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CLINICAL ARTICLE

Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia

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Abstract

Objective: To compare the levels of 3 oxidative stress markers (glutathione peroxidase [GPX], superoxide dismutase [SOD], and malondialdehyde [MDA]) and 2 antioxidants (vitamin C and lycopene) in healthy and pre-eclamptic pregnant women. *Methods:* Circulating levels of GPX, SOD, MDA, vitamin C and lycopene were measured in 50 healthy pregnant women and 50 women with pre-eclampsia (PE) (41 with mild PE and 9 with severe PE) attending the antenatal clinic or admitted to the maternity ward of the All-India Institute of Medical Sciences, New Delhi, India. *Results:* The levels of GPX, SOD and MDA were significantly higher in women with PE than in controls, and the increase was higher in women with severe PE ($P<0.001$ using analysis of variance and the Kruskal Wallis test). The levels of vitamin C and lycopene were significantly lower in women with PE than in controls, with a greater decrease in women with severe PE. *Conclusion:* Increased levels of oxidative stress markers and decreased levels of antioxidants in pre-eclamptic women suggest that oxidative stress markers play a significant role in the pathophysiology of pre-eclampsia, and that supplemental dietary antioxidants may have a beneficial role in the prevention of pre-eclampsia in women at high-risk for this condition.

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1. Introduction

Pre-eclampsia is a pregnancy-specific condition characterized by hypertension and proteinuria that remits after delivery [1]. Although its exact etiology is unknown, maternal symptoms are thought to be secondary to endothelial cell dysfunction [2]. It has been suggested that free radicals are likely promoters of maternal vascular malfunction, as reactive oxygen species, particularly superoxide anions, evoke endothelial cell activation [3]. Markers of lipid peroxidation have been noted to be increased in the plasma of women with pre-eclampsia [4]. Antioxidants, such as caroteinoids, tocopherols, and ascorbic acid, due to their capacity for scavenging free radicals and their function as inhibitors of reactive oxygen species, are lower in women with pre-eclampsia [5]. Some studies, however, have not found raised levels of oxidative stress markers or lowered levels of antioxidants in pre-eclampsia [6]. The present article reports on a prospective study on pre-eclamptic and normal pregnant women to observe the relative changes in oxidative stress markers and antioxidant levels.

2. Materials and methods

A total of 100 women in the third trimester of their pregnancy attending the antenatal clinic or admitted to the maternity ward of All-India Institute of Medical Sciences, New Delhi, were enrolled in this prospective study. The ethical institutional committee approved the study and all participants gave written informed consent.

Of the 100 women, 50 had pre-eclampsia (i.e., high blood pressure [BP] and proteinuria) and 50 had a normal pregnancy. Pre-eclampsia was defined as systolic and diastolic BP greater than 140 mm Hg and 90 mm Hg, respectively, with significant proteinuria (>300 mg per 24 h); mild pre-eclampsia was defined as diastolic BP less than 110 mm Hg, with significant proteinuria; and severe pre-eclampsia as diastolic BP greater than 110 mm Hg, or massive proteinuria (>2 g/24 h), or serum creatinine level greater than 1.2 mg/dL, or when other signs and symptoms of severe pre-eclampsia such as persistent headache, visual disturbances, persistent epigastric pain, and/or thrombocytopenia were present [1]. Detailed patient history was taken and a physical examination performed. Blood pressure was measured in the left arm with a syphygomanometer. Urinalysis was done for proteinuria. A total of 10 mL of venous blood was taken from all women. All blood samples were drawn into tubes free of endotoxins and containing heparin. The tubes were centrifuged for 10 min at 4000 rpm, plasma was separated, and packed erythrocytes were washed 3 times. Whole blood levels of glutathione peroxidase (GPX) and superoxide dismutase (SOD), serum levels of malondialdehyde (MDA)—3 oxidative stress markers—as well as plasma levels of vitamin C and lycopene—2 antioxidants—were measured.

2.1. Oxidative stress markers

2.1.1. Glutathione peroxidase

Whole blood GPX levels were measured using a commercially available kit (Ransel; Randox Laboratories Ltd., UK) according to the method of Paglia and Valentine [7], where GPX catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to its reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm was expressed as units per liter of the hemolysate.

2.1.2. Superoxide dismutase

Whole blood SOD levels were measured using a commercially available kit (Ransod; Randox Laboratories Ltd.). Xanthine and xanthine oxidase were used to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. Superoxide dismutase activity was then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition of the rate of reduction of INT under the condition of the assay [8]. The blood concentration of SOD was expressed as units per milliliters.

