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Serum trace metals in Pre-Eclamptic Nigerians

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ABSTRACT

Background: The role of trace metals in Pathogenesis of Pre-eclampsia has received insufficient attention in Nigeria. **Materials and Methods:** We examined the effect of serum levels of some trace metals; selenium (Se), zinc (Zn), copper (Cu), cobalt (Co), and manganese (Mn) in the development of pre-eclampsia in Nigeria. Blood samples were collected from 59 pre-eclamptic, 150 normal pregnant and 122 non pregnant women. Serum Se, Zn, Cu, Co and Mn were determined by AAS. **Results:** Result shows significantly lower serum Se, Zn, Cu, Cu: Zn ratio, Co and Mn in pre-eclamptics compared to normal pregnant women ($p<0.001$). Comparison of normal pregnant women and controls showed non significant difference in the zinc level ($P>0.05$), significantly lower levels of serum Se, Co, Mn ($p<0.00011$, $p=0.0022$, $p<0.0001$ respectively) and significantly raised copper level ($p<0.001$) in normal pregnant women compared to controls. Serum Se, Zn, Co and Mn were significantly lower ($p<0.001$) while serum copper and Cu: Zn ratio were significantly higher ($p<0.001$) in pregnant women compared to controls. ANOVA shows significant progressive decreases in serum Se, Zn Co and Mn, from controls to normal pregnant women and pre-eclamptics ($p<0.0001$). **Conclusion:** Decreases in serum level of Se, Zn, Cu, Cu: Zn ratio, Co and Mn may play important role in the pathogenesis of pre-eclampsia.

Key words: Trace metals, pregnant women, Pre-eclampsia, Nigeria

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INTRODUCTION

Trace metals are a group of inorganic elements which are present in the body in minute quantities usually in micrograms or picograms per gram wet tissue and whose recommended daily allowance (RDA) is less than 5mg.[1] Trace metals have important influence on the health of man and of pregnant women and the growing fetus in particular.[2] However their possible antioxidant function and contribution in determination of pregnancy disorders where oxidative stress is high has received insufficient attention particular in this environment Nigeria. Pre-eclampsia is one of such pregnancy disorders, and is a major cause of maternal and perinatal mortality and morbidity.[3] Pre-eclampsia is associated with high incidence of obstetrics interventions such as induction of labour, or caesarean section either in fetal or maternal interest or both.[4] Despite several studies on pre-eclampsia, its etiology has not yet been fully elucidated. A previous report shows that approximately 37,000 Nigerian women die annually because of pre-eclampsia related complication,[5] yet the exact mechanism of development of the disorder is not known. Several reports show that various elements might play an important role in etiology of pre-eclampsia,[6,7] but it has not been adequately examined in this environment. There are still many gaps that have not been elucidated, owing to the variations of pregnancy influencing factors which may vary from environment to environment. This study has the potential of contributing to the knowledge of the role of trace metals in pre-eclampsia in this part of the world. It therefore appears instructive to properly investigate the possible relationship of some trace metals level in maternal blood, and to correlate the findings with development of pre-eclampsia in Southern-Nigerian women. This study was conducted to examine this possibility and implications for maternal and fetal health.

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MATERIALS AND METHODS

Study population

A cohort of pregnant women who commenced prenatal care at the antenatal clinic and wards of the Irrua Specialist Teaching Hospital Irrua, Edo state, southern part of Nigeria were enrolled. Each participant signed an Informed Consent Form after the procedure and implications were explained to the subject in English Language or Special English. Research and Ethics Committee of Irrua Specialist Teaching Hospital Irrua, Edo state, granted ethical approval for this study.

We examined 209 pregnant women within the age range of 20 to 35 years. The pregnant women consist of 150 normal pregnant women and 59 women with pre-eclampsia. They were age matched with 122 non pregnant women (the control group), who were selected after screening for pregnancy. The exclusion criterion was history of any chronic disease.

