

# COMBINED IRON AND ZINC SUPPLEMENTATION IMPROVES HAEMATOLOGIC STATUS OF PREGNANT WOMEN IN UPPER WEST REGION OF GHANA

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## SUMMARY

**Background:** Though pregnant women in Ghana routinely receive iron and folic acid supplements, the prevalence of anaemia continues to be as high as 70%.

**Objective:** To determine the impact of zinc deficiency on iron status indicators in pregnant women

**Design:** A double-blind, randomized controlled trial (RCT) of joint iron and zinc supplementation

**Setting:** The study was conducted in the Upper West Region of Ghana, where the prevalence of anaemia is high.

**Participants:** The study population comprised pregnant women who presented themselves for antenatal care (ANC) in the Wa Regional Hospital of the Upper West Region in Ghana.

**Interventions:** The intervention group received a combined supplement of 40 mg zinc as zinc gluconate and 40 mg iron as ferrous sulphate. The control group received 40 mg elemental iron as ferrous sulphate.

**Main outcome measures:** The primary outcome measures were mean and percentage changes in Hb. Serum ferritin and zinc concentrations serve as secondary outcomes.

**Results:** Adjusted mean Hb increase was 0.6g/dl higher among women who were not iron replete (SF ferritin  $\leq 20$   $\mu\text{g/L}$ ) and received the iron-zinc supplement, compared to women who received iron-only supplement,  $F(1, 99) = 4.356$ ,  $p = 0.039$ .

Women who had low plasma zinc levels were 3-fold increased odds of developing iron deficiency at recruitment, (OR 3.41, 95% CI: 1.19-9.76).

**Conclusions:** Iron-zinc supplementation was effective in raising Hb and serum ferritin values among women who were iron deficient in early pregnancy but not among iron sufficient women.

**Keywords:** Iron-zinc supplementation, Ghana, iron-deficiency anaemia, plasma zinc, serum ferritin concentrations

## INTRODUCTION

Maternal anaemia continues to be significant public health problem during pregnancy in Ghana. As a control measure, 60 mg of elemental iron and 400  $\mu\text{g}$  folic acid are prescribed for all pregnant women who attend antenatal services. In spite of this, the prevalence of anaemia (Hb $<11$  g/dl) continues to be as high as 70% among pregnant women.<sup>1</sup> The reasons for the apparent lack of efficacy of routine iron supplementation in reducing anaemia have not been clearly established. The possibility that some other nutrient deficiencies may have direct limiting effect on the effectiveness of iron supplementation does not appear to have been investigated.

The relative contribution of malaria, iron-deficiency and haemodilution to pregnancy anaemia in Ghana does not appear to be known either. Zinc plays a critical role in haemoglobin synthesis and erythropoiesis and may therefore play very important role in the etiology of anaemia. Ghana is classified in the medium risk category of zinc deficiency by the International Zinc Nutrition Consultative Group Steering Committee (IZiNCG SC).<sup>2</sup>

However, very little is known regarding the impact of zinc deficiency on iron status indicators in pregnant women. It is against this background that a combined effect of iron and zinc supplementation on anaemia merited an investigation. In this paper data is presented to show how zinc deficiency may be contributing to the high prevalence of anaemia in Ghana.

### *Hypothesis*

The primary hypothesis was that joint iron and zinc supplementation would result in higher mean haemoglobin concentrations than iron-only supplementation during pregnancy. The aim of the study was to assess the effect of combined iron and zinc supplementation on haematological status of pregnant women in the Upper West Region of Ghana.

The specific objectives of the study were as follows:

1. To investigate whether there was significant relationship between plasma zinc concentrations and iron status indicators during pregnancy.
2. To assess the prevalence of maternal zinc deficiency
3. To determine the effect of combined iron and zinc supplementation on the prevalence of anaemia in malaria-endemic environment.

## METHODS

### *Study Design*

The details of this study have been described elsewhere<sup>3</sup>. The study design was a double-blind, randomized controlled trial (RCT). In this two-arm design, the intervention group received a combined supplement of 40 mg zinc as zinc gluconate and 40 mg iron as ferrous sulphate. The control group received 40 mg elemental iron as ferrous sulphate. Both groups received also sulfadoxine pyrimethamine (SP), and 400 µg folic acid. The iron and zinc supplements were taken every other day from enrolment until delivery.

