Homeostasis status between prooxidants and antioxidants as a potent marker in Iranian preeclamptic patients

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ABSTRACT

Objectives: To measure the plasma oxidant, lipid peroxidation and antioxidants, ascorbate, to magnify the prooxidants and antioxidants status as a marker of pre-eclampsia.

Methods: Included in the study were 50 preeclamptic and 100 normotensive pregnant women of singleton gestations in their third trimester, presented in Allavi Hospitals in Ardabil province of Iran from August 2004 to April 2005. Among these patients plasma malonaldehyde (MDA) and vitamin C concentration were analyzed. Blood samples (5 ml) were collected aseptically in heparin bulb. Spectrophotometric methods were employed to determine the plasma concentrations of vitamin C. The Statistical Package for Social Sciences software was used to analyze the data.

Results: In preeclampsia, significant increase in prooxidant MDA was observed as compared to controls. A significant fall in antioxidants vitamin C was noted in preeclampsia as compared to control. The MDA showed a significant balance to the level of vitamin C.

Conclusion: Administration of regular nutritive supplementation containing reducing systems like vitamin C should help in maintaining the equilibrium during normal pregnancy and theoretically in preeclampsia too but to what extent they help in clinical practice is still being investigated.

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Current concepts of preeclampsia focused on dysfunction of maternal vascular endothelium as the central pathogenetic factor of the disease.[1] Since increased lipid peroxidation has been reported in preeclampsia, involvement of free radicals in preeclampsia may be associated with endothelial dysfunction.[2,3] The human body has developed a defense strategy to minimize the damaging effects of oxidants, which in extracellular fluids rests mainly with sulfhydryl groups and vitamin C (ascorbate).[4] In view of the fact that antioxidants may act synergistically, for instance, when ascorbate regenerates alpha-tocopherol from the tocopherol radical,[5] it is important to evaluate the overall extent of oxidant and effectiveness of antioxidant defense systems in limiting peroxidative damage. This potentially important interaction, between various oxidant and antioxidants, has not been explored in clinical studies.[6,7] In contrast to vitamin E, lower plasma vitamin C concentrations were consistently reported in women with preeclampsia[8-12] but none magnified homeostasis status between the prooxidants and antioxidants.

Usually there should be a state of equilibrium between prooxidants and antioxidants. When this homeostasis is altered by any factor or disease, cell damage ensues.[13] Investigations into oxidative stress and its relevance to the cause and prevention of preeclampsia were reviewed in view of the abnormally low plasma vitamin C concentrations.[14] Blood levels of malondialdehyde (MDA), a marker of lipid peroxidation increased in pregnancy and in preeclampsia. Treatment with antihypertensives did not reduce the levels of MDA.[13] Activation and dysfunction of the maternal and fetal endothelium in preeclampsia may be the consequence of increased oxidative stress associated with circulating lipid peroxides.[15] Since, oxidative stress results from a significant change of the ratio between prooxidant and antioxidant defence, it appears essential to evaluate the involvement of free radicals in any disease.

The present study is therefore aimed in evaluating the exact balance of lipid peroxidation as an oxidant and vitamin C, the most potent antioxidant status in Iranian preeclamptic patients as the relevant biomarkers, which are likely to provide the best predictive potential, particularly in the region.

Methods. This cross-sectional case control study was conducted in the Department of Obstetrics and Gynecology, Ardabil Educational Hospital, Allavi, Iran, biochemical analysis, however, was carried out in the Department of Biochemistry in the same hospital.

The study included 50 pre-eclamptic and 100 normotensive pregnant women of singleton gestation in their third trimester attending or admitted in the Obstetrics and Gynecology Ward of Allavi Hospital, Ardabil province, Iran; between August 2004 to April 2005. The subjects were selected under defined criteria. Informed consent was obtained from these subjects. These subjects were screened for diabetes mellitus, renal disease, and primary hypertension and if present were excluded from the study. Dietary history was taken to rule out any dietary deficiency of vitamin C in these patients. Study samples were taken from these patients before starting any hypertensive medication by venepuncture under aseptic conditions. Preeclampsia patients were at 28 to 42 weeks of singleton gestation, one measurement of diastolic pressure of 110 mmHg or more, or 2 measurements of 90 mmHg or more on 2 consecutive occasions 6 hours or more apart, urinary protein 2+ or more (100 mg/dl; dipstick reagent strip, Boehringer Mannheim, Germany). The exclusion criteria included history of hypertension and proteinuria before conception or before 20 weeks of gestation, any associated medical disorders, a history of antioxidant vitamin therapy during the last one year, and smoking. The patients who developed convulsions were considered to be suffering from eclampsia were excluded. As cohort control, age- and socio-economically matched healthy normotensives at 28-42 weeks of singleton gestation with no urinary protein were recruited by convenience. The controls were matched by group percent of age, education and income. Ethical clearance was taken by the head of the hospital involved and also obtained from human subjects while experimental investigations were involve, and approved by the local human ethics committee.

