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Effect of pre-eclampsia on plasma and erythrocytic divalent cation concentrations and their bioenergetics of transport

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ABSTRACT

Background: Pre-eclampsia is a syndrome of pregnancy-induced hypertension with proteinuria, which has high prevalence rate among blacks. **Methods:** To study the effect of pre-eclampsia on bioenergetics for the transport of erythrocytic cations to plasma, the plasma and erythrocytic levels of magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) were measured by atomic absorption spectrophotometry in 100 pre-eclamptic women matched for gestation age with 100 normal pregnant subjects. These were also age-matched with 100 non-pregnant women. **Results:** The plasma and erythrocytic levels of the minerals were significantly ($p < 0.05$) lower in the pre-eclamptic patients than the healthy pregnant and the non-pregnant women. Although, levels of these minerals were also reduced in the healthy pregnant women relative to the non-pregnant subjects, only the plasma and erythrocytic levels of Ca, Cu, Mn and Se showed significant ($p < 0.05$) depreciations. Pre-eclampsia and pregnancy did not affect significantly ($p > 0.05$) the divalent-cation-based erythrocytic membrane free energy. **Conclusion:** Our results suggest that low levels of these minerals, especially Mg and Cain pre-eclampsia, may have roles to play in the development of hypertension in these patients, and calls for intensified research into mineral supplementation as a key for possible prevention or management of pre-eclampsia.

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INTRODUCTION

Pre-eclampsia, a leading cause of maternal and perinatal mortality, is a syndrome of pregnancy-induced hypertension with proteinuria after the 20th week of pregnancy.[1] Here, hypertension is defined as systolic blood pressure (BP) > 140 mmHg or diastolic BP > 90 mmHg in a woman known to be normotensive before the pregnancy, while proteinuria is urinary protein excretion ≥ 300 mg/24 hr.[2] Pre-eclampsia has an overall incidence of 2-19% of all pregnancies and is associated with pre-term delivery, foetal growth retardation, and maternal morbidity and mortality.[3] Its greatest impact is in developing countries, where it accounts for 20-80% of the strikingly increased maternal mortality.[1,4,5] This syndrome involves many systems in the body, yet the pathophysiological process of the disease development has remained at the stage of hypothesis. These hypotheses have focused on reduced placental perfusion, endothelial cell dysfunction, imbalance between vasodilating and vasoconstricting prostanglandins, decreased circulating antioxidant activity, abnormal divalent cation metabolism and imbalance in the rennin-angiotensin system.[6]

However, a predominant pathophysiological feature of pre-eclampsia is reduced perfusion of virtually all organs especially the placenta due to abnormal implantation or other pathological disorders. This has been characterized as stage 1 of the two-stage model proposed for pre-eclampsia. The second stage is the production, by a maternal response

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to the reduced placental perfusion, of the maternal syndrome.[7] Oxidative stress has been proposed as the linkage of the two stages of pre-eclampsia. It is posited that reduced placental perfusion generates free radicals, which in the appropriate maternal environment generate systemic oxidative stress. This hypothesis is supported by evidence of markers of oxidative stress in blood circulation and the tissues of pre-eclamptic women.[8]

Erythrocytes are distinct from other cells of the body as they have specific characteristics such as the absence of nucleus and mitochondria and, therefore, have particularly low intracellular divalent cation concentrations. The surface membranes of cells generally serve as the recognition site for hormones, drugs, ions and a variety of other chemical transmitters that modulate cellular processes. The binding of calcium ion to, and its displacement from, the cell membrane constitutes a determining factor in the control of membrane structure and potential. Red blood cell (RBC) membranes have been reported to undergo abnormal curling phenomenon under pathological and experimental conditions in the absence of divalent cations. On the other hand, calcium ion has been shown to have a potential role in arresting RBC curling and mediating localized dynamic membrane deformations in intact RBCs.[9] Although, magnesium ion is known to inhibit calcium ion action, the abnormal intracellular and extracellular homeostases of both ions have long been recognized as major icons in the pathogenesis of many diseases, including pre-eclampsia.[10] Several abnormalities of these and other cations' transport and concentration have also been described in the erythrocytes of patients withpre-eclampsia.[11] Although most of these cation transport studies have centered on monovalent cations, the likely impact of divalent cation transport and the variations in their concentrations across cell membranes on the development and progression of pre-eclampsia must not be overlooked. This is made imperative by the physiological roles of these cations in biological enzymatic and antioxidant activities, as well as the successful application of Mg therapy in the treatment of eclamptic seizures. Generally, little but conflicting biomarker information is available on these elements in pre-eclampsia, especially among the black race reported to be at greater risk for pre-eclampsia.[12]

