Impacts of Elevated Prenatal Blood Lead on Trace Element Status and Pregnancy Outcomes in Occupationally Non-exposed Women

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Abstract

Background: Lead toxicity has been reported to affect hematopoietic, nervous, reproductive, cardiovascular and urinary tract systems. Many investigators have so far studied the effects of high blood lead levels on pregnancy outcomes.

Objective: To investigate the effects of elevated maternal blood lead during pregnancy on some trace elements and pregnancy outcomes.

Methods: Blood lead and plasma copper, iron and zinc were measured in 349 pregnant women with a mean±SD age of 27.0±4.8 years, and gestational age of 21.8±3.1 weeks, at recruitment using atomic absorption spectrophotometer. Maternal and fetal outcomes were recorded during follow-up and at delivery, respectively. A blood lead level of ≥10 µg/dL was considered high.

Results: Women with elevated blood lead had significantly higher plasma copper and iron and lower plasma zinc than women with low blood lead level (<10 µg/dL). Blood lead level correlated with maternal hemoglobin concentration (r=-0.1054, p=0.051) and total white blood cell count (r=0.1045, p=0.053). Hypertension, malaria and low birth weight were significantly higher (p<0.05) in women with elevated blood lead than in those with low blood lead level.

Conclusion: Complications of pregnancy may be induced by a high blood lead level possibly through the alterations in trace element metabolism.

Keywords: Lead poisoning; Nutritional status; Fetus; Pre-eclampsia; Diabetes, gestational; Stillbirth.

Introduction

Environmental lead exposure and toxicity remain important global health problems, especially in developing countries where the environment is polluted due to the use of leaded gasoline and where there are widespread micronutrient deficiencies due to consumption of monotonous diet with low contents of minerals and vitamins. The adverse effects of lead on the body systems at both high and low doses are well documented. For instance, lead toxicity has been reported to affect hematopoietic, nervous, reproductive, cardiovascular and urinary tract systems.

National Research Council of the US reported that pregnant women and children are the population most sensitive to lead exposure. The increased nutritional demand during pregnancy and the
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- Environmental lead exposure and toxicity remain important global health problems, especially in developing countries where the environment is polluted due to the use of leaded gasoline.
- There are widespread micronutrient deficiencies in developing countries due to consumption of monotonous diet with low contents of minerals and vitamins.
- The wide nutritional disparities among pregnant women may be due to contamination of water sources or differential bioavailability of trace elements due to nutrient-nutrient interactions.
- Elevated blood lead levels during pregnancy is associated with increased plasma copper and iron but a decreased plasma zinc.
- Extra-lead is released from maternal skeleton during pregnancy due to mobilization of bone lead stores, particularly in women who smoke or women whose calcium intake is low.
- While maternal blood lead level is negatively correlated with hemoglobin concentration, it is positively correlated with total white blood cell count.
- Complications of pregnancy, such as gestational hypertension, malaria and low birth weight deliveries may be induced by elevated prenatal blood lead by altering trace element metabolism.

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homeorhesis associated with pregnancy have been suggested to cause an increase in maternal blood lead (PbB) levels. Again, the absorption and retention of lead in the body have been found to depend on nutritional status of individuals, among other factors. Studies in monkeys and humans have demonstrated that extra-lead is released from maternal skeleton during pregnancy due to mobilization of bone lead stores, particularly in women who smoke or women whose calcium intake is low.

Many investigators have so far studied the effects of high PbB levels on pregnancy outcomes. While some studies reported that maternal PbB levels ≥10 µg/dL may cause complications during pregnancy including increased risk of gestational hypertension, reduced length of gestation, miscarriage, spontaneous abortion and preterm delivery, others reported lack of association between maternal PbB level and some adverse pregnancy outcomes such as spontaneous abortion, low birth weight, intrauterine growth retardation, premature rupture of membranes and congenital anomalies. Although adverse pregnancy outcomes have been reported even at a mean PbB level of <5 µg/dL, the effect of lower PbB levels on birth weight and prematurity has been inconsistent.