Using a standard mercury sphygmomanometer (Accoson, Essex, UK), maternal blood pressure during earlier routine monthly visits and later weekly visits as the pregnancy advances was measured with the subject in supine position. The subjects were also tested for presence of protein and glucose in their urine on each monthly visit, using Medi-Test Combi 2 strips (Macherey-Nagel, Duren, Germany.). Those with glycosuria were excluded from the study. Women were classified as having Pre-eclampsia based on Systolic Blood Pressure (SBP) $\geq$ 140 mmHg and/or Diastolic Blood Pressure (DBP) $\geq$ 90 mmHg and proteinuria,[8] measured during at least two visits after the 22nd week of gestation, such that the elevated measures could have occurred at any point after 22 weeks, hence no diagnosis of Pre-eclampsia was made before 24 weeks. An average of the last two measurements at different periods before delivery was used to determine the values for Systolic Blood pressure (SBP) and Diastolic Blood Pressure (DBP) assigned to each subject. The gestational age was determined from the last menstrual period (LMP). But if pre-eclampsia develops, they were retained in the clinic for closer monitoring and their B.P measured at least twice daily. Medication (diaxapam 10mg intravenous injection) was given and if there was no improvement within 48 hours, the patient is booked for assisted delivery.

Blood sample collection

Seven milliliters of venous blood collected from each participant at delivery was dispensed into plain container, allowed to clot and the serum was separated, stored frozen and analyzed within 4 days for trace metals; Se, Zn, Cu, Co and Mn.

Methods

Atomic absorption spectrophotometer model-200A, (Buck Scientific, East Norwalk, UK.) was employed for serum Se, Zn, Cu, Co, and Mn determination as previously described by Welz.[9] The principle is based on the dissociation of the element (from the flame) from its chemical bonds. This is then placed in unexcited or ground state (neutral atom). Thus, the neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum. The amount of radiant energy absorbed is proportional to the concentration of trace elements present in the serum. The digested samples were analyzed in duplicates by the instrument, and the average reading taken. Repeated analyses of standard solutions confirmed the method's precision. The Cu: Zn ratio was calculated by dividing value of the concentration of Cu by that of Zn.

Statistical analysis

All data generated from this study were subjected to statistical analysis, using SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA). The mean, standard deviations, student's t- test and Pearson Correlation were computed. Results were expressed as Mean $\pm$ SD. The 5% ($P<0.05$) level of significance was adopted to infer significant change.

RESULTS

Table 1; There was significant lower serum Se, Zn, Co and Mn levels in Pregnant women compared to non-pregnant women (controls) ($p<0.001$ in each case), while serum Cu level and Cu: Zn ratio were significantly higher in pregnant women than in non- pregnant women ($p<0.001$). SBP and DBP were significantly increased in pregnant women compared to the non pregnant women (controls) ($p<0.001$ and $p<0.05$ respectively).

Table 2 shows significantly lower serum Se, Zn, Cu, Cu: Zn ratio, Co, and Mn levels in women with pre-eclampsia than in normal pregnant women ($p<0.001$ in each case).

Table 3; Serum Se, Co and Mn were statistically lower in normal pregnant women than in non-pregnant women (controls) ($p<0.0001$, $p=0.0022$, and $p<0.0001$ respectively). While serum Cu, Cu: Zn ratio, and SBP were significantly higher in normal pregnant women than in non-pregnant women ($p<0.001$,$p<0.001$, and $p<0.01$ respectively). SBP in normal pregnant women compared to the non pregnant women ($p>0.05$),

Table 4; ANOVA shows significant progressive decreases in serum Se, Zn, Co and Mn levels ($p<0.001$ in each case) and significant progressive increases in SBP and DBP

Table 1: Serum Se, Zn, Cu, Cu:Zn ratio, Co, Mn, SBP and DBP in pregnant women and non pregnant women (controls)

Variables (mean±SD)	Pregnant women (n=209)	Non-pregnant women (controls) (n=122)	t-value	p-value
Se (µg/dl)	18.3±8.4	27.7±7.2	10.243	0.0001
Lower 95% CI	17.1	26.4		
Upper 95% CI	19.4	29.0		
Zn(µg/dl)	62.0±14.1	95.8±17.3	9.6205	0.001
Lower 95% CI	60.1	92.7		
Upper 95% CI	63.9	98.9		
Cu (µg/dl)	141.0±42.2	106.7±16.5	5.3201	0.001
Lower 95% CI	135.3	103.8		
Upper 95% CI	146.8	109.7		
Cu:Zn ratio	2.26±0.43	1.13±0.18	8.7656	0.001
Lower 95% CI	2.20	1.09		
Upper 95% CI	2.32	1.16		
Co (µg/dl)	2.2±0.8	2.7±0.7	6.202	0.0001
Lower 95% CI	2.1	2.6		
Upper 95% CI	2.3	2.9		
Mn (µg/dl)	0.88±0.29	1.16±0.27	11.631	0.0001
Lower 95% CI	0.84	1.10		
Upper 95% CI	0.91	1.20		
SBP (mmHg)	131.8±31.5	111.7±9.9	6.820	0.0001
Lower 95% CI	127.5	110.4		
Upper 95% CI	136.1	114.4		
DBP (mmHg)	84.0±20.6	67.4±4.3	8.624	0.0001
Lower 95% CI	81.2	66.5		
Upper 95% CI	86.8	69.2		

