

Full Length Research Paper

Evaluation of trace elements in pregnant women with pre-eclampsia

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Trace elements such as zinc, copper, and selenium display antioxidant activity and act as peroxynitrite scavenger while others such as manganese and magnesium are essential micronutrients. The disturbance in the metabolism of these elements may be a contributing factor in the development of certain diseases such as pre-eclampsia. The aim of this study is to determine the status of these elements in pre-eclamptic pregnant women in Osogbo, Western Nigeria. Forty-nine pre-eclamptic patients and 40 age-matched non pre-eclamptic pregnant women (control group) were recruited from Ladoko Akintola University Teaching Hospital, Osogbo, Osun State, Western Nigeria. The concentration of these trace elements in serum was measured by atomic absorption spectrophotometry after acid digestion. The demographic characteristic of the patients and controls, including blood pressure (measured with digitalised sphygmomanometer) were obtained during patient clerking and history taking by the attending physician. In the pre-eclamptic group, all the elements evaluated were significantly lower when compared with the control group ($p < 0.01$). The systolic and diastolic blood pressure showed significant inverse correlation with all the evaluated micronutrient ($p < 0.01$). The findings of this study showed that trace elements were significantly reduced in pre-eclamptic pregnant women when compared to those of control group. Dietary supplementation with these elements may help to prevent pre-elampsia, at least in susceptible women, especially in developing countries.

Key words: Deficiencies, complications, pregnant women, Nigeria, trace elements.

INTRODUCTION

Pre-eclampsia is defined as the onset of proteinuric hypertension after mid-pregnancy; a systemic disease of the later stages of pregnancy that affects about 5 - 7% of all pregnancies and is the most common, yet least understood disorder of pregnancy (Ziael et al., 2008; Lou et al., 2008). It is a rapidly progressive condition characterized by high blood pressure, platelet aggregation, swelling of the lower extremities and protein in urine (Sarsam et al., 2008). Sudden weight gain, headaches

and changes in vision are important symptoms. Typically, blood pressure elevations and pre-eclampsia occur in the late second or third trimesters and gestational outcome is hardly affected (lihan et al., 2002). It has been reported that, pre-eclampsia is a major cause of both maternal and foetal morbidity and mortality (Bringman et al., 2006). Despite its prevalence and severity, the patho-physiology of this multi-system disorder is still poorly understood. Placental oxidative stress has been shown to be a key feature in the pathogenesis of eclampsia (Bringman et al., 2006; James et al., 2006). Despite several studies on pre-eclampsia, its aetiology has not yet been fully elucidated. Some studies have shown that changes in the levels of blood trace elements in pre-eclamptic patients may implicate its pathogenesis (Bringman et al., 2006;

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James et al., 2006) while others have failed to show an association of blood levels of trace elements and prevalence of pre-eclampsia (Caughey et al., 2005).

Nutritional deficiencies are common during pregnancy and pregnant women in developing countries have been reported to consume diets that are low in minerals and vitamins (Begum et al., 2000; Adam et al., 2001). An inadequate dietary intake before and during pregnancy might be a high risk not only for the mother but also for the foetus. Deficiencies of trace elements such as zinc, copper, selenium and magnesium have been implicated in various reproductive events like infertility, pregnancy wastage, congenital anomalies, pre-eclampsia, placental abruption, premature rupture of membranes, still births and low birth weight (Mohamed et al., 2004; Hofmeyr et al., 2007).

