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Original Research Article

A Comparative Study of Oxidative Stress in Preeclampsia and Normal Pregnancy

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Abstract

Objectives: Pre-eclampsia is a human pregnancy-specific disorder. It is an important cause of maternal mortality in Sokoto, Northwestern Nigeria and is associated with a five-fold increase in perinatal mortality. Identifying modifiable factors to decrease oxidative stress in the pregnant woman's environment may be an inexpensive and non-invasive therapy for decreasing the maternal and foetal morbidity and mortality associated with preeclampsia. This study aimed at comparing the levels of 3 oxidative stress markers; glutathione peroxidase (GPX), superoxide dismutase (SOD), and malondialdehyde (MDA) and 4 antioxidants (Catalase, Vitamins A, C and E) in normotensive and preeclamptic pregnant women in Sokoto. ***Methodology:*** This was a prospective cross-sectional study of 199 normotensive and 201 preeclamptic women attending the antenatal clinics or admitted to the pre-eclamptic/eclamptic wards of Usmanu Danfodiyo University Teaching Hospital, Sokoto or Specialist Hospital Sokoto. Structured questionnaires were administered and relevant information obtained. Blood samples were obtained by standard laboratory techniques and assayed for the oxidative stress markers and antioxidants. Statistical analysis was by GraphPad InStat Software (version 3.0) San Diego, USA. ***Results:*** The mean levels of oxidative stress markers MDA and GPX were increased in the preeclamptic women (3.44±1.25 and 71.53 ± 26.02) when compared to controls (3.024±1.08 and 62.58 ± 22.45) respectively (p=0.00) while SOD was decreased in the cases (13.00±15.27) when compared to the controls (57.21 ± 38.08) p=0.00. The antioxidant Vitamins A and C were significantly decreased in the preeclamptic women (1.52±1.68 and 0.14±1.33) p=0.00 while Catalase was increased (50.93±36.22; p=0.00). ***Conclusion:*** The results of this study are in agreement with most previous studies which showed that markers of lipid peroxidation were increased in the plasma of women with preeclampsia. Follow-up studies, are needed to enable us arrive at the certain conclusion that interventions with antioxidants and vitamins may be the panacea to this disease in our sub-region.

Keywords: Preeclampsia, Oxidative stress, maternal mortality, Sokoto.

INTRODUCTION

Pre-eclampsia is a human pregnancy-specific disorder characterized by hypertension and proteinuria that remits after delivery [1]. Although its exact etiology is unknown, maternal symptoms are thought to be secondary to endothelial cell dysfunction [2]. The incidence of preeclampsia is between 3% and 10% of pregnancies, and there is no evidence that this has changed appreciably during the last century. The syndrome is polymorphic in that virtually every organ system can be affected. Preeclampsia is characterized by vasospasm, increased peripheral vascular resistance, and thus reduced organ perfusion [1]. It adversely affects the mother (by vascular dysfunction) and the fetus (by intrauterine growth restriction)[2]. Preeclampsia is diagnosed by the new development of hypertension (usually ≤140/90 mm Hg), significant proteinuria, and remission of these signs after delivery. Preeclampsia/eclampsia is the leading cause of maternal mortality in developed countries and is associated with a five-fold increase in perinatal mortality [2].

Free radicals are thought to be likely promoters of maternal vascular malfunction, as reactive oxygen species, particularly superoxide anions, evoke endothelial cell activation [3]. Markers of lipid peroxidation have been noted to be increased in the plasma of women with preeclampsia [4]. Antioxidants, such as carotenoids, tocopherols, and ascorbic acid, due to their capacity for scavenging free radicals and their function as inhibitors of reactive oxygen species, are lower in women with pre-eclampsia [5].

Oxidative stress in aerobic life can be defined as the imbalance between the generation of reactive oxygen species (ROS) and the rate of their consumption by antioxidants [6]. In normal pregnancies, there is an increase of free radical production and lipoperoxidation towards the end of pregnancy when compared with non-pregnant women. Similarly, total antioxidant capacity (Uric acid, Vitamins C and E) gradually increases during pregnancy [6], leading to an oxidative balance maintained throughout pregnancy.

Evidence in recent years has shown that a biochemical imbalance in pre-eclampsia occurs with an increase of oxidative stress and lipoperoxidation and, at the same time, a deficient antioxidant protection[7].

