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CROSS-SECTIONAL STUDY OF ANTIOXIDANT STATUS IN NORMOTENSIVE AND HYPERTENSIVE PREGNANCY.

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ABSTRACT

Objectives: The study measured the concentrations of antioxidants in women during pregnancy and the Post Partum Period (PPP) with a view to investigating their role in the aetiology of pregnancy induced hypertension (PIH).

Study Design: Informed consent was obtained from 105 women who were divided into three groups: 15 age and parity matched normotensive non-pregnant (control group), 45 normotensive and 45 hypertensive pregnant women. The two groups of pregnant women were divided into three groups of 15 each and studied in the second and third trimesters and PPP respectively. Venous blood was obtained from all the participants for measurements of some antioxidants (uric acid, albumin, catalase and vitamin C). Data were analyzed using descriptive and inferential statistical methods.

Results: The results show that the mean concentrations of catalase, albumin, vitamin C and uric acid for the control were 3.1 ± 0.18 µ/l, 46.6 ± 6.6 g/l, 0.38 ± 0.01 mg/dl and 0.16 ± 0.03 mmol/l respectively. The mean concentration of all antioxidants except uric acid were significantly lower during pregnancy when compared with controls ($t = 2.06$; $p<0.01$). In the normotensive group of pregnant women, vitamin C was the only antioxidant that showed significant higher concentration when the second trimester concentration and third trimester concentration were compared ($t=2.06$; $p<0.05$). Uric acid levels were significantly higher ($t=2.06$; $p<0.05$) and catalase and vitamin C levels were significantly lower in the hypertensive group during the 2nd and 3rd trimesters ($t=2.06$; $p<0.05$). There was a tendency for all antioxidant concentrations to return to normal values during the PPP in the normotensive group; however in the hypertensive group, uric acid levels remained significantly higher ($t=2.06$; $p<0.05$).

Conclusion: In conclusion this study showed that pregnancy generally reduced the concentration of antioxidants but vitamin C levels were higher in late pregnancy of normotensive women. Therefore higher levels of vitamin C may protect against PIH.

Keywords: Antioxidants, Normal Pregnancy, Pregnancy Induced Hypertension (PIH).

INTRODUCTION

Pre-eclampsia is associated with maternal and perinatal morbidity and mortality in the developed and developing economy. Aetiology of pre-eclampsia is yet unknown despite many theories. The role of antioxidant in its etiology is currently receiving attention worldwide. There is increasing evidence that an imbalance of increased oxidative stress, lipid peroxidation associated with deficiency of antioxidant protection contributes to the endothelia cell damage and dysfunction which is associated with this pregnancy disorder[1-3]. Free radicals are highly reactive molecules[4], excess or high levels of it in the body will lead to tissue damage through oxidative stress and lipid peroxidation, which in turn could lead to complications such as hypertension. Antioxidants such as catalase,

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superoxide dismutase, ß-carotene, vitamin C and E, glutathione/glutathione peroxidase, ceruloplasmin, and transferin are present throughout the body in the cells, cell membranes, lipoproteins and extracellular fluids, where they combat oxidative stress[5].

Available evidence confirms that there is increase oxidative stress in normal pregnancy but with increased antioxidant protection[6,7]. Whereas in pregnancy induced hypertension there is no proportional increase in the antioxidants to counteract the level of oxidative stress experienced. [8-10]. Levels of plasma antioxidants are measured as indices of free radical status, since free radicals are difficult to measure directly. This study is designed to investigate the status of ascorbic acid, catalase, uric acid and albumin in pregnancy induced hypertensions (PIH). The study examined the status and role of these antioxidants in patients with pregnancy induced-hypertension by comparing the plasma status in non-pregnant healthy women (control), normotensive pregnant women and PIH patients in Osun state, Nigeria.

