Role of lipid peroxidation and enzymatic antioxidants in pregnancy-induced hypertension

S.B. Patil[1], *Assist. Prof.*; **M.V. Kodliwadmath**[1], *Prof.*; **S.M. Kodliwadmath**[2], *Prof.*

[1]*Department of Biochemistry,* [2]*Department of Obstetrics and Gynaecology, J. N. Medical College, Nehru Nagar, Belgaum (Karnataka)*

Summary

Aims and Objectives: Preeclampsia remains a major cause of maternal mortality and morbidity. It is a leading indication for iatrogenic premature delivery. Oxidative stress is considered to be one of the factors in the disease process. The present study is centered on the concept that elevated levels of lipid peroxidation (malondialdehyde) due to a decline in the efficacy of antioxidant defenses may predispose an individual to preeclampsia. *Material and Methods*: In the present study we measured lipid peroxidation products (MDA) and the counteracting enzymatic antioxidants. The study comprises 25 healthy non-pregnant women as controls, 25 third trimester normal pregnant women and 25 preeclamptic patients of the same trimester. Estimation of lipid peroxidation by thiobarbituric acid (TBARS) and enzymatic antioxidants were carried out by standard methods. *Results*: In the preeclamptic group malondialdehyde, a product of lipid peroxidation, was significantly increased while enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were reduced significantly as compared to normal pregnant and non-pregnant controls. *Conclusion*: Increased levels of lipid peroxides and reduced antioxidant activities clearly demonstrate the presence of oxidative stress in preeclampsia.

Key words: Lipid peroxidation; Malondialdehyde; Oxidative stress; Pregnancy-induced hypertension (PIH).

Introduction

Preeclampsia is one of the leading causes of fetomaternal morbidity and mortality. It accounts for more than 40% of iatrogenic premature deliveries [1]. Increase in the risk of death for women at 20-32 weeks of gestation is more than for those at 36-40 weeks [2]. The age-specific mortality ratio for preeclampsia reflects a slight risk for younger women (under the age of 20) and is markedly increased in older women. Women who develop preeclampsia during pregnancy are at an increased risk of abruptio placentae, acute renal failure, cerebrovascular and cardiovascular complications and maternal death [3].

The vast majority of our patients belong to low a socioeconomic status. They seek medical help in case of serious problems and in a large majority of cases preeclampsia remains asymptomatic and remits spontaneously, since a diagnosis of preeclampsia is missed. Hence these patients never come in contact with the healthcare system and do not figure in the statistical analysis. There are many reported predisposing factors related to preeclampsia like maternal age, familial aggregation, race, smoking, socioeconomic level, diet, climatic and geographical conditions.

Lipid peroxides are produced when free radicals attack polyunsaturated fatty acids (PUFA), cholesterol and lipoprotein in the membranes. Lipid peroxides are highly reactive compounds that may cause cellular dysfunction by several mechanisms, including a direct interaction with cell membranes and an activation of redox sensitive genes [4]. Lipid peroxidation products inhibit prostacyclin synthesis and stimulate smooth muscle contraction. Therefore increased free radical activity causes platelet aggregation resulting in an imbalance between thromboxane, prostacyclin and vasospasm with features of preeclampsia. Thus free radicals can have deleterious effects during pregnancy by triggering preeclampsia.

It is envisaged that increased free radical activity (oxidative stress) arises from increased production of free radicals or a deficiency in the protective antioxidants. Pregnancy-induced hypertension (PIH), is associated with endothelial dysfunction. Such dysfunction could be caused by oxidative stress [5].

The formation of free radicals is a normal physiological process, but increased production of free radicals can act on lipids to cause lipid peroxidation [6]. The cells have evolved a number of counter acting antioxidant defenses. These antioxidant defense mechanisms can be categorized under the heads of free-radical scavenging and chain-breaking antioxidants. The free-radical scavenging mechanisms include enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GHS-Rx) and catalase, which limit the cellular concentration of free radicals and prevent excessive oxidative damage [7].

The aim of the present study is to elicit the biochemical changes with respect to lipid peroxidation and enzymatic antioxidants in normal pregnant women and women with PIH in the third trimester with non-pregnant healthy controls.

Revised manuscript accepted for publication March 13, 2007

Table 1. — *Malondialdehyde (MDA) enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx) and catalase) levels in non-pregnant women, 3rd trimester normal pregnant women and 3rd trimester PIH patients).*

	MDA		SOD		GSH-Px		GSH-Rx		Catalase	
	n mol/ml	p value	IU/g Hgb	p value	IU/g Hgb	p value	IU/g Hgb	p value	IU/g Hgb	p value
Non-pregnant (n = 25)	1.19 ± 0.09		683.9 ± 155.25		31.08 ± 4.45		10.52 ± 4.67		8.13 ± 2.21	
3rd trimester normal pregnancy (n = 25)	1.79 ± 0.14	0.000*	542.64 ± 139.98	0.001*	23.45 ± 4.79	0.000*	7.78 ± 3.40	0.220*	6.20 ± 1.69	0.001*
3rd trimester PIH (n = 25)	2.93 ± 0.54	0.000*	452.07 ± 103.91	0.000*	18.58 ± 4.46	0.000*	6.86 ± 2.33	0.002*	5.07 ± 1.31	0.001*
		0.000+		0.012+		0.001+		0.275+		0.023+

* Comparison with non-pregnant controls; + Comparison with third trimester normal pregnant women.