In normal pregnancies, there is remodeling at the placental vascular bed, where cytotrophoblast invades the maternal spiral arteries feeding intervillous space (involving endothelium and muscular tunica media), making them loose their smooth muscle and become sinusoids, a type of vessels with large capacity and low resistance, thus lacking any contractibility. However, these physiological changes are absent in pre-eclampsia. The trophoblastic invasion is very shallow, keeping the utero-placental circulation in a state of high resistance, which results in reduced utero-placental perfusion, focal regions of hypoxia, and alterations in the pattern of adhesion of molecules produced by endothelial cells. These changes (retention of smooth muscle caused by deficient invasion) may cause blood flow to remain under vasomotor control. The resulting larger fluctuations of intervillous oxygen concentrations might result in an ischemia-reperfusion phenomenon with overproduction of ROS[7].

In pre-eclamptic women, lipoperoxidation products (especially malondialdehyde, MDA) are increased, and enzymatic antioxidants (superoxide dismutase, SOD, and glutathione peroxidase GPx) as well as non-enzymatic antioxidants (vitamin C and vitamin E) are decreased[4].

Some studies, however, have not found raised levels of oxidative stress markers or lowered levels of antioxidants in pre-eclampsia [8].

Preeclampsia is an important cause of maternal morbidity and mortality in Sokoto[9]. There is a lack of proven predictors and prophylaxis for preeclampsia, implying an urgent need for predictors to help identify those who are at risk of contracting the subclinical disease.

The role of oxidative stress in preeclampsia in our environment is an understudied and compelling area for investigation. This study was designed to evaluate whether pre-eclamptic women show higher degrees of oxidative stress than normal pregnancies. Identifying modifiable factors to decrease oxidative stress among pregnant women may be an inexpensive and non-invasive therapy for decreasing the maternal, foetal and neonatal morbidity and mortality associated with preeclampsia in our environment.

MATERIALS AND METHODS

This was a prospective cross-sectional study. From Usmanu Danfodiyo University Teaching Hospital, Sokoto and Specialist Hospital Sokoto, pregnant women diagnosed with preeclampsia were recruited consecutively and matched with normal healthy pregnant women for age, parity and gestational age as controls. The study was carried out between January, 2015 and August, 2017. Of the 400 women recruited, 201 were diagnosed with pre-eclampsia, and matched with 199 with a normal pregnancy.

Pre- eclampsia was defined as systolic and diastolic BP greater than or equal to 140 mm Hg and 90 mm Hg, respectively, with significant proteinuria (300 mg per 24 h); mild pre-eclampsia was defined as diastolic BP less than 110 mm Hg, with significant proteinuria; and severe pre-eclampsia as diastolic BP greater than 110 mm Hg, or severe proteinuria (N 2g/24 h), or serum creatinine level greater than 1.2 mg/dL, or when other signs and symptoms of severe pre-eclampsia such as persistent headache, visual disturbances, epigastric pain, and/or thrombocytopenia are present[1].

Detailed patient history was taken and a physical examination performed. Blood pressure was measured in the left arm with a sphygmomanometer. Urinalysis was done for proteinuria. A total of 10 mL of venous blood was taken from all the women. All blood samples were drawn into tubes free of endotoxins and containing heparin using vacutainers.

Informed consent was obtained from all participants or their relations verbally. Ethical approval for the study was from the Hospital's Ethics Committee.

Chemicals and reagents

Analytical graded chemicals and reagents were used for the research. Glutathione peroxidase kit (item number: 703102), superoxide dismutase kit (item number: 706002), TBARS assay kit (item number: 10009055) and catalase assay kit (item number:

707002) were products of Cayman Chemical Company, USA. Vitamin A, C and E were products of Lab Tech Chemicals, India. DNPH reagent, thiourea, were products from Sigma Company, United States of America.

Equipment

Micropipette reader RT 2100 C Rayfo Lives and Analytical Science Company Limited, China was used to take absorbance of analysis involving kits. Analysis of minerals was carried out using Atomic absorption spectrophotometer (AAS) AA240FS AAS (Varian Medical Systems Inc. USA) while vitamin analysis was carried out using Spectrophotometer (Optima sp-300), United States of America.

Collection of serum samples

Under aseptic precautions 10 ml of blood was drawn in plain vacutainer tubes from the antecubital veins of the patients. The collected blood was allowed to clot and then centrifuged at 4000 rpm for 10 minutes to clear separation of serum. Serum thus extracted was divided into three aliquot samples stored at -20^{o}C for analysis of: Glutathione peroxidase, Super oxide dismutase (SOD), Catalase, Malondialdehyde and Vitamins (A, C and E).

Determination of antioxidant status

Serum glutathione peroxidase (GPX) activity was determined according to the method described by Paglia and Valentine, using hydroperoxide as substrate [10].

