Assessment of Trace Elements, Total White Blood Cells and Platelets Count in Pregnant Ladies with Preeclampsia in Sudan

Akram Elkhair Noor Eldaem¹, Salah Ismael², Elfadil AL ObeidOmer³
1Omdurman maternity hospital, 2 Faculty of Medicine- Omdurman Islamic University, 3 Faculty of Medical laboratory sciences University of Al Neelain Khartoum, Sudan.

Abstract

Preeclampsia is a multisystem disorder of pregnancy, which complicates pregnancy. Maternal mortality with pre-eclampsia/eclampsia in Sudan is ascertained and accounts for 4.2% of the obstetric complications in Sudan. Despite decades of research into the condition, predicting which women are at increased risk of developing preeclampsia remains problematic and we did this study to detect the role of some elements and to correlate them with Preeclampsia. Pregnant ladies with preeclampsia were selected as cases, and age matched with normal pregnant ladies as controls. Venous blood samples were collected from every patients and controls. Trace elements, copper, zinc, and iron were measured. Total white blood cells and platelets were counted also. Levels of copper and zinc were low in patients while they were normal in controls. Iron was normal, total white blood cells were high in patients and platelets were low. The reduction in serum levels of zinc and copper during pregnancy might be possible contributor in etiology of pre-eclampsia.

Keywords: Preeclampsia, Trace elements, Total white blood cells, Platelets.

Introduction

Maternal mortality is high in Sudan with pre-eclampsia/eclampsia which accounts for 4.2% of the obstetric complications in Sudan and mainly in Kassala, Eastern Sudan and represents 18.1% of the direct causes of maternal deaths [¹, ²]. The cause of preeclampsia remains unknown, and the only known cure is delivery of the foetus and placenta. Despite decades of research into the condition, predicting which women are at high risk of developing preeclampsia remains problematic. Furthermore, predicting preeclampsia in women with underlying conditions such as diabetes and chronic hypertension would be of great clinical value. The problem with using these risk factors is that millions of women worldwide have these risk factors but do not develop preeclampsia. Moreover, the majority of them are no modifiable. 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 [³,⁴], the origin of the
condition is recognized as lying in the placenta. This is known to be the case because preeclampsia occurs only in the presence of pregnancy [5], it resolves after delivery of the placenta and it can occur in the absence of a viable foetus, for example, in molar pregnancies [6]. Placental development is a closely regulated process which is essential for normal foetal development and for maintaining a successful pregnancy. Blood supply to the placenta is via the spiral arteries, which, in turn, are branches of the uterine arteries. Early in normal pregnancy, the cytotrophoblastic cells of the developing placenta invade the uterine wall, disrupting the endothelium and tunica media of the spiral arteries [5, 6].

Eclampsia has been found to have more impact in developing countries where pregnant women have been reported to consume diets with lesser amounts of essential minerals and vitamins [7]. The possible role of various nutrient elements like protein, lipids, calcium, magnesium, zinc, and copper have been emphasized in pre-eclampsia [8, 9, 10].

Zinc is a trace element involved in a variety of biochemical pathways to modulate various functions in the body as it is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division [11]. Zinc also supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell [12]. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system. Alteration in zinc homeostasis might have a devastating effect on pregnancy outcome [13, 14]. Several reports had suggested that zinc deficiency may be associated with increased incidence of pre-eclampsia [15, 16].

Copper is an important trace element which takes parts in structure of many enzymes like lysyl oxidase, cytochrome oxidase, tyrosinase, dopamine-β-hydroxylase, peptidylglycine alpha-a mediating monooxygenase, monoamine oxidase, ceruloplasmin, and copper–zinc superoxide dismutase. Many different studies have shown that there is an association between occurrence of preeclampsia and trace elements, while some other studies have not shown such association [17]. Copper can produce the highly reactive hydroxyl radical. The generation of this radical can begin lipid peroxidation process which may cause endothelial cell damage [18]. Many studies have shown that copper concentration increased in preeclampsia patients [19].

