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Evaluation of Pro-oxidants and Antioxidants in Pre-eclampsia

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OBJECTIVE - To study the pro-oxidant and anti-oxidant defence in pre-eclampsia. **METHODS -** One hundred and sixteen pregnant women were subjected to blood analysis for markers of pro-oxidants and anti-oxidants. They were divided into normal healthy, normotensive pregnant women with ≤12 weeks gestation, normotensive women with 24-34 weeks pregnancy, preeclamptic pregnant women without antihypertensive treatment for preeclampsia and preeclamptic pregnant women admitted to the hospital and on treatment with antihypertensives. The biochemical parameters estimated were malondialdehyde for lipid peroxidation, reduced glutathione (GSH), super oxide dismutase (SOD) and catalase. **RESULTS -** Blood levels of malondialdehyde, a marker of lipid peroxidation were increased in pregnancy and in preeclampsia. Treatment with antihypertensives did not reduce the levels of malondialdehyde. Reduced glutathione blood levels were signigicantly decreased during early pregnancy and in preeclampsia. However they were significantly increased after treatment with antihypertensives compared to before treatment during preeclampsia. Antihypertensive treatment improved reduced glutathione levels. Blood level of superoxide dismutase was found to be increased in early pregnancy, late pregnancy and significantly increased in preeclampsia. It was significantly decreased after treatment with antihypertensives compared to before treatment in preeclampsia. Antihypertensive treatment modified superoxide dismutase level and brought it down to that of early pregnancy. Catalase blood levels remained unchanged during early pregnancy compared to non-pregnant state. They were significantly increased in preeclampsia compared to those in early pregnancy and in nonpregnant state. Catalase levels decreased after treatment with antihypertensives compared to those before treatment during preeclampsia. **CONCLUSION -** There is oxidative stress in normal pregnancy and in preeclampsia. Natural endogenous antioxidant enzymes activity is enhanced significantly against this oxidative stress, to maintain the state of equilibrium in favor of antioxidant defense. However, in preeclampsia, the antihypertensive therapy showed little change in oxidative stress and like in normal pregnancy the body still maintained increased antioxidant enzyme activity. This indicates that though the antihypertensives may reduce the effect of oxidative stress, they cannot eliminate its cause.

Key words : prooxidants, antioxidants, oxidative stress, malondialdehyde, reduced glutathione, superoxide dismutase, catalase, pre-eclampsia

Introduction

Oxidative stress may be defined as an imbalance between prooxidant and antioxidant forces resulting in an overall prooxidant insult. Pregnancy is a physiological state accompanied by a high energy demand of many body functions and an increased oxygen requirement. Because of the increased intake and utilization of oxygen, augmented levels of oxidative stress would be expected. Arguments for a role of oxidative stress and oxidative lipid derivatives in the pathogenesis of preeclampsia are documented in many papers and evidence continues to accumulate that oxidative stress is a mediator of endothelial dysfunction and thus contributes to the cardiovascular complications of preeclampsia[1]. Activation and dysfunction of the maternal and fetal endothelium in preeclampsia may be the consequence of increased oxidative stress associated with circulating lipid peroxides[2]. 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 aimed at studying the prooxidant and antioxidant defence in pre-eclampsia. Lipid peroxidation is considered a marker for prooxidant activity and antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase are considered markers for antioxidant defence. We, studied levels of these to draw valid interpretations.

Material and Methods

Clinical inputs were provided from the department of Obstetrics and Gynecology and the biochemical analysis

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was carried out in the department of Pharmacology. One hundred and sixteen women were studied for oxidative stress. These were divided into the following groups:

GROUP-I- Normal healthy, non-pregnant women of peak child bearing age between 19-25 years to serve as controls. [N=25]

GROUP-II- Normotensive pregnant women, (≤12 weeks) not on any hematinics [N=25]

GROUP-III- Normotensive pregnant women (24-34 weeks) with hematinics, multivitamin and calcium preparations as regular nutritive supplementation required during this phase. [N=25]

GROUP-IV- Preeclamptic pregnant women without antihypertensive treatment for preeclampsia [N=17]

GROUP-V- Preeclamptic pregnant women admitted to the hospital and on treatment with anti-hypertensives [N=24]

Blood samples (5ml) were collected aseptically in EDTA bulb from all subjects. All blood samples were preserved under refrigeration but were not chilled. The following biochemical parameters were estimated using Hitachi spectrophotometer within 48 hours of collection of blood samples -

1) Malondialdehyde for lipid peroxidation
2) Reduced glutathione (GSH)
3) Superoxide dismutase (SOD)
4) Catalase

Whole blood sample (1ml) was used for estimation of reduced glutathione and the remaining blood was centrifuged to obtain plasma. This was used for the estimation of lipid peroxidation. Settled blood cells after hemolysis (by addition of hemolysate) were used for the estimation of superoxide dismutase and catalase.