### *Study Participants*

The study population comprised pregnant women who presented themselves for antenatal care (ANC) and whose gestational age had been determined by use of biometric measurements of the foetus (that is, ultrasound scanning) in the Wa Regional Hospital of the Upper West Region in Ghana. The inclusion criteria for the recruitment of participants were all pregnant women who were at no more than their 16<sup>th</sup> week of gestation. Women were excluded if they were identified through interviews to be on any other form of zinc supplements at any dosage level or were severely anaemic (that is, Hb less than 7.0 g/dl).

A sample size of 354, (177 per group) was calculated based on the ability to determine with an  $\alpha=0.05$ ,  $1-\beta=0.90$  a minimum difference in mean haemoglobin concentration of 0.5 g/dl and standard deviation of 1.45 g/dl. Allowing for a 10% loss to follow up, the overall sample was adjusted to 389 pregnant women. A subsample of 170 subjects were required to have a 90% chance of detecting a significance difference at the 5% level, in serum ferritin of 1.0 µmol/L and standard deviation of 2.0 µmol/L. An additional 10% was added to take account of drop-outs. The total estimated subsample size was thus 200.

Another subsample of 86 was needed to detect a significant difference in serum zinc of 1.4 µmol/L and standard deviation of 2.0 µmol/L with an  $\alpha=0.05$ ,  $1-\beta=0.90$ . An additional 10% was added to take account of drop-outs. The total estimated sample size was thus 100.

Ethical clearance was obtained from the Human

Research Ethics Committee of Edith Cowan University (Perth Australia) (ref. 04-276) and Ghana Health Service Ethical Review Committee (ref. GHS-ERC-3). Eligible women for the trial gave a free and informed consent before enrolment.

### *Randomization and Concealment of Random Allocation Sequence*

An independent statistician generated the allocation schedule/sequence using computer-generated random numbers. The treatment allocations generated were put in opaque envelopes and serially numbered. Each envelope contained a card on which the specific treatment was indicated. In order to safeguard the allocation schedule/sequence, envelopes were opened sequentially and only after the participant's name and other details were written on the envelope. After obtaining informed consent for enrolment, the study midwife randomly assigned participants to one of the two study groups.

### *Blinding*

The iron-zinc and iron-only supplements were pre-coded and supplied by Nutricaps pharmaceutical company in the United States of America (USA). The supplements (in the form of capsules) were of the same shape, colour and taste. The iron supplement given to participants in the Control Group was packaged exactly as the iron-zinc supplement.

### *Supplement Administration and Follow-up*

Each enrolled participant was instructed how to take the supplements (that is, dosage and frequency). The research assistants advised participants to take the supplements at least two hours before or after meals, and this translated practically to taking the supplements at night, just before going to bed (that is, in the post-absorptive state). Compliance/adherence was monitored by interviewing all participants after having being enrolled for four weeks. A structured questionnaire was administered at home to check the frequency and dosage of supplement intake.

### *Blood Sample Collection and Storage*

Anti-coagulated whole blood and plasma were collected from fasting morning venipuncture samples using rigorous collection and separation procedures. Fasting blood ( $\geq 8$  hours) samples were collected in the morning before 11.00 AM to avoid diurnal variation in plasma zinc during the day. The blood samples analyzed for zinc were collected and plasma separated, stored at -34 degrees centigrade until they were transported for analysis at the Nuclear Research Institute, Accra.

### Assessment of maternal iron and zinc status

Two assessments were made at recruitment and 34-36 weeks of gestation. Haemoglobin concentrations were measured by the cyanmethemoglobin method. Microparticle Enzyme Immunoassay (MEIA) was used for the determination of serum ferritin concentration<sup>4</sup>. Flame atomic absorption spectrophotometry (AAS) was used to quantify plasma zinc following standardised procedures.

### Diagnosis of malarial infection

Two hospital-based laboratory technologists diagnosed peripheral blood parasitemia through microscopic examination of thin and thick blood smears. Malaria parasite and leucocyte counts were made on the same microscopic fields and a minimum of 200 leucocytes was counted in each blood sample. Malaria infection was defined as the presence of asexual forms of *P. falciparum* parasites (trophozoites, schizonts) in a thick Giemsa-stained blood film<sup>5</sup>. Blood films were classified as negative if no asexual form of *P. falciparum* was detected under 100 x magnifications.

