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Original research article

EFFECT OF ANTIOXIDANTS IN PRE ECLAMPSIA WOMEN AT INCREASED RISK.

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ABSTRACT

Preeclampsia is a leading cause of maternal and neonatal mortality and morbidity. It is a complex syndrome of undetermined etiologic origin, usually diagnosed during second half of pregnancy. Elevated levels of oxidative lipid derivatives and reduced antioxidants in the circulation of preeclamptics may contribute to endothelial damage. There is good evidence for a significant increase in the levels of malondialdehyde in plasma and erythrocytes. **Group I**: Normotensive nonpregnant women (control), **Group II**: Pregnant women with normal blood pressure, **Group III**: Preeclamptic (pretreatment), women Preeclamptic women were subdivided into **Group IIIA**: Pre eclamptic women supplemented with Vitamin E (n=20), **Group IIIB**: Preeclamptic women supplemented with Omega 3 Fatty Acid, **Group IIIC**: Preeclamptic women supplemented with Vitamin E 400mg + Omega 3 Fatty Acid. Serum SOD, MDA, GPx, GSH levels were measured in all groups and compared with pre treatment and post treatments results by using the one way ANOVA followed by the Tukey – HSD procedure. Our results showed that supplementation of Vitamin E, Omega 3 fatty acid alone and in combination has significantly increased antioxidant enzyme status and significantly decreased oxidative stress when compared to unsupplemented patients($P< 0.001$).

Keywords: Preeclampsia, Vitamin E, Omega 3 fatty acids, SOD, MDA, GPx, GSH

INTRODUCTION

Pre-eclampsia is a leading cause of maternal and neonatal mortality and morbidity. It is a complex syndrome of undetermined etiologic origin, usually diagnosed during second half of pregnancy. The hypertensive disorders in pregnancy are common complications of gestation and form one of the great triad of complications that continued to be responsible for the majority of maternal deaths. How pregnancy per se incites or aggravates hypertensive vascular disease remain unsolved despite decades of intensive research. Diffuse vascular endothelial disruption is believed to play a major role in the pathophysiologic mechanisms of pre eclampsia. Thus, spiral arteries display an abnormally high vascular resistance with reduced uteroplacental perfusion as confirmed by Doppler flow velocimetry studies[1].

Pre-eclampsia is a multi organ system disorder that occurs after the 20th week of gestation and is

characterized by: 1. Hypertension i.e. Blood pressure > 140/90 mm/Hg 2. Proteinuria (urine protein > 0.3 g/24 hours) with or without 3.Abnormal oedema (Beaulieu MD, 1994)When the diastolic blood pressure becomes above 110 mm Hg and protein above 3 mg per day the condition is called severe pre-eclampsia[2] and if the occurrences of seizures are superimposed on pre-eclampsia the condition is referred to as eclampsia[3]

Elevated levels of oxidative lipid derivatives and reduced antioxidants in the circulation of preeclamptics may contribute to endothelial damage. There is good evidence for a significant increase in the levels of malondialdehyde (MDA) in plasma and erythrocytes, a marker of lipid peroxidation in normotensive pregnant women, which were further increased in women with pregnancy-induced hypertension[4]. Evidence of increased oxidative lipid derivatives in the decidual placental tissues was observed in women with established preelampsia[5]**.** Elevated levels of oxidative lipid derivatives, conjugated dienes and reduced antioxidant capacity in the maternal circulation have also been reported[6.]

The free radical scavenging mechanisms include enzymatic antioxidants like Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione reductase (GSH) and Catalase, which limit the cellular concentration of free radicals and prevent excessive oxidative damage.

