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Original Research Article

Role of FRIOS (free radical induced oxidative stress) in outcome of pregnancy induced hypertension cases

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ABSTRACT

Background: The anti-oxidant and the per oxidation product levels both are increased in pregnancy. Much recent works has focused on the role of oxidative disturbance in the preeclampsia and eclampsia. Preeclampsia remains a leading cause of maternal and fetal morbidity and mortality.
Methods: This prospective study was conducted in upper India sugar exchange and maternity hospital, department of Obstetrics and Gynecology, GSVM Medical College, Kanpur in Collaboration with department of pathology, GSVM Medical College, Kanpur (UP), India. All the patients selected for the present study, both from control and study group was in detail with regard the clinical history, general examination, local examination, basic investigation and Specific Investigation, Super Oxide Dismutase (SOD). Blood samples would be collected with the informed consent from control as well as study group for assessment of antioxidant status by determining levels of superoxide dismutase.
Results: The mean age and BMI of control as well as study group is almost same. The incidence of pre-eclampsia and eclampsia in primipara was 29.31 and in multiparous it was 9.52 in all groups. It implies that 95% cases will have improvement in SOD value with mean difference in improvement of at least 0.297807. There is statistically significant difference in mode of delivery by LSCS in study group A as compared to study group B and control in PIH patients. The incidence of complications is significantly higher in Study Group A as compared to control group and Study Group B. There is statistically significant difference in the incidence of preterm birth in study group A as well as Study Group B as compared to control. However, there was no statistically significant difference in the incidence of preterm birth after giving intervention to the study group. When cut off value of SOD enzyme as < 0.578U/mg of protein, sensitivity is 66.07% and specificity as 51.85%.
Conclusions: Vitamin-c supplementation will only prevent PIT in PIH with already reduced antioxidant status.

Keywords: Oxidative stress, Preeclampsia, Superoxide dismutase

INTRODUCTION

Normal pregnancy is a physiological process with delivery of single baby, more than 2.5 kg weight and with absence of maternal and fetal complication. High risk pregnancy is defined as one which is complicated by factor or factors that adversely affects the pregnancy outcome either maternal or perinatal or both. The oxygen paradox underpins the biology of the whole free radical system. The role of free radicals can be traced back to the origin of life of earth. When 3-5 billion years ago the basic chemical component of life were produced by free

radical reaction, with the help of solar radiation.[1,2] In a healthy body, ROS (reactive oxygen species) and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, oxidative stress (ROS) occurs. It plays a role during pregnancy and normal parturition and in recurrent pregnancy loss, initiation of preterm labor, anaemia, preeclampsia, eclampsia, intrauterine growth retardation.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, in turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, and it can cause damage or death to the cell. Antioxidants remediates, and inhibit other oxidations are often reducing agent such as thilos, ascorbic acid or polyphenols.[3-5]

The anti-oxidant and the per oxidation product levels both are increased in pregnancy. Higher level of super oxides is reported to be generated from the placental mitochondria. At the same time, elevated levels of lipid peroxide and vitamin E have also been reported in pregnancy.

Lipid peroxide levels are increased during 2" trimester and then it tapers off, lowering further after delivery. During the third trimester of pregnancy levels of intracellular reactive oxygen species in the leucocytes increases. Pregnancy leads to leucocytes activation and this result in inflammatory state. The leukocyte activation response is further exacerbated in pregnancies complicated by pre-eclampsia. Placental oxidative stress has a key role in pathophysiology of spontaneous abortions, pre-eclampsia and in pregnancies complicated by anemia and intrauterine growth restriction.

Much recent works has focused on the role of oxidative disturbance in the preeclampsia and eclampsia. Preeclampsia remains a leading cause of maternal and fetal morbidity and mortality. Despite extensive research, the mechanism that causes pre-eclampsia are unknown and it has been considered to the disease o the theories.

There is substantial evidence to suggest that the diverse manifestation of preeclampsia and discrete pathology in many organ systems are derived from pathologic changes within the maternal vascular endothelium.

Diagnosis of preeclampsia based on the presence of two or more components i.e. hypertension, proteinuria, edema and with the development of convulsions and coma disorder is termed as eclampsia.

The reported evidence of this mysterious disease has varied geographical, epidemiological and seasonal effects than has profound effect upon feto-placental unit as well as on kidney, liver, brain and circulatory systems of the mother. PIH usually occurs in the last. Trimester of pregnancy or early in purperium primigravida is most commonly effected.

