Iron and Zinc deficiency and haematological correlates among anaemic pregnant women attending ante-natal clinic at Babcock University Teaching Hospital, Ilishan-Remo, Ogun State.

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ABSTRACT
Background: Pregnancy is one of the risk factors for the development of iron deficiency anaemia. Minerals and micronutrients are essential for haematopoiesis, normal foetal growth and development. **Aim:** To determine the incidence of zinc and iron deficiency and their relationship to other haematological parameters in pregnancy. **Method:** This is a cross-sectional study involving 50 pregnant and 25 non-pregnant women. Socio-demographic data and blood samples were obtained over 6 months for serum iron and zinc assay and haematological analysis using standard protocols. The results obtained were analysed using SPSS version21. Independent T-test and ANOVA were used to compare the difference between the means of the groups studied. Pearson's correlation was used to determine the relationship between the variables. **Results**: 24/50 (48%) of the pregnant participants were anaemic and zinc deficient (44/50, 88%). All of the iron deficient pregnant women 14/50 (28%) were also zinc deficient, except one. The observed cases of isolated zinc deficiency among the anaemic pregnant women was 13/24 (54.1%). Pregnant women had significantly lower PCV (p=0.025) and higher values of MCH (p=0.016) and MCHC (p<0.001). The PCV (r=0.390, p=0.001), HGB (r=0.343, p=0.004), MCHC (r=-0.329, p=0.005), RBC (r=0.231, p=0.054), Ferritin (r=0.347, p=0.006) and TIBC (r=-0.266, p=0.027) correlated significantly with zinc. **Conclusion:** Iron and zinc deficiency are common in pregnancy and they co-exist among anaemic pregnant women. The practice of co-supplementation of iron and zinc for anaemic pregnant women is justified.

Key Words: Anaemia; Deficiency; Iron; Micronutrient; Pregnancy; Zinc

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INTRODUCTION

Deficiencies of micronutrients during pregnancy remain a problem of considerable magnitude in most developing countries of the world. Each year half a million women died during pregnancy, labour or in the puerperium [1,2]. The World Health Organisation (WHO) estimates that about 80% of maternal deaths are due to direct causes which are haemorrhage, sepsis, eclampsia, obstructed labour, and unsafe abortion. Nearly 20% are due to indirect causes including anaemia, malaria and heart disease, many of these direct causes can be worsened by the presence of maternal anaemia [3].

Iron requirements increased three times in pregnancy over the non-pregnant state, to supplement this; the recommended daily oral intake (RDI) of elemental iron is 30-60mg, to prevent maternal anaemia, puerperal sepsis, low birth weight, and preterm birth [4]. Iron absorption also increases from 1-2mg per day in the non-pregnant to 6mg per day in pregnancy [5].

This increase in iron requirements during gestation is due to an expansion of the blood volume and growth of the foetus and placenta. About 1000mg of iron is required during gestation; 500-600mg for erythrocyte expansion, 300mg for the foetus and placenta, and the rest to balance blood loss at delivery [6].

Zinc plays an important role in cell growth and differentiation. It is required in several complex mechanisms during cell replication and maturation, like RNA and DNA metabolism. Several processes have zinc-dependent enzymes, binding factors, and transporters. It is a key micronutrient during fetal growth and development, embryogenesis, and mammary gland function. Zinc has also been shown to exert a systemic anti-inflammatory effect [7, 8].

An estimated 80% of pregnant women worldwide have inadequate zinc intake which can result in maternal zinc deficiency and compromise the mental development of the foetus [9]. Zinc deficiency can alter the circulating levels of certain hormones that are associated with the onset of labour and also predispose to intrauterine and systemic infections that may result in preterm birth. Unlike iron there is no particular store for zinc, thus when zinc is in excess (zinc toxicity) the body increases its excretion and reduces its absorption [10]. Zinc and iron are required for the formation of haemoglobin in erythrocytes.