Since pregnancy impacts on nutrition, especially the trace elements which in turn affects absorption and retention of lead, we hypothesize that pregnant women with elevated PbB levels will have lower trace element levels and poorer pregnancy outcomes. This study was therefore undertaken to investigate the impact of elevated maternal PbB levels during pregnancy on some trace elements and pregnancy outcomes.

Materials and Methods

Study site

The study was carried out in Abakaliki, the capital of Ebonyi State in the South Eastern Nigeria at the Department of Ob-
stetrics and Gynecology of the Federal Medical Center Abakaliki. Federal Medical Center Abakaliki is one of the tertiary health facilities in the region. Ebonyi State was created out of old Enugu and Abia States in 1996, and it is the youngest state in the South Eastern Nigeria.

Ebonyi State is located on longitude 8° E and latitude 6° N with moderate relief of between 125 and 245 m above sea level. The vegetation characteristic is that of the tropical rain forest with an average annual rainfall of about 1600 mm and average atmospheric temperature of about 30 °C. Two main seasons dominate the climate of the state. These are the rainy season, which usually begins in late April, and ends in early October; and the dry season, which lasts from late November to early April. It has a population of approximately 2.2 million, primarily populated by the Igbo on a land mass of 5935 km², giving a population density of 371 persons/km². The population is concentrated in Abakaliki, the capital city posing pressure on scarce infrastructures.

Ebonyi State is blessed with enormous mineral resources such as salt lakes at Uburu, Okposi and Osiri, zinc and lead deposits at Enyigba, as well as kaolin and limestone at Ishiagu, Afikpo and Nkalagu. Abakaliki and the environs are inhabited mainly by subsistence-level population. Their main occupation is subsistence-level farming—mainly rice, yam and cassava—with some animal husbandry. Other professions including civil service, trading, and artisanry are also practiced. The transmission of malaria is intense and occurs throughout the year (perennial).

Methods

This prospective study was conducted between July 2007 and October 2008 among pregnant women (n=351) who were receiving antenatal care at the Department of Obstetrics and Gynecology of the hospital. The procedures for the study were explained to participants after which oral informed consents were obtained. The Ethics and Research Committee of the Federal Medical Center Abakaliki and Ebonyi State University Teaching Hospital Abakaliki approved the protocol for the study. The women whose age ranged from 15–40 years were recruited at gestational age of ≤25 weeks. Women with chronic disease, women who smoke and those that were occupationally exposed to lead, for example, petroleum attendants, and those involved in active mining/quarrying activities were excluded from the study. Women who were on calcium supplementation were also excluded. Maternal sociodemographic and obstetrics data, such as maternal age, educational level, occupation, living accommodation, gestational age, parity were collected by structured questionnaires while maternal anthropometrics (weight and height) were determined and their body mass index (BMI) was calculated. Maternal gestational age was based on self-reported date of the last menstrual period (LMP) and ultrasonography. Where there was disagreement between the two methods, that of the ultrasound was used. Five millilitres of venous blood was collected once at recruitment during 8:00–10:00 hours into lead-free navy-blue top vacutainer tubes (Becton-Dickinson, Rutherford, NJ, USA) containing sodium heparin (2.0 mL) for lead and hematological analysis and heparinized bottles (3.0 mL) for trace elements and albumin determination. Maternal hemoglobin concentrations and total white blood cell (WBC) counts were determined immediately as previously described while blood for PbB analysis was stored at 4 °C prior to analysis. The blood in the heparinized bottles was centrifuged at 2000 g for five minutes after which plasma was separated and stored frozen till analyses. Atomic absorption
spectrophotometer (AAS) with a detection limit of 1.0 µg/dL was used to analyze PbB and plasma trace elements (copper, iron, and zinc). Each sample analysis was performed in duplicate, and the mean of both measurements was used as the final value. As part of contamination control, all glassware were routinely washed and soaked in two successive dilute nitric acid baths (0.8 mg/L) then thoroughly rinsed in ultra-pure double-distilled deionized water. Additionally all reagent, glassware and sample collection devices were checked for contamination with lead. No contamination was found when randomly selected sample of tubes used to collect and store blood for lead assay were tested for lead. The tubes were washed with 10% nitric acid and the effluent measured by AAS as described by Jacobson, et al., for low lead concentration. Women were grouped into five categories on the basis of their PbB as follows: PbB <10 µg/dL, 10–19.9, 20–29.9, 30–39.9 and ≥40 µg/dL. These categorization was based on varying levels of blood lead reported in the general Nigerian population. Women with PbB <10 µg/dL acted as the control group; the other four groups were termed “lead groups.” Plasma albumin was measured using Randox commercial kits (Randox Laboratories Ltd, UK) and in accordance with manufacturer’s instructions. To control for intra-individual variability, the same laboratory scientist ran the whole samples with quality control sera supplied by manufacturer of the kits (Randox Laboratories Ltd, UK).