Different trace elements have been found to decrease the risk of having pre-eclampsia. Mahomed et al. (2004) reported a low intake of calcium during pregnancy in many different parts of the world such as Asia, Latin-America, Africa as well as developed countries such as Canada, USA and the UK. There was some variation between the countries, with very low intake in India (250 mg/d) to high intakes in Caucasian women in Canada (1256 mg/d). Hofmeyr et al. (2007) suggested that expectant mothers may be able to prevent potentially serious medical problems in themselves and their babies simply by boosting their daily calcium intake. This was as a result of their findings that pregnant women from communities with low dietary calcium who received at least 1.5 g/day of calcium orally during the second half of pregnancy had a lower risk of hypertension and pre-eclampsia than women who received placebo treatment. Since pre-eclampsia involves endothelial dysfunction and oxidative stress, there is interest in supplementation, with minerals such as calcium and antioxidant vitamins such as vitamins C and E, in the second trimester. A preliminary trial of antioxidant vitamins, in high risk women, has reported improvements in biochemical markers of endothelial activation together with a reduction in pre-eclampsia (Chappel et al., 1999).

Adam et al. (2001) reported an increased incidence of pre-eclampsia in zinc-deficient regions and it was later found that zinc supplementation reduced the high incidence of the disease. Furthermore, decreased levels of selenium and copper have been observed in patients with pre-eclampsia (Rayman et al., 2003).

In recent times, there has been an increasing prevalence in the incidence of pre-eclampsia globally but there are conflicting reports on the relationship between trace elements and pre-eclampsia. It is hoped that this study will contribute to the knowledge of the role of trace elements in women presented with pre-eclampsia. The aim of this study is to determine the plasma level of selected trace elements on pregnant women presented with pre-eclampsia. We hypothesise that changes in the status of trace element of pregnant women may contribute to the pathogenesis of pre-eclampsia.

MATERIALS AND METHODS

Study design

This was a cross-sectional randomized study designed to investigate the levels of trace elements in pregnant women with pre-eclampsia.

Study population

The study population involve pregnant women attending the Department of Medicine and Antenatal Clinic of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo (Western Nigeria). The pregnant women were selected following medical examination and laboratory test that determined fasting blood glucose level.

Of the 120 pregnant women recruited, 89 pregnant women (comprising 49 test and 40 control groups) participated in the study. Informed consent was obtained from each of the subjects after the study was explained to them. The study received the approval of the ethical committee of Lautech Teaching Hospital, Osogbo.

Exclusion criteria

These include lactating mothers, smoking and alcoholic individuals. Women with acute and chronic illnesses or taking any other medications that could potentially affect levels of trace elements were also excluded.

Blood sample collection

Blood was drawn from the cubital vein using a sterile needle and syringe into an appropriate tube. The samples in plain tubes were allowed to clot undisturbed and serum were separated by centrifugation for 10 min at 4000 rpm into plain tubes and stored at -20°C until time of analysis.

Biochemical analysis

Zinc, copper, selenium, manganese and magnesium were determined by flame atomic absorption spectrophotometry as described by Kaneko (1999).

Principle

Serum levels of the selected trace elements were estimated by atomic absorption spectrophotometry (Kaneko, 1999). 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.

Procedure

Blood (2 ml) was taken from the thawed samples after ensuring thorough mixing, added to a clean 10-ml centrifuge tube and diluted to 10 ml with hydrochloric acid. The diluted blood sample was then centrifuged (30 s, 3000 rev/min) to remove cellular debris and aspirated directly into the flame for analysis and data recording.

Table 1. Comparison between pre-eclampsia and control group.

Parameters	Patients (mean ± SD)	Control (mean ± SD)	"t"-value	P-value
BMI (kg/m ²)	25.3 ± 2.4	24.4 ± 3.09	1.703	P > 0.05
SBP (mmHg)	144.5 ± 3.9	110.4 ± 6.4	31.052	P < 0.05
DBP (mmHg)	93.0 ± 2.9	73.3 ± 6.6	18.742	P < 0.05
Zinc (µmol/L)	8.6 ± 1.4	9.4 ± 0.8	3.215	P < 0.05
Copper (µmol/L)	7.9 ± 1.9	17.4 ± 3.3	17.273	P < 0.001
Selenium (µmol/L)	0.6 ± 0.1	1.3 ± 0.4	12.415	P < 0.001
Manganese (µmol/L)	0.007 ± 0.001	0.017 ± 0.005	12.416	P < 0.001
Magnesium (mmol/L)	0.5 ± 0.2	1.0 ± 0.2	12.919	P < 0.001

SD = Standard deviation; Mmol/ = millimole per litre; Kg/m² = kilogram per meter square; N = total number of patients; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; mmHg = millimetre mercury; N = 40.