Reduced glutathione, malondialdehyde for lipid peroxidation, superoxide dismutase and catalase were evaluated by standardized methods[3,4,5]. Results were expressed in the following units:

- Malondialdehyde for lipid peroxidation – LPO n mol/L
- Reduced glutathione-HG GSH/mg protein
- Superoxide dismutase-EU/dl
- Catalase K/g HB

Student's T-test has been used as statistical tool to verify significance of the data obtained.

RESULTS:-

Prooxidant:

Table I : Levels Of Malondialdehyde

GROUP	LPO n mol/L
I	6.14+0.27
II	6.94+0.33
III	6.82+0.35
IV	7.35+0.48*
V	8.09+0.43**

Values expressed as Mean ± 2 SEM

Statistical indices calculated as compared to Group-I

* P<0.02

** P<0.001

Blood levels of malondialdehyde, a marker of lipid peroxidation increased in pregnancy (6.94+0.33). There was a significant increase (7.53+0.48) in preeclampsia (P<0.02). Treatment with antihypertensives did not reduce the levels of malondialdehyde. Infact, the levels were significantly more in subjects on antihypertensives as compared to nonpregnant subjects (8.09+0.43 compared to 6.14 +0.27), P value being <0.001. (Table-I). These results suggest that there is a progressive increase in free radical formation during normal pregnancy, significantly more in preeclampsia. Antihypertensives have no effect on free radical formation in preeclamptic patients.

Antioxidant :

Table II : Levels Of Reduced Glutathione

GROUP	GSH hg GSH/mg protein
I	46.49 ± 2.08
II	32.92 ± 2.72**
III	34.35 ± 1.25**
IV	27.14 ± 2.28**
V	33.62 ± 1.98*

Values expressed as Mean ± 2 SEM for each observation

* P<0.05 Compared to Group IV

** P<0.001 Compared to Group-I

Reduced glutathione blood level was significantly decreased (P<0.01) during early pregnancy (32.92+1.25) and in preeclampsia (27.14+2.28) compared to that in nonpregnant women (46.49+2.08). However, it was significantly increased after treatment with antihypertensives (33.62+1.98) compared to that before treatment during preeclampsia (27.14+2.28). (P<0.05). Antihypertensive treatment improved reduced glutathione levels (Table II). These results suggest that there is a significant decrease in reduced glutathione in early pregnancy, late pregnancy and during preeclampsia. However levels of reduced glutathione after antihypertensive therapy are nearly similar to those in non-pregnant subjects.

Table - III : Levels Of Superoxide Dismutase

GROUP	SOD EU/dl
I	109.92 ± 5.12
II	112.32 ± 6.53
III	117.32 ± 7.10
IV	172 ± 15.09**
V	124.36 ± 7.72*

Values expressed as Mean ± 2 SEM for each observation
Statistical indices calculated as
** P<0.001 Compared to Group-I
*P<0. 01 Compared to Group IV

Blood level of superoxide dismutase was found to be increased in early pregnancy (112.32±6.53), late pregnancy (117.12 ± 7.10) and significantly increased (P<0.01) in preeclampsia (172±15.09) compared to that in nonpregnant women (109.92±5.12). However it was significantly (P<0.01) decreased after treatment with antipertensives (124.36±7.72) compared to that before treatment (172.15±15.09) in preeclampsia. (Table III) Antihypertensive treatment modified superoxide dismutase level and brought it to that in early pregnancy.

Table - IV : Blood Levels Of Catalase

GROUP	Catalase K/g Hb
I	474.65+23.83
II	469.02+26.77
III	523.64+14.88
IV	653.96+27.7**
V	624.25+28.52*

Values expressed as Mean ± 2 SEM
** P<0.001 Compared to Group-I
*P<0. 01 Compared with Group IV

Catalase blood levels remained unchanged during early pregnancy (469.02±26.77) compared to nonpregnant state. These were significantly (P<0.01) increased in preeclampsia (653.96±27.7) compared to early pregnancy and nonpregnant women. Catalase level was decreased after treatment with antihypertensives in preeclampsia (624.25±28.52) compared to that before treatment (653.96±27.70). These results suggest an increase in catalase level during pregnancy and significant increase during preeclampsia. Antihypertensives treatment for preeclampsia has no effect on catalase levels.