We have therefore studied the variations in the intracellular and extracellular erythrocytic concentrations of divalent cations, and their transport free energy across the erythrocytic membrane of women with or without pre-eclampsia to ascertain whether there are abnormalities and, if so, to what extents these might be attributable to pregnancy or pre-eclampsia.

PATIENTS AND METHODS

Subjects

Two hundred primigravid women in their third trimester (27-41 weeks), attending antenatal clinic at Federal Medical Centre, Owerri, Nigeria, and General Hospital, Umuguma-Owerri, Nigeria, between January and September 2013, were recruited for this study. They were made up of 100 pre-eclamptic and 100 normal pregnant women within the age range of 20-40 years. The subjects had normal blood pressures in their early trimesters. Proteinuria was detected in the pre-eclamptic patients with the aid of Protein Urine diagnostic strips (products of Human, Germany). The patients were moderately pre-eclamptic and were not receiving any treatment or micro-nutrients supplements before blood collection. The pregnant subjects were age-matched with 100 apparently healthy, non-pregnant women (controls). The control subjects were subjected to pregnancy test (CALTEST Diagnostics Inc., USA) to ensure that they were not pregnant. Informed consent was obtained from all subjects involved in the study. Ethical approval was sought and obtained from the Ethics Committee of the hospitals used.

Inclusion criteria

Pre-eclamptic subjects were included in the study if (1) they had normal blood pressures (systolic 90-120 mmHg and diastolic 70-80 mmHg) in their early trimesters, (2) they had proteinuria at the third trimester, detected with the aid of the Protein Urine strips, (3) they were not receiving any treatment or micro-nutrients supplements as at the time of sample collection, and (4) they consented to be enrolled in the research.

Exclusion criteria

Pre-eclamptic subjects were excluded in the study if (1) they had high blood pressures (greater than 120 mmHg systolic and 80 mmHg diastolic) in their early trimesters, or had normal blood pressures (systolic 90-120 mmHg and diastolic 70-80 mmHg) in their third trimester, (2) they were already placed on micro-nutrients supplements at the time of the study, and (3) they declined to be enrolled in the research.

Blood sample collection

Five milliliters (5.0 ml) of venous blood sample was collected from each subject by venipuncture using disposable 5.0 ml pyrogen-free plastic syringe. The blood sample was immediately dispensed into heparinized container. After centrifugation, red cells were separated from the plasma, washed thrice with physiological saline and lysed with 1.0 ml of distilled, deionized water. The plasma and RBC haemolysates were stored frozen until analysis.

Methods

The Mg, Ca, Zn, Cu, Mn and Se concentrations of the plasma and RBC haemolysates were determined by atomic absorption spectrophotometry (Perkin Elmner, USA). Standardinstrument parameters for the analysis were applied as provided by the instrument manufacturer. Different concentrations (0.5, 1.0, 2.0, 5.0 and 10.0 mmol/l) of trace elements were used for calibration of the standard graphs. Magnesium, calcium, copper, manganese and zinc were determined by the flame atomization technique with an acetylene-air flame. Selenium was determined using the hybrid generation technique as described by Milde *et al.*[13] To verify the assay accuracy and to maintain quality, the standard solutions were run for every 10-test sample.

The heights and weights of the subjects were measured using meter rule and weighing balance respectively. The body mass index, BMI,was calculated as weight (kg) divided by height squared (m^2).[14] Systolic and diastolic blood pressures (BP) were measured with the aid of a sphygmomanometer.

Estimated free energy change

The free energy required to transport the cations across the erythrocyte membrane was estimated using extracellular and intracellular cation concentrations determined above. The first approximation estimates of the free energy change were calculated for each cation using the Nernst equation as earlier described:[15]

$$\Delta G_m = \frac{RT}{n} \ln \frac{[cation]_{intracellular}}{[cation]_{extracellular}}$$

Where R = gas constant = 8.315 JK^{-1} mol^{-1}; n = Number of charge; T = Absolute temperature (approximate temperature of Owerri = 30°C + 273 = 303 K).

