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ORIGINAL RESEARCH

Serum and Red Cell Magnesium and Calcium Concentrations in Normotensive and Pre-Eclamptic Pregnant Women in Lagos, Nigeria

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Abstract

Background: Pre-eclampsia is a significant cause of maternal and foetal morbidity and mortality. This study identified the relationship between serum and red cell concentrations of magnesium and calcium in pre-eclampsia. This is to justify the use of magnesium in the prevention of pre-eclampsia and eclampsia.
Objective: To compare the serum and red cell levels of calcium and magnesium among pre-eclamptic and normotensive pregnant women.
Method: A prospective, comparative study comparing serum and red cell concentrations of magnesium and calcium in sixty pre-eclamptic patients and sixty normotensive pregnant controls.
Result: The mean serum calcium concentration among pre-eclamptic patients was 2.33±0.19 mmol/l, compared with 2.47±0.23 mmol/l in the normotensive group (p = 0.244). The mean serum magnesium concentration was significantly higher among normotensive pregnant women than pre-eclamptic women (1.18±0.31 mmol/l vs 0.91±0.23 mmol/l; p = 0.001). The mean red blood cell calcium concentration was also significantly higher among pre-eclamptic pregnant women compared to the normotensive group (4.34±0.62 mmol/l vs 3.95±0.79 mmol/l; p = 0.003), but the mean red cell magnesium concentration was lower in pre-eclamptic than the normotensive control group but without statistical significance (2.04±0.65 mmol/l vs 2.26±0.79 mmol/l; p = 0.098).
Conclusion: Pre-eclampsia is associated with low concentrations of serum magnesium and calcium. Therefore, magnesium supplements may be beneficial in preventing pre-eclampsia.

Keywords: Calcium, Magnesium, Pre-eclampsia, Pregnancy, Red Cell.

Introduction

Pre-eclampsia is one of the common causes of maternal and foetal morbidity and mortality. Thus, there is a need to prevent the disease

among pregnant women. [1] It causes about 2% to 8% of maternal deaths and 30% of perinatal deaths worldwide. [2] The disease is mainly found in human pregnancy and is characterised by a multisystemic disorder. It is characterised by elevated blood pressure, proteinuria, and end-organ damage after 20 weeks of gestation. [3, 4] Pre-eclampsia causes vasospasm and increased peripheral resistance resulting in reduced organ perfusion leading to organ failure. [5] As a result of the unpredictable progression and the different modes of presentation of pre-eclampsia, there is a need to make a diagnosis promptly and accurately. [1]

The aetiology of the disease is not yet well known. However, several theories have been proposed. Decades of intensive research have examined various possible aetiologies of the disease, but there are still gaps in the pathophysiology and the different clinical presentations. [1] The pathogenesis has been linked to the alteration of several biochemical agents in the maternal serum, even though none has accurately predicted the disease. [6] Calcium and magnesium are two essential elements shown to have a high relationship with pre-eclampsia. [7] Magnesium is mostly an intracellular element, primarily found in red blood cells, with only 1% of the total magnesium in the extracellular fluid. [8] Magnesium is an intracellular cation; extracellular magnesium reflects the magnesium balance state. [7] Magnesium is an enzyme stabiliser required in the ATP-generating reactions necessary for fat, protein and glucose metabolism. [7] It is needed in enzyme functions such as sodium-potassium ATPase and it modulates peripheral vasodilatation, muscle contraction, and relaxation. [9]

Interestingly, a low magnesium level causes vasoconstriction, and an increased magnesium concentration leads to vasodilatation. [10,11] Therefore, magnesium deficiency has been linked to pre-eclampsia in regulating the tonus of arterioles and veins. [10] Physiologically, changes in calcium concentrations cause variations in humans blood pressure. [8] The lowering of serum calcium concentration causes an elevation of blood pressure in pre-eclamptic patients. [12] This is achieved by decreasing prostacyclin production and increasing the vasoconstriction effect of angiotensin II and noradrenaline in the blood vessel wall. [13] Furthermore, calcium indirectly increases magnesium levels causing peripheral vasodilatation. [15] It may not be surprising that hypomagnesaemia is associated with pre-eclampsia. [10]