Discussion

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. Usually there is a state of equilibrium between prooxidants and antioxidants. When this homostasis is altered by any factor or disease, cell damage ensues.

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 reduced glutathione, superoxide dismutase and catalase were considered for oxidative defense. A total of 91 blood samples of pregnant women and 25 samples of non-pregnant women were analyzed during the six months of this study.

Serum lipid peroxides are known to increase in pregnancy and this increase is still higher in preeclampsia[6,7]. This increased lipid peroxides level can increase the susceptibility of polyunsaturated fatty acids to prooxidative damage, presumably by free radicals that may lead to the formation of malondialdehyde (MDA). Hyperlipidemia may contribute to increased lipid peroxidation.

In the present study, significant increase in lipid peroxidation was observed during early pregnancy, late pregnancy and preeclampsia. Results of the present study confirmed increased lipid peroxidation similar to that reported by several other investigations[6,7]. Increased lipid peroxidation is correlated with increased free radical formation. Results of the present study confirm increased oxidative stress during normal pregnancy and preeclampsia.

Oxygen free radical formation and increased lipid

peroxidation may from the link between the hypoblasts and endothelial cells which occur in preeclampsia[8]. Neutrophils metabolize arachidonic acid to several cell components. The cyclooxygenase pathway of arachidonic acid metabolism is also an important source of oxygen free radicals[9].

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. These are in the form of enzymes (superoxide dismutase, glutathione peroxides and catalase) and reducing systems like vitamins A, E and C, which limit the cellular concentration of free radicals and prevent excessive oxidative damage[10].

In the present study, significant increase in the levels of antioxidant enzymes against prooxidant has been observed during normal pregnancy and preeclampsia. Blood levels of reduced glutathione are decreased significantly as they are indirectly correlated with glutathione peroxidase. These results suggest an enhancement of this antioxidant enzyme activity during normal pregnancy and pre-eclampsia.

Superoxide dismutase (SOD) levels during early pregnancy, late pregnancy and preeclampsia have been significantly increased. Significant decrease in SOD levels during pregnancy and preeclampsia was reported recently[7]. Increased SOD levels in the present study are not in line with this observation. Superoxide dismutase catalyzes the dismutation of the superoxide anion to hydrogen peroxide and oxygen, both of which are non-radical products.

Like superoxide dismutase, blood levels of another antioxidant enzyme, catalase, have been significantly increased during normal pregnancy and pre-eclampsia. Catalase functions to prevent free radical formation by decomposition of hydrogen peroxide to water and oxygen. Antioxidant enzyme systems offer only limited protection from direct free radical damage; indeed, their main function is the prevention of free radical propagation. Enhancement of antioxidant enzyme activity during pregnancy and preeclampsia may be due to increased lipid peroxidation (free radical formation) and decreased level of vitamin E [7].

In preeclampsia, the antihypertensive treatment with drugs like methyldopa or nifedipine showed variable effects. Lipid peroxidation is further increased suggesting antihypertensive have no effect on free radical formation. Reduced glutathione levels improved after that antihypertensives therapy in preeclampsia whereas insignificant decrease was observed in SOD and catalase, again suggesting no significant effect of antihypertensive therapy on antioxidant defense in preeclampsia.

The present clinical study confirmed oxidative stress in normal pregnancy and in preeclampsia. Natural endogenous antioxidant enzymes activity is enhanced significantly against this oxidative stress, to maintain the state of equilibrium in favor of antioxidant defense, assuming pregnancy is not a disease. Administration of regular nutritive supplementation containing reducing systems like vitamin A and C should also help in maintaining this equilibrium during normal pregnancy and theoretically in preeclampsia too but to what extent they help in clinical practice is still being investigated. However, in preeclampsia, the antihypertensive therapy showed little change in oxidative stress and like normal pregnancy, body still maintained increased antioxidant enzyme activity. This indicates that though the antihypertensive may reduce the effect of oxidative stress, they cannot eliminate its cause.

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