LIPID PEROXIDATION AND NON-ENZYMATIC ANTIOXIDANTS STATUS IN PREECLAMPTIC AND POSTPARTUM PREECLAMPTIC WOMEN

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ABSTRACT

Context: Preeclampsia is a hypertensive disorder and is one of the most leading cause for maternal and fetal mortality in developing countries.

Aim: Objective of this study was to investigate the lipid peroxidation and non-enzymatic antioxidants status in women with preeclampsia and compare the same parameters in women with normal pregnancy and postpartum preeclampsia women.

Settings and Design: District civil hospital, Case control study.

Methods and Material: The study comprised of 25 normal healthy non pregnant controls, 25 normal healthy pregnant women in 3rd trimester and 25 preeclamptic women in 3rd trimester. The same 3rd trimester preeclamptic women were used for follow up study after delivery and were considered as postpartum preeclamptic group. Whole blood was used to detect Malondialdehyde (MDA) a product of lipid peroxide, non-enzymatic antioxidants like reduced glutathione, vitamin E, vitamin-C and vitamin-A. Statistical significance was determined by ANOVA and multiple comparison tests.

Results: There was a consistent significant increase in lipid peroxidation (MDA) in all the groups as compared to nonpregnant controls (P< 0.001). Elevated levels of malondialdehyde in pre-eclamptic subjects declined significantly (p<0.001) after delivery. A significant decrease (P<0.001) in the level of non-enzymatic antioxidants viz. reduced glutathione, vitamin-E, vitamin -A and vitamin – C was observed in all the groups as compared to nonpregnant controls. In the postpartum preeclamptic group significant increase was noted in all antioxidants except vitamin A as compared to preeclamptic group.

Conclusions: Our study showed clear insight into disturbances associated with normal pregnancy, which are exaggerated in complicated pregnancy like preeclampsia with enhanced lipid peroxidation and decreased antioxidants. Early attention, intensive management and treatment are essential to improve maternal and fetal outcome.

Key-Words: Antioxidants, oxidative stress, malondialdehyde.

INTRODUCTION

Preeclampsia is a hypertensive disorder unique to pregnancy. It is one of the most leading cause for maternal and fetal mortality and it occurs in approximately 0.4% - 2.8% of all pregnancies in developed countries and many more in developing countries, leading to as many as 83,70, 000 cases worldwide per year. [1] Preeclampsia is associated with increased blood pressure accompanied by proteinuria, edema or both. Without intervention pre-eclampsia progress to eclampsia a malignant hypertensive condition. Lipid peroxidation has been suggested as a causative factor and is found to be increased in pregnancy.[2] The pathogenesis of preeclampsia is very complex, the mechanisms that finally trigger the disease is still not clearly elucidated but numerous pathophysiologic factors like elevated homocysteine, oxidative stress, genetic, immunologic, and environmental factors have been implicated.[3-5] There is substantial evidence to suggest that the diverse manifestations of preeclampsia, including hypertension, renal impairment, proteinuria and discrete pathology in many organ systems, are derived from pathologic changes within the maternal vascular endothelium. It is likely that endothelial cell dysfunction is a central feature in preeclampsia, resulting in vascular reactivity, activation of the coagulation cascade, and loss of vascular integrity. Damage to endothelial cells has been assessed by the measurement of factors released by the cells or

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produced for repair. Some of the factors studied are endothelin-1, fibronectin, platelet-derived growth factors, prostaglandins, free radicals, fatty acids, and nitric oxide. [6] Interstitial fluid in many pregnant women with preeclampsia is increased markedly when compared with non preeclamptic pregnant women. [7] Thus, most pre-eclamptic women have tissue edema beyond that associated with normal pregnancy. Their increase in interstitial fluid is primarily because of endothelial damage and capillary leakage of fluid and protein into the interstitium, which is exacerbated by hypertension, and physiologically promoted by low plasma colloidal oncotic pressure. Preeclampsia is found to be associated with endothelial dysfunction, [8] which could be caused by oxidative stress. One of hypothesis receiving increased attention is that, in preeclampsia, there is an imbalance between prooxidant production and antioxidant defenses. [9-10] It is foreseen that elevated free radical activity is either from increased production of free radicals or deficiency of protective antioxidants. Antioxidants involved in free radical scavenger especially glutathione, vitamin A, E and C limit the cellular concentration of free radicals and prevent excessive oxidative damage. Women who develop preeclampsia are prone to development of cerebrovascular, cardiovascular as well as other fetal and maternal complications.[11-13]

