Original Research Article

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

Abstract: Anaemia associated with iron deficiency in pregnant women in South East, Nigeria is a serious medical issue particularly in remote areas due to poor awareness. The aim of this research work was to determine the prevalence and severity of anaemia in pregnant women in first, second and third trimester using haemoglobin and ferritin concentration as an indicator of 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. In this study a total of 127 blood samples from pregnant women within the age of 20 – 49 years, in which 6 were in first trimester, 50 in second trimester and 71 in third trimester were collected. From this study, the prevalence of anaemia in the subjects based on haemoglobin concentration below 11 g/dl was 39.4%, prevalence based on ferritin concentration below 15 µg/l was 27.6%. The result of correlation analysis of haemoglobin concentration on the effect of age shows a negative correlation which is significant (p < 0.05) but positively correlated with ferritin concentration although not significant at (p > 0.05). Gestational age when correlated with haemoglobin and ferritin concentration shows a negative correlation at (p > 0.05). Intake of routine drug when correlated with haemoglobin shows a negative correlation at (p > 0.05)but when correlated with ferritin shows a positive correlation, although not significant at (p > 10.05). This study reveals that more than half of the subjects were anaemic and their children stands the risk of being born anaemic especially when they are exclusively breast fed. Determination of the iron status of these pregnant women will help the scientific community on the correct approach in the treatment, prevention and management during pregnancyTherefore, public health campaign should be carried out to sensitize pregnant women on the need for early antenatal booking and iron supplementation during pregnancy.

Key words: Iron, haemoglobin, Ferritin, Pregnant women, anaemia, trimester, Nsukka

Introduction

Iron is a type of mineral found in all cells of the body. Iron is obtained from the diet, enters the body and is carried throughout the bloodstream by a protein called transferrin, which is produced in the liver (Elzahrani, 2012). In the blood iron helps form haemoglobin which is an important protein in red blood cells. Haemoglobin is responsible for the transport of oxygen throughout the body so it can function normally. Iron is considered an important mineral because haemoglobin cannot be made without it (Elzahrani, 2012).

Iron deficiency is the most frequent nutritional deficiency disorder in the world (WHO, 2015). A recent estimatebased on World Health Organization (WHO) criteria indicate that around 600-

700 million people worldwide have a marked iron deficiency anaemia. Iron deficiency anaemia in pregnancy has been defined by the National Academic of Science Panel on nutrition as ferritin level lower than 15 μ g/l while the World HealthOrganization defined anaemia in pregnancy as haemoglobin level of 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 defined iron deficiency anaemia as serum ferritin concentration less than 15 μ g/l.

Pre-pregnancy body stores are important because during pregnancy there is a marked physiologic increase in the demands for absorbed iron to expand the woman's red blood cell mass and to secure an adequate iron supply for the function of the placenta and developing foetus(Milman, 2006). To complete a normal pregnancy without taking iron supplements and without developing iron deficiency or iron deficiency anaemia, the woman should have body iron stores at conception of \geq 500 mg (WHO,2015), which corresponds to serum ferritin concentration of 70 – 80µg/I (Milman, 2006).

The development of iron deficiency anaemia is associated with increased risk of pre-term birth and low birth weight infants. The physiologic importance of storage iron is that it provides a rapidly available supply in the event of blood loss. To achieve iron balance, towards the end of pregnancy, the absorption of 4 - 5mg/day is necessary. Requirements are higher during periods of rapid growth in early childhood and adolescent. Worldwide, the highest prevalence figures of iron deficiency today are found in infants, children, teenagers, women of childbearing age and pregnant women. The highest prevalence today is observed in pregnant women and menstruating women (Sachan *et al.*, 2013).

Transfer of iron from mother to foetus occurs mainly in the last trimester of pregnancy. Therefore, during this period, a mother's food should contain surplus quantities of iron. During this time, the child is dependent on the iron reserve, received from the mother during pregnancy. In premature babies, the transplacental transfer of iron might not have taken place. Hence such babies are at risk of iron deficiency (Vasudevan*et al.*,2011). Haemoglobin concentrations are used to characterize, and serve as iron benchmark for anaemia and iron deficiency anaemia have been provided by the WHO (WHO,2015). Iron deficiency is commonly caused by the combination of blood loss and insufficient dietary intake. (Milman, 2006).