Participants were followed-up till delivery. At every follow-up, participants were evaluated by the attending obstetricians for anemia (hemoglobin <11.0 g/dL), hypertension (blood pressure >140/90 mm Hg), diabetes (fasting plasma glucose >140 mg/dL), malaria (positive thin or thick film), and other concomitant illness such as dyspepsia, upper respiratory tract infection (cough and catarrh) and urinary tract infection (positive urine protein, nitrite and leucocytes). At delivery, baby’s birth outcomes such as weight, length, head circumference as well as stillbirth, mode of delivery, and gestational age at delivery were 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 were determined by a measuring tape to the nearest 0.1 cm. Baby was considered underweight if the birth weight was <2.5 kg, and preterm if delivered at <37 weeks gestational age.

**Data analysis**

The data collected were analyzed by SPSS® for Windows® ver 16.0 (SPSS Inc., Chicago, IL, USA). Differences in means between the groups were compared using one-way ANOVA. A p<0.05 was considered statistically significant.
Results

Table 1 shows the general characteristics of pregnant women recruited at gestational age of ≤25 weeks. Although 351 pregnant women were recruited, one (0.3%) died early into the study remaining 350 (99.7%) of which data were available but samples were obtained from 349 participants as one participant declined participation. At delivery, data were available for 319 (91.4%) women and their neonates. Data were incomplete or not available for the remaining 30 (8.6%).

Although, in general, the women were deficient in none of the three trace ele-
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Elevated prenatal blood lead (PbB) levels are associated with adverse pregnancy outcomes, including low birth weight and preterm delivery. The present study aimed to investigate the effects of PbB on maternal hematological and biochemical parameters in a cohort of pregnant women.

Methods:

A total of 100 pregnant women were included in the study. PbB levels were measured using atomic absorption spectrometry. Maternal hematological and biochemical parameters were assessed using standard laboratory techniques.

Results:

The study found that maternal PbB levels were significantly associated with lower hematocrit and hemoglobin concentrations, as well as with higher plasma copper and iron concentrations. However, no significant differences were observed in plasma zinc levels.

Partial correlation analysis, after controlling for age, gestational age, BMI and parity, showed that maternal PbB was inversely correlated with maternal hemoglobin concentration (r=-0.1054, p=0.051), but was positively correlated with total WBC count (r=0.1045, p=0.053).

Conclusion:

Elevated PbB levels during pregnancy are associated with adverse maternal hematological and biochemical outcomes. Further studies are needed to investigate the mechanisms underlying these associations and to develop strategies to mitigate the effects of prenatal lead exposure on maternal health.

Figure 1: Scatter plot of plasma copper concentration against blood lead level.

Figure 2: Scatter plot of plasma iron concentration against blood lead level.

Figure 3: Scatter plot of plasma zinc concentration against blood lead level.
prevalent at higher maternal PbB, a significantly (p<0.05) lower rate of maternal gestational diabetes was observed in women with higher PbB in comparison to those with PbB <10 µg/dL (Table 4). However, other concomitant illnesses were comparable (p>0.05) among the studied groups.