Statistical analysis

The statistical package for the social sciences (SPSS) software package was used for statistical analysis and graphical expression by Microsoft Excel software. Values obtained from this study were expressed as mean and standard deviation when compared using the independent t-test. Pearson correlation coefficient was used to measure the level of association between variables. Variables results were regarded as significant at P < 0.05.

RESULTS

Physical parameters

The mean age of all pre-eclampsia patients was not significantly different from those of control subjects (P > 0.05). The mean body mass indexes of the test patients were significantly different from those of control subjects (P < 0.01). There was a significant difference (P < 0.05) between both the systolic and diastolic blood pressures of the test and control groups (Table 1).

Biochemical parameters

The mean serum concentrations of zinc, copper, selenium, manganese and magnesium were significantly lower (P < 0.05) in pre-eclampsia patients in comparison to the control group (Table 1 and Figures 1 – 5).

Pearson product moment correlation analysis was carried out to determine the relationship between variables. There was an inverse relationship between zinc and diastolic blood pressure (r = -0.250, p < 0.01), copper and systolic blood pressure (r = -0.856, p < 0.01), copper and diastolic blood pressure (r = -0.796, p < 0.01), magnesium and systolic blood pressure (r = -0.782, p < 0.01), magnesium and diastolic blood pressure (r = -0.756, p < 0.01), manganese and systolic blood pressure (r = -0.751, p < 0.01), manganese and diastolic blood pressure (r = -0.742, p < 0.01), selenium and systolic blood

pressure (r = -0.752, p < 0.01), selenium and diastolic blood pressure (r = -0.675, p < 0.01) as well as systolic blood pressure and diastolic blood pressure (r = -0.884, p < 0.01).

There was a positive correlation between copper and zinc (r = 0.281, p < 0.01), zinc and selenium (r = 0.258, p < 0.05), zinc and magnesium (r = 0.345, p < 0.01), zinc and manganese (r = 0.301, p < 0.01), copper and selenium (r = 0.702, p < 0.01), copper and magnesium (r = 0.742, p < 0.01), copper and manganese (r = 0.656, p < 0.01), selenium and magnesium (r = 0.656, p < 0.01), selenium and manganese (r = 0.619, p < 0.01), manganese and magnesium (r = 0.645, p < 0.01) as well as systolic blood pressure and diastolic blood pressure (r = 0.884, p < 0.01).

DISCUSSION

Results obtained from this study showed mean systolic blood pressure of 144.3±3.9 mmHg and a diastolic blood pressure of 93.0±2.9 mmHg in pre-eclamptic patients in contrast to a systolic blood pressure of 110.4±6.4 mmHg and a diastolic blood pressure of 73.3±6.6 mmHg in control subjects. This confirms an earlier investigation by Gifford et al. (2000) who reported a systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg. The slight difference in Gifford et al. (2000) result and ours may be due to ethnic differences. The implication of this is that pathogenesis and development of complications may be more severe in pre-eclampsia patients in our environment compared to Caucasians.

