Lipid peroxidation and antioxidant status in hypertensive pregnancies

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Summary

Aims and Objective: The present study was designed to evaluate the lipid peroxidation and non-enzymatic antioxidant status in hypertensive complications during pregnancy (preeclamptic and eclamptic) compared with healthy pregnant and non-pregnant patients as controls. *Materials and Methods:* 25 healthy non-pregnant women as controls, 25 third trimester normal pregnant women and 25 preeclamptic and 25 eclamptic patients of the same trimester. Lipid peroxidation as a thiobarbituric acid reactive substance reduced glutathione, Vitamin E, Vitamin C and Vitamin A. *Results:* Lipid peroxidation was significantly elevated and significantly decreased levels of antioxidants were found in preeclamptic and eclamptic patients as compared with normal pregnant and control subjects. *Conclusion:* Our study gives support to those few studies considering lipid peroxidation as an important factor in the pathogenesis of preeclampsia and eclampsia. The decrease in antioxidants, probably of a compensatory nature responding to the increased peroxide load in preeclampsia and eclampsia, may reflect the severity of the disease.

Key words: Lipid peroxidation; Malondialdehyde, Preeclampsia; Eclampsia.

Introduction

Hypertensive disorders are the most common medical complications of pregnancy, and are the third leading cause of maternal mortality, after thromboembolism and non-obstetric injuries. Preeclampsia/eclampsia is a specific pregnancy syndrome, occurring in approximately 5% of all pregnancies, 10% of first pregnancies and 20-25% of women with chronic hypertension [1]. Lipid peroxidation has been suggested as a causative factor and is found to increase in pregnancy [2]. The metabolic effects of lipid peroxides may be linked to an imbalance between the production of prostacyclin and thromboxane A2 [3] that is well documented in preeclampsia [4]. Lipid peroxides in preeclampsia has been suggested that poorly perfused placental tissue may evoke the free radical process and inception of generalized lipid peroxidation [5]. It is envisaged that increased free radical activity (oxidative stress) arises from increased production of free radicals or a deficiency in the protective antioxidant system. Preeclampsia is associated with endothelial dysfunction [6]. Such dysfunction could be caused by oxidative stress: the unsaturated lipids and thiol containing proteins in cell membranes are susceptible to free radical attack [7]. There is evidence of increased free radical activity in pregnancy-induced hypertension [8]. Specific antioxidants will play a role in diminishing the action of lipid peroxides.

In the present study, we tested the hypothesis that the normal balance between lipid peroxidation and antioxidant levels observed during uncomplicated pregnancies is impaired in preeclampsia and eclampsia. To test this hypothesis we measured plasma antioxidant levels relative to lipid peroxide levels during normal pregnancy and compared this relationship with that of preeclamptic and eclamptic patients.

Materials and Methods

The present study was carried out jointly by the Departments of Biochemistry and Obstetrics and Gynecology from July 2000 to June 2004. The ethical committee of J.N. Medical College and District Civil Hospital of Belgaum approved the study protocol. Informed consent was given by all subjects. The study comprised 100 cases, of which 25 were normal healthy controls, 25 were normal healthy pregnant women in the third trimester, 25 were third trimester preeclamptic women and 25 women were eclamptic in the same trimester. The subjects (aged 20-40 years) selected for the present study were outpatients or patients admitted to the District Civil Hospital of Belgaum. The diagnosis of preeclampsia was based on the definition of ACOG [8]: 1) Systolic blood pressure greater than 140 mm Hgb or a rise of at least 30 mm Hgb or 2) Diastolic blood pressure greater than 90 mm Hgb or a rise of at least 15 mm Hgb (manifested on two occasions at least 6 hours apart), and 3) Proteinuria of 300 mg or greater in a 24-hour urine collection or protein concentration of 1 g/l (on two occasions at least 6 hours apart). Eclampsia was defined as the occurrence of seizures in women with preeclampsia. Subjects with normal pregnancies were normotensive throughout the gestation and had no proteinuria. None of the women had received antihypertensive medication until the study samples were taken. Blood pressure levels and proteinuria were determined at the time of sampling.

The subjects were of low socio-economic status. Obese women, women with diabetes mellitus under medication and untreated diabetes, women with severe anemia (< 6.0 g% of Hgb), alcoholics, and women suffering from any other systemic disorders were excluded from the present study.

Blood samples (5 ml) were drawn by venipuncture and collected in a heparinized tube. Malondialdehyde, a product of

Revised manuscript accepted for publication October 29, 2008

lipid peroxide detectable in blood, was used as an indicator of lipid peroxidation. Malondialdehyde concentrations were determined by using thiobarbituric acid [9]. Plasma was separated to determine the antioxidant levels like reduced glutathione (Beutler E *et al*. [10]), Vitamin E (Quaife and Baker [11]), Vitamin C (Evelyn and Malloy [12]) and Vitamin A (Bessey *et al.* [13]). Statistical data were expressed as mean ± SD. Statistical significance was determined by ANOVA and the Bonferroni multiple comparison test.

