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Maternal Serum Zinc Levels and Fetal Malnutrition of Term Babies in Nigeria

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Abstract: Maternal zinc deficiency is associated with fetal growth retardation and fetal malnutrition (FM). FM is common in developing countries. The main aim of the current study was to determine the association between maternal serum zinc levels and FM. After receiving hospital ethics committee approval and informed consent from the mothers, consecutive pregnant women and their respective live, singleton, term babies delivered between August 2013 and January 2014 at the Federal Teaching Hospital Ido-Ekiti in south western Nigeria were included in our study. Pregnancy and antenatal records were obtained. Maternal serum zinc levels were assayed and babies were examined for FM within 24 h of birth. FM was diagnosed using the Clinical Assessment of Fetal Nutritional Status Score as adapted by Metcoff. Maternal serum zinc levels were stratified and compared among mothers who had babies with and without FM. A total number of 386 babies were studied, 90 (23.3%) had FM. The mean [±standard deviation (SD)] maternal serum zinc concentration was 9.0 (±6.1) μmol/L and 187 (48.4%) mothers had hypozincemia. The mean (±SD) serum zinc concentration of mothers of babies with FM was 7.4 (±5.3) μmol/L compared to 9.5 (±6.2) μmol/L for mothers of babies without FM ($p = 0.003$). Fifty-eight (64.4%) mothers of the 90 babies with FM and 129 (43.6%) of the 296 mothers of babies without FM had hypozincemia ($p = 0.001$). Although low maternal serum zinc levels were associated with FM, not all mothers with low serum zinc levels had babies with FM and not all babies with FM has mothers with low serum zinc levels. This attests to multifactorial etiology of FM. However, maternal hypozincemia may be a significant contributory factor and, therefore, mothers may benefit from zinc supplementation during pregnancy.

Key words: Fetal malnutrition, maternal hypozincemia, CANS score, Nigeria

INTRODUCTION

Zinc is an essential trace element for normal fetal growth and development (Samimi *et al*., 2012). The relationship between maternal zinc levels and pregnancy outcome has been evaluated in the past decades by many (Tamura *et al*., 1997; Shah and Sachdev, 2001; Ugwuja *et al*., 2010). Based on the estimated average requirement of about 12 mg/d of zinc during pregnancy, up to 82% of pregnant women worldwide may have inadequate zinc intake (Caulfield *et al*., 1998). Pregnant women in developing countries reportedly consume diets with lower amounts of minerals and vitamins than those in developed countries (Parr, 1996) and zinc deficiency is one of the most common trace element deficiencies in the developing countries (Pathak and Kapil, 2004). Previous studies have reported a high prevalence of trace element deficiencies, such as zinc, copper and magnesium, among pregnant Nigerian women (Pathak and Kapil, 2004; Ugwuja *et al*., 2011; Pathak *et al*., 2004). Trace element deficiencies, including zinc, are associated with a low birth weight (LBW) (Pathak *et al*., 2004) and are known to play an important role in adverse fetal outcomes (Shah and Sachdev, 2001).

Fetal malnutrition (FM) is defined as failure of the fetus to acquire and/or maintain an adequate quantum of fat and muscle mass during intrauterine growth evident as soft tissue wasting present at birth (Adebami *et al*., 2007; Adebami *et al*., 2007; Fall *et al*., 2003; Deodhar and Jarad, 1999; Metcoff, 1994). It has been reported to be commoner than LBW in Nigeria (Adebami *et al*., 2007) and in other developing countries and contributes to high numbers of LBW babies in developing countries (Fall *et al*., 2003). In FM, subcutaneous tissues and underlying muscles are diminished; the skin over the arm and elbows, the legs and knees and over the interscapular regions is loose. In severe FM, the neonate looks “emaciated” or “marasmic” as the skin appears several sizes too large for the baby (Metcoff, 1994). The buccal fat pads are reduced and the normal full-rounded appearance of the gluteal fat pads is lost. The scalp hair may be coarse, patchy, or “straight and

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starring" as in marasmus or may even have a "flag-sign" as in severe protein-calorie malnutrition (kwashiorkor). Thus, FM is a clinical diagnosis (Metcoff, 1994; Owa and Adebami, 2007) associated with increased neonatal morbidity and mortality (Tamura *et al.*, 1997) and has been identified as a significant health problem in both developed and developing countries (Adebami *et al.*, 2007; Crosby *et al.*, 1977; Salihoglu *et al.*, 2012).