Until recently, the data supporting the efficacy of supplementation with vitamin C and vitamin E for the prevention of preeclampsia have been limited. [7]Vitamin E, a potent lipid soluble antioxidant is considered as the most reactive antioxidant[8.] The most important function of vitamin E in the body is to protect the polyunsaturated fatty acid from oxidation [9]

Although omega-3 fatty acids have been known as essential to normal growth and health since the 1930s, awareness of their health benefits has dramatically increased in the past few years. New versions of ethyl esterized omega-3 fatty acids, such as E-EPA and combinations of E-EPA and E-DHA, have drawn attention as a highly purified and more effective product than the traditional ones. Furthermore, n -3 LCPUFA have a wide range of biological effects, including beneficial effects on lipoprotein metabolism, platelet function, endothelial function, vascular reactivity, cytokine production and coagulation[10]

Supplementation with Omega 3 Fatty Acid during pregnancy lowers the risk of premature birth and can increase the length of pregnancy and birth weight by altering the balance of eicosanoids involved in labour and promote fetal growth by improving placental blood flow.

Intake of Omega 3 Fatty Acid during pregnancy and breastfeeding may facilitate the child's brain development. There is also some evidence that supplementation with Omega 3 Fatty Acid might help to prevent preeclampsia, postpartum depression, menopausal problems, postmenopausal osteoporosis, and breast cancer**.** Habitual consumption of modest amounts of fish and other seafood has been associated with a reduction in the risk of heart disease[11]

MATERIALS AND METHODS

The subjects for this study were from the Obstetrics and Gynaecology Department of Meenakshi Medical College Hospital and Research Institute, Kancheepuram, Tamil Nadu, India. After enrolling in the study, a detailed obstetric history and the informed consent were obtained. A thorough explanation of the procedure of this long term study was explained in detail and blood samples were obtained basally. The study protocol was approved by an Institutional Human Ethical Committee of Meenakshi University

Grouping: The patients were divided into three groups:

Group I: Normotensive nonpregnant women – control (n = 42), **Group II**: Pregnant women with normal blood pressure (n = 50), **Group III**: Pre eclamptic women (n = 60)

Preeclamptic women were subdivided into **Group IIIA**: Pre eclamptic women supplemented with Vitamin E (n=20), **Group IIIB**: Pre eclamptic

women supplemented with Omega 3 Fatty Acid (n=20), **Group IIIC**: Pre eclamptic women supplemented with Vitamin E 400mg + Omega 3 Fatty Acid (n=20)

The supplements were Vitamin E 400mg, Omega 3 Fatty Acid (DHA 120mg and EPA 180 mg), Vitamin E 400mg + Omega 3 Fatty Acid once daily from 22 - 24 weeks for eight weeks. The three above mentioned drugs were obtained from Merck Pharmaceutical India after getting official permission to be used on human subjects and after complete standardisation. The study groups were asked to consume their normal habitual diet, but they were asked to follow the recommendations for pregnant women.

Blood sample collection was done between 22-24 weeks of gestation and after 8 weeks of supplementation of antioxidants along with the regular antihypertensive agents. The patients were requested to be on overnight fasting. Blood samples were collected between 8.00 AM and 10.00 AM. In each case, 10 ml fasting venous blood were drawn into a sodium heparin vacutainer tube for separating plasma and stored at 4°C, until processed. All samples were processed within 20 hours of sampling. All the pregnant women were encouraged to book and to attend regular antenatal checkups.

The blood samples were centrifuged at 1000g for 15 min at 4°C; the isolated red cells were washed 4 - 5 times with 0.154 M NaCl to remove plasma and buffy coat. After the final wash, required packed red cells were lysed by hypotonic stock solution and different dilutions were used as Hemolysates.

Parameters:

Malondialdehyde (MDA) [12]: The assay was estimated according to the method of Nadiger et al (1986). On the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); forming a MDA-TBA_2 adduct that absorbs strongly at 532 nm. The MDA content in plasma was expressed as nmol / ml.