In the view of above findings present study was conducted to investigate the lipid peroxidation and non-enzymatic antioxidants status in postpartum preeclamptic women and compare with that of preeclamptic and normal pregnant women.

SUBJECTS AND METHODS:

The present study was carried out jointly by the departments of Biochemistry and departments of Obstetrics and Gynaecology. The study protocol was approved by ethical committee.

Sample size: The study comprised of 75 subjects out of which 25 were normal healthy non pregnant controls, 25 were normal healthy pregnant women in the third trimester and 25 were in the third trimester with preeclampsia. The same 3rd trimester preeclamptic women were used for follow up study after delivery and were considered as postpartum preeclamptic group. Written informed consent was given by individual subject. Subjects of the present study were attending to or admitted in the district civil hospital. They were aged between 20-40 years.

Inclusion criteria: The study consisted of normal women, normal pregnant women and women with preeclampsia diagnosed based on definition of ACOG: 1) Systolic blood pressure greater than 140 mm Hg or a rise of at least 30 mm Hg or 2) Diastolic blood pressure greater than 90 mm Hg or rise of at least 15 mm Hg (manifested on two occasions at least 6 hours apart), and 3) Proteinuria of 300 mg or greater in 24 hour urine collection or protein concentration of 1gm/Liter (on two occasions at least 6 hours apart). [14] Subjects with normal pregnancy were normotensive and had no proteinuria.

Exclusion criteria: Women with diabetes mellitus under medication and untreated diabetes, Obese women, women with severe anemia (<6.0g% of Hb), alcoholic, and women suffering from any other disorder were excluded from the present study.

None of the women had received antihypertensive medication until the study sample was taken. Blood pressure levels and proteinuria were determined at the time of sampling.

Collection and storage of blood samples: 5 mL of blood drawn by venipuncture was collected in a heparinized tube (10 units of heparin per mL of blood). In case of postpartum group blood sample was collected one month after delivery. The following parameters were analyzed within 10-15 minutes of collecting blood sample. Whole blood was used to measure Malondialdehyde (MDA) levels by using thiobarbituric acid.[15] Non enzymatic antioxidant Vitamin E was measured by Quaife et all method [16] Vitamin-C by Evelyn and Malloy method[17] and Vitamin-A by Bessey et al method [18] and reduced glutathione by Beutler. E method. [19] Results were expressed as mean ± SD. Comparison of mean values of study groups was performed by ANOVA [F test]. To find the intergroup difference Bonferroni post-hoc test was performed. Significance level was kept at 0.05.

RESULTS

The characteristics of the four groups are summarized in

Table no 1. There was a consistent significant increase in lipid peroxidation (MDA) in all the groups as compared to nonpregnant controls (P< 0.001) and MDA in 3rd trimester preeclamptic women was more (P< 0.001)when compared to normal pregnant 3rd trimester women. Elevated malondialdehyde levels were significantly reduced (p<0.001) after delivery in preeclamptic group. A significant decrease (p<0.001) of non-enzymatic antioxidants viz. reduced glutathione, vitamin-E and vitamin- C was observed in 3rd trimester preeclamptic group as compared to non-pregnant controls and 3rd trimester normal pregnant group. Postpartum preeclamptic group showed significant increase of these antioxidants. Vitamin–A levels also showed decrease in 3rd trimester preeclamptic women when compared to non-pregnant control (p<0.001) and 3rd trimester normal pregnant women (p<0.01) but in postpartum preeclamptic women vitamin A level was increased but not significantly.

