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Research Article

Alterations of antioxidant enzymes in preeclampsia

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ABSTRACT

Background: Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to considerable extent and hypertension is the most common problem encountered during pregnancy, complicating 5-10% of pregnancies. Recent reports suggest that free radical induced endothelial damage as an important factor in the pathogenesis of preeclampsia. Such cell injury might in turn is counteracted by the action of several in vivo antioxidants. The aim of present study was to determine the activity of enzymatic antioxidants - glutathione peroxidase and catalase in preeclampsia.
Methods: Thirty cases of preeclampsia and thirty gestational age matched normotensive pregnant women attending Narayana General Hospital, Nellore were included in the study. The antioxidant enzymes - RBC glutathione peroxidase and catalase activities were determined by the respective laboratory methods in preeclampsia cases and compared with that of normotensive pregnant women.
Results: The activity of catalase was significantly decreased (mean ± SEM 5.6 ± 0.26 vs. 8.5 ± 0.32 k/ml) and that of glutathione peroxidase was significantly increased (mean ± SEM 103.9 ± 1.67 vs. 83.8 ± 1.85 U/gm of Hb) in preeclamptic cases when compared with that of normotensive pregnant women.
Conclusions: We conclude that activities of antioxidant enzymes-glutathione peroxidase and catalase are altered in preeclampsia. The increased activity of the antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress while the decreased activity supports that lipid peroxidation is an important causative factor in the pathogenesis of preeclampsia.

Keywords: Glutathione peroxidase, Catalase, Preeclampsia

INTRODUCTION

Preeclampsia is a multisystem disorder characterised by hypertension to the extent of 140/90 mm of Hg or more, proteinuria (≥300 mg/day) and edema induced by pregnancy after twentieth week.[1] Recent studies have shown that oxidative stress plays an important role in the pathogenesis of preeclampsia and may be a final common pathway leading to tissue damage.[2] An adaptive mechanism enhancing the maternal antioxidant defence system to counteract the effect of free radicals through enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and also nonenzymatic antioxidants like reduced glutathione (GSH) can prevent the occurrence of oxidative stress in preeclampsia. However, pregnancy is a state where this adaptation may be easily disrupted. There are conflicting reports on activities of the antioxidant enzymes like Glutathione Peroxidase (GPx), catalase in preeclampsia. Some studies have shown an increase in the activity of glutathione peroxidase,[3,4] whereas few studies reported a reduction in its activity[5-7] in preeclampsia. Similarly, the

activity of catalase has been reported to increase,[7] or decrease[8] in preeclampsia. In this context, the present study was undertaken to determine the activities of RBC glutathione peroxidase (GPx) and catalase in preeclampsia.

METHODS

Thirty cases of preeclampsia and thirty normotensive pregnant women attending antenatal clinic at Narayana General Hospital, Nellore were enrolled for the study after taking informed consent. Both cases and controls were primigravida, between 20-30 years of age and were in their third trimester.

Inclusion criteria: Women with Preeclampsia diagnosed based on definition of American College of Obstetricians and Gynecologists (ACOG)s: 1) Systolic blood pressure greater than 140 mm of Hg or rise of at least 30 mm of Hg or 2) Diastolic blood pressure greater than 90 mm of Hg or rise of at least 15 mm of Hg (manifested on two occasions at least 6 hours apart) and 3) Proteinuria of 300 mg or greater in 24 hours urine collection or protein concentration of 1 gm/litre (on two occasions at least 6 hours apart).[9] Subjects with normal pregnancy were normotensive and had no proteinuria.

Exclusion criteria: Tobacco adductors, illnesses like severe anemia, diabetes mellitus, ischemic heart disease, essential hypertension, renal insufficiency, history of stroke and alcoholics.

5 ml of venous blood was collected into EDTA tube from the study subjects and antioxidant enzyme activities in RBC were determined.

Isolation of erythrocyte and haemolysate preparation

The blood samples were centrifuged at 3000 g for 15 minutes at 4°C and the isolated red cells were washed 4-5 times with 0.154 M NaCl to remove plasma and buffy coat. After the final wash, the required packed cells were lysed by hypotonic shock and different dilutions were used as haemolysate.

Hemoglobin estimation

Haemoglobin content of the erythrocyte was measured using cyamethemoglobin method.