MATERIALS AND METHODS:

The study was conducted at the Department of Obstetrics and Gynaecology, Obafemi Awolowo University Teaching Hospitals Complex (Wesley Guild Hospital, Ilesa and Ife State Hospital, Ile-Ife) Osun State, Nigeria. Informed consent was obtained from 105 women who were divided into three groups: 15 age and parity matched normotensive non-pregnant (control group), 45 normotensive and 45 hypertensive pregnant women. The two groups of pregnant women were divided into three groups of 15 each and studied in the second and third trimesters and three to six days postpartum period (PPP) respectively. Ethics and research committee approval was obtained. The diagnosis of pre-eclampsia and PIH were established in accordance with the ACOG definitions [11]. Diastolic blood pressure was taken with a cuff sphygmomanometer according to the fifth Korotkoff sound. The presence of proteinuria was screened with the dipstick Albustix method (Ames Division, Miles Laboratories Limited, England) and the amount of protein was measured in a 24-hour urine sample. The presence of oedema was established by physical examination.

Uric acid, albumin, catalase and ascorbate antioxidants were investigated. They were estimated in the 2nd and 3rd trimesters and three to six days postpartum period (PPP). Correlation between these measured parameters (antioxidants) and the values of the blood pressure in pregnancy within each trimester were also studied. The results were also compared with that obtained for the 15 non-pregnant normotensive women matched for age and parity.

Patient with chronic hypertension with super imposed pre-eclampsia were excluded from the study, samples were taken before any medications were started and before any attempt at delivery while postpartum samples were taken three to six days postpartum. Peripheral venous blood samples were collected into heparinized vacutainer tubes and centrifuged at 2500rpm for 10minutes at 4°C to separate the plasma. The plasma was then put into plain tubes and frozen at –20°c until analyses. Plasma ascorbic acid concentrations were assayed within 24 h in order to prevent changes of level. All the parameters were estimated in plasma. Plasma total ascorbic acid levels were assayed spectrophotometrically by the 2,4-dinitrophenylhydrazine method described by Henry et al (1974).)[12] Uric acid was estimated by TPTZ method as described by Morin Prox (1973)[13]. Albumin was estimated by the bromocreso green (BCG)/succinate buffer system as documented by Doumas et al (1972)[14]. Catalase assay method was modified from Experimental Biochemistry Laboratory Manual, Department of Biochemistry, University of Wisconsin, (1967)[15]

The data obtained for each of the antioxidant in the different groups were subjected for statistical analyses using SPSS statistical software package (SPSS Inc., Chicago, USA) version 11.0. Analysis of variance (ANOVA), student t-test and the Pearson correlation coefficients were used. The results were accepted to be significant when P value was less than 0.05. All values are expressed as mean and standard deviation.

RESULTS

The clinical characteristics and the mean and standard deviation values of the plasma concentrations of uric acid, albumin, catalase, and vitamin C in the three groups of women are seen

in tables 1&2. The mean maternal age and parity did not differ significantly among the groups. The results show that the mean concentrations of catalase, albumin, vitamin C and uric acid for the control were 3.1 ± 0.18 μ/l, 46.6 ± 6.6 g/l, 0.38 ± 0.01 mg/dl and 0.16 ± 0.03 mmol/l respectively. The mean concentration of all antioxidants except uric acid were significantly lower during pregnancy when compared with controls (t= 2.06; p<0.01). In the normotensive group of pregnant women, vitamin C was the only antioxidant that showed significant higher concentration when the second trimester concentration and third trimester concentration were compared (t=2.06; p<0.05). Uric acid levels were significantly higher (t=2.06; p<0.05) and catalase and vitamin C levels were significantly lower in the hypertensive group during the 2nd and 3rd trimesters (t=2.06; p<0.05). There was a tendency for all antioxidant concentrations to return to normal values during the PPP in the normotensive group; however in the hypertensive group, uric acid levels remained significantly higher (t=2.06; p<0.05).