The serum SOD enzymatic activity was determined according to the method reported by Marklund[11]. Serum MDA levels was determined by the method of Niehans and Samuel [12].

Lipid peroxidation was evidenced by formation of thiobarbituric acid (TBA). Serum catalase activity was determined using Cayman's catalase assay kit according to Johansson and Borg[13]. Serum vitamin E was assayed using the method of Hashim and Schuttrnger[14]. Ascorbic acid (Vitamin C) was assayed by the method of Natelson[15]. Vitamin A was determined by the method of Rutkowski *et al*. [16].

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM by using InStat Software (version 3.0) San Diego, USA. The data was subjected to analysis of variance (ANOVA) and Turkey's t-test was used for comparison of means between two groups. The statistical significance level was set at $p < 0.05$.

RESULTS

The total number of women recruited into the study was 400, made up of 201 preeclamptic women with 199 matched controls of normal healthy pregnant women (matched for age, parity and gestational age).

The ages of the women with preeclampsia ranged between 17 to 50 years with a mean of 29.0 ± 6.4 years, while that of the control ranged between 15 to 40 years with a mean of 24.5 ± 4.8 years.

Age, tribe, religion and educational status showed statistically significant differences between the cases of preeclampsia and the matched controls (Table 1).

Table-1: Socio-demographic characteristics of the preeclamptic and control women

Variable	Preeclampsia (%)	Control (%)	Statistics
Age			
15 – 24	47 (23.4)	117 (58.8)	χ^2= 57.747
25 – 34	91 (45.3)	69 (34.7)	df=4
35 – 44	41 (20.4)	7 (3.5)	p = 0.000
45 – 55	22 (10.9)	6 (3.0)	
Total	**201 (100)**	**199 (100)**	
Tribe			
Hausa/Fulani	136 (67.7)	168 (84.4)	χ^2= 17.060
Yoruba	15 (7.5)	7 (3.5)	df=3
Igbo	24 (11.9)	5 (2.5)	p = 0.001
Others	26 (12.9)	19 (9.6)	
Total	**201 (100)**	**199 (100)**	
Religion			χ^2= 13.060
Islam	160 (79.6)	183 (92.0)	df=3
Christianity	41 (20.4)	16 (8.0)	p = 0.000
Total	**201 (100)**	**199 (100)**	
Occupation			
House wife	142 (70.6)	146 (73.4)	χ^2= 3.498
Civil servant	30 (14.9)	19 (9.6)	df=3
Business	13 (6.5)	18 (9.0)	p = 0.321
Student	16 (8.0)	16 (8.0)	
Total	**201 (100)**	**199 (100)**	

Educational Status			
None	11 (5.5)	5 (2.5)	χ^2= 28.512
Quranic	38 (18.9)	20 (10.1)	df=4
Primary	13 (6.5)	17 (8.5)	p = 0.000
Secondary	64 (31.8)	78 (39.2)	
Tertiary	75 (37.3)	79 (34.5)	
Total	**201 (100)**	**199 (100)**	
Marital Status			
Married	192 (95.5)	199 (100)	χ^2= 6.031
Divorcee	2 (1.0)	0 (0)	df=3
Widow	2 (1.0)	0 (0)	p = 0.110
Single	5 (2.5)	0 (0)	
Total	**201 (100)**	**199 (100)**	

Majority of the women presenting with preeclampsia were primigravidae. Few patients were unbooked. These variables were also statistically significant. (Table 2)

Table-2: Obstetric characteristics of the women

Variable	Preeclampsia (%)	Control	Statistics
Parity			
0	78 (38.8)	97 (48.7)	χ^2 = 42.309
1-4	70 (42.8)	91 (45.7)	df = 4
> 4	53 (18.4)	11 (5.5)	p = 0.000
Total	**201 (100)**	**199 (100)**	
Booking Status			
Booked	137 (68.2)	182 (91.5)	χ^2= 45.086
Unbooked	64 (31.9)	17 (8.5)	df=1
Total	**201 (100)**	**199 (100)**	p = 0.000

The mean levels of oxidative stress markers, malondialdehyde and glutathione peroxidase were significantly higher in the preeclampsia group than in the matched control of normotensive women while superoxide dismutase was significantly higher in the control group.

Also, the mean levels of the antioxidants, Catalase, Vitamin C, and Vitamin E were significantly higher in the control group of normotensive women when compared to the preeclampsia group. However, Vitamin A mean levels were not significantly higher between the two groups. (Tables 3 and 4).