Determiantion of GPx (EC 1.11.1.9) activity

Glutathione peroxidase activity was measured spectrophotmetrically[10] at 340 nm in 50 mM phosphate, 5 mM EDTA buffer, pH 7.0 containing 0.3 mM NADPH, 0.3 U/mL GRx, 5 mM GSH, 4 mM sodium azide, 75µM H_2O_2 and 10µL of erythrocyte lysate in a final reaction mixture of 3 mL. The hemolysate was pretreated with Drabkin's reagent to produce stable cyanmethemoglobin, eliminating methemoglobin-reductase-mediated (or nonenzymatic) oxidation of NADPH. One unit of GPx was considered to be the amount necessary to oxidise 1 µmol NADPH/min. The activity was expressed as U/g of Hb.

Determination of catalase (1.11.1.6) activity

Catalase decomposes H_2O_2 and forms water and molecular oxygen.

H_2O_2 absorbs maximum light at 240 nm. The absorbance decreases as H_2O_2 is being decomposed by catalase. Determination of catalase activity was done by monitoring the rate of H_2O_2 decomposition spectrophotometrically at 240 nm following the procedure of Abei.[11]

RBC lysate was prepared by adding 0.1 ml of RBC to 0.4 ml of milli Q water. 20 µl of RBC lysate was further diluted in 10 ml of phosphate buffer (50 mM KH_2PO_4 and 50 mM Na_2PO_4, pH 7.0). Catalase activity was performed by adding 1.0 ml of 30 mM H_2O_2 to 2 ml of diluted RBC lysate. The decomposition of H_2O_2 was measured by monitoring the decrease in the absorbance at 240 nm for 60 seconds. The catalase activity was expressed as rate constant (k/ml).

Statistical analysis: Data was analysed using statistical software SPSS version 20. Values are expressed as mean ± SEM (standard error of mean). Comparison of values between cases and controls was done using Student's t test. A p value of less than 0.05 was considered statistically significant.

RESULTS

The activity of catalase was significantly decreased (mean ± SEM 5.6 ± 0.26 vs. 8.5 ± 0.32 k/ml) and that of glutathione peroxidase was significantly increased (mean ± SEM 103.9 ± 1.67 vs. 83.8 ± 1.85 U/gm of Hb) in preeclamptic cases when compared with that of normotensive pregnant women.

Table 1 shows comparison of RBC catalase activity between preeclamptic and normal pregnant women.

Table 2 shows comparison of RBC Glutathione peroxidase activity between preeclamptic and normal pregnant women.

Table 1: Comparison of RBC catalase activity between preeclamptic and normal pregnant women.

Parameter	Preeclamptic women (n=30) Mean ± SEM	Normal pregnant Women (n=30) Mean ± SEM	P value
Catalase (k/ml)	5.6 ± 0.26	8.5 ± 0.32	0.000*

*p value <0.05 statistically significant, n=number of subjects

Table 2: Comparison of RBC Glutathione peroxidase activity between preeclamptic and normal pregnant women.

Parameter	Preeclamptic women (n=30) Mean ± SEM	Normal pregnant Women (n=30) Mean ± SEM	P value
GPX (U/g Hb)	103.9 ± 1.67	83.8 ± 1.85	0.000*

*p value <0.05 statistically significant, n=number of subjects

DISCUSSION

Preeclampsia can have significant impact on health of both mother and fetus. In health, oxidation by free radicals and neutralisation by antioxidants remain in balance. In our previous study we demonstrated that uncontrolled lipid peroxidation is a key factor in the etiopathogeneiss of preeclampsia.[12] Elevation of lipid peroxidation products causes impaired antioxidant enzyme defence mechanism and this imbalance may contribute to the pathogenesis of preeclampsia. In the present study, we observed significant alterations in antioxidant enzymes, GPx and catalase in preeclampsia. A statistically significant decrease in the activity of catalase (p value <0.000) was noted in preeclampsia when compared with that of normotensive pregnant women. Similar to our study decreased activity of catalase was noted by others.[6,13,14] Catalase is the enzyme which protects the cells from the accumulation of hydrogen peroxide by dismutating it to water and oxygen or by using it as an oxidant in which it works as peroxidase.[14,15] In contrast to our study, few others reported increased activity of catalase enzyme in preeclampsia.[16-18]

We also observed a statistically significant increase in the activity of GPx (p value <0.000) in preeclampsia compared to that of normotensive pregnant women. Also, few others observed increased GPx activity in preeclampsia, similar to our study.[14,19,20] In contrast to our study, a decrease in GPx activity in preeclampsia was noted by others.[5,6,17] Glutathione peroxidase (GPx), an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes.[21] The rise in the activity of this antioxidant enzyme could be due to its induction to counter the effect of increased oxidative stress.[14] Also increase in GPx activity might be explained on the basis of the lack of vitamin E levels in cases of severe preeclampsia.[3,4]

CONCLUSION

Pregnancy creates oxidative stress and stress level further increases in preeclampsia. This causes an imbalance between lipid peroxidation and antioxidant defences in preeclampsia, leading to free radical mediated endothelial dysfunction. We strongly recommend that all pregnant women should be supplemented with antioxidants to prevent overwhelming effect of oxidative stress.