In a recent year there is growing evidence of possible role of free radical in PIH that are produce continuously either in the intracellular compartment by the mitochondrial respiratory chain and mixed function oxidize system or in the extracellular compartments especially by phagocytes, which cause cellular injury by destroying various cellular components such as lipid peroxides. The pathogenesis of PIH may be associated with the defective free radicals and defensive effects of SOD. It can be hypothesized that placental oxidant, antioxidant intensifies the lipid peroxidation produces into circulation Vascular contact with circulating peroxide products cause dysfunction of vascular endothelium by promoting peroxidative damage of endothelial membrane ultimately resulting in manifestation of the disease. There is now ample proof for the existence of oxygen derived free radicals in human placenta. It is well known that human placenta contains free radicals as well as free radical scavenging enzymes i.e. superoxide dismutase, catalase, glutathione peroxides. Free radicals are highly reactive compounds can act as initiator and/or promoter, cause DNA damage, activate procarcinogens and alter cellular antioxidant defense system.[6-8]

With the above background, the present study was conducted to evaluate the role of FRIOS (free radical induced oxidative stress) in outcome of pregnancy induced hypertension cases.

METHODS

This prospective study was conducted in upper India sugar exchange and maternity hospital, department of Obstetrics and Gynecology, GSVM Medical College, Kanpur in Collaboration with department of pathology, GSVM Medical College, Kanpur (UP), India.

Subject of the present study were divided into two groups

- Control group
- Study group

Control group

The control group included 146 patients with normal SOD level at 20 weeks. The normal value of SOD is taken as >0.702U/mg of protein.

Study group

This group included 180 patients with decreased SOD level at 20 weeks, they were further divided into two groups; Study Group A and Study Group B

Group A - those who did not receive intervention/anti-oxidant

Group B - those who received intervention/anti-oxidant (Vit C 1000mg IOD) for 12 weeks starting from 20 weeks gestation till 32 weeks. SOD was re-measured in control and study group at 32 weeks and intervention continued till delivery.

- Gestational age > 20 weeks
- Pregnancy complicated by: PIH

Exclusion criteria

- Patients of gestational age < 20 weeks
- Patients with diabetes
- Patients with renal insufficiency
- Patients with essential hypertension
- Patients with complication apart from study group

Method of Case study

All the patients selected for the present study, both from control and study group was in detail with regard the clinical history, general examination, local examination, basic investigation and Specific Investigation SOD.

Method of collection of blood samples

Collection of blood plasma, RBC and preparation of hemolysate. Blood samples would be collected with the informed consent from control as well as study group for assessment of antioxidant status by determining levels of superoxide dismutase. Heparinised 5 ml of blood from each subject would be collected from median vein forearm and were sent to department of pathology GSVM Medical college where blood was centrifuged for 10 minutes and 3,000 rpm in refrigerated centrifuge machine (O-50C) So as to collect plasma and plasma free packed cell volume (RBC etc) and processed immediately foe preparation of hemolysate. The plasma (Supernatant) was carefully decanted into plastic tubes Care was taken to ensure that plasma was not contaminated with red blood cells (RBCs) plasma thus obtained was used for the estimation of lipid peroxidation.

Preparation of Hemolysate

Plasma removed PCV (packed Cell Volume) were washed 3 times with normal saline. Cells were lysed by adding 10 ml chilled distilled water for 19 minutes and then would be shaken vigorously for 2 minutes 0.5 ml chloroform would be added as preservative. Mixture would be centrifuged at 3000 rpm for 20 minutes. Mixture would be clearly separated into 3 layers, lower most layers would be chloroform, middle of stroma and upper most layers would be clear hamolysate.

Precautions during blood sampling and investigation

- Haemolysed blood will not be used for estimation
- Centrifugation of Samples will not be used for estimation and speed, for the required period only.
- For all laboratory procedures, high quality plastic was used. Ordinary glassware was totally avoided for all estimation

Biochemical investigation

Estimation of Superoxide dismutase (SOD)

Principle

Superoxide anions will be generated in a system comprised of NADH and phenazine methosuiphate. These superoxide anions reduced the nitro blue terazolium forming a blue formation, which was measured at 560 nm. Superoxide dismutase inhibited the reduction of nitro blue terazolium and thus the enzyme activity is measured by monitoring the rate of decrease in optical density at 560 nm. SOD was determined by using the spectro-photometric method. Indirect assay method for estimation of SOD activity is generally used because the substrate for its enzymatic activity is a free radical in nature or (superoxide anion) is generated by some mechanisms and allowed to react with a detector molecule SOD by removing the O2 inhibits the reaction with the detector molecule thus decreasing the colour intensity

Methodology

- The washed RBC's were haemolysed by adding thrice the volume of water to them.
- The haemolysate was centrifuged at 800 xg for 15 min in the cold.
- The ghost free supernatant of the RBC hemolysate was used as the source SOD.
- A standard curve between concentration of nitroblue netrazolum and absorbance at 560 nm was pltted to determine the concentration to use in the assay system.

