nsukka community. The hospital gives quality healthy service and sensitize pregnant women on how to take care of themselves during the course of their pregnancy.

Equipments and Instruments

Equipment and instrument used for the study were obtained from Shalom Research Laboratory and Projex Laboratory and other scientific shop in Nsukka. They include; centrifuge (model 800D; New Life Medical Instrument, England), Spectrophotometer (model SPM721- 2000, Biodiagnosis Inc., USA), refrigerator (Haier thermo cool, England), non-anticoagulated bottles, EDTA bottles, micropipette (Volume Range 0-1000µl; Swastika Scientific Instrument Private Ltd, Mumbai India), micro titer plate reader (model MR 9602A; Biotech USA).

Data Collection

The following were the inclusive criteria for selection of subject; their age, trimester, routine drug, pregnancy history, resident within Nsukka community, Enugu State, consent to participate in the study, willingness to give 5ml of blood.

Sample Collection

Five milliliter (5ml) of blood sample was collected from each participant by venepuncture using disposable needles and syringes. Then 3ml of the blood sample was transferred into a labeled plane container. The blood was allowed to clot, spun at the speed of 4000rmp for 10 minutes using centrifuge. The serum obtained was carefully separated using Pasteur pipette and transferred to a new plain container, while the whole blood was discarded properly according to the best practice of laboratory waste disposal. The remaining 2ml of the blood sample was transferred into EDTA bottle for haemoglobin determination.

Haemoglobin Determination

Haemoglobin concentration was determined as described by Ochei and Kolhalther (2008) based spectophotometric method. A blood sample of 20 µl was diluted in 8ml of Drakin's solution by 1:250. The tube was then covered and inverted several times and left to stand for 5 minutes to ensure complete conversion. The cyanmethaemoglobin (HiCN) was poured into a cuvette. Concentration of haemoglobin was measured spectrophotometrically at 540nm using Drabkin's solution as blank. The colour intensity measured at 540 nm is proportional to the total haemoglobin concentration A conversion factor of 36.8 was used in multiplication to get to give the actual haemoglobin concentration.

Ferritin determination

Serum ferritin determination was done using enzyme immunoassay method as described by Addison (1972) .A suitable substrate was added to produce a colour effect measurable with the use of spectrophotometer. Prior to assay, the reagent was allowed to stand at room temperature 22^{o}c. The reagent was gently mixed before use. The desired number of coated strips was placed in to the holder for patient's specimen to be assayed in duplicate. A known volume, 25 µl (0.02ml) of ferritin standard, control and samples waspipette into appropriate wells and 100µl of biotin reagent was added into each well. The plate was shaken for 30 minutes. The plate was covered and incubated for 30 minutes at room temperature 22^{o}C. Liquid was removed from all wells and washed well for three times with buffer. The plate was tapped and blotted dry with absorbance paper followed by the addition of 0.100 ml of the enzyme reagent into each well , covered and incubated for 30 minutes at room temperature 22^{o}C. The liquids from all wells were removed and washed for three times with buffer. The absorbance in each well was read at 450nm within 15 minutes after adding the stop solution using wavelength 630nm in a micro plate reader.

Statistical analysis

All data and statistical analyses were conducted using Statistical Package for Social Sciences software, version 27. Descriptive statistics such as means and percentages were calculated and reported by groups. Test of statistical significance was carried out using One-Way Analysis of Variance (ANOVA). Pearson test was performed to assess the correlation between haemoglobin , ferritin concentrations with other stated variables.

Results and Discussion

Anaemia (due to iron deficiency) in pregnancy is a problem of public health concern around the globe (Obianeli et al , 2024). The World Health Organization estimates that 40% of pregnant women worldwide are anaemic and the three major causes include iron deficiency, haemoglobinopathies and malaria (WHO, 2020). The aim of this research was to determine the prevalence and severity of anaemia in pregnant women in first, second and third trimester using haemoglobin and ferritin concentration as bio-indicators for anaemia among the subjects. Effect of age, gestational age (trimester) and intake of routine drug on haemoglobin and ferritin concentration of the subjects were also determined.

Table 1 shows the demographic information of pregnant women. One hundred and twenty-seven pregnant women aged 20 – 49 years participated in the study. The age distributions were 20 – 24 years (26.8%), 25 – 29 years (37%), 30 – 34 years (20.4%), 35 – 39 years (12.6%), 40 -44 years (2.4%) and 45 – 49 years (0.8%). Among the pregnant women used in this study, 23.6% were in their first trimester , 31.5% were in their second trimester and (44.9.9%) in their third trimester. A sample of 44.1% took the routine drugs regularly while 55.9% did not.