Since zinc is an essential trace element for normal fetal growth and development (Adebami *et al.*, 2007) and maternal deficiency can have an adverse effect, it is important to further understand the relationship between maternal zinc status of different people groups and its association with FM. Specifically, our objectives were to determine the prevalence of FM among term neonates, maternal serum zinc levels and the association between these parameters.

MATERIALS AND METHODS

Subjects: The current study had a cross-sectional design. Ethical approval was obtained from the Research and Ethics Committee of the Federal Medical Centre of Ido-Ekiti (now Federal Teaching Hospital of Ido-Ekiti), Ekiti state, Nigeria. The present study was conducted over a period of 6 months (August 2013 to January 2014) at the maternity and neonatal wards of the Federal Teaching Hospital of Ido-Ekiti. This hospital is the main referral health institution providing specialist neonatal and maternity services to the semi-urban population of Ekiti state and adjoining communities in southwestern Nigeria.

Subjects were mothers of consecutive, singleton, live-birth, term (37-42 weeks gestation) and their babies delivered during the study period at the maternity unit of the hospital. All information obtained was recorded in the proforma designed for the current study by one of the researchers. Maternal data included age, date of last menstrual period, parity, place of antenatal care, number of clinic visits, duration of pregnancy and the gestational age (GA) at the time of registration for antenatal care. This information was extracted from antenatal records and cross-checked with each mother's clinical history when necessary. A history of drugs taken during pregnancy, including details of vitamin and mineral supplements, antimalarials, antibiotics and antivirals were recorded. All women who met all inclusion criteria and consented to participate were followed-up till delivery. Women who took zinc-containing supplements during pregnancy and their babies, women exposed to cigarette smoke (passive and active smokers) during pregnancy and babies with obvious congenital malformations and stigmata of chromosomal anomalies were excluded from the study.

Serum zinc sampling and analysis: Using a disposable plastic syringe and taking aseptic precautions, 5 ml of venous blood was collected from the antecubital vein of each mother at the time of delivery and 5 ml of cord blood was also collected. Blood samples were transferred into sterile bottles that were prewashed in 10% nitric acid and rinsed with deionized water to prevent trace element contamination. Blood samples were left to stand for about 30 min to clot and then centrifuged at 3500 rpm for 5 min to separate cells from the serum. The serum obtained was transferred into trace element-free clean sterile polypropylene tubes and stored in the freezer at -20°C until analyzed.

Serum zinc determination was done using the hospital Atomic Absorption Spectrophotometer (UNICO model number 2100, serial no. AO706023) in the hospital's routine laboratory and zinc concentrations were reported in μmol/L. The lower cut-off for maternal serum zinc concentration used in the present study and accepted in previous works was 7.6 μmol/L (IziNCG, 2004; Hotz *et al.*, 2003). Therefore, a maternal serum zinc was categorized as "low" or "normal" if the concentration was $<$ or $>$7.6 μmol/L, respectively (IziNCG, 2004; Hotz *et al.*, 2003).

Assessment of FM using clinical assessment of fetal nutritional status (CANS) score: After all of the babies had their various anthropometric measurements taken, each was assessed and scored for FM within 24 h of birth using a simple, rapid and quantifiable method called the CANS score adapted by Metcoff (Metcoff, 1994). The CANS score consists of nine "superficial," readily detectable signs of FM based on inspection and hands-on estimates of loss of subcutaneous tissue and muscles. The hair, cheeks, neck, chin, arms, back, buttocks, legs, chest and abdomen of neonates were examined and then scored between 1 (worst evidence of malnutrition) and 4 (no evidence of malnutrition). The total rating of the nine CANS signs is the CANS score for the subject and ranges between 9 (lowest) and 36 (highest). Babies with a CANS score $\geq$25 were regarded as normal or having no FM; (Crosby *et al.*, 1977) babies with a CANS score $<$25 were diagnosed with FM. Maternal serum zinc levels were compared between babies with and without FM.

RESULTS

Mothers, GA and gender of babies: A total of 386 mother-infant pairs were included in the current study. The GA at delivery for all 386 mothers ranged between 37 and 42 weeks with a mean [±standard deviation (SD)] GA at delivery of 38.9 (±1.4) weeks. Of the babies, 172 (44.6%) were males and 214 (55.4%) were females (males:females, 0.8:1). The mean (±SD) of GA at delivery for males was 38.7 (±1.5) weeks and 39.1 (±1.4) weeks for females. Though the mean GA was higher for females, the difference was not statistically significant ($t = 0.70$, $p = 0.4$).