For fetal outcomes (Table 4), delivery of babies with low birth weight (LBW) increases with increasing maternal PbB, with mothers in PbB groups 20.0–29.9, 30.0–39.9, and >40 µg/dL having significantly higher LBW newborns (13.9%, 17.6% and 22.7%, respectively) when compared with women in the PbB <10 µg/dL (7.6%) and 10–19.9 µg/dL (11.1%) groups. However, the frequencies of assisted, surgical and preterm deliveries were not affected by maternal PbB, as comparable values were observed among all the groups (Table 4). Again, while stillbirth was lower among women in the higher PbB groups in comparison to women with PbB <10 µg/dL, the least (1.6%) was observed in women with PbB in the 10.0–19.9 µg/dL category. Fetal anthropometrics shows that birth weight of newborns from women in the PbB group 20.0–29.9 µg/dL was significantly lower in comparison to newborns from PbB group <10 µg/dL or other PbB groups (Table 4). Also birth length of newborns born to women in the PbB group 10–19.9 µg/dL was significantly higher when compared with birth length of neonates from women in other PbB groups (Table 4). However, the head circumference of the newborns was not affected by maternal PbB as comparable values were observed in all the groups.

Discussion

The wide variations in the levels of trace elements recorded in this study suggest a wide nutritional disparity among the women. Although the reason for this nutritional disparity remains obscure, contamination of water source may be a probability. Contamination of water supply has been found to be one of the important causes of acute toxicity of trace elements in the general population. Another plausible contributory factor to the wide differences in concentrations of trace elements among these women is differential bioavailability of trace elements due to nutrient-nutrient interactions. This has important public health implications not only for the mothers but also for their newborns as this may reflect in differential concentrations of these elements which may affect maternal and fetal health.

This study has documented that elevated maternal PbB during pregnancy was associated with elevation of plasma copper and iron and a decrease in plasma zinc level. We also found that higher incidence of maternal hypertension, malaria, LBW and lower incidence of gestational diabetes and stillbirths were associated with increased maternal PbB during pregnancy. The significantly higher levels of plasma copper and iron in pregnant women with elevated PbB observed in the present study
is in contrast with decreased iron reported by Jin, et al.,\textsuperscript{29} and an inverse association between PbB and serum iron reported elsewhere.\textsuperscript{30,31} It also contrasted equivocal relationship between PbB and iron status reported by Barany, et al.\textsuperscript{32} However, the lower level of plasma zinc among women with elevated PbB in the present study is in contrast with the findings of Truckenbrodt, et al.\textsuperscript{33} in lead-exposed population where increasing lead concentrations in blood, correlated with elevated levels of zinc in whole blood, erythrocytes and plasma. It is also in disagreement with the finding of Choi and Kim\textsuperscript{30} where serum zinc was found to be significantly higher in individuals with elevated PbB. Although the reason for the increases in plasma copper and iron and a decrease in plasma zinc in pregnant women with elevated PbB is obscure, it may be related to the enhancement of lead absorption associated with trace element deficiencies.\textsuperscript{34} Previously, widespread deficiencies of copper, iron and zinc have been reported in this population.\textsuperscript{2} Even though iron\textsuperscript{35} and zinc\textsuperscript{36} deficiencies have been reported to increase lead absorption in normal individuals, it is not known whether this is applicable in pregnancy. Nonetheless, it has been found

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<tr>
<td>Maternal outcomes</td>
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<td>6/69 (8.7)\textsuperscript{a}</td>
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<td>Fetal outcomes (n=319)</td>
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<tr>
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<td>Head circumference (cm)</td>
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*Values are expressed as proportions with percentages in parenthesis. \textsuperscript{†}LBW: Low birth weight.
In each row, values carrying different superscripts are statistically (p<0.05) different.
that the relationships among the trace metals may be influenced by the characteristics of subjects, including age, gender, nutritional status, degree of exposure to environmental contamination, or behavioral habits.\textsuperscript{37}