Despite the above synergy between zinc and iron metabolism, the role of zinc in the prevention of anaemia is not well established and co-administration with iron in pregnancy is not yet routine.

MATERIALS AND METHODS

Research Design

This is a cross-sectional study of pregnant women who attended aged 18-45, with uncomplicated pregnancies attending the ante-natal clinic of Babcock University Teaching Hospital, Ilishan-Remo, Ogun State. Apparently healthy non-pregnant women attending the gynaecology clinic of the same hospital participated in the research and served as controls. The Research and ethics committee of Babcock University, Ilishan approved the protocols. The case records were retrieved for consenting participants from the records department and each of them responded to a structured questionnaire. We included all women with uncomplicated pregnancies in our center

with the exclusion criteria including women with multiple pregnancies, hypertensive diseases of pregnancy, disorder of zinc metabolism or protein causing enteropathy, renal and cardiac diseases, or other chronic diseases that affect feeding or result in wasting and all the women who bled during the pregnancy.

All the relevant information including their sociodemographic characteristics: age, parity, occupation, gestational age at booking, etc., and were retrieved and analysed using tables and percentages. A total of 75 subjects were recruited into this study using a 2:1 ratio for pregnant women and controls (50 pregnant women and 25 controls).

Sample collection

Five millilitres (5ml) of venous blood was collected from the antecubital fossa of each participant using disposable needles and syringes, between 8 am and 11 am on clinic days. One millilitre was dispensed into ethylene diamine tetra acetic acid (EDTA k3) bottle and the other 4ml into a plain bottle. The samples in the plain bottles were centrifuged for 10 minutes at 4000 rpm and separated into plain bottles for ferritin, serum iron, total iron-binding capacity, and zinc analysis while the samples in the EDTA bottles were analysed for haematological parameters.

Estimation of Serum Iron (Techo diagnostics). *Manufacturer's Manual*

The iron in serum is dissociated from its Fe (III)- transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduced Fe (III) to Fe (II). The chromogenic agent, Ferene, forms a highly colored Fe (II)- complex that is measured photometrically at a wavelength of 560nm.

The unsaturated iron-binding capacity (UIBC) is determined by adding Fe (II) iron to serum so that they bind to the unsaturated iron-binding sites on transferrin. The excess Fe (II) ions are reacted with Ferrozine to form the colour complex, which measured photometrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron-binding. The total iron-binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

Procedure:

1. Test tubes/cuvettes were labelled "Blank", "Standard", "Control", "Sample", etc.
2. 2.5ml Iron Buffer reagent was added to all tubes.
3. 0.5ml (500ul) sample was added to the respective tubes and mixed. NOTE: 0.5ml (500ul) iron-free water (deionised water) to blank.
4. Spectrophotometer was zeroed at 560nm with the reagent blank.
5. Absorbance's of all tubes were read and recorded. (A_1 reading).
6. 0.05ml (50ul) Iron colour reagent was added to all tubes and mixed.
7. All tubes were placed in a heating bath at 37°Cfor 10 minutes.
8. Spectrophotometer was zeroed at 560nm with the reagent blank.
9. Absorbance's of all tubes were read and recorded. (A_2 reading).

Calculation:

Given; A = Absorbance, Std = Standard

$$\text{Total Iron } (\mu g/dl) = \frac{A_2\text{Test} - A_1\text{Test} \times \text{Conc. of Std}}{A_2\text{Std} - A_1\text{Std}}$$

Procedure for unsaturated iron binding capacity (UIBC)

1. Test tubes/cuvettes were labelled "Blank", "Standard", "Control", "Sample", etc.
2. 2.0ml UIBC reagent was added to all tubes.

