Protein carbonylation, Lipid peroxidation and serum alpha Tocopherol activity in Preeclampsia

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Research Article

Abstract: Hypertensive disorders are the most common medical complication of pregnancy and are important cause of maternal and perinatal morbidity and mortality. The etiology of preeclampsia has always remained elusive. More recently, antioxidants have been proposed as a potential preventive strategy on the basis of data suggesting that endothelial dysfunction is fundamental to the development of preeclampsia as a result of increased oxidative stress and deficiency of antioxidant protection. In this context the present study was undertaken to evaluate the intensity of oxidative stress and to investigate a possible correlation between oxidative stress, antioxidant status and severity of preeclampsia. A case control study was performed on 50 normal pregnant and 50 diagnosed preeclamptic women age and trimester matched. Malondialdehyde (MDA), Protein Carbonyls (PCO)and Vitamin E(Vit E) were estimated in the serum of all subjects. Statistically significant increase in levels of PCO ($p< 0.0001$), MDA ($p <0.0001$) and decrease in Vit E ($p<0.0001$) were observed in preeclamptic women as compared to normal controls. A negative correlation between oxidative stress markers (MDA, PCO) and Vit E and positive correlation between oxidative stress markers (MDA, PCO) and blood pressure was observed in preeclamptic women. Increased MDA, PCO and decreased Vit E suggests an imbalance between the oxidative stress and antioxidant status which supports the hypothesis that the oxidative stress is an important causative factor in pathogenesis of preeclampsia and supplementation with antioxidant vitamin may benefit the preeclamptic mothers.

Key words: Preeclampsia, MDA, Protein carbonyls, Vitamin E, Lipid peroxide, Antioxidant.

1. Introduction

In normal pregnancy profound physiological changes occur in the maternal cardiovascular system including increase in blood flow through uterine blood vessels, altered response to vasopressor agents, and reduced peripheral resistance and blood pressure[1].Hypertensive disorders are the most common medical complications of pregnancy and are important causes of maternal and perinatal morbidity and mortality[2].Preeclampsia(PE) is a pregnancy specific disorder, where there is a development of hypertension and proteinuria with or without edema in a normotensive and non proteinuric women with 20 weeks of gestation. The exact causes of these maternal changes in relation to pregnancy are not clear. There is generalized maternal vascular endothelial dysfunction and leukocytic activation[3]. Increase in oxidative stress marker has been implicated to damage the maternal vascular endothelium leading to the elevation in diastolic pressure which further aggravates the condition of preeclamptic patients[4]-[3].Oxidative stress can result in lipid peroxidation (which compromise mitochondrial ATP products and stimulate proapoptic events) protein carbonylation or nitration /nitrosylation (which alter protein conformation and function)[5]. Cumulative evidence in recent years point towards biochemical imbalance in preeclampsia with an increase of oxidative stress and at the same time a deficient antioxidant protection. A number of reports suggested significant elevation of MDA[6]-[9] and protein carbonyl in preeclamptic women[10].A majority of studies agree that PE causes a diminution in serum levels of antioxidant Vit E as well as other lipid soluble antioxidant such as Coenzyme Q10[11]. Some studies did not demonstrate significant difference in plasma Vit E concentration between women who developed PE and those who did not[12] and some results are at variance with the prevailing hypothesis that PE is an antioxidant deficient state, since increased plasma concentration of Vit E among women with in PE as compared with normotensive pregnant women have been reported[13]-[14].The present study was conducted on preeclamptic and normal pregnant women to observe the relative changes in oxidative markers and antioxidant levels and to evaluate their association with severity of the disease.

2. Materials and Methods

With the approval of institutional ethical committee, 50 preeclamptic and 50 gestational age and trimester matched normal pregnant women attending OBG department of Narayana Medical College and Hospital, Nellore were recruited in the study and all patients provided informed consent. Preeclampsia was diagnosed if the patient had blood pressure over 140/90 mmHg on two or more occasions at least 4hr apart after the 20th weeks of gestation with proteinuria on a dipstick

value of more than 1+(30mg/dL)on two separate occasions at least 6hr apart. Both mild and severe PE patients were included in the study. The exclusion criteria included patients those on Vit E or antioxidant supplements or with any other complications other than PE like gestational diabetes, diabetes mellitus, hypertension, multiple pregnancy, renal disease and other chronic diseases that might interfere with the study.Under aseptic precautions venous blood was collected and analyzed for MDA, PCO and Vit E levels. MDA levels were estimated by thiobarbituiric acid reactivitymethod[15], PCO by reacting with DNPH[16] and Vit E by HPLC method. Statistical analysis was done using SPSS version 16. The mean and standard deviation were calculated for all the parameters. The significance between the groups was determined using unpaired Student t- test for Equality of means. The p-value <0.05 was considered significant. Pearson's correlation was done to know the association between prooxidants and antioxidant.

3. Results

A total number of 100 samples were analyzed to evaluate the levels of serum MDA, PCO and Vit E in 50 normal pregnant controls and 50 preeclamptic cases. The preeclamptic group of 50 subjects was divided into 32 mild and 18 severe PE. Blood pressure, one of the diagnostic criteria for PE was analyzed in both groups. A statistically significant increase in BP ($p < 0.0001$) was observed in PE as compared to normal controls as shown in table-1. The oxidative stress markers and antioxidant levels of both groups are shown in table 2. Correlation of various parameters in cases and controls is shown in table 3.The present study showed statistically significant increase in MDA ($p<0.001$) and PCO ($p<0.001$) and decrease in Vit E ($p < 0.0001$) levels in PE as compared to controls .A positive correlation of MDA with both systolic ($r = 0.57$) and diastolic ($r = 0.35$) BP was found in PE which is statistically significant ($p <0.05$). In the present study significant negative correlation between Vit E and MDA ($r = -0.27$), but insignificant negative correlation between Vit E and systolic BP ($r = - 0.11$), diastolic BP($r= -0.01$) and PCO ($r = -0.01$) was observed.