Table 2: Serum Se, Zn, Cu, Cu:Zn ratio, Co, Mn, SBP and DBP in women with pre-eclampsia and normal pregnant women

Variables (mean±SD)	Women with pre-eclampsia (n=59)	Normal pregnant women (n=150)	t-value	p-value
Se (µg/dl)	8.8±3.0	22.0±7.0	13.881	0.0001
Lower 95% CI	8.0	20.8		
Upper 95% CI	9.6	23.1		
Zn(µg/dl)	45.8±9.7	68.2±10.1	8.3626	0.001
Lower 95% CI	42.9	66.8		
Upper 95% CI	47.7	70.0		
Cu ((µg/dl)	86.7±16.5	162.4±27.4	7.9386	0.001
Lower 95% CI	82.2	157.6		
Upper 95% CI	90.5	166.5		
Cu:Zn ratio	1.93±0.36	2.39±0.38	7.8564	0.001
Lower 95% CI	1.83	2.33		
Upper 95% CI	2.02	2.45		
Co (µg/dl)	1.5±0.6	2.5±0.7	9.295	0.0001
Lower 95% CI	1.3	2.3		
Upper 95% CI	1.6	2.6		
Mn (µg/dl)	0.58±0.22	0.99±0.23	11.808	0.0001
Lower 95% CI	0.51	0.95		
Upper 95% CI	0.63	1.03		
SBP (mmHg)	175.2±20.9	114.6±13.1	25.068	0.0001
Lower 95% CI	169.2	110.0		
Upper 95% CI	180.7	116.2		
DBP (mmHg)	110.6±15.8	73.3±9.8	20.564	0.0001
Lower 95% CI	105.8	69.9		
Upper 95% CI	114.4	74.7		

(p<0.001 respectively) from controls to normal pregnancy and to pre-eclampsia.

DISCUSSION

The search for understanding of pre-eclampsia and its prevention continues. The mechanism involved in the pathogenesis of the disorder remains uncertain. Selenium, zinc, copper, cobalt and manganese are essential trace metals, and are required for maintenance of normal health in human populations.[1,10] The observed decreases in serum, Se, Zn, Co, and Mn in pregnant women compared to controls may be as a result of increase in body requirement during pregnancy to meet the mother and baby's need. Also the significant lower serum Se, Co and Mn levels in normal pregnant women compared to controls in this study buttresses that there is primary decrease due to pregnancy. It has been previously reported that whole blood and plasma levels of selenium are lower in pregnant women when compared with non-pregnant women[11] and decrease as gestation proceeds.[12]

However it seems that these essential trace metals are further decreased in pre-eclampsia. This study shows significant lower serum Se, Zn, Cu, Co and Mn levels in women with pre-clampsia than in normal pregnant women. It is likely that the decreases in the antioxidant trace metals; selenium, zinc, copper, cobalt and manganese levels may have contributed to the development of pre-eclampsia in the population of study in this environment. This is buttressed by the observed significant progressive decreases in serum Se, Co and Mn levels from controls to normal pregnancy and to pre-eclampsia as shown by ANOVA in this study. This is consistent with the reports of other investigators from Turkey; a Caucasian population, that there was lower serum manganese[13] and selenium[14] levels in pre-eclampsia than in normal pregnancy and non pregnant controls. The findings from this present study from Southern Nigeria are also consistent with a previous report from Osogho in western part of Nigeria.[15] However there are no previous reports on cobalt in pre-eclampsia.

The mechanism of development of pre-eclampsia is likely to involve increase in oxidative stress. Oxidative stress is defined as an imbalance between the cellular generations of reactive oxygen species (ROS) and the capacity of anti-oxidants to prevent this phenomenon. High burden of reactive oxygen species (ROS), such as superoxide ($O2^-$) and hydrogen peroxide (H_2O_2) are normally produced in the course of oxygen metabolism and are safely disposed of by the antioxidant defense system. However, a variety of pathophysiological conditions result in heightened

production of ROS and/or impaired antioxidant capacity, which culminate in oxidative stress.[16,17]