Maternal serum zinc levels: Maternal serum zinc concentrations ranged between 0.6 and 27.6 µmol/L with (mean±SD, 9.0±6.1 µmol/L). Table 1 shows the proportion of mothers with normal and low serum zinc levels; 187 (48.4%) of the mothers had low serum zinc levels (<7.6 µmol/L), Hotz *et al.* (2003) while 199 (51.6%) had normal zinc levels (>7.6 µmol/L) Hotz *et al.* (2003).

Babies with FM: Ninety (23.3%) of the 386 babies had a CANS score <25 and were diagnosed with FM. The 90 babies with FM consisted of 36 (40.0%) males and 54 (60.0%) females (males:females, 1:1.5). Thirty-six (20.9%) of the 172 males and 54 (25.2%) of the 214 females had FM. Although a higher proportion of females had FM, the difference was not statistically significant (χ^2 = 0.99, df = 1, p = 0. 320).

Maternal serum zinc levels and babies with FM: The mean (±SD) serum zinc concentration for mothers of babies with FM was 7.4 (±5.3) µmol/L, which was lower than that for mothers of babies without FM (mean±SD, 9.5±6.2 µmol/L) and this difference was statistically significant (t = 2.95, df = 384, p = 0.003). Table 2 shows a comparison of the mean serum zinc concentrations of mothers of babies with and without FM in relation to GA; the mean maternal serum zinc concentration was higher in mothers of babies without FM in all GA groups and statistically significant at the GA of 39 weeks (p = 0.026). On the other hand, there was an increase in maternal serum zinc concentration with GA in babies without FM. Table 3 compares the prevalence of low serum zinc levels of mothers of babies with and without FM in relation to GA. There was an overall statistically significantly higher proportion of mothers of babies with versus without FM that had hypozincemia. For example, 58 (64.4%) of the 90 mothers of babies with FM had hypozincemia compared to 129 (43.6%) of the 296 mothers of babies without FM (p = 0.001). In all the GA groups, mothers of babies with FM had a higher prevalence of low zinc than mothers of babies without FM. This was significant at Gas of 37 and 39 weeks (p = 0.011 and 0.049, respectively).

Table 4 shows a comparison of mean maternal serum zinc concentrations using different indices of nutritional status for babies. For each nutritional index, the mean maternal serum zinc concentration was lower for babies with FM versus those without. This difference was statistically significant for the ponderal index (PI) and CANS score.

Table 5 compares the mean values of the anthropometric data of babies of mothers with hypozincemia with those of babies of mothers with normal serum zinc levels. The mean weight, head circumference (HC), mid-arm circumference (MAC), MAC/HC, PI and CANS score of babies of mothers with hypozincemia were lower than those from mothers with normal serum zinc levels. These differences were statistically significant for the CANS score, MAC/HC and PI (p = 0.006, 0.017 and 0.001, respectively). On the other hand, the means lengths and HC of babies of mothers with hypozincemia were higher than that of babies of mothers with normal serum zinc levels, though the differences were not statistically significant (p = 0.2 and 0.5, respectively).

Table 1: Distribution of maternal serum zinc status and mean serum zinc concentration

Variable	Number (%)	Mean (SD) (µmol/L)
Maternal serum zinc level		
Low	187 (48.4)	3.8 (1.7)
Normal	199 (52.6)	13.9 (4.4)
Total	386 (100.0)	9.0 (6.09)

SD: Standard deviation

DISCUSSION

In the present study, 48.4% of the mothers studied had zinc deficiency. This was similar to national-level prevalence estimates of 47.7% reported by the Nigerian Federal Ministry of Health (2005). Both the results of the present study and those of the Federal Ministry of Health suggest a high prevalence of maternal zinc deficiency in Nigeria. A similarly high prevalence of maternal zinc deficiency of 45.8% was reported by Ugwuja *et al.* (2010) in Abakaliki, Nigeria, in 2010. In one of the earliest studies in Nigeria about maternal serum zinc concentrations, (Okonofua *et al.*, 1989) alluded to a rather high proportion of pregnant women in Nigeria having biochemical hypozincemia. A possible reason for the high prevalence of zinc deficiency which was also found in the current study of pregnant Nigerian women has been attributed to diets low in minerals and vitamins. Thus, suboptimal zinc intake may be relatively common (Parr, 1996; Ugwuja *et al.*, 2011).