Table 1: Blood pressure (BP) of subjects*

Blood Pressure	Normalpregnant women(controls) * n = 50	Over allPreeclamptic Women(cases) * n = 50	Mild PE* n = 32	Severe PE * n = 18	p value (control Vscases)
Systolic (mm Hg)	112.8 ± 6.33	156 ± 20.10	145.6 ± 4.96	176.1 ± 22.14	< 0.0001
Diastolic (mm Hg)	73.8 ± 6.60	102.6 ± 9.96	97.81 ± 6.95	111.1 ± 8.74	< 0.0001

*values expressed as Mean and SD

Table 2: oxidative stress markers and antioxidant levels of both groups

Biochemical Parameters	Normal pregnant Women(controls)* n = 50	Over allpreeclamptic Women(cases) * n =50	Mild PE Women* n = 32	Severe PE Women* n =18	p value (controlVs cases)	p value (mild vs severe PE)
MDA (µmol/ L)	2.97 ± 0.97	6.68 ± 2.87	5.3 ± 0.91	8.9 ± 3.5	< 0.0001	< 0.001
PCO (nmol/ gm OfProtein)	2.8 ± 1.1	4.5 ± 1.2	4.4 ± 1.2	4.7 ± 1.3	< 0.0001	>0.05
Vit E (mg/ dL)	1.96 ± 0.24	1.2 ± 0.4	1.24 ± 0.39	1.15 ± 0.42	< 0.0001	>0.05

*values expressed as Mean and SD

Table 3: correlation of various parameters in cases and controls

Correlation Parameters	Controls r value (p value)	Cases r value (p value)
MDA with systolic BP	0.13 (0.36)	0.57 (0.00)
MDA with diastolic BP	0.17 (0.28)	0.35 (0.01)
MDA with Vit E	-0.08 (0.58)	-0.27 (0.05)
Vit E with PCO	0.03 (0.83)	-0.01 (0.94)
Vit E with systolic BP	0.14 (0.33)	-0.11 (0.44)
Vit E with diastolic BP	-0.01 (0.94)	-0.01 (0.94)

4. Discussion

The medical significance of oxidative stress has become increasingly recognized to the point that it is now considered to be a component of virtually every disease. Oxidative damages to protein, lipid or DNA may all be seriously deleterious and may be concomitant. The literature suggest that PE is a wide spread inflammatory state where a number of plasma factors that regulate endothelial functions are altered[17]. Lipid peroxidation has been blamed to be the main causative factor for oxidative stress in PE. Free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membrane [18]. PUFA are important for the normal functioning of most of the cell, MDA an end product of lipid peroxidation induced by reactive oxygen species (ROS), is well correlated with the degree of lipid peroxidation. This may result in a greater potential for endothelial damage ultimately leading to elevated diastolic blood pressure[4]. Thus lipid peroxidation and free radicals may be important in the pathogenesis of PE.The present study revealed an increase in MDA levels in PE ($p < 0.0001$) and more increase in severe PE as compared to normal pregnant women. Present results on MDA levels are consistent with others[4],[6]-[8],[19].Serum MDA levels in PE showed positive correlation with both systolic and diastolic BP and these results are in accordance with studydone by F.F Yaniketal[20],[6],[7]. ROS and oxidative stress causes damage to proteins directly or indirectly leading to formation of protein carbonyl group. These are relatively stable in plasma and used as marker of oxidative damage [21]. In the present study PCO levels are increased significantly ($p < 0.0001$) in PE and these results are in accordance with other studies [22]-[24].Zusterzeel PL in 2000 showed increased PCO levels in normal pregnancy ($p<0.001$) as compared to healthy non pregnant women and also significantly higher levels in preeclamptic women than healthy pregnant women (0.0001). The higher levels of PCO suggest that increased oxygen free radicals occur in normal pregnancy and to a much higher extent in PE.Decrease in the levels of Vit E in PE and more in severe PE as compared to controls were observed in this study. These results are in consistent with others[7], [10], [25], [26].This decrease in antioxidant levels may be due to the increased turn over for preventing oxidative damage[25].Antioxidant vitamins have been reported to have an important function in regulating blood pressure[27]. In the present study statistically significant negative correlation was seen between MDA and Vit E which is in accordance with study done by Mohanty S *et al* [26], and insignificant negative correlation between Vit E and BP which is in accordance with study done by F.F Yanik *et al* [20] .Many reports suggest that antioxidant supplementation in women who were at risk of PE was associated with improvement in biochemical indices of the disease[28] and several reports reviewed by Rodrigo *et al* in 2005[29] suggest that prophylactic use of Vit C and E before the 20th week of gestation , after identifying the risk factors on the basis of history of the patients , would lower the risk of maternal vascular dysfunction and there in the onset of PE.Etiology of PE is still obscure but one of the most favored hypotheses is the endothelial dysfunction secondary to the peroxidation of membrane lipids. Excessive generation of free radicals, depressed antioxidant status or imbalance in peroxidation and cellular dysfunction, which is probably the cause of preeclampsia.

5. Conclusion

Despite increased understanding of the cause of the syndrome, there is currently no accepted method of prevention of PE. Oxidative stress may be the point at which multiple factor converge resulting in endothelial cell dysfunction and the consequent clinical manifestation of preeclampsia. Early diagnosis and treatment with antioxidant vitamin may be essential for improving the maternal and fetal outcome.

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