Table-3: The mean levels of oxidative stress makers in the preeclampsia and control groups

Variable	Preeclampsia mean ± SEM	Control mean ± SEM	Statistics
Malondialdehyde (µ/ml)	3.44 ± 1.25	3.024 ± 1.08	t = 3.54 p = 0.000
Superoxide Dismutase (units/ug)	13.00 ± 15.27	57.21 ± 38.08	t = -15.278 p = 0.000
Glutathione Peroxidase (nmol/min/ml)	71.53 ± 26.02	62.58 ± 22.45	t = 3.680 p = 0.000

Table-4: The mean levels of antioxidants in the preeclampsia and control groups

Variable	Preeclampsia	Control	Statistics
Catalase (nmol/min/ml)	50.93 ± 36.22	12.85 ± 35.30	t = 10.650 p = 0.000
Vit A (mg/dl)	1.52. ± 1.68	2.36 ± 0.98	t = -990 p = 0.234
Vit C (mg/dl)	0.14 ± 1.33	0.84 ± 3.77	t = -2.96 p = 0.008
Vit E (mg/dl)	1.51 ± 1.68	2.36 ± 0.99	t = -17.57 p = 0.000

DISCUSSION

This was a comparative, prospective, cross-sectional study between pregnant women diagnosed with preeclampsia and normal healthy pregnant women matched for age, parity and gestational age.

The mean age of the women with preeclampsia was 29.0 ± 6.4 years, while that of the control was 24.5 ± 4.8 years and this difference was statistically significant. Various studies that looked at the relationship between age and development of preeclampsia concluded that teenage age group and those greater than thirty years were more prone to develop preeclampsia while in those between 20 to 30 years, the chances of developing preeclampsia in normal pregnancy was much less[17, 18, 19]. However, this finding was not corroborated by this study as majority of the women with preeclampsia were between the age range of 24 to 34 years.

In this study, women with high educational level developed preeclampsia. This is not in agreement with the finding from the Generation R Study which concluded that low maternal socioeconomic status was a strong risk factor for preeclampsia. Having adjusted for the confounding effects of age, gravidity and multiple pregnancy, they found that women with low educational level were more likely to develop preeclampsia (odds ratio 5.12; 95% confidence interval: 2.20, 11.93) than women with high educational level[20].

Majority of the women presenting with preeclampsia were primigravidae. This is not surprising as some studies have shown that the incidence of preeclampsia is significantly more in the first pregnancy than in subsequent ones[21,22].

The oxidative stress markers malondialdehyde (MDA) and glutathione peroxide (GPx) mean levels were higher in the preeclamptic women while superoxidase dismutase (SOD) levels were lower. MDA is the end product of non-enzymatic degradation of polyunsaturated fatty acids. Lipid peroxidation has been blamed to be the main causative factor for oxidative stress in preeclampsia[4]. Uncontrolled peroxidation of fatty acids and cholesterol alter membrane fluidity and permeability as lipid peroxides are toxic compounds that damage endothelial cells, increase peripheral vasoconstriction and increase thromboxane synthesis and decrease prostacyclin synthesis[23]. This could ultimately reduce the ability of the endothelium to act as a permeability barrier to plasma components. Exposure of the vascular endothelium to lipid peroxides would begin to shut off production of prostacyclin, increasing the propensity for vasoconstriction and platelet aggregation[24].

In this study, we found that the levels of malondialdehyde was significantly increased in preeclampsia when compared to normal pregnant females. This increase signifies the excessive ongoing lipid peroxidation in preeclampsia and may well be a marker of oxidative stress. This result correlates well with other studies undertaken at different institutions[24 – 27]. However, the lowered levels of SOD in the preeclamptic subjects found in this study is at variance with some studies [26, 28] but in agreement with others [29].

The antioxidant Vitamins (A, C and E) had lower mean levels in the preeclamptic subjects when compared with the control. This may be as a result of increased consumption. On the other hand, catalase (CAT) and glutathione mean levels were higher in the preeclamptic group and this has been attributed to as a compensatory mechanism. Their elevation may be an adaptive response to counter the effect of increased oxidative stress. Other studies on antioxidant status in preeclampsia have also revealed such mixed outcomes [26, 29].

CONCLUSION/RECOMMENDATION

The results of this study are in agreement with most previous studies which showed that established preeclampsia is associated with increased concentrations of oxidative stress markers including lipid peroxidation products, and a reduction in antioxidant concentrations. Further follow up studies, to assay the levels of these markers of oxidative stress, are recommended in preeclamptic women at 2 weeks and 6 weeks postpartum to enable us arrive at the certain conclusion that interventions with antioxidants vitamins may be the panacea to this disease in our resource constrained sub-region.

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Ethical approval

The study was approved by the Institution's Ethics Committee.

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