### Estimation of Parasitemia

Quantification of malarial parasites was done following standard microscopy procedures. For all positive blood films, determination of the degree of parasitemia at the time of diagnosis was carried out. The parasite density count per micro-litre (1  $\mu$ l) of blood was estimated on the assumption that the average leucocyte count (WBC) in one micro-litre of blood is estimated to be 8,000<sup>6</sup>.

### Outcome Measures

The primary outcome measures were mean and percentages changes in haemoglobin concentration (Hb). Changes in serum ferritin and zinc concentrations served as secondary outcomes. Haemoglobin assessment was made on 389 participants at recruitment, and at 34-36 weeks of gestation. However, baseline serum ferritin concentrations were measured in a random sub-sample of 200 (51.4%) children, and baseline zinc concentrations were measured in a random sample of 100 (25.7%) study participants.

At follow-up, there were 27 (13.5 %) missing values for ferritin concentrations and those participants were subsequently not part of the analysis. Similarly, there were 14 (14.0 %) missing values for plasma zinc concentrations at follow-up assessment. Essentially, analyses of biochemical indicators included only subjects with available samples both before and after supplementation.

### Statistical Analysis

The data were edited and coded for statistical analysis using SPSS for windows 14.0 (SPSS Inc, Chicago, IL, USA). Before formal statistical tests were applied, the data set was explored to verify whether conditions for parametric tests were met (e.g. normality and homogeneity of variance). Haemoglobin data were normally distributed. However, serum ferritin and zinc concentrations, malaria parasite load (parasitaemia) were all transformed to logarithms before parametric tests were used. The antilogarithm of the geometric mean of the log-transformed adjusted values was then reported in the report. Analysis of covariance (ANCOVA) was used to adjust group means. Multiple linear regression analysis was used to examine the predictors of haemoglobin concentration. Statistical difference was considered significant if the p-value was <0.05 and 95% confidence intervals (CI) were calculated for all main outcome measures that met the normality and homogeneity assumption criteria.

## RESULTS

### Sample Characteristics

The study groups did not differ significantly in terms of their baseline characteristics except for age where the Iron-zinc Group had older participants,  $F(1, 598) = 4.864, p = 0.028$  (Table 1).

**Table 1** Baseline characteristics and comparability of treatment groups (that is, differential attrition)

Characteristic	Control Group	Iron-zinc Group
Mean Maternal Age (years)	26.4 $\pm$ 5.0	27.4 $\pm$ 5.6
Malaria Rate (Presence of Plasmodium parasites in peripheral blood) n (%)	9.8 % (19/194)	16.4% (32/195)
Mean Malaria Parasite Density (parasites/ $\mu$ l blood)	464 $\pm$ 3.0	342 $\pm$ 4.0
Geometric Mean Plasma Zinc Concentration ( $\mu$ g/dl)	41.4 $\pm$ 2.5	38.2 $\pm$ 2.5
Prevalence of anaemia (Hb < 11.0g/dl)	37.1 % (72/194)	42.1 % (82/195)
Proportion of Zinc-Deficiency (Plasma zinc <60 $\mu$ g/dl) n (%)	85.4 % (134/157)	85.3 % (139/163)
Mean Hb Concentrations (g/dl)	11.3 $\pm$ 1.5	11.2 $\pm$ 1.4
Geometric Mean Serum Ferritin Concentration ( $\mu$ g/L)	33.2 $\pm$ 2.4	34.8 $\pm$ 2.3
Proportion of Maternal Iron-deficiency (SF<12 $\mu$ g/L)	58.5 % (48/82)	50 (47/94)
Mean Height (cm)	159.1 $\pm$ 5.6	159.2 $\pm$ 6.0
Mean Weight (Kg)	63.2 $\pm$ 12.1	63.1 $\pm$ 10.9
Mean Body Mass Index (BMI) kg/m <sup>2</sup>	25.0 $\pm$ 4.5	25.0 $\pm$ 4.0
Mean Gestational Age at recruitment (weeks)	13.7 $\pm$ 2.9	13.8 $\pm$ 2.9
Mean Diastolic Blood Pressure (mmHg)	59.0 $\pm$ 10.0	59.0 $\pm$ 10.0
Mean Systolic Blood Pressure (mmHg)	103.5 $\pm$ 12.7	102 $\pm$ 13.2

### Prevalence of Anaemia and Iron Deficiency Anaemia

At baseline, the prevalence of all-cause anaemia (Hb < 11.0 g/dl) was 39.6% (154/389). The overall prevalence of iron deficiency (ID) (SF<35 µg/L) at recruitment was 54.0% (95/176). Iron deficiency anaemia (SF<12 µg/L and Hb < 11.0 g/dl) was only 5.1% (9/176) at recruitment. Gestational age was a very strong predictor of Hb during pregnancy. Haemoglobin levels at recruitment were inversely correlated with gestational age at recruitment ( $r = -0.356$ ,  $p = 0.001$ ).