We observed a significant decrease in all the trace elements and magnesium in pre-eclamptic women when compared to the control group. This suggests a possible involvement of zinc, copper, selenium, manganese and magnesium in the development and pathogenesis of pre-eclampsia. However, the result must be interpreted with caution. This is because, we did not analyse the dietary intake of pre-eclamptic women to confirm whether the

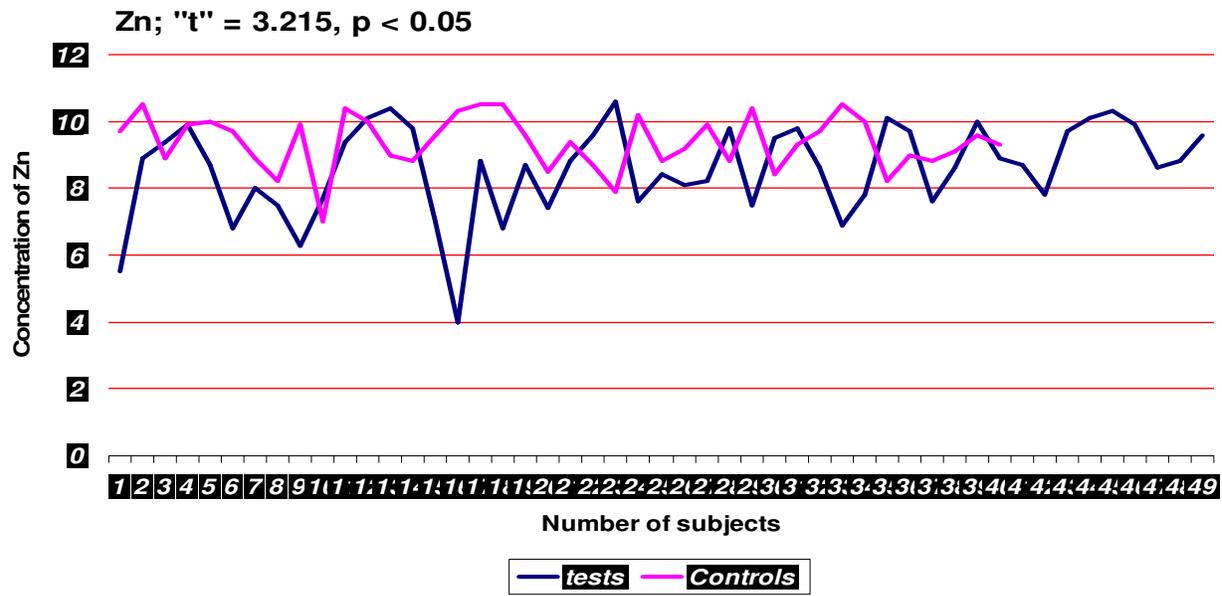


Figure 1. Distribution of zinc in pre-eclampsia and control groups. The zinc level of pre-eclampsia patients was significantly lower (P < 0.05) than the control group.