Materials and Methods

The present study was carried out jointly by the Department of Biochemistry and Obstetrics and Gynecology from July 2000 to June 2004 and the ethical committee of J.N. Medical College and Belgaum District Civil Hospital approved the study protocol. Written informed consent was given by individual subjects. The study comprised 75 cases, of which 25 were normal healthy controls, 25 normal healthy pregnant women in the third trimester and 25 were in the third trimester with PIH. The subjects selected for the present study were attending and/or admitted to the district hospital and ranged in age from 20-40 years. The preeclamptic patients were diagnosed by the presence of persistent hypertension (more than 140/90 mmHg) gross proteinuria (urine protein heat coagulation test) and pathological edema.

The subjects were of low socioeconomic status which was based on low income. Women who suffered from obesity, diabetes mellitus (under medication and untreated), alcoholism, severe anemia (< 6.0 g % of Hgb) and any other systemic disorders were excluded from the study.

Collection and Storage of Blood Samples

A blood sample (5 ml) was drawn by venipuncture and collected in a heparinized tube (5 units/ml of blood). Malondialdehyde, a product of lipid peroxides detectable in blood, was used as an indicator of lipid peroxidation. Malondialdehyde concentrations were determined by using thiobarbituric acid [8]. Hemolysate was prepared to determine antioxidant acivities like superoxide dismutase (Misra Fridovich [9]), glutathione peroxidase, glutathione reductase and catalase by Beutler [10] *et al.*, and hemoglobin by Drabkin [11].

The results are presented as mean ± SD. The data was analyzed using the unpaired 't' test.

Results

A statistically significant increase in the levels of circulating malondialdehyde (a lipid peroxidation marker) was observed in the third trimester of normal pregnant women and in PIH patients as compared to non-pregnant controls. A further increase was observed in PIH patients when compared to normal pregnant women.

Significant decreased activity of all the enzymatic antioxidants (SOD, GSH-Px, GSH-Rx and catalase) was observed in normal pregnant women as well as in PIH patients as compared to non-pregnant controls. Further decreased activity of all the enzymatic antioxidants was observed in PIH patients compared to normal pregnant women (Table 1).

Discussion

Oxidative stress during pregnancy and PIH was evaluated in the present study by analyzing prooxidant and enzymatic antioxidants. Lipid peroxidation was considered as a marker for prooxidant, whereas superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were considered as enzymatic antioxidants.

Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. Markers of lipid peroxidation (MDA) are increased during the progression of normal pregnancy and further aggravated in PIH patients [12].

The human RBC has an effective mechanism to prevent and neutralize this oxidative stress-induced damage which is accomplished by antioxidant enzymes like glutathione peroxidase, catalase and superoxide dismutase. These enzymes are present as metalloenzymes. Superoxide dismutase is a metalloprotein present as copper-zinc-superoxide dismutase in which copper is the catalytic metal and zinc helps to maintain the enzyme structure. Catalase is a hemoprotein catalyzing the decomposition of hydrogen peroxide to water and oxygen. Glutathione peroxidase is a seleno-enzyme, which catalyzes the degradation of hydrogen peroxide and hydroperoxides at the expense of reduced glutathione [13].

Hubel *et al.* [14] and Kharb *et al.* [15] have noticed that serum lipid peroxides are known to increase in pregnancy and that this increase was exaggerated in preeclampsia. Increased lipid peroxide levels can increase the susceptibility of polyunsaturated fatty acids to peroxidative damage, leading to the formation of malondialdehyde (MDA).

The present study showed significantly increased lipid peroxide (MDA) levels in the third trimester of normal pregnant women compared to non-pregnant women and a further increase was observed in PIH patients when compared to normal pregnant women as well as non-pregnant women. Our findings are in accordance with a few reports that indicated lipid peroxidation may be an important factor in the pathogenesis of PIH [16, 17].

Wisdom *et al.* [18] and Davidge *et al.* [19] noticed that superoxide activity was reduced in the gestation period of normal pregnancy and was lowest in PIH with proteinuria. This could be due to reduced enzyme production or

enzyme inactivation by lipid peroxides. Our study showed a significant decrease in the activity of superoxide dismutase and catalase in normal pregnant women as compared to non-pregnant controls. A further decrease was observed in PIH patients when compared to normal pregnant women and controls, which were supported by Kumar and Das [12].

