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Original Research Article

Comparison of the level of lipid peroxidative marker and antioxidants between preeclamptic and normotensive pregnant women

Sonal Sogani[1,*], Suman Jain[1]

[1]*Dept. of Biochemistry, Pacific Institute of Medical Sciences, Udaipur, Rajasthan, India*

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ABSTRACT

Background: Preeclampsia is a complex multisystem disorder that is characterized by hypertension and proteinuria which is exclusively seen in human species. This disorder is a leading cause of maternal and fetal morbidity and mortality. It is seen that placental oxidative stress is involved in the etiopathogenesis of preeclampsia. This oxidative stress induces endothelial cell injury which in turn causes lipid peroxidation. Although the lipid peroxidation is counteracted by the action of several antioxidants.
Aim: The aim of this study was to elucidate the changes in the level of lipid peroxidation markers and antioxidants in preeclamptic pregnant women that may contribute to the pathogenesis in preeclampsia.
Materials and Methods: Fifty (50) pregnant women clinically diagnosed as preeclampsia were taken as subjects and an equal number of age matched, parity matched and gestation age matched healthy normotensive pregnant women were taken as controls for the study. This is the case-control hospital based study carried in the Department of Biochemistry, Pacific Institute of Medical Sciences (PIMS), Umarda, Udaipur (Raj.).Blood samples collected were estimated for Malondialdehyde (MDA) and Superoxide dismutase (SOD) using standard spectrophotometric method and Vitamin C and Uric acid using standard colorimetric method. Comparison between both the groups was done by Student's t-test.
Results: The levels of MDA and uric acid were significantly elevated ($p<0.001$), and the levels of Vitamin C and SOD were significantly decreased ($p<0.001$) in preeclamptic pregnant women as compared to the normotensive pregnant women.
Conclusions: The results of our study concluded that the increased levels of lipid peroxidation product (MDA) and depletion of antioxidant such as Vitamin C and SOD except uric acid in preeclamptic women may suggest that oxidative stress plays a key role in inducing oxidative stress and expression of preeclampsia.

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1. Introduction

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent.[1] Preeclampsia is a pregnancy-specific hypertensive disorder sometimes progressing into a multiorgan cluster of varying clinical features affecting some 5-8% of pregnant women worldwide as a result of vasospasm and endothelial activation.[2,3] In India the incidence of preeclampsia is reported to be 8-10% of the pregnancies.[4,5] It is characterized by hypertension to the extent of 140/90 mm Hg or more, proteinuria (>300 mg/day) and pathological edema after 20^{th} weeks of gestation.[6] Despite many research, the cause of preeclampsia is not clear. Maternal symptoms are thought to be secondary to endothelial cell dysfunction. Reduced perfusion as a result of abnormal placentation leads to ischemia perfusion injury to the placenta. Placental oxidative stress, resulting from the ischemia perfusion injury, is said to be involved in the etiopathogenesis of preeclampsia.[7]

Preeclampsia is characterized as a state of oxidative stress resulting from increased generation of free radicals

* *Corresponding author.*
E-mail address: sonal.sogani246@gmail.com (S. Sogani).

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and decreased levels of antioxidants which scavenge free radicals. Oxidative stress occurs when generation of reactive oxygen species (ROS) increases and overwhelms the body's antioxidant defenses. Reactive oxygen species is highly reactive metabolites which are derived from molecular oxygen and nitrogen. These ROS attack the phospholipids of cell membranes and react with polyunsaturated fatty acids to form lipid peroxides resulting in cellular injuries. ROS have been proposed as a promoter of lipid peroxidation and endothelial cell dysfunction.[7] Thus vascular endothelial dysfunction in preeclampsia may be caused by uncontrolled lipid peroxidation. Lipid peroxidation is an oxidative process which occurs at low levels in all cells and tissues. Variety of antioxidant mechanisms serve to control this peroxidative process under normal conditions.[8] Antioxidants are enzymes or compounds that scavenge and reduce the presence of free radicals. These naturally occurring antioxidants usually protects the cells and tissues from the effects of lipid peroxidation. Cumulative evidences in recent years has shown that in preeclampsia, there are an increase in lipid peroxidation and a decrease in antioxidants protection leading to oxidative stress.[9] Nowadays measurement of lipid peroxidation has become an acceptable trend in medicine to consider at oxidative stress at molecular level. For this reasons, the present study was conducted to evaluate the changes in the level of lipid peroxidation markers and antioxidants in preeclamptic pregnant women and its comparison with normotensive pregnant women.

