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Impact of Maternal Copper and Zinc Status on Pregnancy Outcomes in a Population of Pregnant Nigerians

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Abstract: Micronutrient deficiencies, especially during pregnancy have been identified as important public health problem especially in economically disadvantaged settings. To determine the effect of maternal deficiencies of copper and zinc on pregnancy outcomes in a population of pregnant Nigerians, 349 pregnant women aged 15-40 years (mean; 27.04±2.75 years) recruited at gestational age of ≤25 week (mean; 21.8±3.14 wks) were evaluated for plasma copper and zinc using Atomic Absorption Spectrophotometer. The women were followed-up till delivery during which maternal morbidity and foetal outcomes were recorded. Both maternal sociodemographic and obstetric data were obtained by questionnaire. One hundred and sixty (45.8%) women were zinc deficient (mean = 2.65±1.16 µmol/l), 58.2% were deficient in copper (mean = 3.26±1.80 µmol/l), 23.8% were deficient in both copper and zinc while 18.6% were not deficient in either copper or zinc. There was comparative prevalence of illness in copper-deficient and copper-adequate mothers, except for hypertension which was significantly ($p = 0.021$) higher in the former. Significantly ($p = 0.026$) higher proportion of zinc adequate pregnant women suffered upper respiratory tract infections and malaria when compared with their zinc deficient counterparts. However, the prevalence of diabetes mellitus was found to be significantly ($p<0.05$) higher in mothers who were zinc deficient. Both plasma copper and zinc status had no significant effect on foetal outcome. The reason for the lack of effect of copper and zinc deficiencies on foetal outcomes in the presence of adverse maternal outcomes remained unknown.

Key words: copper and zinc deficiencies, pregnant Nigerian women, public health

INTRODUCTION

Micronutrient deficiencies are common during pregnancy, especially in pregnant women from economically disadvantaged settings where diets with low content of minerals and vitamins are consumed. Zinc is an essential trace element with wide range of functions in the body including the synthesis of enzymes and nucleic acids (WHO, 1996). Studies of pregnant women in African countries such as Nigeria, Egypt, Zaire and Malawi have shown lower plasma or hair zinc concentrations than in pregnant women from developed countries (Okonofua *et al*., 1990; Kirksey *et al*., 1994; Anaud *et al*., 1994; Gibson and Huddle, 1998).

Also, several studies have reported that maternal plasma zinc decreases during pregnancy from 24-33 week of gestation (Perveen *et al*., 2002; Ajose *et al*., 2001; Izquierdo *et al*., 2007; Martin-Lagos *et al*., 1998). However, Meran *et al*. (2003) while reporting comparable plasma zinc in pregnant and non pregnant women documented decreased plasma zinc in pregnant women greater than 35 years old.

Like zinc, copper is involved in the functions of several cuproenzymes that are essential for life (Goel and Misra, 1982). Copper plays a role in the mobilization of iron to plasma from the tissue stores (Raman and Leela, 1992) and copper deficiency during embryonic and foetal development has been found to cause numerous gross structural and biochemical abnormalities. It has been reported that more than 50% of human conception fail to implant and of those implanted, approximately 30% fail to reach term due to copper deficiency (Ebbs *et al*., 1984). Significantly higher mean serum copper had been reported in healthy pregnant Nigerian women than their non-pregnant counterparts (Ajose *et al*., 2001). Similar increase in serum copper had been reported in Spanish (Izquierdo *et al*., 2007) and Turkish women (Meran *et al*., 2003).

Also deficiencies of trace elements; copper and zinc have been implicated in various reproductive events like infertility, pregnancy wastage, congenital abnormalities (Black, 2001), pregnancy induced hypertension, placental abruption, premature rupture of membranes,

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still birth and low birth weight (Pathak and Kapil, 2004). In Nigeria, there is paucity of data on the impact of plasma zinc and copper levels on pregnancy outcomes. Therefore the present study is aimed at evaluating the effect of maternal plasma levels of copper and zinc during pregnancy on pregnancy outcomes.

MATERIALS AND METHODS

The study was carried out at the Department of Obstetrics and Gynaecology of the Federal Medical Centre, Abakaliki, one of the referral tertiary health institutions in the South eastern part of Nigeria. Abakaliki and the environs are inhabited mainly by subsistence population whose main occupation is farming (mainly, yam and cassava) with some animal husbandry and other professions and/or activities such as civil service, trading, artisan and stone quarrying. Malaria transmission is intense and occurs throughout the year (perennial).