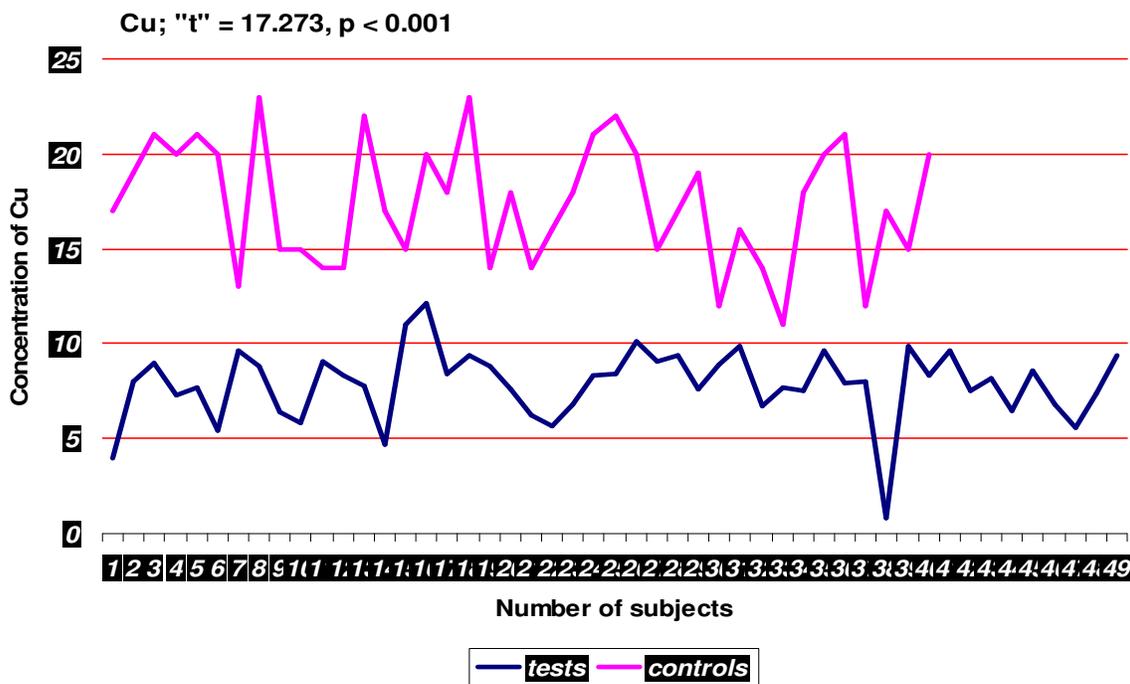


Figure 2. Distribution of copper in pre-eclampsia and control groups. All the pre-eclampsia patients investigated in this study have serum copper levels significantly lower than (P < 0.001) than the control group.

reduced levels of trace elements reported here is due to nutritional deficiencies. However, our data agrees with that of Mahomed et al. (2004) who reported reduced zinc concentration in women with eclampsia and Fawcett and Haxby (1999) who reported a relationship between magnesium deficiency and pre-eclampsia.

Decrease in the level of selenium is known to enhance pre-eclampsia by its resultant effect on glutathione peroxidase. This is important as increased oxidative stress as a result of low density lipoproteins has been confirmed in pre-eclampsia and this complication is strictly regulated by antioxidants of which selenium forms