The negative correlation between maternal PbB and hemoglobin concentration observed in the present study is in corroboration with the findings of Fahamida, \textit{et al.}\textsuperscript{38} where PbB levels in the range 10–40 μg/dL had inverse correlation with hematocrit, erythrocyte count and hemoglobin level. It is also in keeping with significant associations between maternal PbB and hematocrit reported by Schell, \textit{et al.}\textsuperscript{39} This has been partly attributed to inhibition of hemoglobin synthesis and shortened red cell survival.\textsuperscript{40,41} On the other hand, it is also possible that iron deficiency, which is one of the common causes of anemia, must have caused increased lead absorption, resulting in high PbB.\textsuperscript{42} Probably, the anemia reported among pregnant women exposed to air pollution by Stankovic, \textit{et al.}\textsuperscript{43} may have been related to augmented lead absorption due to iron deficiency. However, this association has been refuted in some studies.\textsuperscript{41,42}

Furthermore, the positive correlation between PbB and maternal mean total WBC count observed in the present study is in tandem with the finding of Choi and Kim\textsuperscript{30} among healthy adolescents, thus showing the important relationship between PbB and leukocytes counts and iron parameters.\textsuperscript{30} Previously, it has been reported that WBC counts were significantly high in dogs and cats with increased PbB levels.\textsuperscript{44}

Higher incidence of hypertension in women with elevated PbB recorded in the present study is in agreement with previous findings.\textsuperscript{15,16,17,45} However, it contrasted the earlier findings of Angell and Lavery\textsuperscript{46} who recorded no relationship between concentrations of lead in cord blood and the incidence of pre-eclampsia among other obstetrics outcomes. The disparity in this finding and theirs could be attributed to differences in sample used (maternal blood \textit{vs.} cord blood). Studies have shown that placenta provides no barrier for maternal lead transfer to the fetus, and that maternal lead, cadmium, selenium and copper levels have been found to be significantly higher than those in the cord blood.\textsuperscript{47} However, levels of metals in umbilical cord blood have been reported to be sensitive indicators of female reproductive toxicity as compared with those in mother whole blood.\textsuperscript{15}

Although the exact mechanism by which lead induced hypertension is unknown, studies suggest multifactorial approach. These include functional deficiency in nitric oxide due to inactivation of endogenous nitric oxide and down-regulation of soluble guanylate cyclase by reactive oxygen species, heightened sympathetic activity and plasma norepinephrine together with depressed vascular and elevated renal β-adrenergic receptor density, elevated plasma angiotensin-converting enzyme (ACE) activity, plasma rennin activity, angiotensin II and aldosterone levels, increased kininase I and II activities, a rise in cellular Na\textsuperscript{+} and hence Ca\textsuperscript{2+} due to lead-induced inhibition of vascular muscle Na\textsuperscript{+}-K\textsuperscript{+} ATPase, and a possible rise in endothelin and thromboxane generation.\textsuperscript{48} Also studies suggest that lead affects blood pressure by altering kidney functions.\textsuperscript{49} For instance, in human lead nephrotoxicity, alterations of kidney functions have been found to precede the development of hypertension.\textsuperscript{50,51} However, studies in animals and humans\textsuperscript{50,52,53} have not been able to provide conclusive evidence of how the kidney mediates association between PbB and blood pressure.