DISCUSSION

Our study has shown that the plasma concentrations of catalase and vitamin C were significantly decreased in second trimester normotensive pregnant women than non–pregnant normotensive. The significant decreases in catalase and vitamin C may suggest that in normal pregnancies, these antioxidants may have been excessively consumed because of their activity in removing excess free radicals. Several studies[16-18] have confirmed that decreased antioxidants in maternal circulation show that women with normal pregnancies have increased oxidative stress, lipid peroxidation and also increased antioxidant protection that offsets the oxidative stress when compared with non pregnant women. The mean plasma catalase and ascorbate antioxidants levels of the second and third trimesters and postpartum period of hypertensive pregnant women were significantly (p<0.001) lower than those of second and third trimesters and 3-6 day postpartum period in normotensive pregnant women respectively. This result further confirms the findings of other workers that antioxidants are highly reduced in hypertensive than in normotensive subjects and that as the hypertensive pregnancy progresses, the decreases in plasma levels of catalase and vitamin C become worse[9-10, 19-20].

There was no significant change in plasma albumin concentrations in non-pregnant normotensive women when compared with 2nd trimester normotensive pregnant women. There is a decrease in albumin in second and third trimester hypertensive pregnancy respectively but the difference is not significant. This result does not agree with that found earlier by Thomas et al (2003)[21], which say there is a significant difference. The fact that plasma albumin was decreased, but not significantly in these two groups supports the report of Burtis and Ashwood (2001)[22] that plasma values of albumin in normal pregnancies, decreased as a result of pregnancy induced physiological changes by an average of 3.4g/dl in late pregnancy. The significantly higher plasma uric acid level in second and third trimester hypertensive pregnant women than in normotensive pregnant women seen in this study confirms similar findings of Madazli et al (2002)[23]. In the plasma, uric acid level is significantly higher in three to six days post partum hypertensive than three to six days post partum normotensive. The high plasma uric acid level may have contributed to proteinuria and oedema seen in most of the severe hypertensive subjects[22, 24].

Vascular endothelial cell dysfunction in pre–eclampsia may be caused by uncontrolled lipid peroxidative damage of endothelial cell membranes as a result of vascular contact with placenta–originated circulating peroxidation products, and overwhelms the protective mechanisms of the antioxidants, which in turn may lead to the observed hypertension in these pregnant women. Such event may establish a cycle leading ultimately to manifestations of pre–eclampsia. The phenomenon of significantly lower antioxidants in hypertensive pregnant women than normotensive pregnant women as confirmed in this study; may lead to accumulation of free radicals that mediate lipid peroxidation, thereby involved in the endothelial damage seen in pre-eclampsia[25-26]. It also confirms the report indicating that maternal circulating and placental tissue levels and production rates of lipid peroxides are increased in pre–eclampsia as compared with normal pregnancy[26-28]. The

increase in oxidative stress and lipid peroxidation may explain the mechanisms of various clinical, biochemical and pathophysiological peculiarities of pre–eclampsia. The severity of the pathological process determines the clinical manifestation of pre-eclampsia syndrome[30].

There is a significant increase in plasma concentrations of albumin, catalase and vitamin C in both 3-6 days postpartum normotensive and 3-6 days postpartum hypertensive when compared with their matched 3rd trimester normotensive pregnant women and 3rd trimester hypertensive pregnant women respectively. Uric acid showed a significant decrease. Labor involves expelling of the baby and placenta from a woman's womb. Since the syndrome disappears after delivery, it may further confirm that the fetoplacental unit may be the origin of oxygen free radicals and lipid peroxides. Since the immune response normally comes into play when there is a pathogen or an agent which the immune system regards as foreign gets into the body, it may be clear that some abnormalities may have occurred at the fetoplacental level as seen with high activation of the immune system, and this could have been the onset of the increasing free radical concentration in the body.

The body antioxidants both exogenous and endogenous scavenge these free radicals and repair small damages they cause. As the pregnancy progresses a point is reached when the increased free radicals super-cede the antioxidants of the body. The increase in oxidative stress might not be so important if there was a compensatory increase in antioxidant protection, but the opposite occurs, since this present study has shown a significant decrease in the concentration of antioxidants, a finding consistent with those of Mikhail et al, (1994)[9]; Poranen et al, (1996)[19]; Madazli et al, (1999)[29], where in pre-eclamptic women, several important antioxidants such as vitamin A, C and E, erythrocyte levels of thiols and superoxide dismutase were reported to be decreased.