Table No 1. Malondialdehyde (MDA) non enzymatic antioxidants reduced glutathione, vitamin E, vitamin-C and vitamin-A levels in the normal non pregnant controls, 3rd trimester normal pregnant, 3rd trimester preeclamptic and postpartum preeclamptic women.

GROUP	A	B	C	D	
	Non – Pregnant Controls (n= 25)	3rd Trimester normal Pregnant Women (n=25)	3rd Trimester Pre-eclamptic Women (n=25)	Postpartum Pre-eclamptic Women (n=25)	F value
MDA n mol/ml p values	1.19 ± 0.09 Range (1.04-1.38)	1.79 ± 0.14 Range(1.41-1.92) <0.001*	2.93 ± 0.54 Range (1.83-4.01) <0.001*†	2.07 ± 0.51 Range (1.44-3.75) <0.001‡	F 3,96 = 86.900
Reduced glutathione mg% p values	59.97 ± 1.3 Range (58.00-62.00)	50.77 ± 6.91 Range (41.54-55.38) <0.001*	41.35 ± 4.23 Range (32.31-50.77) <0.001*†	50.95 ± 6.84 Range (32.31-60.00) <0.001‡	F 3,96 = 48.665
Vitamin-E mg% p values	1.42 ± 0.19 Range (1.20-1.80)	0.87 ± 0.15 Range (0.52-1.11) <0.001*	0.57 ± 0.16 Range (0.40-0.93) <0.001*†	0.72 ± 0.21 Range (0.32-1.22) <0.01‡	F 3,96 = 110.194
Vitamin-C mg% p values	1.11 ± 0.24 Range (0.66-1.66)	0.96 ± 0.33 Range (0.50-1.50) NS*	0.60 ± 0.24 Range (0.33-1.17) <0.001*†	0.9 ± 0.26 Range (0.33-1.33) <0.001‡	F 3,96 = 15.025
Vitamin-A µg% p values	28.62 ± 3.45 Range (24.10-33.50)	22.84 ± 5.17 Range (14.40-31.80) <0.001*	18.93 ± 5.28 Range (11.50-30.30) <0.001* <0.01†	19.22 ± 5.21 Range (12.00-31.80) NS ‡	F 3,96 = 19.718

* Comparison with non-pregnant controls
†Comparison with third trimester normal pregnant
‡Comparison with third trimester pre- eclamptic subjects

DISCUSSION

The present study evaluated the oxidative stress by analyzing the pro oxidants and non-enzymatic antioxidants in non-pregnant, normal pregnant, 3rd trimester preeclamptic and postpartum preeclamptic women. Lipid peroxidation (MDA) was considered as a marker for pro oxidant whereas reduced glutathione, vitamin A, vitamin E and vitamin C were considered as non-enzymatic antioxidants. Unstable and transient nature of free radicals makes it difficult to measure them directly but their tendency to cause lipid peroxidation has been used as an indirect measure.

Table no. 1 depicts the blood levels of malondialdehyde in different study groups. MDA was observed to be increased remarkably at IIIrd trimesters of pregnancy as compared to non-pregnant women and the increase in IIIrd trimester pre eclamptic was more remarkable than that of IIIrd trimester of normal pregnancy. It was noticed that increased levels of malondialdehyde in pre-eclamptic subjects declined significantly ($p<0.001$) after delivery. Kharb S., et al [20] have shown with similar results that serum lipid peroxides are known to increase in pregnancy and this increase was still higher in pre-eclampsia. This increased lipid peroxide level can increase the susceptibility of polyunsaturated fatty acid to peroxidative damage, presumably by free radicals that may lead to the formation of malondialdehyde (MDA) revealing an increase in lipid membrane damage in pre-eclamptic patients as compared with healthy pregnant subjects. Walsh et al [21] showed that the metabolic effects of lipid peroxides leads to an imbalance between the production of prostacyclin and thromboxane A_2, a well demonstrated phenomenon of pre-eclampsia. Placental production of lipid peroxides and thromboxane are abnormally increased in pre-eclampsia. Thromboxane A_2 is a potent vasoconstrictor of the placental vasculature and plays an initial role in pre-eclampsia. Lipid peroxidation products inhibit prostacyclin synthesis and stimulate smooth muscle contraction. The vasoconstriction thus produced can worsen hypertension causing ischemic injury to the cells and subsequent peroxidation, leading to a vicious cycle. Our findings clearly indicate and support some studies [22] that peroxidation may be important factor in the pathogenesis of preeclampsia.