In the presence of oxidative stress, uncontained ROS cause tissue damage and dysfunction by directly attacking and denaturing functional and structural molecules and by activating redox-sensitive transcription factors and signal transduction pathways, thus playing a critical part in the pathogenesis of many acute and chronic illnesses, including hypertension.[16, 18]

Table 3: Serum Se, Zn, Cu, Cu:Zn ratio, Co, Mn, SBP and DBP in normal pregnant women and non pregnant women (controls)

Variables (mean±SD)	Normal pregnant women (n=150)	Non-pregnant women (controls) (n=122)	t-value	p-value
Se (µg/dl)	22.0±7.0	27.7±7.2	6.554	0.0001
Lower 95% CI	20.8	26.4		
Upper 95% CI	23.1	29.0		
Zn(µg/dl)	68.2±10.1	95.8±17.3	1.2892	>0.05
Lower 95% CI	66.5	92.7		
Upper 95% CI	69.8	98.9		
Cu (µg/dl)	162.4±27.4	106.7±16.5	5.6721	0.001
Lower 95% CI	158.0	103.8		
Upper 95% CI	166.8	109.7		
Cu:Zn ratio	2.39±0.38	1.13±0.18	6.9983	0.001
Lower 95% CI	2.33	1.09		
Upper 95% CI	2.45	1.16		
Co (µg/dl)	2.5±0.7	2.7±0.7	3.096	0.0022
Lower 95% CI	2.3	2.6		
Upper 95% CI	2.6	2.9		
Mn (µg/dl)	0.99±0.23	1.16±0.27	8.870	0.0001
Lower 95% CI	0.95	1.10		
Upper 95% CI	1.03	1.20		
SBP (mmHg)	114.6±13.1	111.7±9.9	0.1109	>0.05
Lower 95% CI	112.4	109.8		
Upper 95% CI	116.7	113.3		
DBP (mmHg)	73.3±9.8	67.4±4.3	5.600	0.0001
Lower 95% CI	70.5	66.2		
Upper 95% CI	74.4	68.7		

Selenium; an essential trace metal (micronutrient) is an essential component of theantioxidant selenoproteins, including glutathione peroxidases. These remove the products of peroxidation by hydroperoxidesand oxidized lipoproteins[19] and so limit adverse effects of ROS onthe endothelium.[20] Furthermore, the expression and activity of important anti-oxidant proteins are decreased in placental tissues from pre-eclamptic mothers, resulting in an imbalance between pro-oxidants and anti-oxidants leading to oxidative stress. Two of these anti-oxidant proteins are glutathione peroxidase and thioredoxin reductase, enzymes that have selenocysteine within their active site and are selenium-dependent for activity.[21] Besides, Manganese is a component of the antioxidant enzyme; manganese superoxide dismutase (MnSOD), and is the principal antioxidant enzyme of the mitochondria. It has been reported that MnSOD deficiency enhanced 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative stress in mice.[13]

Therefore, it seems that as there is increase in oxidative stress, selenium and manganese get involved in the antioxidant mechanism and is reduced in the process, thus explaining the decrease in selenium and manganese levels in women with pre-eclampsia compared to the normal pregnant women as observed in the present study.

Table 4: Analysis of variance (ANOVA) of serum Se, Zn, Cu, Co, Mn, SBP and DBP in pre-eclampsia, normal pregnancy and controls

Variables (mean±SD)	Women with pre-eclampsia (n=59)	Normal pregnant women (n=150)	Non-pregnant women (controls) (n=122)	F-value	p-value
Se (µg/dl)	8.8±3.0	22.0±7.0	27.7±7.2	160.26	<0.0001
Lower 95% CI	8.0	20.8			
Upper 95% CI	9.6	23.1			
Zn(µg/dl)	45.8±9.7	68.2±10.1	95.8±17.3	313.04	<0.0001
Lower 95% CI	42.9	66.5			
Upper 95% CI	47.7	69.8			
Cu (µg/dl)	86.7±16.5	162.4±27.4	106.7±16.5	38.604	<0.0001
Lower 95% CI	82.2	158.0			
Upper 95% CI	90.5	166.8			
Co (µg/dl)	1.5±0.6	2.5±0.7	2.7±0.7	66.38	<0.0001
Lower 95% CI	1.3	2.3			
Upper 95% CI	1.6	2.6			
Mn (µg/dl)	0.58±0.22	0.99±0.23	1.16±0.27	110.91	<0.0001
Lower 95% CI	0.51	0.95			
Upper 95% CI	0.63	1.03			
SBP (mmHg)	175.2±20.9	114.6±13.1	111.7±9.9	483.15	<0.0001
Lower 95% CI	169.2	112.4			
Upper 95% CI	180.7	116.7			
DBP (mmHg)	110.6±15.8	73.3±9.8	67.4±4.3	377.21	<0.0001
Lower 95% CI	105.8	70.5			
Upper 95% CI	114.4	74.4			