Another factor that may be responsible for the high prevalence of maternal zinc deficiency is the presence of high amounts of fiber and phytate in the diet although dietary intake was not assessed in the present study. High fiber and phytate diets are factors known to affect zinc bioavailability, especially considering phytate is one of the greatest inhibitory factors of zinc absorption (Caulfield *et al.*, 1998; IziNCG, 2004). The degree to which phytate inhibits zinc absorption has been defined by the ratio of phytate:zinc in the diet (Awadallah *et al.*, 2004). Diets with unrefined, unfermented and ungerminated grains which are consumed by many women in developing countries like Nigeria have a high phytate:zinc ratio (IziNCG, 2004). Moreover, intake of animal proteins which are rich sources of zinc is negligible for many of these women (Ugwuja *et al.*, 2011; IziNCG, 2004)

However, the mean (±SD) maternal serum concentration of zinc in the present study was 9.0 (±6.1) µmol/L, which is higher than the accepted lower cutoff for normal

Table 2: Comparison of mean maternal serum zinc concentration between babies with and without fetal malnutrition (FM)

	Babies with FM		Babies without FM		
Gestational age (weeks)	N	Mean (SD) maternal zinc (µmol/L)	N	Mean (SD) maternal zinc (µmol/L)	p-value
37	20	6.8 (5.4)	55	8.6 (5.2)	0.184
38	21	7.9 (4.7)	82	9.6 (5.9)	0.225
39	15	4.3 (2.9)	55	8.8 (7.4)	0.026
40	17	9.6 (6.5)	58	9.8 (5.9)	0.914
41	17	7.8 (5.7)	35	10.8 (7.6)	0.154
42	0	-	11	11.1 (3.7)	
Total	90	7.4 (5.3)	296	9.5 (6.2)	0.003

t = 2.95, df = 384, p = 0.003, SD: Standard deviation

Table 3: Comparison of maternal serum zinc status between mothers of babies with and without fetal malnutrition (FM)

	Mothers of babies with FM		Mothers of babies without FM		
Gestational age (weeks)	Maternal low zinc n (%)#	N	Maternal low zinc n (%)#	N	p-value
37	15 (75.0)	20	25 (45.5)	55	0.011
38	12 (57.1)	21	32 (39.0)	82	0.475
39	13 (86.7)	15	30 (54.5)	55	0.049
40	9 (52.9)	17	24 (41.4)	58	0.398
41	9 (52.9)	17	16 (45.7)	35	0.625
42	0 (0.00)	0	2 (18.2)	11	-
Total	58 (64.4)	90	129 (43.6)	296	0.001

#Figures in parenthesis are percentages of the total in each row

Table 4: Comparison of mean maternal serum zinc concentration using different indices of nutritional status

Variable	Category	Mean (SD) maternal serum zinc concentration µmol/L	t	P
CANS score	FM	7.4 (5.3)	2.95	0.003*
	No FM	9.5 (6.2)		
MAC/HC	FM	8.1 (7.2)	0.78	0.817
	No FM	9.1 (6.0)		
PI	FM	8.4 (4.7)	0.55	0.036*
	No FM	9.1 (6.2)		

FM: Fetal malnutrition, CANS: score, Clinical Assessment of Nutritional Status score, MAC/HC: Mid-arm circumference, HC: Head circumference, PI: Ponderal index, SD: Standard deviation

Table 5: Comparison between the mean anthropometric data of babies of mothers with normal serum zinc levels and those with hypozincemia

	Mean (SD) of variables of the babies			
Variables in babies	Mothers with low Zn	Mothers with normal Zn	t	p-value
Weight (kg)	2.97 (0.63)	3.01 (0.64)	0.5	0.5
Length (cm)	48.3 (3.3)	47.9 (4.1)	1.2	0.2
HC (cm)	34.1 (1.7)	34.0 (2.2)	0.6	0.5
MAC (cm)	10.3 (1.3)	10.5 (1.3)	1.5	0.1
MAC/HC	0.30(0.03)	0.31 (0.03)	2.4	0.017
PI (g/cm^3)	2.63 (0.41)	2.80 (0.59)	2.4	0.001
CANS score	26 (4)	27(3)	2.8	0.006

CANS: Score, Clinical Assessment of Nutritional Status score, HC: Head circumference, MAC: Mid-arm circumference, PI: Ponderal index, SD: Standard deviation

serum zinc concentration (7.6 µmol/L). The mean maternal serum zinc concentration obtained herein is similar to that reported by a study conducted in Malawi, (Gibson and Huddle, 1998) wherein a high phytate intake along with frequent pregnancies and malarial infections were major factors in the etiology of suboptimal zinc levels of pregnant women in that region (Gibson and Huddle, 1998). These factors may be the same for many pregnant women living in Nigeria. Malaria, for instance, is associated with the prevalence of FM (Fall *et al*., 2003) and may be a risk factor for low serum zinc levels in pregnant Nigerian women.