3. 1.0ml iron-free water was added to "blank".
4. 0.5ml (500ul) iron-free water and 0.5ml standard were added to standard and mixed.
5. 0.5ml (500ul) of samples and 0.5ml iron standard were added to "test" respectively and mixed.
6. Spectrophotometer was zeroed at 560nm with the reagent blank.
7. Absorbance's of all tubes were read and recorded. (A_1 reading).
8. 0.05ml (50ul) Iron colour reagent was added to all tubes and mixed.
9. All tubes were placed in a heating bath at 37°Cfor 10 minutes.
10. Spectrophotometer was zeroed at 560nm with the reagent blank.
11. Absorbance's of all tubes were read and recorded. (A_2 reading).

Calculation:

$$\frac{\text{Conc. of Std} - [A_2\text{Test} - A_1\text{Test}] \times \text{Conc. of Std}}{[A_2\text{Std} - A_1\text{Std}]} = \text{UIBC (ug/dl)}$$

TIBC (ug/dl) = Iron level + UIBC

Estimation of Serum Zinc (Fortress Diagnostics). *Manufacturer's Manual*

Zinc present in the sample is chelated by 5-Br-PAPS, 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)- phenol in the reagent. The formation of this complex is measured at a wavelength of 560nm.

Procedure:

1. Test tubes/cuvettes were labelled "Blank", "Standard", "Control", "Sample", etc.
2. 1000ul Zinc reagent were added to all tubes.
3. 50ul of sample and standard were added to respective tubes and mixed.
4. Tubes were incubated at 37^0C for 5 minutes.
5. Spectrophotometer was zeroed at 560nm with the reagent blank.
6. Absorbance's of all tubes were read and recorded.

Calculation

$$\text{Zinc in umol/l (ug/dl)} = \frac{\text{Abs of Sample} - \text{Abs of Blank}}{\text{Abs of Sample} - \text{Abs of Blank}} \times \text{Conc. of Std mol/l (ug/dl)}$$

Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 21.0. Numerical data were expressed as mean± standard deviation (SD). Independent T-test and Analysis of Variance (ANOVA) was used to compare the difference between the means of the groups studied. Pearson's correlation was used to investigate the relationship between the biochemical parameters (ferritin, serum iron, total iron binding capacity, and the outcome variables; zinc and iron) and haematological parameters studied. The level of statistical significance was set at a p-value of less than 0.05.

RESULTS

The present study assessed the prevalence of zinc and iron deficiency and their relationship to other haematological parameters in pregnant women. The socio-demographic characteristics of the participants is presented in Table 1. The baseline bio-data of the pregnant participants were comparable to those of the non-pregnant participants: Age (p=0.127), Tribe (p=0.398), Educational level (p=0.567) and Parity (p=0.481). There was

no significant difference in dietary habits (p=0.810) and fruit intake (p=0.134). The proportion of pregnant women (45/50, 90%) on iron supplements (Fersolate) were significantly higher (p<0.001) compared to their non-pregnant counterparts. None was on zinc supplements. Also, none of the participants had received a blood transfusion within the preceding 6-months of the study.

Table 1: Socio-demographic characteristics of the participants

Variables	**Pregnant (n=50)**	**Non-pregnant (n=25)**	***p-Value***
Age (years)			
21-25	5(10%)	5 (20%)	0.127
26-30	18(36%)	3 (12%)	
31-35	21(42%)	10 (40%)	
36-40	5 (1%)	6 (24%)	
>40	1 (2%)	1 (4%)	
Tribe			
Hausa	2 (4%)	0	0.398
Igbo	10 (20%)	8 (32%)	
Yoruba	34 (68%)	17 (68%)	
Educational level			
Secondary	11 (22%)	7 (28%)	0.567
Tertiary	39 (78%)	18 (72%)	
Parity			
0	27 (54%)	17 (68%)	0.481
1-2	16 (32%)	5 (20%)	
>2	7 (14%)	3 (12%)	
Dietary habit			
Vegetarian	7 (14%)	3 (12%)	0.810
Non-vegetarian	43 (86%)	22 (88%)	
Fruit intake(weekly)			
Once	2 (4%)	2 (8%)	0.134
Twice	14 (28%)	12 (48%)	
≥Thrice	34 (68%)	11 (44%)	
Supplement usage			
No	5 (10%)	18 (72%)	<0.001
Yes	45 (90%)	7 (28%)	
Other medications			
No	48 (90%)	21 (72%)	0.071
Yes	2 (4%)	4 (28%)	