In severe cases, the uric acid, with its low water solubility, easily becomes deprotonated and in this form, it easily combines with sodium ions to form sodium urate crystals in subcutaneous tissues, known as tophi, it later precipitates leading to nephropathy and gouty arthritis[4]. When these crystals settle at the glomerulus, it provokes immune responses and leads to glomerulonephritis. All these result in proteinuria and oedema. Furthermore, the nephropathy may lead to renal sodium retention and or decreased filtration surface. These in turn lead to increased fluid volume and venous constriction, which leads to an increased cardiac output, and further increases in blood pressure. This explains why with increased proteinuria, there is further increase in severity of the pre–eclamptic patient. Since there is a decrease in albumin, to maintain the blood osmotic balance, water moves to the interstitial cell spaces and this is the cause of oedema. However, the decrease in plasma albumin may not be due to free radicals, but due to proteinuria caused by renal complications that was not considered in this work. The high uric acid could also be due to renal complications. Since catalase and vitamin C have no source of alteration that could lead to their decrease, other than pregnancy, their significant decrease confirms significant increases in free radicals in PIH when compared with pregnant normotensive women, who in turn have higher levels of free radicals than non-pregnant normotensive women.

In conclusion this study showed that pregnancy generally reduced the concentration of antioxidants but vitamin C levels were higher in late pregnancy of normotensive women.

Therefore higher levels of vitamin C may protect against PIH, further research in this area is necessary in our environment.

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Table 1:
Physical and biochemical parameters in non-pregnant compared to 2nd Trimester pregnant women

Groups	Normotensive Non-pregnant	Normotensive Pregnant	Hypertensive Pregnant	Comparison	
Variable	A (n=15)	B (n=15)	C (n=15)	A vs B	B vs C
Age (years)	28.3±3.8	29.1±4.1	29.5±4.7	NS	NS
Parity	1.5±1.3	2.1± 1.2	2.2±1.7	NS	NS
SBP	108±10.3	107±8.2	194±24.6	NS	<0.001
DBP	71.0±9.9	68.6±8.8	94.0±7.0	NS	p<0.001
Uric acid	0.16±0.03	0.194±0.10	0.30±0.11	NS	p<0.022
Albumin	46.6±6.6	39.0±9.1	39.6±4.8	p<0.027	NS
Catalase	3.1±0.18	2.7±0.32	1.1±0.30	p<0.018	p<0.0001
Vitamin C	0.38±0.10	0.11±0.05	0.07±0.03	p<0.0001	p<0.018

NS= Not Significant

Table 2
Comparison of physical and biochemical parameters between normotensive and hypertensive in the 3rd Trimester and days 3-6 postpartum

Groups	Normotensive Pregnant	Hypertensive Pregnant	Comparism
Variable	D(n=15)	E (n=15)	
Age (years)	29.0±4.0	33.5±5.7	NS
Parity	1.7±1.3	3.1± 2.3	NS
SBP	104±9.7	177±27.1	p<0.001
DBP	63.0±9.5	107.6±16.4	p<0.001
Uric acid	0.16±0.03	0.22±0.06	p<0.031
Albumin	38.8±6.1	36.8±4.6	NS
Catalase	2.5±0.25	0.86±0.33	p<0.0001
Vitamin C	0.38±0.10	0.06±0.03	p<0.001
Days 3-6 Postpartum			
Age (years)	27.3±3.9	27.1±5.4	NS
Parity	1.9± 1.6	2.1±2.1	NS
SBP	107.0±13.4	160.0±10.5	p<0.001
DBP	68.0±10.3	101.0±11.0	p<0.001
Uric acid	0.17±0.03	0.41±0.16	p<0.0001
Albumin	48.4±2.2	26.5±5.5	p<0.0001
Catalase	2.9±0.18	1.5±0.44	p<0.0001
Vitamin C	0.36±0.07	0.10±0.06	p<0.0001

NS= Not Significant