Unit of superoxide dismutase activity

The unit of enzyme activity is defined here as the amount of enzyme activity was express as units per mg protein. The activity of the enzyme was measured following the decrease in optical density. I minute during oxidation of NADPH spectrophotometrically at 340 nm.

Statistical analysis

Data was analyzed statistically using appropriate tools of statistical inference using medcalc Software, SPSS software. The statistical significance of observed difference between control and study group was determined by mean with SD, Independent 't' test, ROC curve analysis logistic regression.

- > 0.05 not significant
- Value between 0.05 and 0.01 significant.
- < 0.001 highly significant

RESULTS

Table 1: Age distribution of study patients.

Group	Mean age with SD
Control	24.03+2.61
Study	
Without intervention	24.54+2.83
With intervention	24.61+2.78

The mean age of control as well as study group is almost same. Thus, age cannot be a confounding factor.

Table 2: BMI distribution of study patients.

Group	Mean BMI with SD
Control	22.84+1.278
Study	
Without intervention	22.59+1.303
With intervention	22.81+1.396

The mean BMI of control as well as study group are almost same. Thus, BMI cannot be confounding factor.

The incidence of pre-eclampsia and eclampsia in primipara was 29.31 and in multiparous it was 9.52 in all groups. 't' test was applied on above tables to compare improvement in SOD values after giving intervention (vitamin C 1000 mg).

Table 3: Distribution of PIH patients according to parity.

Patients	Primigravida	Multigravida
Control	10	07
Study		
Without intervention	19	09
With intervention	05	04
Total	34	20
Incidence rate of PIH in different group	29.31%	9.52%

Table 4: Mean SOD with standard deviation in study group.

	Mean value of SOD at 20 weeks	Mean SOD at 32 weeks
Control	0.73366+ 0.005205	0.63791+ 0.153091
Study (A) Without intervention	0.50631	0.36529+ 0.05579
Study (B) With intervention	0.51523	0.66310+ 0.02266

Table 5: Independent samples test.

		Levene's test for equality of variances		t-test for equality of mean		
		F	Sig.	T	Df	Sig.(2-tailed)
SOD	Equal variances assumed	5.671	0.018	-47.258	178	0.000
	Equal variances not assumed			-46.877	114.872	0.000

Table 6: Comparing significant improvement in SOD value after giving intervention.

Independent samples test		t- test for equality of means			
		Mean difference	Std. error diff.	95% confidence interval of the difference	
				Lower	Upper
SOD	Equal variances assumed	-0.297807	0.006302	-0.310242	-0.285371
	Equal variances assumed	-0.297807	0.006353	-0.310391	-0.285223

't' Value-46.877, 'p' Value-0.0001, 95% Confidence interval -0.285223-0.310391, Mean difference 0.297807. This is statistically highly significant. It implies that 95% cases will have improvement in SOD value with mean difference in improvement of at least 0.297807 which is statistically highly significant. Regression coefficient-1.504 ($p<0.0001$), Odds Ration - 4.5, C.I. (95%) 1.99-10.18. The above values are statistically highly significant. From this it can be interpreted that intervention when given at early weeks of gestation has a role in reducing incidence of PIH in patients with already reduced antioxidant (SOD) status. The above analysis also shows that odds in control of PIH through intervention (Vit. C-1000 mg OD) are at approximately 2 times higher than those who were not given intervention.

Table 7: Comparing mode of delivery in patients of PIH between study group and control group.

Patients	LSCS	Vaginal	P value
Control	61(41.7%)	85(58.3%)	0.0008
Study group A	52(52.8%)	38(42.20%)	
Study group B	59(54.5%)	41(45.50%)	
Total	162	164	

There is statistically significant difference in mode of delivery by LSCS in study group A as compared to study group B and control in PIH patients.

The incidence of complications is significantly higher in Study Group A as compared to control group and Study Group B.

Table 8: Complications in control and study group.