The body mass index (BMI), a widely used method of calculating the percentage of body fat as a continuum rather than simply by conventional classification units, was premeditated in the present study.

Elevated risk of preeclampsia was curvilinear, meaning that the risk of preeclampsia rose sharply as pre-pregnancy BMI increased. Compared to women with a "normal" pre-pregnancy BMI of 21 (56 Kg for a 65 cm woman), women with a pre-pregnancy BMI of 26 (70 Kg), considered "overweight" with a high ratio of body fat to muscle, were twice as likely to develop preeclampsia. Women with a BMI of 30 (81 Kg), considered "obese," were three times as likely to develop preeclampsia as the normal BMI women. Even small increases in BMI were significant. Woman with a pre-pregnancy BMI of 17, classified as "underweight," was only half as likely to develop preeclampsia as a woman with a BMI of 21, considered "normal weight.

A venous blood samples (5 ml) were collected from antecubital vein of each of the case and control in heparin bulb. They were kept undisturbed for at least 10 minutes and protected from ultraviolet light, on wet ice and processed within 30 minutes. The samples were centrifuged at 3000 rmp for 10 minutes in a cooling centrifuge. The plasma decanted into cryovials was preserved with metaphosphoric acid/dithiothreitol solution and was stored at -24°C for analysis. The concentration of ascorbic acid in the plasma was determined by spectrophotometric within 48 hours of collection of blood samples as described by Ayekyaw.[16] Absorbance was measured against a reagent blank at 700 nm by a spectrophotometer (UV-VIS, Eppendorf Co., W. Germany). Plasma vitamin C is categorized as deficiency (less than 0.2 mg/dl) marginal deficiency (0.2-0.4 mg/dl), and normal (more than 0.4.0 mg/dl). The same samples were assayed for MDA.[17]

The Statistical Package for Social Sciences software version 10.0 (SPSS Inc. Chicago, USA) was used to analyze the data. The results were statistically analyzed using student's t-test and regression analysis was carried out. Descriptive statistics were calculated for all variables. Values were expressed as percentage and mean±SE. A p value of <0.01 is considered significant.

Results. Clinical parameters of the patients and normotensive controls are shown in **Table 1**. The mean maternal and gestational ages of the patients and control were found to be similar. They had different gravida distribution and had nearly equal proteinuria. As anticipated by definition, the systolic and diastolic pressures of preeclampsia patients were also significantly (p<0.001) higher than those of pregnant control (**Table 1**).

Table 2 shows plasma MDA and vitamin C status of the case subjects and normotensive control. It was observed that lipid peroxidation was enhanced in preeclamptic women as judged by elevated levels of MDA as compared to control (p<0.001), (**Table 1**). A significant reverse correlation was noted between the levels of MDA and vitamin C concentration in preeclampsia, which was found to be significantly lower than that of pregnant control (p<0.001). The MDA and vitamin C levels in preeclampsia were found to be influenced by maternal age (p<0.01) (**Table 2**). The correlation between the age, MDA and vitamin C level were determined and it was found to be positively related with (p<0.001). In pregnant control, gestational age showed positive influence on MDA and vitamin C level (p<0.001).

Table 1 - Age and clinical characteristics of preeclampsia and normotensive pregnant (control).

Clinical characteristic	Case subject	
	Normotensive pregnant	Preeclampsia
Maternal age (year)	22.05 ± 1.41	27.11 ± 0.93
Gestational age (week) at sampling	34.64 ± 0.95	37.23 ± 1.64
Gravida (%)		
Primi	12 (30)	28 (35)
Multi	7 (17.5)	12 (15)
Nulliparous	21 (52.5)	40 (50)
Proteinurea		
Primi	0	2+ (26)[1]
Multi	0	3+ (18)
BMI (kg/m^2)	22.5 ± 0.4	25.7 ± 0.6[2]
Systolic blood pressure (mmHg)	109.86 ± 9.27	160.68 ± 22.61[3]
Diastolic blood pressure (mmHg)	73.43 ± 8.81	109.16 ± 15.21

Values are expressed in mean ± SE
Compare Means: Independent-samples t-test
1. p<0.001; 2. p<0.01; 3. p<0.001
Odds ratio 2.1 (95% confidence interval: 1.4, 3.4)

Table 2 - Serum concentrations of MDA and vitamin C in preeclampsia and normotensive pregnant (control).