Protective antioxidant mechanisms are complex and multifactorial. Over all imbalances between the degree of oxidative stress and antioxidant defense increases the susceptibility of cell to oxidative stress. Reduced glutathione is an effective reductant and plays an important role in a variety of detoxification processes. It readily neutralizes the hydroxyl radicals, which are considered a major source of free radical damage.[23] A drop in glutathione levels in maternal whole blood might indicate decreased detoxification or free radical scavenging in pre-eclampsia.[24] Pyska W, et al [25] noticed that significantly decreased levels of reduced glutathione and increased levels of malondialdehyde in pre eclamptic subjects as compared to normotensive pregnant subjects. Our findings are in consistent with the above workers. The level of reduced glutathione was significantly decreased in 3rd trimesters women as compared to non-pregnant women. There was statistically significant decreased levels ($p<0.001$) in pre-eclampsia as compared to IIIrd trimester pregnant and normal pregnant women. Reduced Glutathione level was found to be elevated in postpartum preeclamptic women.

Vitamin-E is the most important chain breaking antioxidant and prevents lipid peroxidation of membranes. Kharb S, et al [20] showed that, as pregnancy advances the levels of lipid peroxides increased and Vitamin-E level was decreased significantly when compared to non-pregnant controls. Yanik FF et al [26] reported that, pre-eclamptic patients had higher malondialdehyde and lower Vitamin-E levels as compared with controls. In our finding showed normal pregnant women exhibited significantly diminished levels of Vitamin-E in the IIIrd trimesters as compared to non-pregnant. Further decrease of vitamin-E was observed in pre-eclamptic patients as compared normal pregnant and non-pregnant controls. Decreased levels of vitamin–E elevated significantly in postpartum pre-eclamptic subjects. Above results was supported by Wang et al [27] who suggested that the decreased levels of Vitamin-E in pre-eclampsia are attributable to elevated levels of lipid peroxides signifying its role as an antioxidant.

Ascorbic acid has been described as the major front line water-soluble antioxidant and provides major defense against oxidative stress. Significantly lower levels of vitamin A and vitamins C were observed in preeclamptic women as compared to controls. Our study indicates that

normal pregnant women had markedly reduced plasma vitamin A whereas vitamin C was not significantly reduced as compared to non-pregnant control. A further decrease is observed in preclamptic patients when compared to normal pregnant women. Vitamin C was observed to be raised in preeclamptic women after delivery but the rise in level of vitamin A was not significant. Some studies have shown that delivery does not eliminate the risk for preeclampsia and its complications. [28] By the above findings it is understood that, increased levels of lipid peroxides and reduced antioxidant status clearly demonstrates the presence of oxidative stress. Hence imbalance between lipid peroxides and reduced antioxidants may result in decreased prostacyclin synthesis and endothelial cell injury in preeclampsia and in addition leading to increased thromboxane production with severity of preeclampsia.

CONCLUSION

The present study shows clear insight into disturbances associated with normal pregnancy, which are exaggerated in complicated pregnancy like preeclampsia with enhanced lipid peroxidation and decreased antioxidants. Early attention, intensive management and treatment may be essential to improve maternal and fetal out come as well as in preventing impending complications in postpartum preeclamptic women. However a larger sample size is justified for proper conclusion.

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