Given the above, examining the relationship between low serum magnesium and calcium concentrations and the development of pre-eclampsia is germane. In addition, magnesium and calcium supplements in women of reproductive age or in pregnancy may become necessary to prevent pre-eclampsia. It is important to note that red cells are readily available for studies of intracellular magnesium and calcium concentrations. [16] This study aimed to determine the correlation between serum and red cell concentrations of magnesium and calcium and the development of pre-eclampsia. This can provide valuable evidence in preventing and managing pre-eclamptic and eclamptic patients.

Methods

The study was conducted at the Obstetrics and Gynaecology Department of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos State, Nigeria, between October 2013 and February 2014. The Health Research Ethics and Committee of the Lagos University Teaching Hospital approved the study protocol with Ethical Approval Number: HREC 275216. The magnesium and calcium status was assayed at

the Prenatal Diagnosis Laboratory, College of Medicine, University of Lagos.

Study Design

This prospective comparative study compared serum and red cell concentrations of magnesium and calcium in sixty pre-eclamptic women and sixty normotensive pregnant controls. [17] Consenting pre-eclamptic women aged 18 to 45 were randomly selected from 20 weeks gestation onwards. They served as the subjects while consecutive consenting normotensive pregnant women, matched for age and gestational age, were recruited as controls. Excluded from the study were women with multiple gestations, diabetes mellitus, renal failure, heart diseases, chronic/essential hypertension, collagen vascular disease and women on alcohol, magnesium, calcium supplementation or diuretic agents during pregnancy.

The sample size determination formula for the comparison of two means was used: [17]

$n = 4\sigma^2 (z_{crt}+z_{pw})^2 / D^2$

σ - the assumed standard deviation of each group from a previous study, 0.47[7]

Z_{crt} – confidence interval in a 2-tail test is 1.96.

Z_{pw} – standard normal deviate = 80%.

D – the minimum expected difference between the two means = 0.37.

Using the above formula, the sample size in each group was 50. Twenty per cent was added to the calculated minimum sample size to allow for non-response, making 60.

There a total of 120 subjects were recruited.

All subjects recruited in the study fulfilled the eligibility criteria. Data were collected from selected eligible participants using well-structured questionnaires administered by the investigator. The subjects were educated on the study methodology and were allowed to make an informed decision to participate, and after that, they signed the informed consent form. Blood pressure was measured with the subject in a semi-recumbent position using a standard sphygmomanometer (Acusson®). Urinary albumin was examined using an acid test strip Combur- 10 (Combes® manufactured by Roche) urinary dipstick, which tested for ten different parameters, including albumin. [18] The subjects were counselled to collect midstream urine into a universal bottle. The Combur-10 test strip was then dipped into the bottles of urine for 1-2 seconds, the strip was taken out, and after another 1 second, the colour code was read off. The colour of the detection pad of the strip was matched with the colour scale label on the container. The strips were placed downwards during the operation, and fingers were kept clear from the samples throughout the process.

Pre-eclampsia was diagnosed in women with systolic blood pressure equal to or greater than 140 mmHg and or diastolic blood pressure equal to or greater than 90mmHg on at least two occasions measured four hours apart in a previously normotensive woman accompanied by significant proteinuria (2+or more on urinary dipstick).

Afterwards, the subjects were counselled and allowed to rest. Five millilitres of venous blood was drawn from them under aseptic conditions by venepuncture using a five-millilitre sterile disposable syringe and needle. The blood sample was poured into lithium heparin bottles. A trained laboratory assistant took the blood samples following standard techniques in blood sample collection. The samples were drawn from cubital fossae within two to five minutes of a tourniquet and were then transported to the laboratory. In the laboratory, blood samples were centrifuged at 3000 rpm for 15 minutes within six hours of collection. Red blood cells and serum samples were separated and stored at 2-8^{0}C.