### Haematological Response to Iron-zinc Supplementation

Haemoglobin response to iron supplementation was dependent on the iron status at recruitment. Not all women appeared to have benefited from iron supplementation because only iron deficient and

anaemic women had a steady improvement in mean Hb (that is, a positive response) whilst non-anaemic women rather had reduced Hb levels by late pregnancy (a negative response). Haemoglobin response ( $\geq 1.0$  g/dl) was observed in 52.6% (81/154) of anaemic pregnant women but only 5.5% (13/235) of non-anaemic women (OR 0.05, 95% CI: 0.028-0.10).

Among women who were anaemic at recruitment, a trend in steady increase in mean Hb was observed (from 9.7 to 10.8 g/dl),  $F(1, 153) = 130.37$ ,  $p = 0.001$  but there was a decline among women who were not anaemic (from 12.2 to 11.0 g/dl),  $F(1, 233) = 148.236$ ,  $p = 0.001$ . Similar trends were observed in the study groups (Table 2).

**Table 2** Response in Hb to iron-zinc supplementation according to treatment group stratified by maternal anaemia at recruitment

Gestation period	Iron-zinc Group		Standard Treatment Group	
	Sub-groups		Sub-groups	
	Anaemia	Non-anaemia	Anaemia	Non-anaemia
	Mean Hb (g/dl)	Mean Hb (g/dl)	Mean Hb (g/dl)	Mean Hb (g/dl)
Recruitment	9.7±0.7	12.2±0.9	9.7±0.7	12.2±1.1
34-36 weeks gestation	10.9±1.1	10.9±1.3	10.7±1.1	11.1±1.1
Mean change (g/dl)	1.3±0.1	-1.3±0.1	1.0±0.1	-1.1±0.1
% point change	13.5	-10.7	10.3	-9.0

The proportion of iron-deficient women (SF ferritin  $\leq 20$  µg/L), whose mean Hb increased ( $\geq 1.0$  g/dl) was 10.5 percentage points higher in the Iron-zinc Group compared to the iron-only Group (45.1% versus 34.6%), (OR 0.65, 95% CI: 0.29-1.43).

Iron-zinc supplementation was effective in raising Hb levels among women who were iron deficient but not among iron replete women (serum ferritin > 20 µg/l). Positive response in mean Hb was more pronounced among women who were initially iron deficient. Adjusted mean Hb increase after 18±5.6 weeks of supplementation was 0.6g/dl higher among women who were not iron replete (SF ferritin  $\leq 20$  µg/L) and received the iron-zinc supplement, compared to women who received iron-only supplement,  $F(1, 99) = 4.356$ ,  $p = 0.039$ . The mean Hb was adjusted for gestational age at recruitment and season of delivery.

### Treatment Effect on Iron Deficiency Anaemia (IDA)

The odds of developing IDA was lower in the Iron-zinc Group compared, to the iron-only group (OR 0.72, 95% CI: 0.33– 1.56). By the 34-36 weeks of gestation, the prevalence of IDA from among iron deficient women (SF <35µg/L) who took the iron-zinc supple

ments increased from 14.5% (8/55) to 17.6% (3/17) giving an absolute difference of 3.1%. The rise in the prevalence of IDA in the iron-only group increased from the initial 7.7% (4/52) to 19.0% (4/21) giving an absolute difference of 11.3%. The relative additive effect in the Iron-zinc Group was therefore 21.4% compared to 146.8% among women who received iron-only.

### Effect of Iron-zinc Supplementation on Mean Serum Ferritin Concentrations

At the end of the supplementation period, (an average of 18±5.6 weeks), adjusted geometric mean serum concentration in the Iron-zinc Group was significantly higher than in the iron-only group (22.9 µg/L versus 16.9 µg/L),  $F(1, 156) = 6.265$ ,  $p = 0.013$ . The mean values were adjusted for maternal age, anaemia at 34-36 weeks gestation, preterm delivery, and log parasitaemia at 34-36 weeks of gestation, socio-economic class, gravidity and parity groupings.