Statistical analysis

The data obtained were analyzed using One-way Analysis of Variance (ANOVA) and Pearson Correlation Analysis with the aid of GraphPad Prism 5.3 (GraphPad Inc., USA). Values for $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Figure 1 shows that the body mass index (BMI), systolic and diastolic blood pressures (BPs) of the pre-eclamptic subjects were significantly ($p < 0.05$) raised in comparison with those of the normal pregnant and non-pregnant women. It has been documented that pre-eclampsia is associated with a raise in systolic and diastolic BPs.[4,16] Thus, pre-eclampsia is presently diagnosed as a significant raise in systolic BP of ≥ 140 mmHg and diastolic BP of ≥ 110 mmHg after 20 weeks of pregnancy, in the presence of proteinuria. This may explain why there was no observed significant ($p > 0.05$) difference in the systolic and diastolic BPs between the normal pregnant and non-pregnant as also reported by Adamoya *et al.*[2]

The mean BMIs of the pregnant women were significantly ($p < 0.05$) higher than those of the non-pregnant controls. This was because of the significantly conspicuous rise in body weight commonly associated with pregnancy, whose increase is usually directly proportional to the advancement of the pregnancy.

The plasma and erythrocytic levels of the divalent cations were significantly ($p < 0.05$) lower in the pre-eclamptic patients than the healthy pregnant and non-pregnant women (Table 1). Although, the levels of some of the minerals were also reduced in the healthy pregnant women in comparison with the non-pregnant controls, only the plasma and erythrocytic levels of Ca, Cu, Mn and Se showed significant ($p < 0.05$) depreciations. The observed reductions in the levels of almost all the minerals in pregnancy may be attributed, not only to pre-eclampsia, but to increase in blood volume, poor mineral supplementation in pregnancy as well as their utilization for fetal growth.[5,17,18]

Although biomarker information on Ca, Cu and Zn in pre-eclampsia are conflicting and supplementation trials with the elements have failed to prevent the disorder, the need for consistent micro-elemental supplementation during pregnancy cannot be ruled out given our present observed low levels, and their reported significant reductions in blood concentrations during normal pregnancy and pre-eclampsia.[18-21]

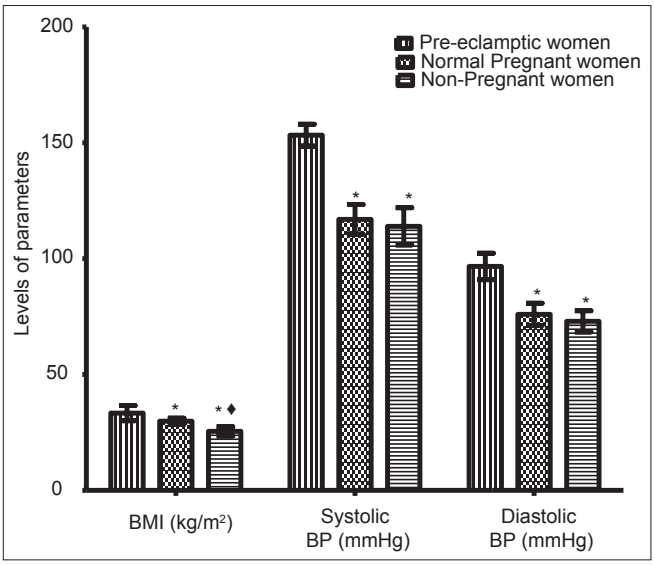

Figure 1: The body mass index (BMI), systolic and diastolic blood pressures (BPs) of the subjects. *Values significant (p<0.05) in comparison with those of the pre-eclamptic subjects; ♦Value significant (p<0.05) in comparison with that of the normal pregnant subjects

Table 1: Plasma and erythrocytic concentrations of divalent cations in the subject groups*

