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Oxidative stress as a significant contributor in the pathogenesis and white cell changes in pre-eclampsia: A study in Owerri, Nigeria.

Authors:
[1,2]Alisi P. Nc,
[1]Buseri FI and
[3]Alisi CS.

Institution:
[1]Department of medical Laboratory Science Rivers State University of Science and Technology Port Harcourt, Nigeria

[2] Federal medical centre PMB 1010 Owerri, Nigeria

[3]Department of Biochemistry Federal University of Technology PMB 1526 Owerri.

Corresponding author:
Alisi, Precious. Nc.

ABSTRACT:

Haeomatological and oxidative stress parameters were measured in Pre-eclamptic [PE] women attending clinic in owerri South Eastern Nigeria. The effect of Pre-eclampsia on these parameters was assessed by juxtaposition with similar indices in normal pregnant and normal non-pregnant women. Result showed that pre-eclampia resulted in significant decrease (P< 0.05) in Lymphocyte count. There was a significant increase in total white blood cell count, neutrophil count and mixed differential count. Pre-eclamptic resulted in significant increase (P<0.05) in erythrocyte lipid peroxidation, met-haemoglobin concentration and percentage hydrogen peroxide-induced haemolysis. The result indicate that oxidative stress is a significant contributor in the pathogenesis and white cell changes seen in pre-eclampsia.

Keywords:
Pre-eclampsia, oxidative stress,met-haemoglobin, lipid peroxidation.

Email:
preshngo.alisi@yahoo.com

Phone No:
+2348060966075

Web Address:
http://jresearchbiology.com/Documents/RA0033.pdf.

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INTRODUCTION

Pre-eclampsia is a pregnancy complication recognized by new-onset gestational hypertension and proteinuria. The disorder affects both mother and their infants. Once the disease is evident clinically, it can be cured only by delivery. The indicated delivery of women to prevent the progression of pre-eclampsia is responsible for 15% of all preterm births (Golderberg and Rouse, 1998). The cause of pre-eclampsia remains largely unknown, but poor placentation is an important predisposing factor. A shallowly implanted placenta which becomes hypoxic, leading to an immune reaction characterized by secretion of up-regulated inflammatory mediators from the placenta, and acting on the vascular endothelium. The shallow implantation is thought to stem from the maternal immune system's response to the placenta. Not all women with reduced placental perfusion develop pre-eclampsia. Other conditions recognized to increase the risk of pre-eclampsia include obesity (Sibai *et al*., 1995), hypertension (Caritis *et al*., 1998), diabetes (Caritis *et al*., 1998), hyperhomocysteinemia (Power *et al*., 2001), increased androgens (Laivuori *et al*., 1998), and black race (Eskenazi *et al*., 1993). Of course, these are all risk factors for cardiovascular disease in later life. Appreciation that pre-eclampsia is a multi-systemic syndrome characterized by vasoconstriction, metabolic changes, endothelial dysfunction, activation of the coagulation cascade, and increased inflammatory response, to mention only some of the organ systems involved, has redirected research (Roberts *et al*., 2003). As a result, progress in understanding the disorder has accelerated greatly with attendant optimism for potentially effective treatments (Roberts *et al*., 2005). Pre-eclampsia is associated with haematological complications. Altered erythrocyte aggregation has been observed in severe pre-eclampsia than in normal pregnancy (Sengupta, 1995). In some other stress associated diseases there has also been dramatic alteration in neutrophil and monocyte values but this has not been investigated in PE (Oishi *et al*., 1999).

It has also been documented that PE is accompanied by oxidative stress that contributes to vascular dysfunction (Shaarawy *et al*., 1998). In the present paper we studied systemic oxidative stress *vis-a-vis* pre-eclampsia by assessing lipid peroxidation, hydrogen peroxide induced haemolysis, methaemoglobin concentration and some haematological parameters in patients of pre-eclampsia.

MATERIALS AND METHODS

Study design

It was a cross sectional case control study conducted prospectively among antenatal women attending clinic at Holy Rosary, Federal Medical Centre and General Hospitals Owerri. The study included fifty non-pregnant, fifty PE and fifty normotensive pregnant women of singleton gestation in their third trimester.

Selection criteria

The subjects were selected under defined criteria. PE patients were at 28 to 42 wks of single-diastolic pressure of 110mm hg or more, or two measurements of 90mmhg or more on two consecutive occasions of 6 hours or more apart, urinary protein 2+ or more. The exclusion criteria include history of hypertension and proteinuria before conception or before 20wks of gestation, a history of antioxidant vitamins therapy during the last one year and smoking.