Results

Table 1 summarizes the characteristics of the four groups. A statistically significant increase in the levels of circulating malondialdehyde, a marker of lipid peroxidation, was observed in the third trimester in normal pregnant women and in preeclamptic and eclamptic patients as compared to non-pregnant controls. Further increase was observed in preeclamptic and eclamptic patients when compared to normal pregnant women.

Non-enzymatic antioxidants like reduced glutathione, Vitamin E, Vitamin C and Vitamin A differed significantly in each group by analysis of variance.

Table 1. — *Malondialdehyde (MDA) enzymatic antioxidants reduced glutathione, Vitamin E, Vitamin C, and Vitamin A levels in the non-pregnant, 3rd trimester normal pregnant and 3rd trimester toxemic (preeclamptic and eclamptic) women.*

Groups	MDA n mol/ml	Reduced glutathione mg %	Vitamin E mg %	Vitamin C mg %	Vitamin A µg %
Non-pregnant women (n = 25)	1.19 ± 0.09	59.97 ± 1.3	1.42 ± 0.19	1.11 ± 0.24	28.62 ± 3.45
3rd trimester normal pregnancy (n = 25)	1.79 ± 0.14	50.77 ± 6.91	0.87 ± 0.15	0.96 ± 0.33	22.84 ± 5.07
3rd trimester pre-eclamptic (n = 25)	2.93 ± 0.54	41.35 ± 4.23	0.57 ± 0.16	0.66 ± 0.24	18.93 ± 5.28
3rd trimester eclamptic (n = 25)	4.80 ± 0.61	36.92 ± 4.71	0.48 ± 0.14	0.48 ± 0.22	16.11 ± 3.41
F value	347.849	113.075	167.781	30.891	36.729
p value	0.0005	0.0005	0.0005	0.0005	0.0005

Discussion

Oxidative stress during pregnancy and preeclampsia and eclampsia was evaluated in the present study by analyzing pro-oxidant and non-enzymatic antioxidants. Lipid peroxidation was considered as a marker for pro-oxidant, whereas reduced glutathione, Vitamin E, Vitamin C and Vitamin A were considered as 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 (such as MDA) have been increased during the progression of a normal pregnancy [14]. Non-enzymatic antioxidants like reduced glutathione, Vitamin E, Vitamin C and Vitamin A oppose the toxic actions of lipid peroxides and oxygen radicals by limiting the amount of lipid peroxide formation. In the lipophilic environment tocopherol acts as a protective chain-breaking antioxidant. Vitamin C acts as an antioxidant by scavenging superoxides, hydroxyl radicals and various lipid hydroperoxides. In addition it can also restore the antioxidant properties of oxidized Vitamin E.

Hubel *et al.* [2] and Kharb [15] have shown that serum lipid peroxides increase during pregnancy and this increase was exaggerated in preeclampsia. 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.

We found a significant increase in lipid peroxide (MDA) levels in the third trimester of normal pregnant women compared to non pregnant women, and a further increase was observed in preeclamptic and eclamptic patients when compared to normal pregnant women as well as non-pregnant women. Our findings are in accordance with some researchers that show lipid peroxidation may be an important factor in the pathogenesis of pregnancy-induced hypertension [16].

Glutathione, an effective reductant, plays an important role in a variety of detoxication processes. It readily neutralizes the hydroxyl radicals, which are considered to be a major source of free radical damage. Decreased levels of glutathione in maternal blood might indicate decreased detoxification of free radical scavenging in preeclampsia [17]. Our study showed a significant decreased level of reduced glutathione and increased levels of MDA in preeclamptic and eclamptic patients as compared to normotensive pregnant patients as well as non-pregnant controls, which was supported by Pyska *et al.* [18].

Kharb [19] reported that vascular endothelial damage has been implicated in the pathophysiology of preeclampsia. Significantly lower levels of Vitamin E and Vitamin C were observed in preeclamptic women as compared to controls. Our study indicates that normal pregnant women had markedly reduced plasma alpha tocopherol, ascorbic acid, and retinol as compared to non-pregnant controls. A further decrease was observed in preeclamptic and eclamptic patients when compared to normal pregnant women.

Hypertensive complications of pregnancy are not the only disease group where enhanced lipid peroxidation and decreased levels of antioxidants are reported. Early attention, intensive management and better treatment with antioxidant vitamins are essential to improve the maternal and fetal outcome in pregnancy-induced hypertensive patients.

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