Three hundred and fifty-one (351) consecutive women aged 15-40 years (Gestational age ≤25 weeks) who gave their consent to participate in the study were recruited between July 2007 and September 2008. Those excluded from the study were women with chronic disease, women that were HIV-seropositive and those with multiple pregnancies. The protocol for this study was approved by the Ethics and Research Committee of the Federal Medical Centre, Abakaliki. The sociodemographic data of the participants were collected by structured questionnaires. Height and weight were measured with the subject in light clothes without shoes and BMI (Kg/m^2) was calculated.

Five millilitres (5.0 ml) of non-fasting venous blood collected between 08.00-10.00 hours were dispensed into trace element-free heparinized plastic bottle (3.0 ml) and EDTA bottle (2.0 ml) for biochemical and haematological analyses respectively. Plasma was separated by centrifugation at 2000 g for five minute. The plasma samples were frozen until they were analyzed.

Participants were followed-up till delivery. At every follow-up, participants were evaluated by the attending Obstetricians for anaemia (Hb < 11.0 g/dl), hypertension (blood pressure > 140/90 mmHg), diabetes (fasting plasma glucose > 7.8 mmol/l), *H. pylori* infection (Seropositive to *H. pylori* antibody) and concomitant illness such as malaria (positive thin or thick film), upper respiratory tract infection (cough and catarrh), urinary tract infection (positive urine protein, nitrite and leucocytes).

At delivery, baby's birth outcomes such as weight, length, head circumference as well as still birth, mode of delivery, gestation age at delivery was recorded. Birth weight was determined using electronic weighing balance and recorded to the nearest 0.05 Kg with the scale checked periodically throughout the study for accuracy while birth length and head circumference was determined by a measuring tape to the nearest 0.1 cm. Baby was considered underweight if the birth weight was ≤2.5 Kg (Fawzi *et al*., 2007), preterm if delivered at ≤37 weeks and post-term if delivered at > 42 weeks.

Plasma copper and zinc were determined in duplicates using flame Atomic Absorption Spectrophotometer and the mean was recorded as the absolute value of the elements while plasma albumin was determined by bromocresol green method as previously described (Hill, 1985).

Maternal haemoglobin concentration was determined by Cyanmethaemoglobin method as described previously (Dacie and Lewis, 1994). The World Health Organisation (1992) criteria for typing anaemia in pregnancy were adopted in this study. Plasma copper < 8.0 mmol/l and zinc < 5.0 mmol/l were considered deficiencies (Robets *et al*., 2006).

Statistical analysis: The data obtained were analyzed using Statistical Package for Social Science (SPSS) version 7.5. The results were expressed and mean ± S.D or proportion. Mean plasma levels of zinc and copper were compared among groups by Chi-square and the level of significance set and $p<0.05$.

RESULTS

Although three hundred and fifty one (351) pregnant women were recruited, one (0.3%) died early into the study remaining three hundred and fifty (99.7) of which data was available but samples were obtained from 349 participants as one participant declined participation. At delivery, data was available for three hundred and nineteen (91.4%) women and their neonates. Data was incomplete or not available for the remaining thirty (8.6%).

Table 1 shows the general characteristics of pregnant women recruited at ≤25 weeks gestation. Although, in general, the women were deficient in either of the two trace elements (Mean: 9.59±9.42 and 9.19±9.16 mmol/l for copper and zinc respectively) evaluated, the ranges of the elements vary from very low levels to very high concentrations, with copper and zinc concentrations from 0.89 and 0.70 mmol/l respectively to values as high as 45.36 and 67.32 mmol/l respectively. However, the participants were generally anaemic with mean haemoglobin concentration of 10.21±1.26 g/dl.

One hundred and sixty (45.8%) women were zinc deficient (mean = 2.65±1.16 µmol/l) and 203 (58.2%) were deficient in copper (mean = 3.26±1.80 µmol/l), 83 (23.8%) were deficient in both copper and zinc while 65 (18.6%) were not deficient in either copper or zinc.

Figure 1 and 2 shows the prevalence of maternal concomitant illnesses in relation to copper and zinc status. There was comparative prevalence of illness in copper-deficient and copper-adequate mothers, except for hypertension which was significantly ($p = 0.021$) higher in the former. However, there appeared to be generally non-significant higher prevalence of concomitant illness in the copper deficient groups.