The overall geometric mean serum ferritin levels at recruitment and at 34-36 weeks of gestation were 33.7±2.3 µg/L and 19.5±2.3 µg/L respectively. This represented a 42.1% fall in serum ferritin concentration levels.

The fall in serum ferritin levels in the iron-zinc and iron-only groups were 37.7% and 46.5% respectively. The depletion in maternal serum ferritin concentrations (iron stores) in each group was significant as shown by main time effect  $F(1, 78) = 11.334, p = 0.00$ .

#### *Determinants of Serum Ferritin in Late Pregnancy*

Maternal ferritin concentration is primarily a reflection of maternal iron status, and the important determinants of serum ferritin among iron deficient and/or pregnant anaemic women are shown in Table 3. Using the Standardized B- coefficients, women who received the

iron-only treatment had 0.22 standard units of ferritin concentration less than women who received the combined iron and zinc supplement. Parity was the strongest predictor of serum ferritin concentrations in late pregnancy. Multiparous women had 0.46 standard units of ferritin concentration more, compared to nulliparous women. Similarly women who delivered preterm babies (less than 37 weeks gestation) were of more serum ferritin concentrations, compared to women who delivered full-term babies.

**Table 3 Determinants of serum ferritin at 34-36 weeks gestation**

Model	Standardized Coefficients	T	Sig.	95% Confidence Interval for B	
	Beta			Lower Bound	Upper Bound
(Constant)		-0.91	0.36	-3.07	1.13
Supplement Type	-0.22	-2.70	0.008	-0.26	-0.04
Parity Group of participant	0.46	3.55	0.001	0.08	0.29
Gravidae Group of Participant	-0.32	-2.43	0.017	-0.42	-0.04
Gestational age in weeks on delivery	0.28	2.48	0.014	0.014	0.12
Preterm delivery	0.28	2.52	0.013	0.066	0.54

#### *Prevalence of Maternal Zinc Deficiency*

The overall proportion of women with low plasma zinc concentrations (<60 µg/dl) at recruitment (mean gestation = 13.0 weeks) was 70.9% (61/86). The geometric mean plasma zinc concentrations at recruitment and at 34-36 weeks of gestation were 26.3±2.5 µg/dl (95% CI: 24.1-28.6) and 40.1±2.7 µg/dl (95% CI: 32.5-49.4) respectively.

#### *Relationship between plasma zinc and iron status parameters*

Plasma zinc concentrations correlated negatively with Hb ( $r = -0.165, p = 0.001$ ) in early pregnancy. However, plasma zinc concentrations in early pregnancy were positively correlated with serum ferritin ( $r = 0.137, p = 0.051$ ) and serum iron ( $r = 0.261, p = 0.001$ ). Women who had low plasma zinc levels were 3-fold increased odds of developing ID at recruitment, (OR 3.41, 95% CI: 1.19-9.76).

Women with low plasma zinc levels (<60.0µg/dl) at recruitment also had significantly higher Hb but lower serum ferritin and iron concentrations, compared to women with normal values ( $\geq 60$  µg/dl). Low plasma zinc concentrations in early pregnancy were protective of anaemia (OR 0.57, CI: 0.33-0.99).

## **DISCUSSION**

The key finding in the study population was the low prevalence of IDA, though more than 50% were iron deficient. Additionally, joint iron-zinc supplementation reduced the risk of low Hb among iron-deficient but not iron sufficient pregnant women. Gestational age was a very strong predictor of Hb during pregnancy. Haemoglobin levels at recruitment were inversely correlated with gestational age at recruitment ( $r = -0.36, p = 0.001$ ). The older the gestation, the lower the Hb. This may relate to increased plasma volume as gestation increases and the subsequent haemodilution of haemoglobin.

#### ***Prevalence and Determinants of Total Anaemia, Iron Deficiency and Iron Deficiency Anaemia***

Of 82 all-cause anaemia cases at recruitment, 43.9% (36) were iron deficient. This meant that anaemia was more frequent among iron sufficient women. This further suggests that causes other than iron deficiency were responsible for anaemia among the study participants. The data showed more than half of the anaemia associated with pregnancy was not due to iron deficiency but to other causes (for example, inflammation, haemo-dilution and infection such as malaria).

It has been documented that anaemia due to other factors other than iron are not associated with low serum ferritin.<sup>7</sup> Additionally, the prevalence of iron deficiency anaemia (IDA) was relatively low compared to the prevalence of iron deficiency (ID); finding which meant most women with low iron stores did not suffer from iron deficiency anaemia.