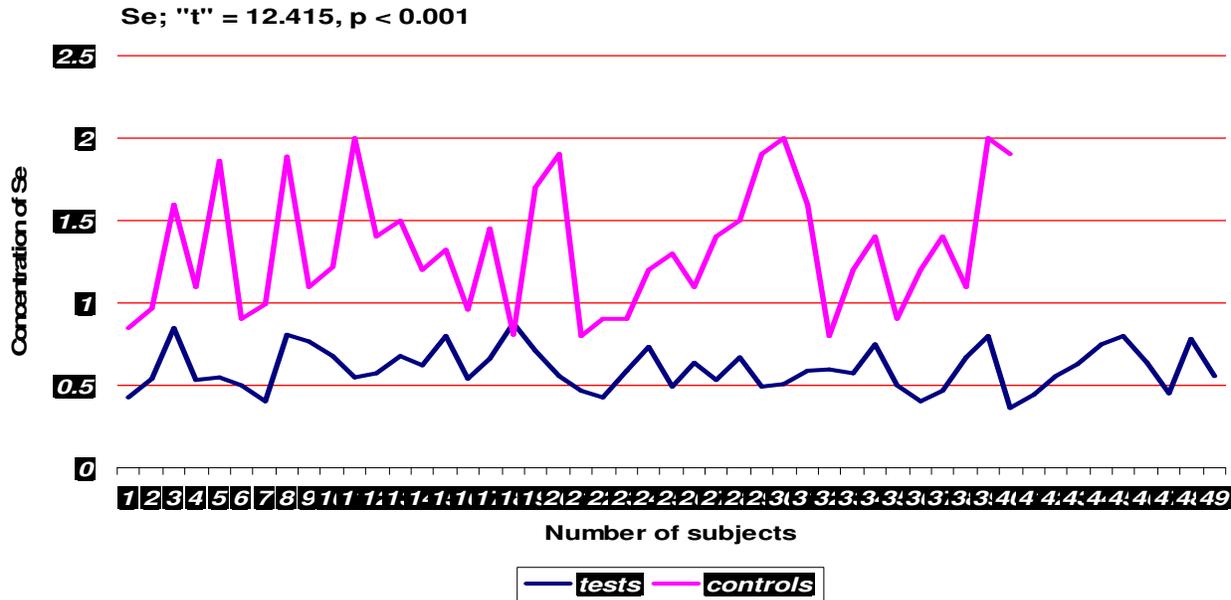


Figure 3. Distribution of selenium in pre-eclampsia and control groups. The distribution of selenium in the pre-eclampsia patients fell within a narrow range which was significantly lower than the control group.

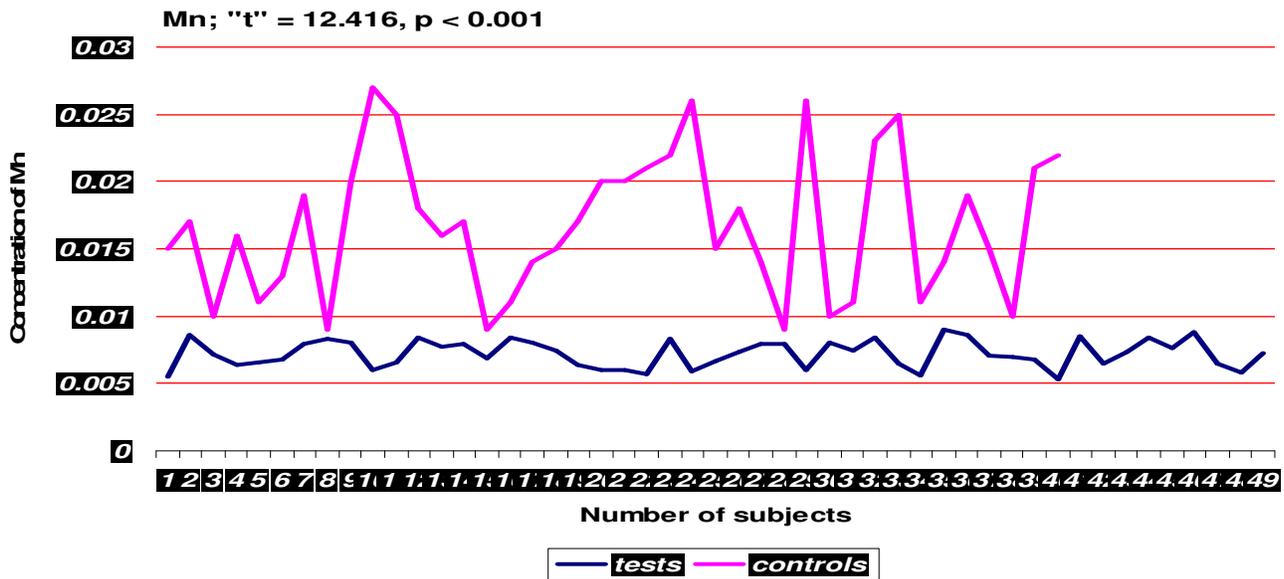


Figure 4. Distribution of manganese in pre-eclampsia and control groups. The distribution of manganese in pre-eclampsia patients fall within a narrow range and shows significantly lower ($P < 0.001$) level when compared to the control group.

a part (Witztum, 2001). Selenium is known to behave as an antioxidant and peroxynitrite scavenger when incorporated into selenoproteins. It is the main element in glutathione peroxidase, an active enzyme in oxidative stress that reduces the formation of free radicals and peroxidation of lipoproteins. The low concentration of selenium in serum exposes the subject to an accumulation of free radicals and its associated pathogenesis of disease such as pre-eclampsia. On the other hand, low concentration of this

element in blood might be an indication of active production of free radical and increased scavenging activity of either selenium or glutathione peroxidase. This study suggests that pre-eclampsia is associated with oxidative stress. This is in consonance with the view of Witztum (2001) who reported that increased oxidative stress as a result of peroxidation of low density lipoproteins is a common phenomenon in pre-eclampsia. Supplementation with this trace element may help reduce the pathogenesis of