Laboratory procedures

Magnesium concentrations in serum were assayed by a manual spectrophotometric method

using the Xylidyl Blue Colorimetric technique. [18] Red cells were pipetted into a labelled test tube. The tube was three-quarters filled with saline to re-suspend the cells. The tube was centrifuged at 4000rpm for 15 minutes until a clear supernatant was obtained. The supernatant was decanted, and the process was repeated. Red blood cells were washed thrice with sodium chloride and centrifuged at 4000rpm. Two hundred microlitres of red cells were pipetted into a centrifuge tube. Then three millilitres of distilled water were added and centrifuged till all the red corpuscles were lysed. Four hundred microlitres of sodium tungstate were added with 400µl of 0.1% sulphuric acid and centrifuged for five minutes. One hundred microliters of the supernatant were added to 1 ml calmagite reagent.

Calcium in serum and red cell

Calcium in the serum and red blood cells were assayed using the spectrophotometric manual method, using O-cresol phthalein – complexone. [19] To ensure quality control, samples were analysed in duplicate to eliminate errors. The samples were stored in lithium heparin bottles to ensure quality control. All blood samples were drawn and immediately spun and prepared for storage at 2-8^{o}C to maintain the viability of red blood cells and allow for at once analysis in batches.

Data analysis

The data were analysed using the Statistical Package for Social Sciences (IBM SPSS) 19.0. Demographic and baseline variables were summarised using descriptive statistics expressed as mean (± standard deviation) or median and range for continuous variables and percentages for categorical variables. Continuous variables were compared using the Student's t-test, and categorical variables were compared using the appropriate Chi-Squared or Fisher's exact test. Statistical significance was defined as a P-value level less than 0.05.

Results

The mean age of the normotensive pregnant women was 31.37±4.5 years (range 17-42 years). Fifty-nine (98.3%) were married, 33 (55%) had primary education, and 35 (58.1%) were of Yoruba ethnicity. The mean parity was 1.20±1.3 (range 0-5), and the mean gestational age at delivery was 38±1.39 weeks (range 36-41 weeks). A majority had a vaginal delivery (73.3%), as shown in Table I.

In comparison, the pre-eclamptic pregnant women had a mean age of 30.98±4.66 years (range 21-42). Forty (66.7%) had primary education, 38 (63.3%) belonged to Igbo ethnic group, and all of them were married. The mean parity was 1.1±0.37 (range 0-5), and the mean gestational age at delivery was 35±3.85 weeks (range 26-40 weeks). A majority had a caesarean section (71.7%). The mode of delivery was significantly different from that of the normotensive group, as shown in Table I.

The mean birth weight for the babies of normotensive women was 3.1±0.47 kg compared to 2.5±0.89 kg for pre-eclamptic women ($p<0.001$). Table II shows that only five babies had an APGAR score less than seven at the first minute, and in the fifth minute, none had an APGAR score of less than seven. On the other hand, the mean birth weight of the babies of pre-eclamptic women was 2.5±0.89 kg. For these babies, the APGAR score at the first minute of life was less than seven among 14 babies, while 7 babies had a fifth-minute APGAR score of less than seven. The difference in the distribution of the babies in both groups according to birth weight and APGAR scores showed no statistical significance. A total of 29 babies had low birth weight; 22 (36.7%) of these belonged to mothers with pre-eclampsia and 7 (11.7%) to normotensive women.