Pregnant women are particularly vulnerable to iron deficiency which is the most prevalent global nutrition deficiency and the most common cause of anemia worldwide. There is a current concern that irrespective of worldwide campaign against anaemia in pregnancy, women of reproductive age still enter into pregnancy without proper iron stores and could give birth to children with inadequate iron deposit. Anaemia associated with iron deficiency in pregnant women in South East Geopolitical zone of 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 on the need for 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 different trimester in Nsukka, Enugu State, South East, Nigeria.

MATERIALS AND METHODS

MATERIALS

Subjects

One hundred and twenty-seven pregnant women at different trimester (6 were in the first trimester; 50 were in the second trimester and 71 were in the third trimester), all living at Nsukka community, Enugu State, Nigeria, were recruited for this study. 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).

Chemicals/Reagent

Analytical grade chemicals were used in this study. The reagents and chemicals used for haemoglobin determination were Darbkin's reagent which contain potassium cyanide, potassium ferricyanide, and sodium bicarbonate. Cal biotech kits for determination of ferritin which contain biotin reagent, streptavidin coated microwells, ferritin reagent (horseradish peroxide), ferritin calibrator, substrate A (tetramethylbenzidine), substrate B (hydrogen peroxide) and stop solution (hydrochloric acid).

METHODS

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

A known volume, 5ml of blood sample was collected from each participant by venepuncture using disposable needles and syringes. Thereafter, 3ml of the blood sample were 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 ethylene diaminetetracetic acid (EDTA bottle). It was properly kept for malaria parasite and haemoglobin determination.

HAEMOGLOBIN DETERMINATION

Haemoglobin determination was done using standard method as described by Ochei and Kolhalther (2008).Thehaemoglobin determination is based on light intensity principle on the dilution of blood (from EDTA) in a solution called Drabkin's solution, which contains potassium cyanide and potassium ferricyanide. Red blood cells are haemolysed and haemoglobin is released which is converted to methaemoglobin by reacting with potassium ferricyanide. Themethaemoglobin is converted to cyanmethaemoglobin (which has maximum absorbance of 540nm) by potassium cyanide. The colour intensity measured at 540 nm is proportional to the total haemoglobinconcentration. The blood sample (20 μ l) was diluted in 8ml of Dakin'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. The spectrophotometer was set to 100% transmitter at 540nm using Drabkin's solution as blank. The haemoglobin value was then determined by multiplying by a factor of 36.8 to give the actual haemoglobin value.

FERRITIN DETERMINATION

Serum ferritin determination was done using enzyme immunoassay method as described by Addison (1972) and as contained in Calibiotech ferritin kit. The principle is based on enzyme reaction where streptavidin coated micro well react with ferritin biotin reagent (monoclonal biotinylated antibody) and the serum containing the native antigen from an antibody-antigen complex. The biotin attached to the antibody binds to streptavidin on the micro well resulting in immobilization of the complex. After incubation the antibody-antigen bound fraction is separated from unbound antigen by decanting. Another antibody that is directed to a different epitome labeled with an enzyme was added leading to an enzyme labeled antibody-antigen-biotinylated complex. Excess enzyme is washed out using the washing buffer. A suitable substrate is added to produce a colour effect measurable with the use of spectrophotometer. Prior to assay, the reagent was allowed to stand at room temperature $(20 - 25^{\circ}C)$. 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. Thereafter the plate was shaken for 10 - 30 minutes. The plate was covered and incubated for 30 minutes at room temperature (20 - 25° c). Liquid was removed from all wells and washed well for three times with 30x wash buffer. The plate was tapped and blotted dry with absorbance paper.After which 100µl (0.100 ml) of the enzyme reagent was added into each well and was covered and incubated for 30 minutes at room temperature $(20 - 25^{\circ}c)$. The liquids from all wells were removed and washed for three times with 300x wash buffer. The plate was tapped and blotted dry using absorbance paper. The substrate, 100µl (0.100ml) was added into all wells and incubated for 15 minutes at room temperature. Thereafter 50µl (0.050ml) of stop solution was added into all well and was carefully mixed for 10 -20 minutes at room temperature. The absorbance in each well was read at 450nm within 15 minutes after adding the stop solution (using wavelength of 620 - 630nm) in a micro plate reader.