Superoxide dismutase (SOD)[13]: Superoxide dismutase activity was estimated according to the method of Hartz et al 1983**.** The assay procedure is based on the autoxidation of SOD reagent to an adrenochrome at alkaline pH by measurement of changes in the absorption at a wavelength of 480 nm. Superoxide dismutase activity is evaluated and expressed as IU/gHb

Glutathione peroxidase (GPx)[14]: Glutathione peroxidase activity was determined by the nonenzymatic method by Paglia and Valentine (1967). Glutathione peroxidase enzymes, catalyses the reduction of H_2O_2 and organic peroxides to water and the corresponding stable alcohol thus inhibiting the formation of free radicals. Enzyme activity can be decreased by negative feedback from excess substrate or from damage by oxidative modification.

Glutathione reductase (GSH) [15] : Glutathione Reductase activity was measured according to the method of Goldberg et al 1983. Glutathione Reductase catalyses the reduction of glutathione in the presence of NADPH, which is oxidised to NADP+. The decrease in absorbance at 340nm is measured.

Statistics: The data was analayzed by one way ANOVA (Analysis of variance) followed by the Tukey – HSD procedure. The SPSS software package version 17 was used to test the significance of the experiment performed. In the present study Group II was compared with Group I (i.e., compared pregnant women with normal women). Group III compared with Group II to find out the effect of the preeclampsia effect on antioxidant status. Group IIIA, Group IIIB, Group IIIC was compared with Group III (i.e., comparison between pre and post treatment).

RESULTS

The results were shown in Figures 1 and 2. In comparison among the three groups, the mean values of lipid peroxidation product malondialdehyde and the enzymatic antioxidants superoxide dismutase, glutathione peroxidase and glutathione reductase are among the three groups were significant. In addition, the mean value of pregnancy induced hypertension (Group III) was statistically more significant ($P< 0.001$).

Table.1: Comparison of Lipid Peroxidation Product and Antioxidant Enzymes in Group I, Group II & Group III.

Parameters	Group I [n=42]	Group II [n=50]	Group III [n=60] Pretreatment
MDA (nmol/L)	1.75± 0.41	2.76±0.4	3.74±0.4
SOD (IU/gmHb)	683.76±58.2	597.21±61.3	466.85±37.5
GPx (IU/gmHb)	30.35± 2.2	25.99±1.5	19.74±1.2
GSH (IU/gmHb)	12.55± 1.9	9.07±0.8	5.81±0.7

Table.2: Comparisons of Lipid Peroxidation Product and Antioxidant Enzymes among Subgroups Group III A, Group III B, Group III C with Group III (Pretreatment)

Parameters	Group III [n=60] Pretreatment	Post treatment (N=20 in each group)		
		Group III A Vit.E	Group III B Omega 3 Fatty acids	Group III C Omega 3 Fatty acids+Vit E
MDA (nmol/L)	3.74±0.4	3.20± 0.2	3.27± 0.4	3.296± 0.4
SOD (IU/gmHb)	466.85±37.5	511.36±27.0	512.79±50.6	520.53±31.27
GPx (IU/gmHb)	19.74±1.2	23.68 ± 0.9	22.00 ± 1.4	24.8 ± 0.93
GSH (IU/gmHb)	5.81±0.7	7.86 ± 0.6	6.59 ± 0.88	8.87 ± 0.53

Our results showed that supplementation of Vitamin E, Omega 3 fatty acid alone and in combination has significantly increased antioxidant enzyme status and significantly decreased oxidative stress when compared to unsupplemented patients (P< 0.001).

DISCUSSION

In preeclampsia the oxidant/antioxidant balance is tipped in favour of oxidants at the expense of antioxidants, because in plasma, elevated levels of maternal lipid peroxides were measured.[16] Antioxidant enzymes like Superoxide Dismutase, Glutathione Peroxidase, and Glutathione Reductase form the first line of defense against ROS and the decrease in their activities contributes to the oxidant assault on the cells. Dismutase converts superoxide oxygen into Hydrogen Peroxide and in preeclamptic oxidative stress conditions oxidative balance system is disturbed. During normal pregnancy there is an increase in oxidative stress and therefore a parallel increase in antioxidant activity is seen. The findings implicate oxidative stress in the disease and cite the biochemical rationale for clinical trials of antioxidants to prevent and treat pregnancy induced hypertension. Supplementation of antioxidants along with polyunsaturated fatty acids, particularly omega-3 fatty acids, may be useful in the management of preeclampsia.