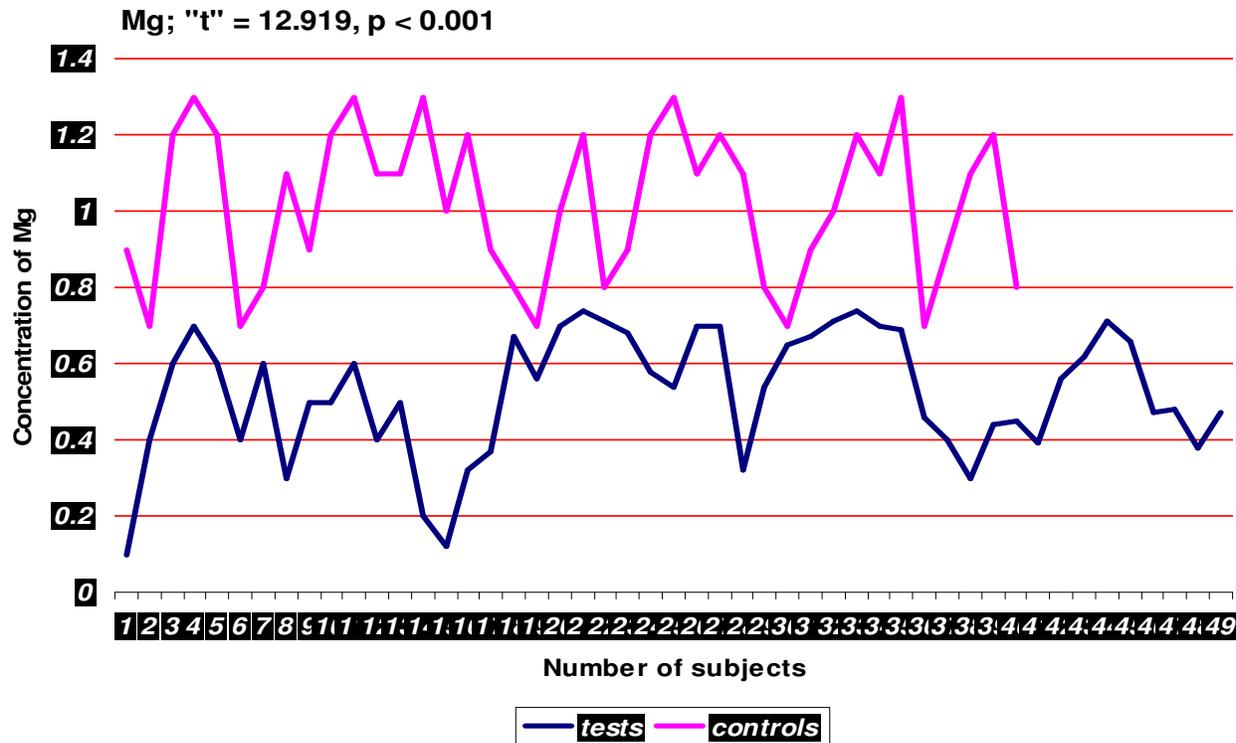


Figure 5. Distribution of magnesium in pre-eclampsia and control groups. All the pre-eclampsia patients investigated in this study have serum magnesium levels significantly lower ($P < 0.001$) than the control group.

this disease and its complications (Rayman et al., 2003). Manganese is found as a free element in nature (often in combination with iron), and in many minerals. It is an important trace element in all forms of life and is a cofactor for a wide range of enzymes including oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins, and integrins. It is also a component of the polypeptide arginase and Mn-containing superoxide dismutase (Mn-SOD) (Begum et al., 2000).