Decrease in antioxidant trace metals level may cause excessive accumulation of reactive oxygen species (ROS), whichresults in oxidative stress, playing a critical role as a possible mediator of endothelial cell dysfunction,[22] hypertension, and thus clinical manifestations of pre-eclampsia.[16, 23] Two previous studies have shown decreases in maternal serum or toenail selenium concentrations in pre-eclamptic patients compared to normal pregnant controls.[24,25] This is consistent with our present report.

It has been demonstrated that when taken as a supplement, selenium modulates the cellular response to oxidative stress, inducing a faster restoration of the endogenous antioxidative defense system against the production of reactive oxygen species.[26] However, correlation studies in the present report revealed inverse relationship of selenium with SBP and DBP. This suggests that the lower the selenium level, the higher the oxidative stress as may be manifested through multiple step pathways.[16,22,23]

This study shows significant decreases in serum Zn, Cu and Cu: Zn ratio levels of women with pre-eclampsia compared to normal pregnant women. Copper and zinc are essential components of the antioxidant enzymes copper-zinc superoxide dismutase (Cu-Zn SOD) which is involved in destruction of free radicals/ROS.[27] Copper and zinc deficiency might cause insufficiency of Cu-Zn SOD,[28] thereby exposing the women to free radicals/ ROS accumulation and oxidative stress, which may lead to the development of pre-eclampsia. Besides, it has been reported that Zn deficiency can increase the susceptibility of neurons to oxidative tress.[29]

The potent vasodilator, nitric oxide (NO), is critical to normal vascular function and endothelial integrity. Nitric oxide is essential to the normal adaptive vasodilation of pregnancy in animals and women, and decreased NO bioavailability is thought to contributeto the development of pre-eclampsia.[30] Copper is also very essential for the release of NO from S-nitrosoalbumin (SNO-Alb.) Robin et al[31] reported significant elevations in plasma concentrations of SNO-Alb in women with pre-eclampsia; suggesting a deficiency in copper –mediated release of nitrogen oxide (NO)from SNO-Alb.[31] This corroborates the decreased copper status observed in women with pre-eclampsia as opposed to normal pregnancy in this present study.

Furthermore, in the present study significantly higher serum Cu level in normal pregnant women compared to non pregnant women (controls)and women with pre-eclampsia was observed. Serum copper level increases from about 80-155 mg/dl before pregnancy to about 118-302mg/dl by the end of the third trimester. The physiologic increase in copper concentration in pregnancy is in part associated with estrogen induction of copper carrying protein (ceruloplasmin).[32] However this present study show lower serum Zn level in pregnant women than controls, and also lower Zn level in pre-eclamptic women than normal pregnant women (table 1 & 2 respectively), thus buttresses that Zn deficiency may be a major factor in the development of pre-eclampsia in this environment. This corroborates previous report from Saudi Arabia that Cu and Zn are decreased in Pre-eclampsia,[33, 34] but is conflicting with a report from Iran that serum Zn in pre-eclamptics had no significant difference with normotensive subjects.[35] The present study reveals that decreases in some serum trace metals levels may in part contribute to development of pre-eclampsia in this environment. The Implication/Strength of the present study is that this result may be important to other populations with low levels of these trace metals and the overall Nigerian population in the prevention of pre-eclampsia and other trace metals deficiency-related disorders. The present study has limitation; serial serum trace metals in various trimesters were not included.

CONCLUSION

Decrease in serum selenium, zinc, copper, Cu: Zn ratio, cobalt and manganese levels may play a critical role in the pathogenesis of pre-eclampsia in this environment. There may be need for increase in dietary intake of these essential trace metals during pregnancy to prevent pre-eclampsia in this environment.

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Authors Contribution:
ICI - Concept and design of the study, reviewed the literature, data collection, statistically analyzed and interpreted the data, manuscript preparation and critical revision of the manuscript; **MNC -** Concept of the study, collected data and review of literature and manuscript preparation and helped in correction of manuscript; **AJI -** Co-supervised the study, conceptualized study, designed the study, collected data, and prepared first draft of manuscript and critical revision of the manuscript; **OIAO -** Supervision of the study, Concept and design of study, collected data, manuscript preparation and review of study.

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