2.1.3. Malondialdehyde

Serum MDA levels were estimated by the method of Beuge et al. [9] using thiobarbituric acid (TBA). The acid reacts with MDA to form a stable pink color with maximum absorption at 535 nm. According to this method, 375 mg of TBA was dissolved in 2 mL of 0.25 N chlorhydric acid (HCl), followed by 15 g of trichloroacetic acid (TCA) for a total volume of 100 mL. The solution was heated in a water bath at 50 °C to dissolve TBA properly. Then, 1 mL of serum was combined with 2 mL of TCA—TBA—HCl and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculant precipitate was removed by centrifugation. Sample absorbance was then determined at 535 nm against a blank that contained all reagents except the serum sample. Serum MDA concentration was expressed as nmol/mL.

Table 1 Circulating levels of oxidative stress markers in healthy pregnant women and pregnant women with pre-eclampsia[a]

Oxidative stress marker	Control group ($n=50$)	PE group			P value (control vs. overall PE)
		Overall PE group ($n=50$)	Mild PE ($n=41$)	Severe PE ($n=9$)	
GPX (U/L)	156.80 ± 27.18 (118–206)	309.36 ± 59.88 (210–467)	290.85 ± 43.05 (210–391)	393.67 ± 54.27 (302–467)	<0.001
SOD (U/mL)	199.94 ± 28.48 (158–260	332.74 ± 85.59 (203–528)	306.98 ± 67.65 (203–437)	450.11 ± 55.60 (370–526)	<0.001
MDA (nmol/mL)	2.35 ± 0.76 (1.09–4.02)	6.68 ± 1.75 (3.82–9.84)	6.49 ± 1.83 (3.82–9.84)	7.55 ± 0.97 (6.18–9.04)	<0.001

Abbreviations: GPX, glutathione peroxidase; MDA, malondialdehyde; PE, pre-eclampsia; SOD, superoxide dismutase.
[a] Values are given as mean ± SD (range) unless otherwise indicated.

2.2. Antioxidants

2.2.1. Vitamin C

Plasma ascorbic acid levels were estimated calorimetrically using a DTCS reagent prepared by mixing dinitrophenyl hydrazine, thiourea, and copper sulfate in a 1:1:20 ratio, according to the method developed by Teitz [10]. Ascorbic acid in plasma is oxidized by Cu_2^+ to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenylhydrazine to form a red *bis*-hydrazone, which was measured at 520 A. Ascorbic acid concentration was expressed as mg/dL.

2.2.2. Lycopene

Plasma lycopene was extracted using absolute alcohol and petroleum ether, and analyzed by high-performance liquid chromatography. The levels of lycopene were measured as a single peak containing all *trans*- and *cis*-isomers using an absorbance detector set at 472 nm. An external standard of lycopene (Sigma Chemicals Co., USA) was used as reference [11]. Lycopene concentration was expressed as nmol/L.

2.3. Statistical analysis

Oxidative stress markers and antioxidants levels were compared between groups using analysis of variance, the Kruskal Wallis test, and the Mann-Whitney *U* test. Pre-eclampsia was diagnosed and managed as per hospital protocol.

3. Results

Levels of 3 oxidative stress markers and 2 antioxidants were measured in blood samples from 100 pregnant women, 50 healthy women and 50 women with pre-eclampsia (41 with mild and 9 with severe pre-eclampsia).

Mean ± SD age was 27.77 ± 4.61 years (range, 21–34 years) in the study group vs. 26.53 ± 3.54 (range, 19–38 years) in the control group ($P=0.14$). There were 14 primigravidas and 36 multigravidas in the study group vs. 31 primigravidas and 19 multigravidas in the control group ($P=0.001$).

Gestational age was 36.10 ± 3.21 weeks (range, 26.20–42.00 weeks) in the study group vs. 36.88 ± 2.91 (range, 27.40–41.20 weeks) in the control group ($P=0.22$). There was no significant difference in cesarean section rates between the study and control groups (14% vs. 16%). Birth weight was 2590 ± 0.560 g (range, 0.884–3700 g) in the study group vs. 2660 ± 490 g (range, 610–3800 g) in the control group ($P=0.51$). There were 3 and 2 neonates with Apgar scores less than 7 in the study and control groups, respectively ($P=0.52$).

The levels of oxidative stress markers GPX, SOD, and MDA in the 2 groups are shown in Table 1. The mean levels of the 3 stress markers were significantly higher in the pre-eclampsia group than in the control group, and they were the highest among women with severe pre-eclampsia.

The levels of antioxidants vitamin C and lycopene are shown in Table 2. They were

Table 2 Circulating levels of antioxidants in healthy pregnant women and pregnant women with pre-eclampsia[a]

Antioxidant	Control group($n=50$)	PE group			P value (control vs. overall PE)
		Overall PE($n=50$)	Mild PE($n=41$)	Severe PE($n=9$)	
Vitamin C (mg/mL)	0.80 ± 0.07 (0.69–0.97)	0.86 ± 0.08 (0.72–1.02)	0.86 ± 0.08 (0.73–1.02)	0.86 ± 0.10 (0.72–1.00)	<0.05
Lycopene (nmol/L)	43.72 ± 14.68 (7.78–368.4)	7.05 ± 1.39 (0.24–21.85)	7.53 ± 1.17 (5.51–10.95)	6.85 ± 1.81 (0.24–21.85)	<0.01

[a] Values are given as mean ± SD (range) unless otherwise indicated.

significantly lower in the pre-eclampsia group than in the control group, and the decrease in mean levels was the highest among women with severe pre-eclampsia.