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Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Pradnya Phalak, Jyoti Kulkarni, Mona Tilak, A. P. Thorat. Role of lipid peroxidation and antioxidant status in pathogenesis of preeclampsia. Indian J Basic Appl Med Res. 2013;2(6):536-9.
2. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2005;3:28.
3. Llurbha E, Gratacos E, Martin-Gallan P, Cabero L, Dominguez C. A comprehensive study of oxidative stress and antioxidant status in preeclampsia and normal pregnancy. Free Radic Biol Med. 2004;37:557-70.
4. Orhan H, Onderoglu L, Yiicel A, Sahin G. Circulating biomarkers of oxidative stress in complicated pregnancies. Arch Gynecol Obstet. 2003;267:189-95.
5. Yildirim A, Altinkaynak K, Ahsoy H, Sahin YN, Ahay F. Plasma xanthine oxidase, superoxide dismutase and glutathione peroxidase activities and uric acid levels in severe and mild preeclampsia. Cell Biochem Funct. 2004;22:213-7.
6. Chamy VM, Lepe J, Catalan A, Retamal D, Escobar JA, Madrid EM. Oxidative stress is closely related to clinical severity of preeclampsia. Biol Res. 2006;39:229-36.
7. Atamer Y, Kocyigit Y, Yokus B, Atamer A, Erden AC. Lipidperoxidation, antioxidant defence, status of trace metals and leptin levels in preeclampsia. Eur J Obstet Gynecol Biol. 2005;119:60-6.
8. Dordević NZ, Babić GM, Markovic SD, Ognjanovic BI, Stajn AS, Zikic RV, et al. Oxidative stress and changes in antioxidant defence system in erythrocytes of preeclampsia. Reprod Toxicol. 2008;25:213-8.
9. Patil SB, Kodliwadmath MV, Kodlwadmath SM, Patil MB. Lipidperoxidation and nonenzymatic antioxidants status in preeclampsia and postpartum preeclmaptic women. Natl J Basic Med Sci. 2012;3(1):39-43.
10. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidise. J Lab Clin Med. 1967;70:158-69.
11. Abei H. Catalase *in vitro*. Methods Enzymol. 1984;105:121-6.
12. T. Sharmila Krishna, D. Raja Rajeswari, E. Venkata Rao, R. R. Aparna, J. N. Naidu. Correlation between lipid peroxidation product - malondialdehyde (MDA) and reduced glutathione (GSH) in

preeclampsia. Int J Appl Biol Pharmaceut Technol. 2015;6(2):196-9.
13. Dave J, Vaidya M, Sharma M. A comparative study of degree of oxidative stress in preeclamptic and healthy pregnant women in Madhya Pradesh. Int J Adv Pharm Biol Chem. 2012;1(3):397-401.
14. Krishna Mohan S, Venkataramana G. Study of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with pregnancy induced hypertension. Indian J Physiol Pharmacol. 2007;51(3):284-8.
15. Lenzi A, Cualosso F, Gandini L, Lombardo F, Dondero F. Placebo controlled double blind cross over glutathione therapy in male infertility. Hum Reprod. 1993;8:1657-62.
16. Suhail M, Suhail MF, Khan H. Role of vitamin C and E in regulating antioxidant and prooxidant markers in preeclampsia. J Clin Biochem Nutr. 2008;43:210-20.
17. 16. Suhail M, Suhail MF, Khan H. Alterations in antioxidant and prooxidant balance in preeclampsia - impact on erythrocyte osmotic fragility. Biochem Med. 2008;18(3):331-41.
18. Ciragil P, Kurutas EB, Gul M, Kilinc M, Aral M, Gueven A. The effects of oxidative stress in in urinary tract infection during pregnancy. Mediators Inflamm. 2005;5:309-11.
19. Sharma JB, Sharma A, Bahadur A, Vimala N, A. Satyam, S. Mittal. Oxidative stress markers and antioxidant levels in normal pregnancy and preeclampsia. Int J Gynecol Obstet. 2006;94:23-7.
20. Cebovic TN, Maric D, Nikolic A, Novakov-Mikic A. Antioxidant status in normal pregnancy and preeclampsia upon multivitamin supplementation in the region of Vojvodina. Int J Biosci Biochem Bioinform. 2013;3(2):138-43.

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