Data Analysis

Datawas expressed as mean \pm standard error of mean (SEM) and test of statistical significance was carried out using One-Way Analysis of Variance (ANOVA). Pearson test was performed to assess the correlation between Haemoglobin concentration, serum ferritin with other variables.

Ag

e **RESULTS**

(ye Demographical Information on Pregnant Women.

ars) Table 1 shows the demographical 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, six
20 - (4.7%) were in their first trimester, fifty (39.4%) were in their second trimester and seventy-one (55.9%) in their third trimester. A total number of fifty-six(44.1%) took the routine drugs

34 regularly, while seventy-one (55.9%) did not.

26. Prevalence of Anaemia Using Haemoglobin Concentration Alone

- 8 Table 2 shows the result of prevalence of anaemia using haemoglobin concentration alone. From the table 39.4% of the pregnant women were anaemic based on haemoglobin less than 11g/dl. In
- 25- first trimester, 0.79% were anaemic, second trimester 18.89% were anaemic while 19.69% were
- anaemic in the third trimester.

47 Prevalence of Anaemia Using Ferritin Concentration Alone

- 37 Table 3 shows the prevalence of anaemia using ferritin concentration alone. From the result 27.6% of the subjects were anaemic based on ferritin concentration less than 15μ l. In first
- 30- trimester 3.94% were anaemic, 11.81% were anaemic in second trimester while 11.81% were anaemic in third trimester.
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Table 1: Demographical Information on Pregnant Woman

1 st Participant(n=127)		Nu	mber	Percentage (%)	
35-39	16	1	2.6		
40-44	3	2.	4		
45-49	1	0.	8		
Trimester	6	4.	7		
2 nd Trimester		50	39.4		
3 rd Trimester 71	55.9				
Intake of Iron Supplement	S				
Regular		56	44.1		
Irregular		71	55.9		
 Trimester 2nd Trimester 3rd Trimester 71 Intake of Iron Supplementa Regular Irregular 	6 55.9 s	4. ⁷ 50 56 71	7 39.4 44.1 55.9		

Gestation age (n=127)	Number	Percentage (%)
First trimester	1	0.79
Second trimester	24	18.89
Third trimester	25	19.69
Total 50	39.40	

Table 2: Prevalence of Anaemia using Haemoglobin Concentration alone (Anaemia =Hb<11 g/dl).</td>

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

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Gestation age (n=127)	number	percentage (%)	
ũ, v		1 0 7	
First Trimester	5	3.94	
Second Trimester	15	11.81	
Third Trimester	15	11.81	
Total 35 27.60			

Prevalence of Anaemia using CombineHaemoglobin and Ferritin Concentration in pregnant women in first, second and third trimester.

Table 4 shows the prevalence of anaemia and non-anaemic pregnant women both in first, second and third trimester. From the result 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 while 23.9% in third trimester were anaemic based on low haemoglobin and ferritin level respectively.

Effect of Age on Haemoglobin and Ferritin Concentration

Table 5 shows the effect on haemoglobin and ferritin concentrations. From the table, mean haemoglobin concentration has a significant different (p < 0.05) when age range of 40 – 45 years is compared to other age range. However, the age range of 30 – 34 years are non-significantly (p > 0.05) when compared to age range of 35 -39 years and 40 – 44 years. Mean ferritin concentration shows that the age range of 40 – 44 years and 45 – 49 years are significantly (p < 0.05) different when compare to the age range of 20 – 24 years, 25 – 29 years, 30 – 34 years and 35 – 39 years. However, the age range of 20 – 25 years and 35 – 39 years are significantly (p < 0.05) lower than the age range of 30 – 34 years.

The Effect of Gestation Age (first, second and third trimester) on Haemoglobin andFerritin Concentrations

Table 6 shows the of effect gestational age on haemoglobin and ferritin concentrations. from the results, the mean concentration of haemoglobin in the first trimester (11.60 ± 1.86) shows no significant (p> 0.05) difference when compared to second (11.03 ± 1.56) and third trimester (11.06 ± 1.64) . The mean value of ferritin concentration in the first trimester (16.24 ± 3.10) was significantly (p< 0.05) lower when compare to the mean ferritin concentration in the second trimester (75.72 ± 9.50) and third trimester (66.53 ± 5.09) .