Table I: Comparison of the socio-demographic characteristics of subjects

Variables	*Groups*	*Normotensive (n = 60)*	*Pre-eclampsia (n = 60)*	*p-value*
Age (Years)	15-19	5 (8.3)	0 (0.0)	0.645
	20-24	9 (15.0)	9 (15.0)	
	25-29	15 (25.0)	11 (18.3)	
	30-34	19 (31.6)	30 (50.0)	
	35-39	10 (16.7)	7 (11.7)	
	≥40	2 (3.3)	3 (5.0)	
Marital status	Male	59 (98.3)	60 (100.0)	1.000
	Female	1 (1.7)	0 (0.0)	
Level of Education	Primary	33 (55.0)	40 (66.7)	0.854
	Secondary	16 (26.7)	9 (15.0)	
	Tertiary	11 (18.3)	11 (18.3)	
Ethnicity	Hausa	0 (0.0)	3 (5.0)	0.006
	Yoruba	35 (58.3)	19 (31,7)	
	Igbo	25 (41.7)	38 (63.3)	
Gravidity	1	0 (0.0)	2 (3.4)	0.911
	2-4	51 (85.0)	51 (85.0)	
	≥5	9 (15.0)	7 (11.0)	
Parity	≤1	38 (63.3)	41 (68.3)	0.678
	2-4	22 (36.7)	17 (31.0)	
	≥5	0 (0.0)	2 (0.1)	
Gestational age (weeks)	≤27	0 (0.0)	3 (5.0)	0.001
	28-36	7 (11.7)	23 (3.3)	
	37-42	53 (88.3)	34 (56.7)	
Mode of delivery	Vaginal delivery	44 (73.3)	17 (28.3)	0.005
	Caesarean section	26 (26.7)	43 (71.7)	

The biochemical profile of the two groups were compared in Table III. The mean serum calcium concentration was higher in normotensive women than pre-eclamptic women but without statistical significance (2.47±0.23 mmol/l vs 2.33±0.19 mmol/l; p = 0.244). The mean serum magnesium concentration was statistically significantly higher in normotensive pregnant women compared to pre-eclamptic women and was statistically significant (1.18±0.31 mmol/l vs 0.91±0.23 mmol/l; p = 0.001). The mean red blood cell calcium concentration was significantly higher in pre-eclamptic women compared to the normotensive group (4.34±0.62 mmol/l vs 3.95±0.79 mmol/l; p = 0.003). The red blood cell magnesium concentration was also higher in normotensive women compared to the pre-eclamptic group but without statistical

significance (2.26±0.79 mmol/l vs 2.04±0.65 mmol/l; p = 0.098).

Table II: Comparison of the outcomes of delivery

Variables	*Groups*	*Normotensive (n = 60)*	*Pre-eclampsia (n = 60)*	*p-value*
Birthweight (kg)	<2.4	7 (11.7)	22 (36.7)	0.001
	2.5-3.9	51 (85.0)	37 (61.7)	
	>4.0	2 (3.3)	1 (1.7)	
	Mean	3.1±0.46	2.5±0.89	
	Range	1.55-4.20	1.09-3.80	
First-minute APGAR score	0-3	0 (0.0)	3 (5.0)	0.01
	4-6	5 (8.4)	11 (18.3)	
	7-8	32 (53.3)	31 (51.7)	
	9-10	23 (38.3)	15 (25.0)	
	Mean	8.02±1.10	7.23±2.02	
	Range	4-9	0-9	
Fifth-minute APGAR score	0-3	0 (0.0)	3 (5.0)	0.002
	4-6	0 (0.0)	4 (6.7)	
	7-8	6 (10.0)	8 (13.3)	
	9-10	54 (90.0)	45 (75.0)	
	Mean	9.48±0.62	8.50±2.22	
	Range	8-10	0-10	
Placental weight (g)	500	13 (21.7)	14 (23.3)	0.103
	>500	47 (78.3)	46 (76.7)	
	Mean	567.28±165.9	523.05±123.20	
	Range	100-850	200-750	

Thirty-nine babies were admitted into the Special Care Baby Unit (SCBU); only seven babies in the normotensive group were admitted for low birth weight, and all the babies were discharged to their mothers. Among the babies of pre-eclamptic mothers, 22 (64,7%) were hospitalised for low birth weight, 7 (20.5%) for birth asphyxia and 3 (8.8%) for prematurity, as shown in Figure 1. Two (5.8%) had early neonatal death from complications of severe birth asphyxia in the form of hypoxic-ischaemic encephalopathy (HIE) Stage III and chemical pneumonitis.