The occurrence of anaemia, iron and zinc deficiencies among the participants is presented in Table 2. A significant proportion of the pregnant participants (24/50, 48%) were anaemic, whereas only 2/25 (8%) of the non-pregnant participants were anaemic. 15/50 (30%) of the pregnant participants had iron deficiency alone, while 44/50 (88%) had zinc deficiency alone. 14/50 (28%) had zinc and iron co-deficiency.

Furthermore, the occurrence of Iron and zinc deficiencies among anaemic study participants is presented in Table 3. None (0/24) of the anaemic pregnant women had isolated iron deficiency, but 13/24 (54.1%) had isolated zinc deficiency. 10/24 (41.7%) had zinc and iron co-deficiency. Meanwhile, only one of them (1/24, 4.2%) was free from both zinc and iron deficiencies.

The comparison of mean values of iron, zinc, and haematological parameters between the pregnant and non-pregnant participants is presented in Table 4. Mean serum iron was non-significantly lower ($p=0.203$) in pregnant study participants (125.55±58.75µg/dl) compared to the non-pregnant (142.95±21.63µg/dl).

Regarding iron-related biochemical indices, while TIBC (371.18±52.73 µg/dl) was significantly elevated ($p<0.001$) on one hand; ferritin (18.52±8.02 ng/dl) was significantly lower ($p<0.001$) in pregnant study participants on the other hand. In addition, the serum zinc level (53.42±11.60µg/dl) was observed to be significantly lower in the pregnant study participants ($p<0.001$) compared to their non-pregnant counterparts (66.55±9.97µg/dl). The outcome of this study also show that the pregnant women had significantly lower PCV levels ($p=0.025$), higher red blood cell indices: MCH ($p=0.016$), MCHC ($p<0.001$) and higher WBC ($p=0.003$). Although not statistically significant, the lower MCV levels ($p<0.810$) recorded among the pregnant participants in conjunction with other red cell indices suggest microcytic anaemia, which is typical of iron deficiency. The effects of iron supplements on zinc, iron and haematological parameters of the pregnant participants is presented in Table 5.

The difference in iron, zinc and haematological parameters between the pregnant participants that were taking iron supplements and those who did not, was statistically non-significant ($p>0.05$). The effects of gestational age on zinc, iron and haematological parameters of the pregnant study participants is presented in Table 6. The mean serum iron was found to be non-significantly ($p=0.362$) lower in the second trimester compared to other trimesters; while, the mean serum zinc level was found to be non-significantly ($p=0.857$) higher in the second trimester compared to other trimesters. On the other hand, the mean PCV ($p=0.922$) and WBC ($p=0.997$) levels were found to be non-significantly higher in the third trimester when compared to other trimesters.

Table 7 shows the relationship between serum zinc and serum iron to other biochemical and haematological parameters using Pearson's correlation. PCV ($r=0.390$, $p=0.001$), HGB ($r=0.343$, $p=0.004$), MCHC

(r=-0.329, p=0.005), RBC (r=0.231, p=0.054), Ferritin (r=0.347, p=0.006) and TIBC (r=-0.266, p=0.027) were significantly correlated with zinc. There was a strong positive correlation between PCV, HGB, RBC, Ferritin and zinc; however, there was no significant correlation between serum iron and the other biochemical and haematological parameters.

Table 2: Occurrence of anaemia, Iron and zinc mono- and co-deficiency among the study participants.