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

Parameters

Prevalence (%)

First Trimester Second Trimester Third Trimester

	(n=71)				
itin	1(16.7)		3(6)		8(11.3)
1	(0)		13(26)		8(11.3)
4(66.6)		23(46)		38(53.5)	
	1(16.7)		11(22)		17(23.9)
Table 5: The Effect of Age on Haemoglobin and Ferritin Concentrations					
Mean Hb (g/dl)	M	ean Ferrit	in ($\mu g/l$)	
11.57	± 1.37	24.87	± 5.72		
11.20	± 1.91		86	$.84 \pm 11.0$	01
10.69	9 ± 1.03		90	$0.06 \pm 12.$	20
10.58	3 ± 1.75		80.99 ±	10.41	
10.38 ± 0.100	.58 8.32	2 ± 1.36			
.64 ± -	8.78	3 ± -			
	$\begin{array}{r} \text{it in} \\ 0 & 4(66.6) \\ \hline 0 & \mathbf{Haemor} \\ 0 & 0 \\ \text{Mean Hb (} \\ \hline 11.57 \\ 11.20 \\ 10.69 \\ 10.58 \\ 10.38 \pm 0. \\ .64 \pm - \end{array}$	(n=71) itim 1(16.7) n (0) o 4(66.6) 1(16.7) on Haemoglobin ar Mean Hb (g/dl) 11.57 ± 1.37 11.20 ± 1.91 10.69 ± 1.03 10.58 ± 1.75 10.38 ± 0.58 8.32 .64 ± - 8.78	(n=71) itim 1(16.7) n (0) 0 4(66.6) 23(46) 1(16.7) on Haemoglobin and Ferriti Mean Hb (g/dl) M 11.57 ± 1.37 24.87 11.20 ± 1.91 10.69 ± 1.03 10.58 ± 1.75 10.38 ± 0.58 8.32 ± 1.36 .64 ± - 8.78 ± -	(n=71) itim 1(16.7) 3(6) n (0) 13(26) p 4(66.6) 23(46) 1(16.7) 11(22) on Haemoglobin and Ferritin Concer Mean Hb (g/dl) Mean Ferriti 11.57 ± 1.37 24.87 ± 5.72 11.20 ± 1.91 86 10.69 ± 1.03 90 10.58 ± 1.75 80.99 ± 10.38 ± 0.58 8.32 ± 1.36 .64 ± - 8.78 ± -	(n=71) itim 1(16.7) 3(6) n (0) 13(26) o 4(66.6) 23(46) 38(53.5) 1(16.7) 11(22) on Haemoglobin and Ferritin Concentrations Mean Hb (g/dl) Mean Ferritin (µg/l) 11.57 ± 1.37 24.87 ± 5.72 11.20 ± 1.91 86.84 ± 11.0 10.69 ± 1.03 90.06 ± 12. 10.58 ± 1.75 80.99 ± 10.41 10.38 ± 0.58 8.32 ± 1.36 .64 ± - 8.78 ± -

Result are expressed in mean \pm standard deviation.

Hb = haemoglobin

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

Gestation age	Mean Hb (g/d	l) Mean Ferritin (μ g/l)	
First Trimester (n=6)	$11.60 \pm 1.8616.24 \pm 3$.10	
Second Trimester (n=49)	11.03 ± 1.56	75.72 ± 9.50	
Third Trimester (n=72)	11.06 ± 1.64	66.53 ± 5.09	

Result are expressed in mean \pm standard deviation.

Hb = haemoglobin

The Effect of Routine Drugs on HaemoglobinandFerritin Concentrations

From the result, the mean haemoglobin concentration of those that received routine drug (11.30 \pm 1.73 g/dl) was slightly higher compared to those who didn't receive the drug (10.59 \pm 1.49 g/dl). However, the mean ferritin concentration of pregnant women who receive routine drug (58.53 \pm 4.08 µg/l) was significant higher (p < 0.05) when compared to the concentration of those that didn't receive (75.17 \pm 9.10 µg/l).