Table 1: General characteristics of pregnant women at ≤25 weeks gestation

Parameters	N	Mean	SD	Range
Age (yrs)	350	27.04	4.75	15-40
BMI (Kg/m^2)	350	27.3	4.3	17.8-42.6
Parity (n)	350	1.41	1.46	0-4
Haemoglobin (g/dl)	349	10.21	1.26	6.5-13.3
Albumin (g/dl)	349	3.45	0.80	1.80-5.50
Copper (mmol/l)	349	9.59	9.42	0.89-45.36
Zinc (mmol/l)	349	9.19	9.16	0.7-67.32
Antenatal attendance (n)	343	7.01	2.52	1-14

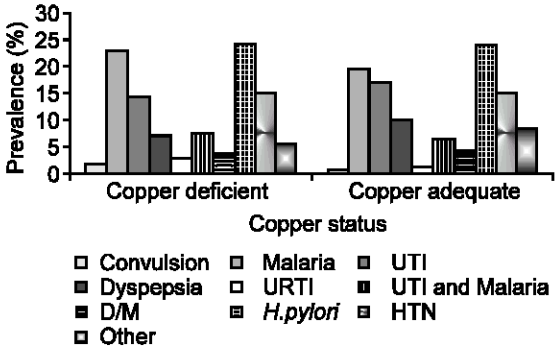

Fig. 1: Maternal morbidity in relation to copper status. UTI: Urinary tract infection; D/M: Diabetes Mellitus; URTI: Upper Respiratory Tract Infections; HTN: Hypertension. P<0.05 for Hypertension

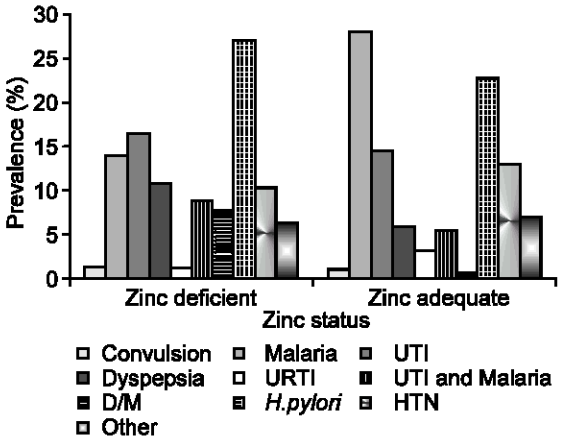

Fig. 2: Maternal morbidity in relation to zinc status. UTI: Urinary Tract Infection; D/M: Diabetes Mellitus; URTI: Upper Respiratory Tract Infections; HTN: Hypertension. P<0.05 for URTI, Malaria and D/M

For plasma zinc, significantly (p = 0.026) higher proportion of zinc adequate pregnant women suffered upper respiratory tract infections and malaria when compared with their zinc deficient counterparts. However, the prevalence of diabetes mellitus was found to be significantly (p<0.05) higher in mothers who were zinc deficient than in mothers who had adequate plasma zinc concentrations.

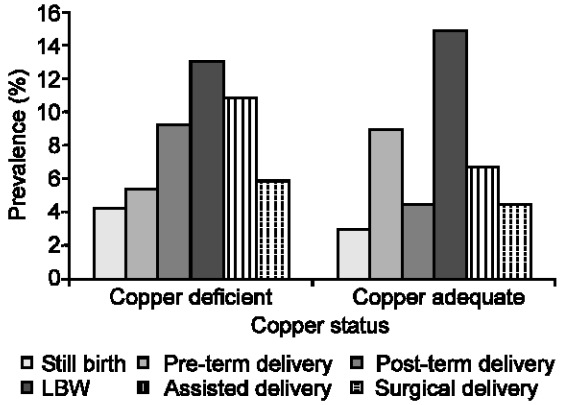

Fig. 3: Foetal outcomes relative to maternal copper status. LBW: Low Birth Weight

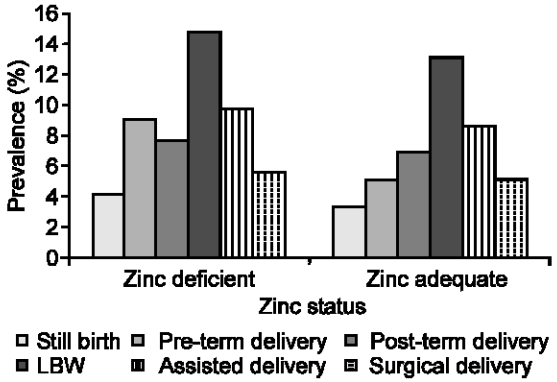

Fig. 4: Foetal outcomes relative to maternal zinc status. LBW: Low Birth Weight

Plasma copper status was found to have non-significant effect on foetal outcome (Fig. 3). Although while lower proportions of pre-term and LBW infants were delivered by copper deficient pregnant women, higher proportions of post-term infants, still birth infants and infants delivered through instrument assisted/or caesarean sections were recorded in women who were not copper deficient, although these were found to be statistically non-significant.