Table 1: Demographical Information on Pregnant Woman

Age (years	Number	Percentage
20-24	34	26.8
25-29	47	37
30-34	26	20.4
35-39	16	12.6
40-44	3	2.4
45-49	1	0.8
Stages of Pregnancy		
1st Trimester	30	23.6
2nd Trimester	40	31.5
Third Trimester	57	44.9
Intake of Iron Supplements		
Regular	56	44.1
Irregular	71	55.9

n = 127

Based on the WHO criterion of haemoglobin level less than 11g/dl as definition of anaemia in pregnancy, 39.4% of the pregnant women used in this study were anaemic, with a total number of 0.79% in first trimester, 18.89% in second trimester and 19.69% in third trimester (Table 2). Our finding is in consonant with that of McMahon (2013) but lower than the value of 64% reported in Enugu State (Ezugwu et al ., 2013).Okoroiwu et al., (2021) also reported in their study thatanaemia was predominant in regnant women at their second trimester. However, our value was higher than that reported by Alem et al., (2013) in North West Ethiopia. This may be attributed to the differences in feeding habits, routine drug intake or some other factors not considered in this research.

The important and commonly used assay for iron status is serum or plasma ferritin as it is a standard measure for iron deficiency (WHO, 2020). The results in Table 3 revealed thatexactly 27.6% of women were anaemic based on ferritin concentration below 15 μg/l . None of the subjects were anaemic in first trimester, 26% of the subjects were anaemic in second trimester while 11.3% of the subjects were anaemic in third trimester. Based on ferritin level alone, 66.6% of the subjects were anaemic in first trimester, 46% in second trimester while 53.5% of the subjects in third trimester were anaemic. Late antenatal booking may have contributed to a rise in prevalence, since most of the subjects in this study started their antenatal booking during the second and third trimester (Owolabi and Olalorum, 2014).

Table 2:Prevalence of Anaemia using Haemglobin Concentration (Anaemia = Hb <11g/dl)

Gestation age	Number	Percentage
First trimester	1	0.79
Second trimester	24	18.89
Third trimester	25	19.69
Total	50	39.4

n = 127

Table 3: Prevalence of Anaemia using Ferritin Concentration alone (Anaemia = ferritin<15µl).

Gestation age	Number	Percentage (%)
First trimester	5	3.94
Second trimester	15	11.81
Third trimester	15	11.81
Total	35	27.60

n = 127

The result of effect of haemoglobin on gestational age (Table 3) revealed higher value (11.60±1.86)in first trimester when compared to second (11.03±1.56) and third (11.06±1.64) trimester respectively. This result is in accordance with Kumar *et al.* (2013) and Goswami *et al.* (2014), which reported that increase in gestational age does not affect haemoglobin concentration. Also Hogue (2016) reported that the prevalence of anaemia among pregnant women of in Bida, Niger State based on haemoglobin level shows no difference when compared with other trimester. This could be as a result of greater expansion of plasma volume with increase in red blood cell volume (Perez *et al.*, 2005). Plasma volume expands nearly 50% and consequently haemoglobin level decreases. However, ferritin level in second trimester was higher when compared to first and third trimester.According to Bencarova and Breymann (2014), serum ferritin concentration is maximum in second trimester and then falls with advance gestational age in third trimester. This could be as a result of iron supplement consumption by the subject in second trimester since they normally start their prenatal care in second trimester. By third trimester, the iron demand by the foetus increases leading to a general decline in ferritin levels. Gestational age when correlated with haemoglobin and ferritin concentration shows a negative correlation ($p > 0.05$). This implies that as gestational age of the subject increases, there was a decrease in maternal haemoglobin and ferritin concentration. This result agrees with the report of Demmouche*et al.* (2011) and Nik *et al.* (2012).

Table 4: Prevalence of Anaemia using Combine Haemoglobin + Ferritin Concentrations in first, second and third trimester.

Parameters	**Prevalence (%)**		
	1st trimester (n=6)	**2nd trimester (n=50)**	**3rd trimester (n=71)**
Normal Hb + Normal Ferritin	**1 (16.7)**	**3 (6)**	**8 (11.3)**
Low Hb + Normal Ferritin	**0**	**13 (26)**	**8 (11.3)**
Normal Hb +Low Ferritin	**4 (66.6)**	**23 (46)**	**38 (53.5)**
Low Hb + Low Ferritin	**1 (16.7)**	**11 (22)**	**17 (23.9)**

Table 5: The Effect of Age on Haemoglobin and Ferritin Concentrations

Age range	**Mean Hb (g/dl)**	**Mean Ferritin (µg/l)**
20-24 years (n=34)	**11.57 ± 1.37**	**Mean Ferritin (µg/l)**
25-29 years (n=47)	**11.20 ± 1.91**	**86.84 ± 11.01**
30-34 years (n=26)	**10.69 ± 1.03**	**90.06 ± 12.20**
35-39 years (n=16)	**10.58 ± 1.75**	**80.99 ± 10.41**
45-49 years (n=1)	**8.64 ± -**	**8.78 ± -**

Result are expressed in mean ± standard deviation.