Variable	Pregnant (n=50)	Non-pregnant (n=25)
Anaemia	24 (48%)	2 (8%)
Iron deficiency	15 (30%)	1 (4%)
Zinc Deficiency	44 (88%)	0
Zinc and Iron deficiency	14 (28%)	0

Table 3: Occurrence of Iron and zinc mono- and co-deficiency among anaemic study participants

Variable	Pregnant (n=24)	Non-Pregnant (n=2)
Iron deficiency alone	0	1 (50%)
Zinc deficiency alone	13 (54.1%)	0
Zinc and iron deficiency	10 (41.7%)	0
Non zinc, non-iron deficiency	1 (4.2%)	1 (50%)

Table 4: Comparison of mean values of iron, zinc, and haematological parameters between the participants.

Parameter	Pregnant (N=50)	Non-Pregnant (N=25)	F-Value	P-Value
Ferritin (ng/dl)	18.52±8.02	33.95±10.95	-6.521	0.000*
TIBC (µg/dl)	371.18±52.73	306.80±17.01	5.328	0.000*
Serum iron(µg/dl)	125.55±58.75	142.95±21.63	-1.285	0.203
Zinc (µg/dl)	53.42±11.60	66.55±9.97	-4.443	0.000*
PCV (%)	31.68±3.16	33.39±1.68	-2.294	0.025*
HGB (g/dl)	10.60±0.82	10.59±0.46	-0.108	0.914
MCV (fL)	84.72±6.53	85.13±5.94	-0.242	0.810
MCH (pg)	28.82±2.63	27.10±2.61	2.469	0.016*
MCHC (%)	33.95±1.22	31.78±1.21	6.728	0.000*
RBC (1012/L)	3.78±0.41	3.95±0.43	-1.541	0.128
WBC (109/L)	7.64±2.74	5.61±1.77	3.062	0.003*

* Considered statistically significant.

Table 5: The effects of iron supplements on zinc, iron and haematological parameters of the pregnant study participants

Parameter	Supplement	No supplement	F-Value	P-Value
Ferritin (ng/dl)	18.72±8.40	16.74±2.75	0.521	0.605
TIBC (µg/dl)	373.30±54.06	352.60±38.32	0.829	0.411
Serum Iron (µg/dl)	129.84±58.27	86.90±53.46	1.574	0.122
Zinc (µg/dl)	53.82±11.49	49.80±13.39	0.732	0.468
PCV (%)	31.76±3.20	30.92±3.08	0.561	0.578
HBG (g/dl)	10.63±0.81	10.40±0.90	0.591	0.557
RBC (1012/L)	3.78±0.40	3.75±0.54	0.168	0.867
WBC (109/L)	7.69±2.72	7.26±3.23	0.327	0.745

Table 6: The effects of gestational age on zinc, iron and haematological parameters of the pregnant study participants

Parameter	1st Trimester (n=2)	2nd Trimester (n=12)	3rdTrimester (n=36)	F-Value	P-Value
Ferritin (ng/dl)	10.08±12.62	18.97±7.99	18.52±8.03	1.161	0.322
TIBC (µg/dl)	379.50±91.22	350.42±29.52	377.83±56.54	1.246	0.297
Serum Iron (µg/dl)	162.25±28.64	107.54±50.39	129.51±61.69	1.038	0.362
Zinc (µg/dl)	49.00±4.24	54.00±13.00	53.47±11.56	0.155	0.857
PCV (%)	31.60±2.12	31.36±2.13	31.79±3.52	0.081	0.922
HBG (g/dl)	10.85±1.20	10.63±0.74	10.59±0.85	0.100	0.905
RBC (1012/L)	3.99±0.40	3.79±0.41	3.76±0.42	0.303	0.740
WBC (109/L)	7.60±1.69	7.59±2.07	7.66±3.02	0.003	0.997