Pathak *et al.* [20] observed a progressive fall in the activity of glutathione peroxidase and superoxide dismutase in normal pregnancy. Decreased activity of glutathione peroxidase and significantly increased levels of MDA were observed in women with preeclampsia versus women with normal pregnancies. Glutathione peroxidase is one of the primary antioxidants present in tissues and it inactivates lipid peroxides thereby limiting their levels. Our study showed a significant decrease in the activity of glutathione peroxidase and glutathione reductase in normal pregnant women as compared to non-pregnant women. Further decreased activity was seen in PIH patients when compared to normal pregnant women and non-pregnant controls.

A negative correlation was observed between lipid peroxidation and enzymatic antioxidant activities with increased lipid peroxide levels.

Conclusion

It is evident from the present study that increased oxidative stress in PIH leads to decreased activity of antioxidants. Hence natural antioxidants like Vitamin E and C supplementation in the treatment protocol could be beneficial to overcome impending complications like PIH.

References

[1] Chapell L.C., Seed P.T., Briley A.L. *et al.*: "Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomized trial". *Lancet,* 1999, *354,* 810.

[2] Meis P.J., Goldenberg R.L., Mercer B.M. *et al.*: "The preterm prediction study: risk factors for indicated preterm births". *Am. J. Obstet. Gynecol.,* 1998, *178,* 562.

[3] Abdella T.N., Sibai B.M., Hays J.M. *et al.*: "Relationship of hypertensive disease to abruption placentae". *Obstet. Gynecol.,* 1984, *63,* 365.

[4] Tsuktani E.: "Etiology of EPH-gestosis from the viewpoint of dynamics of vasoactive prostanoid, lipid peroxides and vitamin E". *Acta Obstet. Gynecol.*, 1983, *35,* 713.

[5] Roberts J.M., Taylor R.N., Musci T.J. *et al.*: "Preeclampsia: an endothelial cell disorder". *Am. J. Obstet. Gynecol.,* 1989, *161,* 1200.

[6] Cheesman K.H., Slater T.F.: "An introduction to free radical biochemistry". *Br. Med. Bull.,* 1993, *49,* 481.

[7] Scott W.: "Lipid peroxidation in pregnancy". *Hypertension in pregnancy*, 1994, *13,* 1.

[8] Yagi K.: "Assay for lipid peroxide level and its clinical significance". In: Yagi K. (ed.). Lipid Peroxide Level in Biology Medicine. New York, Academic Press, 1982, 223.

[9] Mishra H.P., Fridovich I.: "The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase". *J. Biol. Chem.,* 1972, *247,* 3170.

[10] Beutler E., Blume K.G., Kaplan J.C. *et al.*: "International Committee for Standardization in Haematology: recommended methods for red cell enzyme analysis". *Br. J. Haemat.*, 1977, *35,* 331.

[11] Drabkin D.L., Austin J.H.: "Spectrometric constants for the common hemoglobin derivatives in human drug and rabbit blood". *J. Biol. Chem.*, 1932, *98,* 719.

[12] Wickens D.: "Free radical oxidation (peroxidation) products in plasma in normal and abnormal pregnancy". *Ann. Clin. Biochem.*, 1981, *18,* 158.

[13] Gutteridge J.M.C.: "Free radicals in disease process a compilation of cause and consequence". *Free Radic. Res. Commun.,* 1993, 19, 141.

[14] Hubel C.A., James M., Robert M.D. *et al.*: "Lipid peroxidation in pregnancy: New perspective on preeclampsia". *Am. J. Obstet. Gynecol.*, 1989, *161,* 1025.

[15] Kharb S.: "Evaluation of oxidative stress in pre-eclampsia". *J. Obstet. Gynecol. India,* 2000, *50,* 56.

[16] Kumar C.A., Das U.N.: "Oxidant stress in pre-eclampsia and essential hypertension". *J. Assoc. Phys. India*, 2002, *50,* 1372.

[17] Desai P., Rathod S.P., Garge V., Mansuri Z.: "Evaluation of prooxidants and antioxidants in preeclampsia". *J. Obstet. Gynecol. India*, 2003, *53*, 445.

[18] Wisdom S.J., Rhoda W., James H. *et al.*: "Antioxidant systems in normal pregnancy and pregnancy induced hypertension". *Am. J. Obstet. Gynecol.*, 1991, *165,* 1701.

[19] Davidge S.T., Hubel C.A., Brayden R.D. *et al.*: "Sera antioxidant activity in uncomplicated and pre-eclamptic pregnancies". *Obstet. Gynecol.*, 1992, *79,* 897.

[20] Pathak S.S., Shetty D.N.: "Essentials of zinc in pregnancy to maintain antioxidant status". *Indian Pract.,* 2001, *54,* 766.

Address reprint requests to:
S.B. PATIL, Ph.D.
Department of Biochemistry
J.N. Medical College
Nehru Nagar
Belgaum, 5900010 (Karmataka)