As cohort control, age and socio-economically matched healthy normotensives at 28 to 42 wks of singleton gestation with no urinary protein, were recruited by convenience. The non-pregnant controls were consisted of 50 healthy normotensive subjects. They were matched by group percent of age, education and income. Ethical clearances were obtained from the Heads of the three hospitals involved.

Blood Collection

4mls of venous blood was collected from each case and control subjects and added into a bottle (8ml) containing 80µl of ethylene diamine tetra-acetic acid (EDTA). Samples were used for the various analyses. Analysis was done within 1hour of blood collection. The full blood count was done with the Erma automatic multi-parameter blood cell counter for in vitro diagnostic use in clinical laboratories.

Measurement of Hydrogen peroxide (H_2O_2) induced haemolysis

Equal volumes of blood were mixed with H_2O_2 and the fraction of red cells lysed is determined spectrophotometrically as reported by Liu *et al*., 1990. Briefly, A 5% suspension of washed, packed erythrocytes in buffer saline was mixed with the same volume of 1% H_2O_2 solution and the final mixture was incubated at 37^{o}C for 2 hrs. At the end of incubation the extent of haemolysis was determined by measuring released haemoglobin into the supernatant by absorbance measurement in a spectrophotometer (Turner Model390) at 540nm and was expressed on the

basis of maximum absorbance (100%) in the aliquots of erythrocyte completely haemolysed in distilled water.

Measurement of Methaemoglobin (Hi) **in blood**

Methaemoglobin has a maximum absorption at 630nm. When cyanide is added this absorption band disappears and the resulting change in absorbance is directly proportional to the concentration of Hi. Total Haemoglobin in the sample is then measured after complete conversion to HiCN by the addition of ferricyanide-cyanide reagent using the method of Evelyn and Malloy (1938).

Briefly, 0.2mls of blood was lysed in a solution containing 4ml of buffer and 6ml of nonidet P40. The lysate was divided into two equal volumes (A and B). The absorbance of A was measured in a spectrophotometer at 630nm (D_1). 1 drop of KCN solution was added and absorbance was measured after mixing (D_2). 1 drop of $K_3Fe(CN)_6$ was added to solution B and after 5mins absorbance was measured at the same wavelength (D_3). Then 1 drop of KCN was added to solution to B and absorbance was measured after mixing (D_4). Measurements were made against a blank containing buffer and detergent in the same proportion as present in the sample. % methaemoglobin concentration was calculated thus:

$$\% \text{ Methaemoglobin} = \frac{D_1 - D_2}{D_3 - D_4} \text{ C } 100$$

Measurement of Lipid Peroxidation

Lipid peroxidation in the erythrocyte was determined spectrophotometerically by assessing the level of thiobarbituric acid reactive substance (TBARS) as described by Liu et al. (1990). Absorbance was measured at 532 nm using a spectrophotometer to know Malonyldialdehyde (MDA) an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen–thiobarbituric acid reactive substance. Calculations were made using a molar extinction coefficient of 1.56×10^5M−1 cm−1and expressed as micromoles /l.

Statistical Analysis

Data obtained from the study were analyzed by the use of two-way analysis of variance (ANOVA) and values for $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Pre-eclampsia is associated with a five-fold increase in prenatal mortality and its socioeconomic impact on developing countries is huge, its pathophysiological changes include elevated systemic vascular resistance, generalized vasoconstriction, and activation of the coagulation cascade, all of which may be explained by disruption of normal maternal endothelial function (López-Jaramillo *et al.,* 2001).

Effect of pre-eclampsia on hydrogen peroxide induced haemolysis in erythrocytes (figure 1), shows that erythrocytes from pre-eclamptic patients underwent more lysis than erythrocytes from the normal pregnant control (PC) and the non-pregnant control (NPC). It is known that the increased lysis results from oxidative damage to the erythrocyte membrane, causing a decrease in membrane fluidity and reducing its ability to withstand osmotic changes. Hydrogen peroxide participates in the Haber–Weiss reaction: $O_2\bullet^- + H_2O_2 \rightarrow O_2 + \bullet OH + OH^-$. Increase in the H_2O_2 induced haemolysis in pre-eclampsia could indicate a lesser ability to neutralize hydrogen peroxide ($H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$). Our observation could mean that there is a decreased antioxidant capacity in pre-eclampsia. Increase in osmotic lysis have been reported in pre-eclampsia in the united kingdom and the Netherland (Courinne et al., 1998; Armutcu, 2005).