Mineral	Mineral concentrations of the subject groups*		
	Pre-eclamptic	Normal pregnant	Non-pregnant
Plasma (extracellular)			
Mg (mmol/l)	0.48±0.10[a]	0.77±0.12[b]	0.83±0.16[b]
Ca (mmol/l)	1.14±0.33[a]	1.99±0.17[b]	2.36±0.27[c]
Cu (µmol/l)	5.75±1.13[a]	12.45±1.53[b]	18.14±1.66[c]
Zn (µmol/l)	6.03±0.92[a]	6.84±0.74[b]	7.13±0.85[b]
Mn (µmol/l)	0.21±0.07[a]	0.36±0.05[b]	0.48±0.08[c]
Se (µmol/l)	0.22±0.05[a]	0.36±0.08[b]	1.09±0.17[c]
Erythrocyte (intracellular)			
Mg (mmol/l)	0.65±0.14[a]	1.05±0.20[b]	1.12±0.91[b]
Ca (mmol/l)	0.76±0.18[a]	1.15±0.18[b]	1.64±0.16[c]
Cu (µmol/l)	10.86±1.10[a]	17.54±1.23[b]	24.64±1.78[c]
Zn (µmol/l)	8.55±0.72[a]	10.25±0.96[b]	10.91±1.00[b]
Mn (µmol/l)	0.26±0.05[a]	0.62±0.08[b]	0.71±0.09[c]
Se (µmol/l)	0.43±0.11[a]	0.49±0.09[a]	1.54±0.13[b]

* Values are mean±SD of 100 determinations per group. Values with different superscript letter per row are significantly different (p<0.05)

High doses of intravenous magnesium sulphate have been the treatment of choice for preventing severe pre-eclampsia and eclamptic associated seizures. This is because Mg is believed to relieve cerebral blood vessel spasm, thereby increasing blood flow to the brain.[22] The need to infuse Mg for the management of pre-eclampsia may indicate or explain the significantly reduced plasma and erythrocyte concentrations of Mg observed in this study amongst the pre-eclamptic patients. This observation corroborates earlier reports of low Mg levels in the plasma and erythrocytes of Caucasian and Asian pre-eclamptic women, supports the hypothesis on the role of Mg deficiency in pre-eclampsia pathophysiology, and suggests the usefulness of its assessment in the early diagnosis of the disorder.[11,21]

As stated earlier, oxidative stress has been proposed as the linkage of the different, yet unspecified stages of pre-eclampsia.[8] Selenium and Mn, as essential constituents of superoxide dismutases and glutathione peroxidases, take part in the reduction of hydrogen and lipid peroxides as well as the potential deleterious effects of superoxide anion free radicals within the body.[23] Thus, Se and Mn play an indirect role in the body's prevention and management of oxidative stress. However, reports on Se and Mn as biomarkers of pre-eclampsia are conflicting. The results of this study showed that Se and Mn concentrations in the plasma and erythrocytes of the pre-eclamptic and normal pregnant women were significantly lower than those of the non-pregnant women. This differs with the report of Sekine *et al.*[24] which stated that Se concentration in red blood cells of full term pregnant women were significantly higher than in non-pregnant controls. However, it corroborates the reported observations of decreased Se concentration in blood during normal pregnancy[25] and pre-eclampsia.[26] Similarly, Beline and Wolters[27] reported a decrease in plasma Se contents of pregnant women but with no significant change in its erythrocytic contents between pregnant and non-pregnant women. The decreased plasma and erythrocytic Se and Mn contents in pre-eclamptic women observed in this study may be due to their mobilization and utilization, their associated use in the antioxidant arsenal of the body to tackle the effects of free radicals and lipid peroxidation which has been shown to increase in activity with gestation age as well as the presence of pre-eclampsia.[25] Furthermore, oxidative stress has been proposed as one of the hypotheses for the pathogenesis of pre-eclampsia which resolves after delivery. This indicates that there will be a continuous use and hence depletion of the above-mentioned arsenal until the source of the assault, which is suspected to be the placenta has been removed. Furthermore, the reduction in the plasma and erythrocytic contents of Se and Mn in all the pregnant subjects may be attributed also to changes previously stated to be associated with pregnancy.[18]