4. Discussion

Oxidative stress is the presence of reactive oxygen species in excess of the buffering capacity of available antioxidants [3]. It has been implicated in atherosclerosis, cancers, pre-eclampsia, and many other human diseases [6]. Ascorbate oxidizing activity and levels of known circulating markers of oxidative stress (e.g., nitrosothiols, lipid oxidation products, and antibodies to low-density lipoproteins) are increased in women with pre-eclampsia [12]. Oxidative stress markers are also increased in the decidua, placenta, and other maternal tissues [13,14].

There is an imbalance between lipid peroxidation and antioxidant defenses in pre-eclampsia, leading to endothelial dysfunction and free radical-mediated endothelial cell injury [4 5]. It could be due to their consumption in pre-eclampsia [14]. Many studies confirm that levels of antioxidants such as vitamin C, vitamin E, and other antioxidants are reduced in the sera and placentas of pre-eclamptic women [3—5]. Sagol et al. [15] observed impaired antioxidant activity in women with pre-eclampsia. Palan et al. [3] found significantly lower levels of β-carotene, lycopene, and canthaxanthin in the sera and placentas of pre-eclamptic women than in the sera of normotensive women. Williams et al. [16] observed that the risk of pre-eclampsia decreased with increasing concentration of α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin, and they noted a 50% decrease in the risk of pre-eclampsia in women whose β-carotene concentration was in the highest quartile compared with women whose concentration was in the lowest quartile. The higher levels of oxidative stress markers and reduced levels of antioxidants may persist after delivery. Ozan et al. [17] investigated total plasma antioxidant status, plasma lipid profile, and uterine artery Doppler velocity waveform in nonpregnant women with a history of pre-eclampsia, and observed that the mean total plasma antioxidant status was subnormal in 72% of the formerly pre-eclamptic group, in contrast to 35% in the control group. However, there was no significant difference between the 2 groups regarding uterine Doppler velocity waveforms and plasma lipid levels.

Although some studies seem to have demonstrated that oxidative stress plays a major role in the etiopathogenesis of pre-eclampsia, other studies have found no evidence of lipid peroxidation or reduced antioxidant activity in these women [6,18, 19]. The best test will be the successful prevention of pre-eclampsia by antioxidant therapy, and many trials have been completed with promising results.

Gulmezoglu et al. [20], who conducted the first of these trials, administered 1000 mg of vitamin C, 800 IU of vitamin E, and 200 mg of allopurinol daily between the 24th and the 32th week of pregnancy to 56 women with severe pre-eclampsia. Unfortunately, they found no benefit of this antioxidant therapy, probably because the condition was already too advanced. In women with a diastolic notch in uterine artery, who therefore are at high risk for pre-eclampsia, Chappell et al. [21] found the incidence of pre-eclampsia to be 8% in the group that received vitamin C and vitamin E, compared with 17% in the placebo group. In a further study, Chappell et al. [22] concluded that antioxidant supplementation in women who were at risk of pre-eclampsia was associated with improvement in biochemical indices of the condition. The authors of the present study also found a significant reduction in the incidence of pre-eclampsia in primigravida women who were given 2 mg of lycopene given twice daily (LycoRed; Jagsonpal Pharmaceuticals Ltd., India) between the 16th and the 20th week of pregnancy (8.6% vs. 17.7%, or a 57.4% reduction; $P=0.04$) [23]. Other antioxidants such as selenium, zinc, magnesium, coenzyme Q, and melatonin have been used with encouraging results [24].

There are on going trials in women at low risk for pre-eclampsia in Canada, Mexico, and the USA. The largest of these trials, which is conducted in the USA by the National Institute of Child Health and Human Development Maternal—Fetal Medicine Network for clinical trials, with support from the National Heart, Lung and Blood Institute (NICHD/NHLB), is enrolling 10,000 women [25]. The results are likely to be available in 2008; if antioxidant supplementation is proven beneficial, this trial will be a landmark in the prevention of pre-eclampsia.

The results of the present study clearly show that circulating levels of oxidative stress markers (GPX, SOD and MDA) are statistically elevated in women with pre-eclampsia, but the difference in antioxidant levels was not so marked—as has also been reported by other authors [18,19]. As advocated by Chappell et al. [22], there is a need to perform larger, multicenter trials to (1) measure levels of oxidative stress markers and antioxidants, (2) administer antioxidant therapy to all women with abnormal levels, and (3) determine whether these levels can be improved with antioxidant therapy.

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