The elevated serum iron levels are due to hemolysis caused by physical destruction of RBC as a result of vasosapam or abnormal endothelial cell erythrocyte interactions. Excess iron is a causative factor of oxidative stress (i.e., in its radical form) involved in the pathogenesis of pre eclampsia [20, 21]. In addition to this the damaged placenta is a
site for release of free radicals (FR) in pre eclampsia. The elevation or excess iron can also react with these released FR of placenta and can initiate and propagate lipid peroxidation both in placenta and vasculature. This is one of the significant etiologic factors in the endothelial cell damage of pre eclampsia [22]. The doubling of percent transferrin saturation is due to raised serum iron and decreased serum transferrin levels.

Leukocytosis occurs during normal pregnancy and leukocyte activation plays a significant role during the disease process in preeclampsia [23]. Activated leukocytes release a variety of substances such as cytokine interleukin-8 and tumor necrosis factor-α, which are capable of mediating endothelial function. Interactions between activated leukocytes, platelets, and vascular endothelium are believed to contribute to the vascular injury in preeclampsia. Furthermore, neutrophil activation is believed to be a major component of exaggerated inflammatory responses in the maternal vascular system during preeclampsia [23]. However, reports on leukocyte count and differentials associated with the severity of preeclampsia are scarce. So the current study was conducted to assess serum levels of zinc, copper, total white blood and platelets count in preeclampsia patients compared to normal pregnant ladies.

Materials and Methods
The study was case-control laboratory based study, presented to the Ethical Committee at Al Neelain University for ethical clearance. Patients were consented before their enrolment. Pregnant women were selected, their age was between 18-35 years old and gestational age was from 30-40 weeks. Preeclampsia was diagnosed as a blood pressure 140/90 mm Hg or more on two or more occasions at their follow up. The patients with any chronic illness, diabetes mellitus or other endocrine disorder, renal disease, and other secondary causes of hypertension, malignancy, etc., which were likely to affect the serum level of our measured parameters were excluded from the study. Two hundred venous blood samples were collected from pregnant women. Hundred samples were from patients having proteinurea, and hundred from healthy pregnant females. Each sample was divided in to two parts, one was left to be clotted in plain container, and then the serum was separated for the assays of copper, zinc, and iron, which were carried out by using atomic absorption spectrophotometer by deproteinization with trichloroacetic acid and standards. The other part of the sample was dispensed in EDTA anticoagulant container for total white blood cells and platelets count which was done by SYMEX KX 21 N automatic analyzer.

Results
The assays of copper, zinc, and iron estimation were significantly varied between patients and controls (Table 1-Figures 1). Serum level of copper and zinc were low in patients compared to controls. P values less than 0.05. Surprisingly iron was significantly more in patients than in controls with a P value equals 0.05.

Table (1). The measured parameters, the mean of concentration in patients, the mean of concentration in controls, and reference values of the parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean in patients</th>
<th>Mean in controls</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>84.82653</td>
<td>135.435</td>
<td>70 – 160 µg/dl</td>
</tr>
<tr>
<td>Zinc</td>
<td>2.71665</td>
<td>15.1752</td>
<td>10.7 – 24.5 mol/l</td>
</tr>
<tr>
<td>Iron</td>
<td>105.928</td>
<td>91.835</td>
<td>45 – 150 µg/dl</td>
</tr>
<tr>
<td>TWBCs</td>
<td>10.152</td>
<td>6.48</td>
<td>4.5 – 10 X103/µL</td>
</tr>
<tr>
<td>Platelets</td>
<td>163.3838</td>
<td>385.8586</td>
<td>150 – 450 X103/µL</td>
</tr>
</tbody>
</table>

Total white blood cells count was high in patients compared to the normal count in controls, while platelets count was found to be low in patients compared to controls which they were found to be normal, P values less than 0.05 (Table 1-figure2).
Figure (2) a: Copper concentration (µg/dl) in the serum samples of preeclampsia patients and healthy controls. The error bars represent mean ± standard deviation (s.d.). P = 0.039. b: Zinc concentration (µMOL/L) in preeclampsia patients and healthy controls. P = 0.031. c: Iron concentration (µg/dl) in preeclampsia patients and healthy controls. P = 0.05.
Figure (1) The error bars represent mean ± standard deviation (s.d.). \( P \leq 0.05. \) \( a \): Copper concentration (µg/dl) in the serum samples of preeclampsia patients and controls. \( b \): Zinc concentration (µMOL/L) in preeclampsia patients and controls. \( c \): Iron concentration (µg/dl) in preeclampsia patients and controls. **Figure (2)** The error bars represent mean ± standard deviation (s.d.). \( P \leq 0.05. \) \( a \): Total white blood cells count (Cell X10\(^3\)/µL) in the serum samples of preeclampsia patients and controls. \( b \): Platelets count (Cell X10\(^3\)/µL) in preeclampsia patients and controls.