2. Materials and Methods

The present case control study was conducted in the Department of Biochemistry at Pacific Institute of Medical Sciences (PIMS), Umarda, Udaipur (Raj.). The 50 pregnant women were taken as subjects clinically diagnosed as preeclampsia during third trimester (28-40 weeks) with the age 18-35 years visiting obstetrics OPD and wards of M.Y. Hospital. This was done on the basis of blood pressure, (both systolic and diastolic) proteinuria and pathological edema, which are the diagnostic criteria of preeclampsia. As a control group 50 healthy normotensive pregnant women were taken who were also in the third trimester (28-40 weeks) of their pregnancy with the age 18-35 years along with the same parity and gestational age. Inclusion criteria for preeclamptic pregnant women included in the study were: should not be using any kind of oral contraceptives, anticoagulant drugs, should be non-smokers and non alcoholics and exclusion criteria was: past history of diabetes, systemic or endocrine disorder, chronic infection, chronic renal disease and hypertension women in the labor pains were excluded from the study.

Preeclampsia was diagnosed according to American college of Obstetrics and Gynecology (ACOG) criteria: a blood pressure higher than 140/90 mm Hg and proteinuria more than 300mg/24hr were observed on at least two occasions more than 6hrs apart after the 20^{th} weeks of pregnancy.

2.1. Sample collection

Fasting ante-cubital venous blood (10ml) were obtained with aseptic measure. After clotting, the blood was centrifuged for 30 minutes and the supernatant (serum) was taken in the separate test tube. Serum for MDA, SOD, Vitamin C and Uric acid estimation was ready.

Serum MDA levels were estimated by manual method of Beuge et al.[10] using TBARS method (Thiobarbituric acid reactive substances). Serum SOD levels were measured using a commercially available kit (Ransod; Randox Laboratories Ltd.) Serum Vitamin C levels were estimated calorimetrically using a DTCS reagent according to the method developed by Teitz.[11] Serum Uric acid levels were estimated calorimetrically using enzymatic PAP method with uricase-peroxidase.[12]

2.2. Statistical analysis

All the data were expressed as mean ± SD. The statistical significance was evaluated by Student's t-test using SPSS software, version 20. The level of significance was set at < 0.05.

3. Results

Table 1 shows the Anthropometric factors of healthy normotensive pregnant women andpreeclamptic pregnant women. When comparison of maternal age and body mass index was done between both the groups, no significant difference was observed between the groups ($p>0.05$, Table 1). Gestational age, systolic and diastolic blood pressures were significantly higher in preeclamptic pregnant women as compared to healthy normotensive pregnant women ($p<0.001$, Table 1).

Table 2 shows the comparison of levels of Oxidative stress markers between healthy normotensive pregnant women and preeclamptic pregnant women. The levels of MDA was found to be elevated in preeclamptic pregnant women when compared with healthy normotensive pregnant women and the difference was found to be highly significant between both the groups ($p<0.0001$, Table 2).

Table 3 shows the comparison of levels of Antioxidants between healthy normotensive pregnant women andpreeclamptic pregnant women. The levels of SOD and Vitamin C was found to be decreased and the levels of uric acid was found to be increased in preeclamptic pregnant women when compared with healthy normotensive pregnant women and the difference was found to be highly significant between both the groups ($p<0.0001$, Table 3).

Table 1: Comparison of mean and standard deviation of Anthropometric factors of control and subjects

Anthropometric factors	Healthy normotensive pregnant women (n=50)	Preeclamptic pregnant women (n=50)
	Mean ± SD	Mean ±SD
Age (yrs)	23.36 ± 2.50	22.82 ± 2.82[@]
BMI (Kg/m^2)	23.92 ± 1.61	24.12 ± 1.46[@]
Gestational age (wks)	38.76 ±2.92	35.74 ±2.74[#]
Systolic blood pressure (mm of Hg)	114.6 ± 4.98	140.2 ± 5.47[#]
Diastolic blood pressure (mm of Hg)	75.4 ± 5.73	91.88 ± 6.46[#]

[@] p>0.05-comparedwith healthy normotensive pregnant women, [#] p<0.001-compared with healthy normotensive pregnant women

Table 2: Comparison of mean and standard deviation of level of oxidative stress marker of control and subjects

Oxidative stress marker	Healthy normotensive pregnant women (n=50)	Preeclamptic pregnant women (n=50)
	Mean ± SD	Mean± SD
MDA (nmol/mL)	4.09 ± 1.46	8.16 ± 1.26[*]

[*] p<0.001-compared with healthy normotensive pregnant women

Table 3: Comparison of mean and standard deviation of levels of antioxidants of control and subjects

Antioxidants	Healthy normotensive pregnant women (n=50)	Preeclamptic pregnant women (n=50)
	Mean ± SD	Mean± SD
SOD(U/mL)	4.44 ± 0.99	2.37 ± 0.64[**]
Vitamin C	0.88 ± 0.208	0.30 ± 0.19[**]
Uric acid	4.37 ± 1.30	5.49 ± 1.49[**]

[**] p<0.001-compared with healthy normotensive pregnant women

4. Discussion

Preeclampsia is considered a disorder which is strongly related to oxidative stress.[12] Evidences proves that in preeclampsia there is a biochemical imbalance with an increase of oxidative stress and a deficient antioxidant protection.[13] It is difficult to measure free radicals as they are transient in nature and are unstable. They have a tendency to cause lipid peroxidation and have been used as an indirect measure.[8] Free radicals released from the poorly perfused fetoplacental unit initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes, converting them to form lipid peroxidase by direct reaction of oxygen and lipids.[9]