Excessive formation of methaemoglobin was seen in the erythrocytes of the preclamptic patient (figure 2) in comparison with the control groups. This could be as a result of the oxidation of erythrocyte protein (haemoglobin) which leads to accumulation of methaemoglobin. The above is similar to accumulation of methaemoglobin seen in

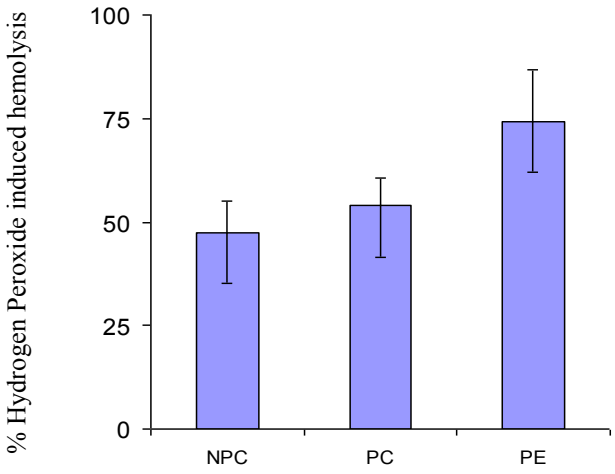

Figure 1: Effect of pre-eclampsia on hydrogen peroxide-induced haemolysis

oxidative damage by drugs and chemical agents. Increases observed in methaemoglobin concentration in pregnancy and pre-eclampsia implied a shift in pro-oxidant/antioxidant equilibrium in favour of the pro-oxidant, resulting in stress. Increased concentration has been postulated in diseases associated with oxidative stress (Dacie and Lewis 2006).

In this study, erythrocyte lipid peroxidation (LPO) as seen from the malondialdehyde concentration were significantly higher in the blood of pre-eclamptic women (figure 3) than the controls. However, the RBC LPO in the pregnant control was also higher than the non-pregnant control. This shows that during uncomplicated pregnancy, there is an increased production of pro-oxidants that is balanced by the synthesis of antioxidants (Scholl *et al.,* 2005). Sëimsë *et al.,* (1998) also reported that serum lipid peroxide concentrations in pregnant subjects were significantly higher than those in non-pregnant subjects. The significant variation seen in the pre-eclamptic group and pregnant control is suggestive that pre-eclampsia resulted from an imbalance between pro-oxidant production and probably the antioxidant defenses. This imbalance could contribute to vascular dysfunction (Shaarawy *et al*., 1998). The nature and extent of oxidative injury contributing to vascular dysfunction would depend on the concentration of free radicals/ reactive oxygen species. Increases in lipid peroxidation products like malondialdehyde correlates directly with increased imbalance in pro-oxidant/antioxidant concentrations.

The total white cell count (table 1) in pre-eclamptic women was significantly elevated than the NPC. A significant increase in total white cell count was also found in PE when compared to PC women. This observation implied that pre-eclampsia further exacerbated leukocytosis as pregnancy is universally known to induce leukocytosis. This is in agreement with Balloch and Cauchi (1993). Schuiling *et al.,* (2000) found an increased activated white blood cell in preeclampsia than in normal pregnancy, while Sargent *et al.,* (1998) found an increase in activated white blood cells both in normal pregnancy and preeclampsia which was more in preclampisa resembling sepsis condition. On the other hand Ceyhan *et al.,* (2006) and Heilman *et al.,* (2004) recorded in their study an increase in total white cell count in preeclampsia that was not statistically significant. Correlation has been found to exist between stress conditions and higher total white cell count (Allsop *et al* 1992). This could lead one to say that white blood cells are activated in pre-eclampsia and that pre-eclampsia may be partly an inflammatory disorder since the activation of white cells were similar to that seen in inflammatory disorder (Sargent *et al.,*1998).