CONCULSION

Based on the results of the present study, it is clear that in PIH there is an altered oxidant status and decreased antioxidant enzyme status suggesting that there is an indirect evidence for the presence of oxidative stress in pre eclampsia. In conclusion Vitamin E and Omega 3 Fatty acid, alone or its combination protects the human placenta against the deleterious effects of reactive oxygen species. Our data support this hypothesis

that, over generation of free radicals at the initial stage is promptly scavenged by supplementation with vitamin E and Omega 3 fatty acids thereby reinforcing placental resistance to oxidative injury. The role of antioxidants in the prevention of preeclampsia is controversial. From our study it was found that, supplementing women with antioxidants, combination of Vitamin E with Omega 3 fatty acids (eicosapentanoic acid and docosohexanoic acid) during preeclampsia may help to counteract oxidative stress and thereby prevent or delay the onset of preeclampsia and therefore, improve the health of the mother and baby.

REFERENCES

1. Aquilina J, Harrington K. Pregnancy hypertension and uterine artery Doppler ultrasound. CurrOpinObstet Gynecol. 1996; 8: 435-40.
2. Helewa ME, Burrows RF, Smith J, Williams, Brain P, Rabkin SW. Definitions, evaluation and classification of hypertensive disorders in pregnancy: Report of the Canadian Hypertension Society Consensus Conference: Can Med Assoc. J. 1997; 157 (6): 715-25
3. Chapell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt BJ et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: A randomised trial. Lancet 1999; 354: 810-16
4. Kabi BC, Goel N, Rao YN, Tripathy R, Tempe A, Thakur AS. Levels of erythrocytic malondialdehyde, vitamin E, reduced glutathione, and G6PD activity and plasma urate in patient of pregnancy induced hypertension. Indian J Med Res 1994;100:23-5.
5. Henriksen T. The role of lipid oxidation and oxidative lipid derivatives in the development of precelampsia. Semin Perinatol 2000; 24:29–32.
6. Uotila J, Tuimala R, Aarnio t, Pyykko K, Ahotupa M. Lipid peroxidaiton products, selenium – dependent glutathione peroxidase and vitamin e in normal pregnancy. Eur J ObstetgynecolReprodBiol 1991; 42; 95-100.
7. Jeyabalan A, Caritis SN. Antioxidants and the prevention of preeclampsia unresolved issues. N Engl J Med 2006; 354: 1841–3.
8. Sandhya P, Thomas GA, Donald DH Vitamin E supplementation in the prevention of coronary heart disease. Mayo.Clin .Proc. 2001;76:1-7.
9. Kleijnen J, Knipschild P, RietgT. Vitamin E and Cardiovascular disease. Eur.J.Clin. Pharmacol.1989;37:541-44.
10. Connor SL, Connor WE. Are fish oils beneficial in the prevention and treatment of-coronary artery disease? Am J ClinNutr 1997; 66:1020- 31.
11. Harris WS: Extending cardiovascular benefits of omega-3 fatty acids. CurrAtherosclerosis Rep, 2005; 57: 375-80.
12. Nadiger M.A., Chandrakala M.V. et al Malondialdehyde level in different organs of rats subjected to acute alcohol toxicity. Indian J.Clin.Biochem.1986; 1; 133.
13. Hartz, J.W., Funakoshi, S., and Deutsch, H.F., The levels of superoxide dismutase and catalase in human tissues determined immunochemically. Clin. chimActa. 1973; 46; 125-32
14. Paglia, D.E and Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase J. Lab.Clin.Med., 1967; 70: 158-69.
15. Goldberg D.M and Spooner RJ. Glutathione reductase. In Bergmeyer HU ed. Methods Enzymol Basel VerlagChemie In methods of Enzymatic analysis (Bergmeyen, H.V.Ed) 3rd edn. 1983;3: 258-26