Discussion

There is a relationship between serum and red blood cell calcium and magnesium concentrations and pre-eclampsia. [20] The demographic data in the two groups were similar. The obstetric history, consisting of the gravidity, parity and gestational age at delivery, showed that only the gestational age differed significantly. This suggests that pre-eclamptic pregnant women tend to be delivered preterm more than normotensive pregnant women to save the lives of the mothers and babies. Ethnicity showed statistical difference as the majority of the pre-eclamptic were Igbos from the southeastern part of the country Nigeria. This

may be difficult to explain as it may have been a coincidence; no significant explanation can be attributed to this finding, and it may need to be evaluated in further studies.

Table III: Comparison of the mean values of serum and red blood cell concentrations of calcium and magnesium

Variables	*Biochemical substrates*			
	Serum Calcium	**Serum Magnesium**	**Red Blood Cell Calcium**	**Red Blood Cell Magnesium**
Normotensive	**mmol/L**	**mmol/L**	**mmol/L**	**mmol/L**
Mean+ SD	2.47±0.23	1.18±0.31	3.95±0.79	2.26±0.79
Range	2.0-3.4	1.00-1.40	2.50 – 5.90	2.00 – 5.00
Pre-eclampsia				
Mean+ SD	2.33±0.19	0.91±0.23	4.34±0.62	2.04±0.65
Range	2.0 – 2.80	0.50-0.97	3.20 – 5.70	1.10 – 4.20
P-value	0.244	0.001	0.003	0.098

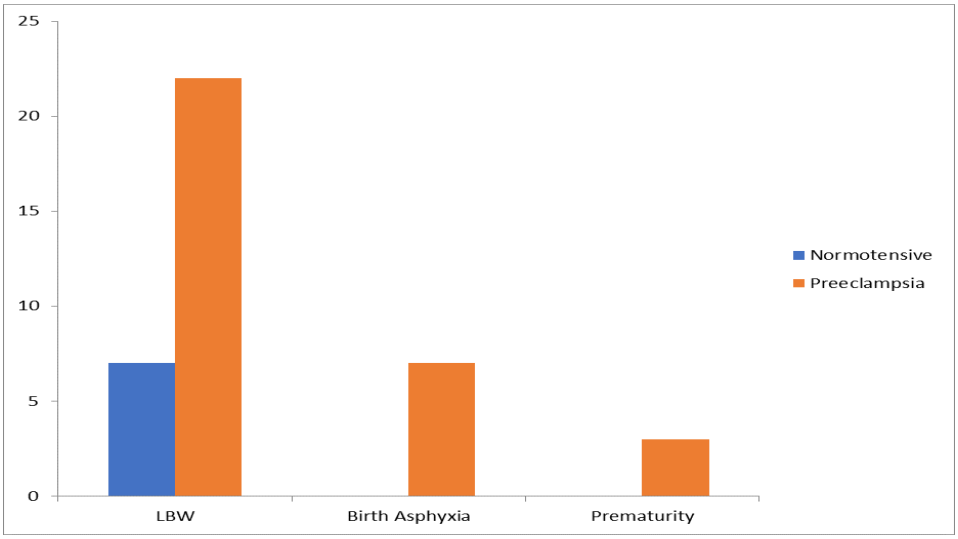

Figure 1: Pattern of hospitalisation among babies of normotensive and pre-eclamptic mothers

The mode of delivery among the two groups was significantly different as most pre-eclamptic subjects were delivered through the faster mode of delivery to reduce maternal and perinatal morbidity and mortality. Delay in delivery may lead to the development of maternal complications of pre-eclampsia, foetal distress, eclampsia, placental abruption and other morbidities that may jeopardise the lives of both mother and neonate. [21]

This study showed a significantly lower serum magnesium concentration in pre-eclamptic patients than in normotensive subjects. This finding was similar to many other studies conducted by Idogun and other researchers, where the plasma concentration of serum magnesium was lower in a pre-eclamptic group compared to a normotensive group. It was found that hypomagnesaemia is associated with pre-eclampsia. [7, 16, 20-23] Saunders *et al.* found the level of magnesium in the serum of pre-eclamptic

patients to be higher than normotensive women. [24] The different results may stem from the small sample size used in their study. Kehdun *et al*., however, found no significant difference in the concentrations of magnesium in the normotensive and hypertensive groups. [25] Low magnesium has been associated with a reduction in nitric oxide, which is needed to dilate the vessels. The low concentration of magnesium in the serum exposes the subject to pregnancy complications, including pre-eclampsia and preterm delivery. [21] Magnesium is known to be involved in blood pressure regulation in most cells. [20]