**Discussion**

A large number of novel biomarkers that reflect a broad range of pathological events involved in the progression of preeclampsia. Procedures for risk assessment of chemical mixtures, combined and cumulative exposures are under development, but the scientific database needs considerable expansion.

In particular, there is a lack of knowledge on how to monitor effects of complex exposures, and there are few reviews on biomonitoring complex exposures. Many of these biomarkers, alone or in combination, can play important role in prediction of risks, its types, and status of morbidity. As emerging risks are found to be affiliated with minor and micro level factors and its diagnosis at an earlier stage could find preeclampsia, hence, there is an urgent need of new more authentic, appropriate, and reliable diagnostic and therapeutic markers to confirm disease well in time to start the clinical aid to the patients.

In this study we assessed the role of trace element; we measured the concentrations of copper, zinc, and iron. Normally there was a decrease in serum iron and ferritin during the third trimester of pregnancy as their stores of iron are depleted because of fetoplacental demand and required expansion of red cell mass [24, 25]. However, elevated level of serum iron is observed in pre-eclamptic as compared to normal pregnant women, and this is
supported by [26, 27, 28, 29]. Local iron excess and iron mediated oxidative stress have been demonstrated in the intestinal mucosa, liver spleen, bone marrow and placenta and the production of hydroxyl and methoxyl radicals in both the luminal and mucosal contents of the gastrointestinal tract verify the role of iron in free radical damage [30, 31].

In contrast to our study Sarwar et al [32], found a low iron, but it could be due to a random selection of study population and mismatched inclusion criteria but they agree with our results in zinc and copper concentrations which tend to decrease in patients compared to controls. Regarding zinc and copper extra supportive reports also were reviewed and in parallel with our results [27, 33, 34, 35, 36]. Zinc is required for the proper functioning of enzymes like superoxide dismutase, which is required for scavenging free radicals. Deficient concentrations of these elements during pregnancy may cause impairment of antioxidant potential of cells by decreasing superoxide dismutase activity, as well as increased lipid peroxidation [37], leading to increase in blood pressure [38]. Copper level was also found to decrease in preeclamptic group compared to controls. Hypozincemia is related to hemodilution, increased urinary excretion and the transfer of this mineral from mother to the growing fetus. In pregnant women with preeclampsia, low serum zinc may be partly due to reduced concentrations of transport proteins and estrogen caused by increased lipid peroxidation [39], this leads us to hypothesize that zinc may play a role in preeclampsia through an increase of lipid peroxidation. In contrast to our findings, Ohad et al reported higher levels of Cu and Zn in preeclampsia cases [40]. These results indicate that reduction in serum levels of zinc during pregnancy might be possible contributors in aetiology of preeclampsia, and supplementation of these elements to diet may be of value to prevent preeclampsia. Intake of supplements may help in the reduction of incidence of preeclampsia especially in a population of a developing country like ours where the nutrition is poor.

Preeclampsia evokes inflammatory response so we measured the count of total white blood cells to detect the proliferation of cells. Leukocyte count was higher in females with moderate to severe preeclampsia compared to normotensive ones. Pregnancy induced hypertension is a multisystem disorder and may involve inflammatory response [41]. Thus higher leukocyte count could be one of the clinical manifestations of preeclampsia. Our results are in concordance with earlier findings [41, 42, 43]. However, Sivakumar S et al have not observed any difference in total leukocyte count and differential neutrophil count in pregnancy induced hypertensive mothers compared to normotensive mothers [44]. Platelets were found to be low in preeclamptic females compared to controls and this is in consistency with previous
reports [45, 46, 47]. This result could reflect platelet consumption and could be used as an indicator of poor maternal progress in preeclamptic cases. Not many studies have been done in developing countries to assess the role of these elements in pre-eclampsia.

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