The Mn-SOD enzyme deals with the toxic effect of superoxide, formed from the electron reduction of dioxygen. The low concentration of manganese in serum leads to an accumulation of superoxides which could consequently trigger pre-eclampsia and its complications (Mahomed et al., 2004; Hofmeyr et al., 2007; Lou et al., 2008). A distinct involvement of low serum manganese concentration in the pathogenesis of pre-eclampsia is in the impairment of endothelial function. Arginine which is the precursor of the key determinant of endothelial function, nitric oxide, due to its function in the increase of smooth muscle relaxation, endothelial cell proliferation, decrease in endothelin-1-release, leukocyte adhesion, platelet aggregation, superoxide production, expression of monocytes chemotactic peptides, proliferation of smooth muscle cells and endothelial cell apoptosis contains manganese as an active component (Lou et al., 2008). Thus, reduction in serum manganese concentration in the blood of pre-eclamptic pregnant women as reported in the

current study may be more of a cause than a resultant effect. Supplementation with this trace element may boost the management of pre-eclampsia and thus reduce its complications.

Zinc, an important trace element has been shown to function as a co-factor for the synthesis of a number of enzymes, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Magri et al., 2003; Kumru et al., 2003). Zinc deficiency has been associated with complications of pregnancy part of which is pre-eclampsia. During pregnancy, there is a decline in circulating zinc and this increases as the pregnancy progresses possibly due to decrease in zinc binding and increased transfer of zinc from the mother to the foetus (Tamura et al., 2000). This study showed a decrease in the serum zinc concentration of pre-eclampsia patients compared to the controls and this supports the report of Mahomed et al. (2004) on an association between zinc and pre-eclampsia although attempts to modify the frequency of pre-eclampsia with zinc supplementation has not been successful.

Magnesium has established its role in obstetrics with its relationship to both foetal and maternal wellbeing. The low concentration of magnesium in serum exposes the subject to a risk of pregnancy complications which includes pre-eclampsia. This is usually due to a defect in an enzymatic process which occurs as a result of low circulating magnesium to function as a co-factor. We report a decrease in serum magnesium in pregnant

women with pre-eclampsia. This is in accordance with the view of Lu and Nightingale (2000) who reported a reduced mortality rate from 16% to 8% in Bangladesh as a result of the introduction of a low dose of magnesium sulphate in pre-eclampsia patients. Thus, the success of magnesium therapy as a treatment for eclamptic seizures and the known effect of magnesium on vascular responses *in vitro* suggest that magnesium might be deficient in women with pre-eclampsia.

Copper has been shown to be involved in the function of several cuproenzymes that are essential for life. Cerruloplasmin contain copper which catalyses the conversion of ferric ion to its ferrous form and favours the absorption of iron from the gastro-intestinal tract. It also plays a role in the mobilization of iron to plasma from the tissue stores (Ziael et al., 2008). Our findings indicated a decrease in the serum concentration of copper in pre-eclamptic patients when compared to control subjects. Previous studies have related copper to foetal pregnancy complications like birth defects than to maternal pregnancy complications like pre-eclampsia. Studies conducted in the developed nations have documented a significant correlation between low copper in drinking water and the occurrence of neural tube defects, with the implication being that a primary deficiency of copper could result in birth defects in humans (Ziael et al., 2008).

The importance of the deficiencies of trace elements in pre-eclampsia relates to the fact that they are present in metalloprotein (zinc), ceruloplasmin (copper), superoxide dismutases (copper, selenium and zinc) and glutathione peroxidase (selenium). Unavailability of this element due to deficiency or decrease concentration may be a predisposing factor in the development of pre-eclampsia or a contributing factor in its pathogenesis.

Conclusion

This study established that the levels of zinc, copper, selenium, manganese and magnesium are significantly altered in pregnant women with pre-eclampsia. In the light of the reduction in the concentration of these elements, dietary supplementation or direct replacement therapy of these trace elements is suggested for women with pre-eclampsia.

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