The prevalence of low plasma zinc concentrations in this study sample was striking though similar findings were reported from Peru, where 60% of pregnant women had low levels of plasma zinc.<sup>8</sup> A prevalence of marginal zinc deficiency of more than 20% is of a magnitude that warrants urgent public health interventions.<sup>9</sup> In developing countries such as Ghana, poor dietary quality is often a major determinant of inadequate micronutrient intakes. Poor quality diets, predominantly plant based, often contain very small amounts of expensive animal source foods. Low intake of animal protein could therefore affect plasma zinc concentrations, as about 70% of zinc is transported in the serum bound to albumin. Changes in serum albumin will, in turn, affect plasma zinc concentrations.<sup>10</sup>

A number of factors such as infections, not necessarily related to nutritional zinc status reduce plasma zinc levels. Low plasma zinc concentrations during pregnancy may not necessarily reflect zinc deficiency as infections<sup>11,12</sup>, haemodilution and other metabolic changes can contribute to low zinc levels.<sup>13</sup> These factors therefore further complicate the diagnosis of real zinc deficiency during pregnancy and the assessment of the efficacy of iron-zinc supplementation.

Response to iron supplementation depended on the iron status at recruitment. Iron-zinc supplementation was effective in raising Hb and serum ferritin values among women who were iron deficient in early pregnancy but not among iron sufficient women. Iron-zinc supplementation increased mean Hb levels to  $1.3 \pm 0.1$  g/dl among women who were anaemic. An increase of at least 1.0 g/dl in haemoglobin in response to iron supplementation is indicative of iron deficiency.<sup>14</sup>

Similar responses to combined iron and zinc supplementation in Peru and Japan have earlier been reported.<sup>15,16</sup> Increased erythropoiesis in iron deficient women may account for the increase in mean Hb change. However, in Nepal, a large study that involved 4926 pregnant women reported an insignificant effect of zinc on iron indicators including the prevalence of severe anaemia among the study groups.<sup>17,18</sup> The ratio of iron to zinc used in that study (60 mg: 25 mg) was

different from what was used in this study and may explain the negative finding.

By virtue of its unique relationship with over 300 enzyme systems, zinc is reported to have an important role in normal haematopoiesis.<sup>19</sup> Additionally, zinc-finger transcription factor, (GATA-1), increases erythropoiesis<sup>20</sup>. Nishiyama and colleagues have also reported increases in mean Hb and attributed to zinc derived insulin-like growth factor 1 (IGF-1) following zinc administration.<sup>21</sup> Some possible reasons why women in the iron-zinc supplemented group who were initially anaemic at baseline had higher mean Hb and serum ferritin changes in late pregnancy may therefore be attributed to one or both of the following:

- Iron absorption was improved in the absence of infections such as malaria.
- Zinc promotes the formation of insulin-like growth factor 1 (IGF-1) which together with erythropoietin is required for haematopoiesis.<sup>22,23</sup>

Findings from this and similar studies provide sufficient evidence to suggest that a joint supplementation of iron and zinc best facilitates the production of red blood cells and thus prevents anaemia.

There were strong relationships between maternal zinc status and iron status indicators (that is, Hb, and serum iron) in early pregnancy. There was in particular, a strong positive association between maternal zinc and iron status in this study population; a relationship that has also been consistently shown in the United States.<sup>24,25</sup>

This study found a negative association between plasma zinc and Hb concentrations in early pregnancy. This negative association are inconsistent with what had been reported from Japan where a positive association between plasma zinc and Hb concentrations was found<sup>15</sup>. In another study of pregnant Filipino women, Hb and serum zinc positively correlated ( $r = 0.22$ ,  $p < 0.001$ ,  $n = 271$ ) at 24 weeks of gestation but not at 36 weeks of gestation.<sup>26</sup>

The apparent differences in the findings from these studies may be explained by the fact that blood samples in the Filipino study were drawn in the second trimester whereas blood draw in this study was in the first trimester. The Japanese study collected blood samples from the middle of the second trimester in a relatively small sample size ( $n = 52$ ). A study among pregnant Malawian women had reported lack of correlation between serum zinc and Hb.<sup>27</sup>

The association between plasma zinc and Hb has therefore been inconsistent in the literature. The inconsistencies may be attributed to extraneous factors that can influence both Hb and plasma zinc (for example infections, stage of gestation). This study also found a drastic reduction in plasma zinc concentrations up to ten weeks of gestation; however, thereafter it rose gradually up to around 16 weeks of gestation. There was no linear relationship between plasma zinc levels and gestational age.