Antioxidant vitamin (mg/dl)	Control			Preeclampsia		
	N	(%)	mean ± SE	N	(%)	mean ± SE
MDA level (µmol/ml)	100		1.15 ± 0.34	50		2.89 ± 0.65*
Vitamin C (mg/dl)						0.11 ± 0.30*
≤ 0.2	5	(5)	0.34 ± 0.11	47	(48.5)	
≥ 0.2	95	(95)		3	(1.5)	

MDA - malondialdehyde,
Values are expressed in mean ± SE, *p<0.001

The BMI of the 2 groups were compared that was significantly increased on enhancement and reduction of vitamin C (**Table 3**).

Discussion. Oxidative stress plays an important role in the pathophysiology of preeclampsia. Free radicals are specific reactive oxygen species, which play an important role in health and disease. They have been implicated in pathophysiology of various clinical disorders. Free radicals mainly include superoxide anion, hydrogen peroxide and hydroxyl. These are very unstable reactive species capable of inducing cell injury by damaging proteins, lipids and nucleic acids and by inducing lipid peroxidation. Oxidative status during pregnancy and preeclampsia was evaluated in the present study by analyzing prooxidant and antioxidant levels. Lipid peroxidation was considered as a marker for prooxidant, whereas vitamin C level was considered for oxidative defense. A total of 100 blood samples of normotensive pregnant women and 50 samples of preeclampsia women were analyzed during the 9 months of study from August 2004 to April 2005.

Serum lipid peroxides are known to increase in pregnancy and this increase is still higher in preeclampsia.[18,19] This increased lipid peroxides level can enhance the susceptibility of polyunsaturated fatty acids to prooxidative damage, presumably by free radicals that may lead to the formation of MDA.

In the present study, significant increase in lipid peroxidation was observed during preeclampsia. Results of the present study confirmed increased lipid peroxidation similar to that reported by several other investigations.[8,18,19] Increased lipid peroxidation is correlated with increased free radical formation. Results of the present study confirm increased oxidative stress during normal pregnancy and preeclampsia.

Peroxidation may outline the link between the hypoblasts and endothelial cells, which occur in preeclampsia.[20] Neutrophils metabolize arachidonic acid to several cell components. The cyclooxygenase pathway of arachidonic acid metabolism is also an important source of oxygen free radicals.[21]

Table 3 - Serum concentrations of vitamin C and BMI in preeclampsia and normotensive pregnant (controls).

Antioxidant vitamin (mg/dl)	Control		Preeclampsia		BMI (kg/m^2)	
	N	mean ± SE	N	mean ± SE	Control	Preeclampsia
Vitamin C (mg/dl)						
≤0.2	3	0.34 ± 0.11	39	0.11 ± 0.30*	22.5 ± 0.4	25.7 ± 0.6**
≥0.2	77		1		28.5 ± 0.4	35.7 ± 0.6

BMI - body mass index, values are expressed in mean ± SE
*p<0.001; **p<0.01

Free radical injury is usually caused when antioxidant defense system present in the cell cytosol is overwhelmed. Therefore, in order to survive, aerobic organisms have to develop efficient antioxidant systems to protect themselves from the effect of free radicals. This non-enzymatic reducing systems vitamin C limit the cellular concentration of free radicals and prevent excessive oxidative damage.[22]

In the present study, significant decrease in the levels of potent antioxidant, "vitamin C" against prooxidant has been observed during preeclampsia as they are indirectly correlated with MDA. These results suggest an enhancement of this antioxidant non-enzymatic activity during preeclampsia, which corporate with other studies[13,19,23] and confirmed oxidative stress in preeclampsia. Natural endogenous antioxidant non-enzymatic activity is declined significantly against this oxidative stress to maintain the state of equilibrium in favor of antioxidant defense, assuming this can be a suitable marker for preeclampsia even if this contrasts with the recent findings of Rumbold et al[24] who proved that supplementation of exogenous antioxidant non-enzymatic activity does not reduce the risk of preeclampsia.

In conclusion, it is well known that in preeclampsia, the antihypertensive therapy may show little change in oxidative stress and like normal pregnancy, the body still maintained increased antioxidant non-enzymatic activity. This indicates that though the antihypertensive may reduce the effect of oxidative stress, it cannot eliminate its cause. Significant fall in prooxidants/antioxidants is noted in preeclampsia as compared to control to maintain the state of equilibrium in favor of antioxidant defense. Magnified prooxidants and antioxidants status as a marker of oxidative stress in preeclamsia may provide the best potential predictor for severe ones.

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