Correlation studies of the elemental levels of Mg, Ca, Zn, Cu, Mn and Se with the BMI, systolic and diastolic blood pressures in pre-eclampsia showed general inverse relationships. Significant inverse correlations were observed between both the plasma and erythrocytic Mg levels and the systolic ($r = -0.897$; $p = 0.000$ and $r = -0.730$; $p = 0.049$, respectively) and diastolic ($r = -0.813$; $p = 0.011$ and $r = -0.770$; $p = 0.043$, respectively) blood pressures of the pre-eclamptic patients. In the same vein, plasma Ca levels were also found to be significantly and inversely correlated with the systolic ($r = -0.763$; $p = 0.027$) and diastolic ($r = -0.791$; $p = 0.021$) blood pressures of the pre-eclamptic subjects. There were no observed correlations between all the elements and BMI levels, indicating the higher importance of raised blood pressures than BMI in the pathophysiology and diagnosis of pre-eclampsia. These observations further buttress the reported importance of Mg therapy for prevention/treatment of severe pre-eclampsia and eclamptic associated seizures.[28] The recorded significant correlation observed for Ca may be indirectly dependent on the effects of Mg, since Mg is known to be a significant antagonist of Ca ion activity across cellular membranes.[29]

Table 2 shows the effect of pre-eclampsia on estimated free energy change required forthe transport of the divalent cations across erythrocytic membranes. The regulated movements of ions into and out of cells across plasma membranes via 'gated' ion channels are necessary for the maintenance of the polarized nature of cell membranes, commonly described as resting membrane potential. The ionic current that maintains this resting membrane potential of cells and the changes that occur in response to the

Table 2: Free energy change (ΔG_m; $Jmol^{-1}$) for divalent cations transport across erythrocytic membrane

Mineral	ΔG_m ($Jmol^{-1}$) of the subject groups*		
	Pre-eclampsia	Normal pregnant	Non-pregnant
Magnesium	0.38±0.08	0.39±0.03	0.38±0.01
Calcium	−0.51±0.07	−0.69±0.04	−0.46±0.03
Copper	0.60±0.35	0.43±0.21	0.39±0.12
Zinc	0.44±0.17	0.51±0.12	0.54±0.23
Manganese	0.27±0.12	0.68±0.26	0.49±0.24
Selenium	0.64±0.39	0.39±0.13	0.44±0.26

*Values are mean±SD of 100 determinations per group

presence of a disease condition, pharmacologic or signaling molecules constitute the complex electrophysiologic network that controls the pathophysiological process of any given disease, the contractile activity of smooth muscle, signal transduction of a nerve cell, among others.[29,30]

The results of this study (Table 2) showed that neither pre-eclampsia nor pregnancy significantly affected the calculated free energy changes associated with divalent cation transport. This observation could be attributed to the absence of certain subcellular organelles such as sarcoplasmic reticulum and plasmalemma in erythrocytes which are necessary for storage of these cations, especially calcium, within cells. This meant that as extracellular divalent cation concentrations drop due to pre-eclampsia and/or pregnancy-associated hypervolaemia and utilization, the intracellular concentrations also follow in due course. Unlike the other cations studied, a negative erythrocyte membrane free energy was obtained for calcium which corroborated similar observation with normal erythrocytes from albino rabbits.[29] This indicates that membrane free energy studies using changes in calcium concentrations may offer better empirical evidence than the use of the other divalent cations. Interestingly, the negative membrane free energy values obtained using calcium was not affected by both pre-eclampsia and pregnancy. This gives insight into understanding of the earlier report that, although Mg is a unique calcium antagonist because it can act on most types of calcium channels, $MgSO_4$ infusions used in the treatments of pre-eclampsia cause increases in ionized magnesium (Mg^{2+}) levels without concomitant changes in serum and erythrocyte ionized calcium (Ca^{2+}) concentrations. This, Euser and Cipolla[31] suggested is because $MgSO_4$ effect is not exerted via modulations of Ca^{2+} levels.

CONCLUSION

In conclusion, the results of this study suggest that low levels of these elements, especially Mg and Ca, in pre-eclamptic women may have roles to play in the development of hypertension in these patients. This calls for intensified research into mineral supplementation as a key for possible prevention or management of pre-eclampsia, especially amongst expectant mothers in developing countries majority of who are already overburdened by poverty and several other infectious and non-infectious diseases.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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Authors Contribution:
This was carried out in collaboration between all the authors**. CUI, PAO and COI -** Designed the study and wrote the protocol; **CUI, PAO and JEO -** Managed the analyses of the study; **CUI and JEO -** Managed the literature searches; **while CUI -** Wrote the first draft of the manuscript and incorporated all corrections from co-authors. All authors read and approved the final manuscript.
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