The prevalence of FM in the present study was 23.3%, which was higher than that observed by Adebami *et al.* (2007) in Ilesa, Nigeria (18.8%) and (Deodhar and Jarad, 1999) India (19.6%), about a decade ago. A 23.3% prevalence of FM in the present study was also much higher than that observed by Metcoff (1994) among the American newborns (10.9%). Notably, a significantly higher proportion of mothers of babies with

FM had hypozincemia versus those without. In other words, higher proportions of babies with FM were delivered by mothers with low serum zinc levels. The mean serum concentration of zinc of mothers who had babies with FM was 7.4 µmol/L, which is lower than both the overall mean obtained herein and recommended lower cutoff. The mean maternal zinc level of babies with FM was also significantly lower than that for mothers of babies without FM.

To our knowledge, the present study is the first to examine the relationship between maternal serum zinc deficiency and prevalence of FM in Nigeria. However, researchers in Ugwuja *et al.* (2011) had found that delivery of LBW babies was more common in women with low plasma levels of zinc than those with normal serum levels. Similarly, Neggers *et al.* (1990) also reported that maternal serum zinc levels were a strong predictor of birth weight, with a threshold for maternal serum zinc concentration below which the prevalence of LBW increases significantly. Neggers *et al.* (1990) also showed that the positive association between maternal serum zinc concentration and birth weight was independent of other known risk factors of LBW. Furthermore, their study showed that low maternal serum zinc was a more significant predictor of LBW compared to other known risk factors (Neggers *et al.*, 1990).

However, the current results showed that not all mothers with low levels of zinc had babies with FM and some mothers with normal levels of zinc had babies with FM. Regarding the former situation, it is well known that the fetus behaves like a "parasite" (Naismith, 1969). Therefore, it takes fairly extreme forms of maternal malnutrition to significantly affect the quantity and quality of nutrients transferred to the fetus; this idea was also supported by Caulfield *et al.* (1998) and Neggers *et al.* (1990). The fact that FM was found among babies of mothers without hypozincemia also attests to its multifactorial etiology, which may include factors such as pregnancy-induced hypertension, antepartum hemorrhage and some maternal infections previously implicated in FM etiology (Adebami *et al.*, 2007). Furthermore, though the present results showed a lower maternal serum zinc concentration for those with FM babies versus those who had babies without FM, it is important to note that no singular factor is the absolute cause of FM, rather it is a culmination of several factors which determine the incidence of FM.

Nonetheless, present results may help shape current perspectives about maternal hypozincemia and fetal growth. According to a study done in Ile-Ife (Okonofua *et al.*, 1989) about two decades ago, maternal hypozincemia may not be responsible for fetal growth in Nigerian women simply because the birth weight of neonates in that study population was normal. The apparent conflicts when relating maternal zinc level to LBW rate on one hand and to FM on the other may just reflect the fact that the exact mechanism of maternal zinc deficiency in FM etiology has not been fully elucidated. It is also possible that the effect of zinc on fetal growth goes beyond reduction of anthropometric parameters that have previously been used to access fetal growth. Low maternal serum zinc levels may affect fetal body tissue composition negatively resulting in features of FM. Findings of Padmavathi *et al.* (2009) in rats showed that body mass composition of offspring were negatively affected by maternal zinc restriction. Their study Padmavathi *et al.* (2009) showed a reduction in the percentage of body fat, lean body mass and fat-free mass in the offspring. These results are similar to those assessed using the CANS score, which assessed the amount of fat and/or muscle mass present in the newborns.

Previous studies have also demonstrated that zinc deficiency during the early weeks of pregnancy can produce abortions and congenital malformations (Dey *et al.*, 2010; Mukherjee *et al.*, 1984). If this deficiency persists during pregnancy without adequate homeostatic adjustments, the growth of the fetus may be compromised. The present study showed that compromised fetal growth affects not only proportionality indices, like PI, but may actually affect body composition in terms of fat and muscle as assessed by CANS score.

Conclusion: In conclusion, the results of the present study showed that a high prevalence of low maternal serum zinc, as well as FM, exists in southwestern Nigeria. A causal relationship may exist between low maternal serum zinc levels and FM as evidenced by differences in the mean maternal serum zinc concentration of babies with FM assessed by CANS score and other indices of nutritional status. Along with other known causes of FM, maternal zinc deficiency likely contributes to the high prevalence of FM in Nigeria. A reduction in the prevalence of FM will reduce the associated complications neonates, thereby reducing neonatal morbidity and mortality and one obvious way to circumvent these issues may be by maternal zinc supplementation during pregnancy.

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