The percentage lymphocyte in pre-eclamptic women was significantly decreased when compared to the normal pregnant controls. The result of this study agreed with reports from Israel (Lurie *et al*., 1998) but reports from Louisiana (Canzoneri *et al*., 2009) was different, no statistical significant difference was seen in lymphocyte between the PE and normal PC. The variation in this parameter could come from the increase in the percentage

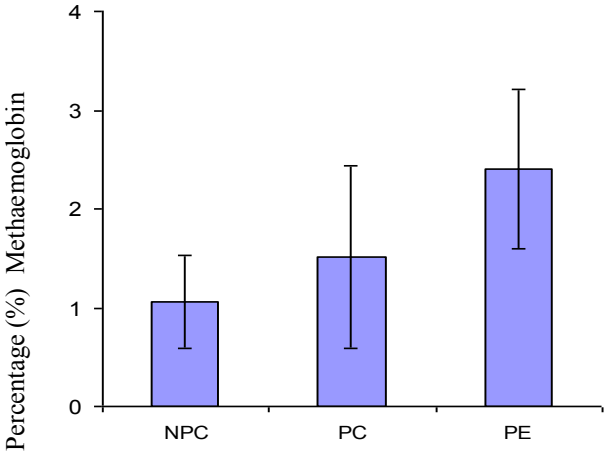

Figure2: Effect of Pre-eclampsia on Methaemoglobin concentration in Humans

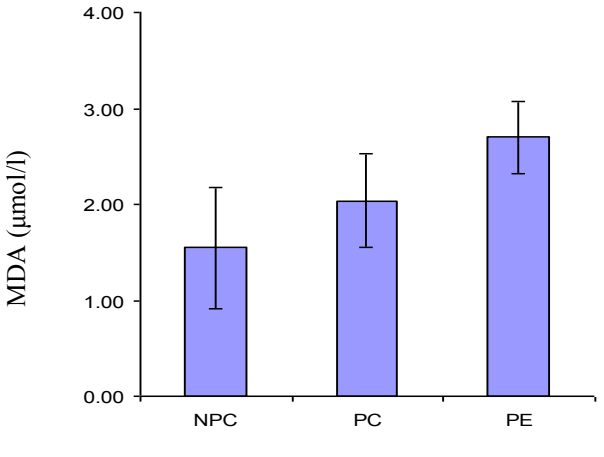

Figure3: Effect of Pre-eclampsia on Lipid peroxidation

Table 1: **Effect of pre-eclampsia on white blood cell parameters**

	Non pregnant controls	Pregnant controls	Pre-eclampsia	P-value
Total White Blood Cell ($x10^9$/L)	7.57 ± 3.06	15.09± 2.40	17.56 ± 2.18	3.48E-43***
Neutrophils (%)	46.08 ± 7.82	63.44 ± 7.01	71.46 ± 7.53	3.12E-27***
Lymphocytes (%)	42.78 ± 8.25	26.71±13.72	14.25 ± 8.49	7.18E-27***
Mixed Differential count (%)	11.14 ± 5.10	9.85± 6.68	14.29 ± 3.50	0.00406**
Absolute lymphocyte count	3.24 ± 1.57	4.03 ± 2.13	2.52 ± 1.56	5.64E-7***
Absolute Neutrophil count	3.73 ± 2.09	9.61 ± 2.05	12.52 ± 1.92	2.72E-30***
Absolute Mixed Differential count	0.86 ± 0.53	1.49 ± 1.04	2.57 ± 0.79	4.93E-10***

neutrophil in pregnancy**.**

The percentage neutrophil count in pre-eclamptic women was significantly elevated when compared to the normal pregnant controls. This finding agrees with the observations of Canzoneri *et al*., (2009) and Lurie *et al*., (1998) who are of the opinion that leukocytosis seen in PE could be due to an increase in neutrophil count. This observation could imply that pregnancy causes neutrophilia. Roberts and Gammill (2005) had opined that endothelial activation could be one component of a generalized activation of inflammatory responses that is characteristic of pregnancy. This could be further accentuated in pre-eclampsia. Neutrophilia is seen in inflammation especially neutrophils with increased granules and staining density (toxic granulation). Neutrophils, are known to produce reactive oxygen species (ROS), and may play a role in mediating endothelial damage in women with pre -eclampsia.

The mixed differential count of this study was significantly raised in pre-eclamptic group when compared with pregnant control. Lurie *et al*., (1998) used a five part autoanalyzer that distinguished the different white cells and got a significant decrease in eosinophil between the PE and PC but found no significant difference in their monocyte and basophil. Canzoneri *et al.,* (2009) also found no significant difference in monocyte between PE and PC. Although the reason for the result is not known but increases in the mixed differential count could mean increase in precursors of white blood cells.

In conclusion, oxidative stress is a significant contributor in the pathogenesis and white blood cell changes seen in pre-eclampia. It is suggested that the antenatal routine diagnosis should be expanded to accommodate oxidative stress parameters including erythrocyte lipid peroxidation, met-haemoglobin concentration and percentage hydrogen peroxide induced haemolysis.

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