The mean concentration of red cell magnesium was higher among the normotensives (2.26±0.79mmol/l) than pre-eclamptic (2.04±0.65mmol/l). This finding of reduced concentration of intracellular magnesium in hypertensive pregnant women compared to normotensive women is similar to the report of Kehdun *et al*. [25]. A low magnesium concentration in red cells may reflect the serum concentration since magnesium is stored more intracellularly. [8] Saunder *et al.* found that intracellular magnesium concentrations in mononuclear blood cells and erythrocytes were elevated in normotensive women compared to the pre-eclamptic group due to intracellular inhibition of nitric oxide synthase in endothelial cells. [24]

In the present study, the serum calcium concentrations between the two groups were not significantly different, though it was slightly lower in pre-eclampsia. A similar study by Idogun and Mittal *et al.* found that plasma concentration of calcium was lower in the pre-eclamptic group than in the normotensive group. [21, 23] The mean concentration of red cell calcium was significantly higher in the pre-eclampsia group than in normotensive patients. There is an accumulation of calcium intracellularly. Kosh *et al.* reported that membrane calcium content was significantly increased in pre-eclamptic patients. However, the results were within normal ranges. [26] This may be responsible for the vasospasm leading to elevated blood pressure observed in pre-eclampsia. This finding suggests an imbalance in the transfer of these cations between the cell membrane and may be of importance in the pathogenesis of pre-eclampsia.

In the present study, serum magnesium concentrations in pre-eclamptic women were significantly lower than in normotensive women, suggesting some role of these elements in the rise of blood pressure. These findings may reflect what is seen in the smooth muscles. The smooth muscle of blood vessels needs calcium to contract. Still, they also require sufficient magnesium to dilate and lower blood pressure. [27, 28] Therefore, magnesium acts as a calcium channel blocker by opposing calcium-dependent arterial constriction and antagonising the increase in intracellular calcium concentration leading to vasodilatation. [28-30] This shows that hypomagnesemia increases calcium within the cell leading to vasospasm in the smooth muscle wall of vessels and subsequent elevated blood pressure. [29,30]

There are suggested reasons for the progressive decline in the concentration of calcium and magnesium in maternal serum, possibly due to increased metabolic demand, physiological haemodilution in pregnancy, reduced uptake from the gastrointestinal tract and increased renal loss of the cations. [20] Similarly, increased urinary excretion and increased transfer of these minerals from the mother to the growing foetus are known to occur. [30, 31] The study showed that both hypomagnesemia and hypocalcaemia might be responsible for the manifestation of pre-eclampsia.

Adverse perinatal events were generally few in this study. The mean birthweight for the

normotensive group was higher compared to the pre-eclamptic group. The fifth-minute APGAR scores in the two groups showed that babies of the majority of the normotensive subjects had significantly better scores compared to babies of the pre-eclamptic group. Though early intervention by expediting delivery in pre-eclamptic women was instituted, the babies were already exposed to the distressful foetal complications associated with hypertension and pre-eclampsia in pregnancy. Therefore, babies of pre-eclamptic mothers had poorer outcomes when compared to their counterparts.

Conclusion

The serum magnesium concentration in the pre-eclamptic group was significantly lower than that obtained in the normotensive subjects in the present study. Magnesium regulates the cellular placement of other cations, such as calcium, which is also essential in blood pressure regulation. Consequently, the study showed that patients with pre-eclampsia have hypomagnesemia and hypocalcaemia. Elevated blood pressure is associated with magnesium deficiency and disturbance in calcium metabolism. Magnesium supplements may play a beneficial role in preventing pre-eclampsia. A limitation of this study is that the adequacy of dietary intake may not always be easy to ascertain among pregnant women, especially in a developing country like Nigeria.

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