The higher incidence of malaria observed in women with elevated PbB in comparison to women with PbB <10 μg/
dL in this study needs further evaluation. Although we did not come across any study on the association between PbB and malaria infection in pregnant women, one study in pediatric population reported a significant negative association between PbB and malaria.\textsuperscript{54} We speculate that the higher susceptibility of pregnant women with elevated PbB to malaria infection may be mediated through hemoglobin concentration. Lead is known to inhibit hemoglobin synthesis by preventing the incorporation of iron into the protoporphyrin nucleus leading to the development of anemia. One study has shown that anemic women were more likely to have malaria infection than non-anemic women.\textsuperscript{55} It is quite intriguing to find significantly lower incidence of gestational diabetes and stillbirth among women in the higher PbB groups in comparison to women that have PbB <10 µg/dL in the present study. This is because significantly higher prevalence of diabetes mellitus had previously been reported in zinc-deficient than in zinc-adequate pregnant women. If this is true, higher incidence of diabetes ought to be found in women with elevated PbB as lower plasma zinc was found in these groups. Although results from micronutrients (including vitamins and minerals) studies in diabetes mellitus have been conflicting\textsuperscript{56-58} and data is scarce on the study of lead metabolism during pregnancy complicated with diabetes mellitus, the present findings require further investigations. However, the longitudinal decline of renal function among middle-aged and elderly individuals reported by Tsaih, \textit{et al},\textsuperscript{59} which appeared to depend on both long-term lead stores and circulating lead, was found to be most pronounced among diabetics and hypertensives. Again, although it is not possible to say whether elevated PbB is the cause of stillbirths in the present study or that high PbB was consequential to the fetal death, higher lead concentrations had been found in stillbirth tissues.\textsuperscript{60}

The present study also recorded lack of significant effect of maternal PbB on preterm delivery which is in contrast with the report of Jellife-Pawlowski, \textit{et al},\textsuperscript{61} where prenatal PbB >10 µg/dL was associated with significant decreases in total days of gestation and increased risk of preterm and small-for-gestational age (SGA) birth. It is also in conflict with the recent findings by Vigeh and colleagues in Iranian women,\textsuperscript{62} where PbB levels were found to be significantly higher in mothers who delivered preterm babies than in women who delivered full-term babies. In that study, a unit increase in PbB levels was associated with increased risk of preterm birth. The discrepancies in the two findings may be partly attributed to the study populations. In Iran, the maternal mean PbB was 3.8 µg/dL—almost one-tenth the value 36.4±18.5 µg/dL recorded in the present study. Suggested pathways through which lead induces preterm delivery include lead-induced reproductive hormone disruption\textsuperscript{63-65} and induction of uterine contractibility,\textsuperscript{66} by inducing the production of reactive oxygen species or effecting nervous system inhibition/stimulation. Many experimental studies support effects of lead on female reproductive functions, both at high and moderate levels; it has been found that in pregnant rodents which were given lead at moderate doses (PbB: 10–40 µg/dL), serum progesterone levels were decreased in dams and hypothalamic levels of gonadotrophin releasing hormone (GnRH) and somatostatin were suppressed in both dams and fetuses.\textsuperscript{67} Also studies in monkeys, rats and humans have shown the adverse effects of lead exposure during pregnancy.\textsuperscript{68-72}

Significant relationship has been reported between fetal anthropometrics (birth weight and length) and maternal blood lead. While Kaul, \textit{et al}, reported that increments in maternal PbB were ac-
accompanied by significant decrements in neonate birth weight; maternal PbB has been recognized as a negative explanatory variable for birth weight and child BMI. These findings are consistent with the current finding of significantly higher incidence of LBW deliveries and lower mean birth weight among women with elevated PbB. Most recently, low-level PbB has been associated with small risk of decreased birth weight with supra-linear dose-response relationship, but was not related to preterm birth or SGA. This observations were in conflict with our findings of higher birth length among newborns of women with PbB >10 µg/dL, but it however corroborated the lack of effect of PbB on fetal head circumference.

We conclude that complications of pregnancy such as hypertension, malaria and LBW may be induced by higher prenatal PbB possibly by altering trace element metabolism. While clinical implications of reduced incidence of gestational diabetes and stillbirths in pregnant women with elevated PbB is unclear, further studies are desired to confirm these findings.

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Conflicts of Interest: None declared.

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Built by Shah Jahan in memory of his wife, Montaz Mahal, on the shore of the river Yamuna, Agra, India, Taj Mahal is among the most recognizable and most beautiful world structures. The construction was mainly built by Iranian architects. (Photo courtesy of Marjan Bayat Maku, Shiraz)