Therefore plasma zinc levels during pregnancy are unlikely to be a reliable and valid diagnostic criterion of maternal zinc deficiency during pregnancy. What then are the possible explanations for the negative association between Hb and plasma zinc levels in early pregnancy observed in this study? High maternal Hb may be associated with one or more of the following:

- Erythropoiesis may be suppressed and that may lead to reduced availability of functional zinc pool.
- Poor plasma volume expansion as a consequence low plasma zinc concentrations can result in apparently high Hb concentrations.
- Increased foetal erythropoiesis may result in reduced plasma zinc but stimulate maternal erythropoiesis with a resultant increase in maternal Hb.

Therefore, there are at least two possible mechanisms that may help explain the negative relationship between plasma zinc and Hb concentrations in this study. The first is that low plasma zinc may be associated with inadequate plasma volume expansion and therefore little haemodilution effect that can lead to high Hb concentrations. Poor nutritional profile in early pregnancy is reported to be positively associated with reduced plasma volume<sup>28,29</sup> and it is possible low plasma zinc concentrations were partly reflective of poor nutritional status of the women.

The negative association between Hb and plasma zinc concentrations in early pregnancy (mean gestation of 13 weeks) may just be a reflection of inadequate plasma volume expansion vis-à-vis increased red cell mass that had taken place. Another possible explanation for the negative relationship between plasma zinc and Hb concentrations in early pregnancy may be attributed to increased foetal erythropoiesis that takes place in early pregnancy.

Foetal erythropoiesis requires the active involvement of IGF-1. Synthesis of IGF-1 in turn requires zinc. Foetal erythropoiesis will necessarily require in addition to erythropoietin, other factors including zinc

and oxygen. Adequate supply of oxygen for foetal erythropoiesis will mean increased maternal erythropoiesis. As maternal erythropoiesis increases, Hb will also increase. Women with high levels of plasma zinc had also low Hb concentrations.

It may be that erythropoiesis was suppressed with the resultant small amount of zinc being used in the synthesis of IGF-1 and that presupposes a relatively greater amount of zinc will be available in maternal plasma, but because demand for oxygen is not great, maternal erythropoiesis was also not triggered. This might have led to low Hb and higher plasma zinc concentrations co-existing.

## CONCLUSION AND RECOMMENDATIONS

Iron-zinc was more effective than iron-only supplementation in raising Hb and serum ferritin values among women who were iron deficient in early pregnancy but not among iron sufficient women. The rationale of routine iron supplementation in non-anaemic pregnant women in Ghana needs to be re-examined.

Combined Iron-zinc supplementation for iron deficient pregnant women in Ghana therefore deserves attention in order to avoid the needless negative impact of iron and zinc deficiencies.

### *Implications for Policy and Practice*

The results have shown that mass iron-zinc supplementation during pregnancy does not appear to be beneficial. Efficacy (response) to iron-zinc supplementation was greater among women with poor iron-zinc nutriture. Iron-replete women did not appear to have benefited from iron-zinc supplementation. Such women need selective iron supplementation based on their iron status during pregnancy.

A reduction in the amount of supplemental iron for non-anaemic women as and when necessary, together with early infection detection and treatment may help prevent adverse pregnancy outcomes and maternal anaemia.

### *Limitations of the Study*

In this study, for ethical reasons it was not possible to have a placebo control group for iron supplementation, it cannot be concluded with complete certainty that the improvement in iron status was due to the iron supplements. A placebo control group would also have made it possible to establish the prevalence of iron deficiency, based on the proportion of women who responded

to iron supplementation compared with those receiving placebo.

However, both arms of the study were supplemented with iron, folic acid and given intermittent preventive treatment (IPT) with sulfadoxine pyrimethamine. The intervention group had in addition to these zinc supplements. So any difference in outcome may be attributed to the zinc.

The results obtained in the study suggest zinc deficiency may be a public problem in Ghana. This needs to be confirmed in a study specifically designed to assess the magnitude of zinc deficiency among pregnant women in all regions of Ghana.

The results also point to the fact that it still remains unclear whether Iron-replete women should continue to be supplemented with iron supplements at the same dosage level as iron deficient women in Ghana.

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