Table 6:The Effect of Gestation Age (first, second and third trimester) on Haemoglobin and Ferritin Concentrations

Gestation age	Mean Hb (g/dl)	Meam Ferritin (μg/l)
First Trimester (n=6)	11.60 ± 1.86	16.24 ± 3.10
Second Trimester (n=49)	11.03 ± 1.56	16.24 ± 3.10
Third Trimester (n=72)	11.06 ± 1.64	66.53 ± 5.09

Result are expressed in mean ± standard deviation., Hb = Haemoglobin

The result of the effect of age (Table 6) showed slightly low levels of haemoglobin concentration among subjects within the age of 20 – 24 years and 25 – 29 years, but lower levels among subjects within the age of 30 -34 years and 45 – 49 years. This implies that haemoglobin level decreases as age increases. This could be as a result of the physiological function of the cells (Finberg, 2011). This result agrees with the findings reported by Marieb and Hoehn, (2013) and Galti *et al.* (2012) in Ethiopia. However, ferritin concentration increased among subjects within the age of 25 – 29 years and 35 – 39 years but decreased among subjects within the age of40 – 44 years and 45 – 49 years. An increase in ferritin concentration among the subject could be as a result of high intake of food that contain iron while a decrease in ferritin concentration among the subject could be as a result of the inability of the subject to absorb iron (Sharp and Srai, 2007). This result agrees with the report of Kozuki *et al.* (2012).

The result of correlation analysis (Table 8) on the effect of age on haemoglobin concentration shows a negative correlation but positively correlated with ferritin concentration. This means that as the age of the subjects was increasing, their haemoglobin level was decreasing. This decrease was statistically significant ($p < 0.05$). However,ferritin concentration of the subject was increasing with an increase in age, although not significant ($p > 0.05$). this result is in accordance with the results of previous study done by Sanku*et al.* (2010).

Report from most developing countries shows that iron supplement intake during the time of pregnancy has limited effect. This could be as a result of poor adherence, infection, inefficient health care systems and high rate of pre-existing anaemia (Alizadeh *et al.*, 2014). Prevalence of anaemia among pregnant women in this study is high despite the fact that they received an average intake of routine drug. This finding is similar to that of Alizadeh *et al.* (2014) . The result of haemoglobin concentration of the subject who had the routine drug (Table 7) inthis study showed a slight difference on effect of intake of routine drug among pregnant women when compared to those that did not. Although there was a decrease in ferritin concentration of those that took the routine drug, there was an increase in ferritin concentration of those that are not regularly taking the routine drug. This could be that the subjects were already iron deficient before taking the routine drug. This result is in accordance with that reported by Mcmahon, (2013) which said that the level of serum ferritin falls early in the development of iron deficiency and it is not affected by recent iron ingestion while an increase in the ferritin level of those that are not taking the routine drug could be because the subjects were taking other supplementsapart from the one issued to them. This result varies with the previous reportof Arise, (2017). Intake of routine drug when correlated withhaemoglobin concentration showed a negative correlation at (p>0.05) but when correlated with ferritin concentration shows a positive correlation at (p>0.05). This implies that increase in intake of routine drug seems to reduce the haemoglobin level but increase ferritin level among the subjects.

Table 7: TheEffect of Routine Drug on Haemoglobin and Ferritin Concentrations

Groups	Mean Hb (g/dl)	Mean Ferritin (µg/l)
Regular (n=57)	11.30 ± 1.73	58.53 ± 4.08
Irregular (n=70)	10.59 ± 1.49	75.17 ± 9.10

Result are expressed in mean ± standard deviation, Hb = Haemoglobin

Table 8: Correlation Analysis of Age range, Gestationage and Routine Drugs on the HaemoglobinandFerritin Concentrations ofPregnant Women.

Groups	**Mean Hb (g/dl)**	**Mean Ferritin (µg/l)**
Age	**- 0.260****	**0.083**
Stage of Pregnancy	**- 0.36**	**- 0.021**
Intake of Supplements	**- 0.126**	**0.057**

Correlation is significant at p- value (p <0.05)
*** Correlation is significant at the 0.01 level**
**** Correlation is significant at the 0.05 level**

Conclusion

Maternal iron status is heavily affected by pregnancy even in a high-resource, generally iron-supplemented population. Our study revealed that anaemia during pregnancy was highly prevalence in Nsukka, Enugu State because more than halve of the subjects screened were anaemic in both first, second and third trimester. Early screening of pregnant women for iron status using serum or plasma ferritin standard measure of iron deficiency according to the world Health Organization is highly encouraged . There is a need to educate and implement strategies that will improve iron status of pregnant women. This will lead to sufficient placenta iron transfer to the unborn child.

Conflict of Interest

We declare that there was no conflict of interest.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.

2.

3.

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