From Fig. 4, both zinc-deficient and zinc-adequate pregnant women had comparable foetal outcomes, although non-significantly (p>0.05) higher proportions of infants with these outcomes were delivered by zinc deficient mothers when compared with zinc-adequate mothers.

DISCUSSION

Pregnancy has been associated with increased demands of all nutrients including copper and zinc and deficiency of any of these could affect the course and outcomes of pregnancy (Upadhyaya *et al*., 2004). Although no study has identified the role of copper

deficiency in pregnancy-induced hypertension, significantly higher prevalence of hypertension in pregnant women deficient in copper when compared to their copper-adequate counterparts in the present study needs further investigation.

While copper-containing protein, ceruloplasmin has been found to promote LDL oxidation *in vitro* (Fox *et al*., 2000) leading some researchers to propose that increased plasma copper rather than deficiency could increase coronary heart disease, another cuproenzyme, Superoxide Dismutase (SOD) and ceruloplasmin possesses antioxidant activities, leading experts to believe that copper deficiency rather than excess copper increased the risk of CHD (Jones *et al*., 1997). However, both epidemiological (Malek *et al*., 2006; Leone *et al*., 2006) and experimental studies (Turley *et al*., 2000; Rock *et al*., 2000) have failed to unequivocally confirm the role of copper in the aetiology of pregnancy-induced hypertension.

Zinc is an important trace element involved in a number of metabolic reactions where they acts as cofactor and deficiency of zinc has been associated with many diseases. However, in the present study, the prevalence of both URTI and malaria were found to be higher in zinc-adequate than in zinc-deficient pregnant women. This is in contrast to the role of zinc in the maintenance of the immune system (Baum *et al*., 2000). Studies have shown that zinc supplementation reduced the incidence of respiratory tract infections such as pneumonia (Bhutta *et al*., 1999), diarrhoea (Fischer and Black, 2007) and malaria (Black, 1998) in children. However, a randomized controlled trial in over 42,000 children showed that zinc supplementation did not significantly reduce the mortality associated with malaria and other infectious diseases (Sazawal *et al*., 2007). Nevertheless, the role of zinc in the prevention of malaria and URTI during pregnancy is yet to be ascertained.

The prevalence of Diabetes Mellitus (D/M) was also found to be significantly ($p<0.05$) higher in zinc-deficient than in zinc-adequate pregnant women. Although results from micronutrients (including vitamins and minerals) studies in diabetes mellitus have been conflicting (O'Connel, 2001; Bo *et al*., 2008; Hussain *et al*., 2009) and data is scarce on the study of zinc metabolism during pregnancy complicated with diabetes mellitus, the present finding requires further investigations. However, *in vitro* studies have found that zinc enhances the effectiveness of insulin in non-insulin dependent D/M (Arquilla *et al*., 1978). Again, the development of glucose intolerance in animals deprived of zinc together with the occurrence of zinc deficiency in type 2 D/M suggests a role for zinc deficiency in the pathogenesis of gestational D/M.

Comparable foetal outcomes observed in pregnant women deficient in copper and zinc in the present study contrasts several studies where independent deficiency of copper or zinc was associated with adverse pregnancy outcomes. The reason for the present observation is not clear but it may not be unconnected to the fact that the deficiencies were of the mild type. In the presence of such mild deficiencies, the foetal plasma copper and zinc levels as well as their functions may not be affected as these may be maintained at the expense of maternal plasma levels (Balai *et al*., 1992).

Therefore it is concluded that maternal trace elements status was associated with maternal morbidity such as hypertension (copper), infections and D/M (zinc), without a significant effect on foetal outcomes. The reasons for the non-significant impact on foetal outcomes of these trace elements deficiencies in the presence of significant effect on maternal outcomes need to be investigated further.

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