Our study concluded that the levels of MDA were significantly elevated (p<0.0001) in preeclamptic pregnant women as compared to normotensive pregnant women. These findings were in consistence with the findings of other authors who also observed increased MDA levels in their studies.[14–16] Lipid peroxidation is of destructive character and so its uncontrolled nature in preeclampsia is consider as an etiological factor. The products of the lipid peroxidation are of candidate factors which causes disturbance in the maternal vascular endothelium. They also inhibit prostacyclin synthesis and stimulate smooth muscle contraction resulting in widespread vasospasm, a prominent feature of preeclampsia.[9]This endothelial damage ultimately increases diastolic blood pressure which further aggravates the condition of preeclamptic patients.[17]

Pregnancy has been characterized as an inflammatory state, and these changes are exacerbated in preeclampsia.[18] A generalized activation and increased leukocytes numbers due to delayed neutrophils apoptosis contribute to leukocytosis or neutrophilia during pregnancy and preeclampsia. It contributes to endothelial dysfunction or activation in preeclampsia.[19] Increased activation of WBC also exaggerates placental response which results in elevated formation of reactive oxygen species like superoxide radical which will cause increase in lipid peroxidation that damage the vascular endothelium, membrane of cells and organelles which is proved by elevated MDA level.[20]

Preeclampsia is a hypertensive disorder of pregnancy in which antioxidant defenses fail and tissues are injured. It has been suggested that there is a deficiency in protective antioxidant systems or increased utilization of antioxidants in preeclamptic pregnant women when compared with normotensive pregnant women. Significant decrease (p<0.001) in serum SOD level in preeclampsia has been found in the present study as compared to normotensive pregnant women. Our studies also reveal similar findings.[14,21,22]

Decreased activity of SOD is one of the hall marks of preeclampsia.[23] SOD are important part of the

defense system. It protects and revitalizes cells and reduces the rate of cell destruction. It is one of the most important antioxidant enzyme which is capable of neutralizing some of the most dangerous free radicals, the superoxide radicals thus preventing its excessive accumulation and may contribute to the continuation of pregnancy.[8,9,24] It scavenges the superoxide radical by catalyzing its dismutation into H_2O_2 and O_2.[25]

Several studies have demonstrated decreased serum levels of vitamin C in preeclamptic pregnant women as compared to normotensive pregnant women ($p<0.0001$).[16,26,27] Our findings were in accordance with these authors. Vitamin C plays an important role in preventing lipid peroxidation. Reduced ascorbate is effective in protecting plasma lipids and susceptible molecules from peroxidation. It is observed that in preeclamptic pregnant women, antioxidants scavange the increased free radicals thus resulting in lowered levels of antioxidants.[26,28]

In preeclamptic pregnancy, the oxidant/antioxidant imbalance characterizes oxidative stress which is indicated by low vitamin C levels. Vitamin C is the most important aqueous phase chain-breaking antioxidant. Antioxidant defense systems include the chain breaking antioxidants that directly scavenges radicals in membranes and lipoprotein particles and prevents lipid peroxidation. Chain breaking antioxidants present in the aqueous compartment.[29,30]

Uricacid is a marker of oxidative stress, tissue injury and renal dysfunction. Abnormal tropbhoblast invasion is seen in preeclampsia due to which placenta receives less blood supply from uteroplacental artery. Placenta thus becomes hypoxic which causes placental tissue breakdown and provides additional purines source. These damaged placental tissues and placenta are the rich sources of purines for generation of uric acid by xanthine oxidase.[31] This may lead to decrease in the renal tubular excretion. Thus the altered renal clearance of uric acid is due to renal dysfunction and increased xanthine oxidase activity.[32]

Hyperuricemia in preeclampsia is primarily due to decreased renal clearance and increased tubular reabsorption of uric acid as the glomerular filtration rate is decreased.[33] Hyperuricemia also contributes to impaired endothelial integrity and contribute to the pathogenesis of preeclampsia. It has an important role in vascular damage and oxidative stress.[31]

5. Conclusions

The present study has shown a significant increase in serum MDA levels, the indicator of lipid peroxidation which suggests that lipid peroxidation plays a role on the pathogenesis of preeclampsia. Increased lipid peroxidation causes increased consumption of antioxidant free radical scavenging system. Also decreased level of SOD that causes oxidative stress, some endothelial disturbing factors like uric acid and decreased level of vitamin C is one of the pathophysiological causes. An imbalance between lipid peroxides and the antioxidant systems in preeclampsia is observed. Thus estimation of serum MDA, SOD, vitamin C and uric acid are useful in predicting the extent of endothelial damage and preventing the complications in preeclampsia. As antioxidant stress can provoke endothelial dysfunction, pregnant and preeclamptic pregnant women should be given antioxidant supplementation to prevent the overwhelming effect of oxidative stress.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Sonal Sogani, Associate Professor

Suman Jain, Associate Professor

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