Table 7: Correlation of zinc and iron to other haematological parameters

	Serum Zinc		Serum Iron	
Parameter	R-value	P-value	R-value	P-value
PCV (%)	0.390	0.001	-0.109	0.368
HBG (g/dl)	0.343	0.004	-0.074	0.544
MCHC (%)	-0.329	0.005*	-0.007	0.955
RBC (1012/L)	0.231	0.054	0.59	0.630
WBC (109/L)	-0.204	0.090	-0.195	0.106
Iron (µg/dl)	-0.008	0.951		
Ferritin (ng/dl)	0.347	0.006	-0.055	0.654
TIBC (µg/dl)	-0.266	0.027	-0.026	0.834
Zinc (µg/dl)			-0.008	0.951

***** Considered statistically significant.

DISCUSSION

The lower MCV and higher MCHC levels observed among the pregnant women are features of microcytic anaemia caused by of iron deficiency [11]. In addition, the higher mean TIBC level also adds to the evidence in favour of iron deficiency; a higher TIBC in pregnancy was also reported in an earlier similar study [12].

Significantly lower levels of serum ferritin were observed among the pregnant women in comparison with the non-pregnant. Ferritin is an essential protein in the body which is responsible for storing iron, a reduction in serum ferritin levels thus indicates a reduction in iron stores in the body which may predispose women to adverse pregnancy outcomes [13, 14].

A significantly lower level of mean serum zinc concentration was observed in pregnant women; a finding which is consistent with some earlier reports which have suggested a relative deficiency of zinc in pregnancy [15-18].

Iron deficiency occurred in 30% of pregnant women in this study, a finding which is much lower than the 75.4% reported from a study in the Northern part of Nigeria [19]. The World health organization data show that iron deficiency anaemia in pregnancy is a significant problem throughout the world with a prevalence ranging from an average of 14% of pregnant women in industrialized countries to an average of 56% in developing countries [3].

Dietary practices, lower parity and older maternal age of child bearing than in the North in addition to the use of iron supplements by most pregnant participants may be responsible for this lower incidence of iron deficiency in this study. The observed incidence of zinc deficiency in Pregnancy in this study is 88%, which is higher than the globally reported value of 80% [9].

Among the anaemic pregnant women; combined zinc and iron deficiency was found in 41.7%, while isolated zinc deficiency occurred in 54.1%. All anaemic pregnant participants with iron deficiency were also found to be zinc deficient. An earlier study had suggested a link between low zinc levels in pregnancy and iron deficiency anaemia [20].

The mean serum iron, PCV and WBC were lowest in the second trimester than the first and third. The mean PCV and WBC levels in the third trimester were greater than observed in the first although there was no statistically significant difference. The second trimester is the time of marked plasma volume expansion and high dilution anaemia while the second and a greater part of the third trimester correspond to periods of rapid foetal growth and trans-placental transfer [21].

The WBC has been well reported to be higher in the third trimester especially in labour or immediate pre-labour states. Higher zinc levels were observed in the second trimester than the first or third, this finding is not in consonance with an earlier report where zinc levels decreased in pregnancy reaching a nadir at term with levels as low as 35% of the non-pregnant state [22].

There was no significant correlation between serum iron and the other biochemical parameters as well as haematological parameters. It was however reported in an earlier similar study that a significant correlation existed between TIBC and serum iron in pregnancy [12]. Serum

zinc level showed a significant correlation with PCV, HGB, MCHC, RBC and Ferritin. The relationship between zinc and haemoglobin corroborates an earlier finding, where the similarity in dietary sources and the role of zinc in erythropoiesis were suggested as mechanisms for this finding of co-prevalent deficiency states [20].

CONCLUSION

Iron and zinc levels are lower in pregnancy as a result of an increased demand in pregnancy. Iron deficiency co-exists with zinc deficiency in anaemic pregnant women. The practice of co-supplementation of iron and zinc for anaemic pregnant women is thus justified.

CONFLICT OF INTEREST

The authors have declared no conflict of interest exist.

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