Correlation Analysis of age range, Gestation age and Intake of Routine Drugs on the Haemoglobin and Ferritin Concentrations of Pregnant Women.

The correlation table below, shows that age is negatively correlated with haemoglobin but positively correlated with ferritin. This means that as age of the subjects was increasing, their haemoglobin level was decreasing. This decrease was statistically significant (p < 0.05). however, the ferritin level was increasing with an increase in the age of the subjects, although not significant (p > 0.05). Gestational age when correlated with the haemoglobin and ferritin concentration shows a negative correlation (p > 0.05). This implies that increase as gestational age of the subjects' increases, there was a decrease in haemoglobin and ferritin concentrations. Also, from the result, intake of routine drugswhen correlated with the haemoglobin concentration shows a negative correlation (p > 0.05), which means that intake of routine drug seems to have reduce the haemoglobin level but increase ferritin level of 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 \pm standard deviation.

Hb = haemoglobin

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

DISCUSSION

Iron deficiency is one of the most important nutritional disorder among pregnant women worldwide. It could directly or indirectly contribute to the high rate maternal, perinatal morbidity and mortality seen in Nigeria (Rodger *et al.*, 2015).

Iron deficiency anaemia was highly prevalent among pregnant women used in this study. Using the World Health Organisation criterion of haemoglobin level below 11g/dl to define anaemia in pregnant women, 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. This result is in support with the data obtained from previous studies in developing countries showing a range from 35.5% to 75.0% (Omigbodum, 2004). The prevalence of anaemia in this study based on haemoglobin level (39.4%) is within the range with the findings of other studies carried out in Nigeria, Mcmahon, 2013 (40.2%); Anorlu*et al.* 2006 (35.3%); and Barker and Greer, 2010 (51.8%). The 39.4% from this study is however lower than 64.1% reported by Ezugwu *et al.* (2013) in Enugu state. This could be as a result of educational and economic status of the subjects. However, the prevalence of 39.4% is higher than the 10.4% obtained at Ibadan in south-West Nigeria (Pasricha *et al.*, 2010) and 21.6% obtained by Alem *et al.* (2013) in Northwest Ethiopia. This may be due to the differences in feeding habits, routine drug intake or some other factors not considered in their research.

The prevalence of anaemia using ferritin concentration alone from this study shows that 27.6% of the subjects were anaemic. This is based on ferritin concentration below $15\mu g/l$, with 3.94% in first trimester, 11.8% in second trimester and 11.8% in third trimester. 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).

The result of haemoglobin on gestational age (Trimester) from this study shows higher value (11.60 ± 1.86) in first trimester when compared to second (11.03 ± 1.56) and third (11.06 ± 1.64) trimester. 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 Demmoucheet al. (2011) and Nik et al. (2012).

The result of the effect of age on this study shows a slightly 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 is to say 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 of 40 - 2044 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 also be as a result of inability of the subject to absorb iron (Sharp and Srai, 2007). This agrees with the report of Kozuki et al. (2012). The result of correlation analysis 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 Sankuet al. (2010).

Report from most developing countries shows that iron supplement intake during the time of pregnancy has limited effectiveness. 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) and Hemmininki and Rimpela (1991). The result of haemoglobin concentration of the subject who had the routine drug in this study shows a slight difference on 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 while there was an increase in ferritin concentration of those that are not regularly taking the routine drug. This could be that the subjects are already iron deficient before taking the routine drug. This result is in accordance with results as 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 as a result of the subjects taking other supplement apart from the one issued to them, although this result varies with the previous report of Arise, (2017). Intake of routine drug when correlate with haemoglobin concentration shows a negative correlation at (p>0.05) but when correlate 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.

CONCLUSION

The study reveals that anaemia during pregnancy is highly prevalence. More than halve of the subjects was anaemic in both first, second and third trimester. This implies that children born by anaemic mothers stand the risk of being anaemic especially during exclusive breast feeding. Report obtained from previous studies have shown that iron reserve received by children as foetus during pregnancy runs out within 4 to 6 months after child birth thereby exposing infants to the risk of anaemia. Hence, public awareness campaign is recommended to create awareness on the need for early antenatal booking to provide opportunity for early detection and treatment

of anaemia. 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.

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