Patients	Mild pet	Severe pet	Abruptio placental	Antepartum eclampsia
Control	16 (10.96%)	1(0.68%)	4(2.7%)	0%
Study group A	25(27.78%)	3(3.33%)	6(6.67%)	1(1.11%)
Study group B	8(8.89%)	1(1.11%)	3(3.33%)	0%
Total	49	5	13	1

Table 9: Comparing incidence of preterm birth in patients of PIH in control and study group.

Patients	≥ 37 weeks	< 37 weeks	P value
Control	100(68.5%)	46(31. %)	
Study group A	78(86.7%)	12(13.3%)	
Study group B	81(90%)	9(10%)	0.0018
Total	259	67	

There is statistically significant difference in the incidence of preterm birth in study group A as well as Study Group B as compared to control. However, there was no statistically significant difference in the incidence of preterm birth after giving intervention to the study group. ROC curve analysis shows that, Area under Curve (AUC): 0.618, 95% CI 0.563 -0.671, 'p' - 0.0034. Curve shows that when cut off value of SOD enzyme as < 0.578U/mg of protein, sensitivity is 66.07% and specificity as 51.85%. This implies that SOD enzyme is not an excellent but only good discriminator between control and study group in PIH.

Table 10: ROC curve analysis of SOD in PIH.

Variable	SOD	< 2.5 kg	P value
Classification variable	PIH	19(13%)	0.6723
Sample size		326	
Positive group	PIH = 1	56	
Negative group	PIH = 0	270	
Disease prevalence (%)		Unknown	

Table 11: Cutoff value of SOD to diagnose pre-eclampsia

Area under the ROC curve (AUC)	0.618
A standard Error	0.0403
95% Confidence interval	0.563 to 0.671
Z statistic	2.926
Significance level P (Area = 0.5)	0.0034
Youden index J	0.2098
Associated criterion	< 0.507

Table 12: Criterion values and coordinates of the ROC curve.

Criterion	Sensitivity	95% CI	Specificity	95% CI	+ LT	- LR
≤ 0.578	66.07	52.2-78.2	51.85	45.7-57.9	1.37	0.65

DISCUSSION

Results from more recent trials stand in contrast to the earlier promising results regarding antioxidant supplementation during pregnancy for the prevention of preeclampsia. One early trial reported that antioxidants significantly reduced the risk of preeclampsia. However, neither the more recent trials nor one of the systematic review could substantiate this finding.[9-13]

The Survival of aerobic organism in an oxygen environment involves a complete interplay between the biological generations of very reactive chemical species the "Free radicals" and the ability of organisms to curb these damaging substances. Free radical interaction has been implicated in large number of disease states which include inflammation, radiation injury and ischemia, the elucidation of the relationship of individual free radical species to the bio-molecular and tissue injury which occurs during disease date may not only and in the understanding of these processes but also in their control.

ROS are double edged sword-they serve as key signal molecules in physical processes but also have a role in pathological processes involving female reproductive tract.

Body also got various antioxidant defense systems to protect against tellur injury and tissue damage caused by free radicals.[14]

Mean age of control (24.03+2.6) as well as study group a (24.54+2.83) and group B (24.61+2.78) are almost same. Thurs age is not a confounding factor. Thus, this finding is in accordance with study of Pandey S et al.[15] Mean BMI of control (22.84+1.278) as well as study group A (22.59+1.303) and group B (22.81+1.396) are almost same thus BMI is not a confounding factor in our study. In our study, it was found that the incidence of pre-eclampsia and eclampsia in primiparous was 29.3% and in multiparous, it was 9.52% this is in accordance with study of Pandey S et al Mean level of SOD at 20 wks in study group was much less as compared to control group (0.702). It also shows that SOD value of SOD in group A at 32 weeks is much less (0.36529+0.05579) as compared to group B in which intervention was given (0.6631+ 0.02266). ['p' value <0.0001- highly significant]. These results are in accordance with the prior studies done by Wiktor H, Kharbs et al.[16,17]

In our study, it is seen that SOD enzyme levels improve significantly at 32 wks after giving intervention. It is seen that SOD enzyme levels are strongly correlated with development and severity of PIH. So, patient outcome given antioxidant (In our study Vit - C - 1000 mg IOD) in already reduced antioxidant states (Decreased levels of SOD) was studied. Intervention was given in patients with reduced level of SOD at 20 weeks in form of Vit C 1000 mg IOD for 2 months, starting from 20 weeks up to 28 weeks and patient was reassessed at around 32 weeks by changes in USG- Doppler and measurement of SOD form our Study.

Patient who received intervention in an already low antioxidant status show only 10% cases developing into PIH. However, the study group in which intervention was not given patients who developed PIH was 31.11%. Logistic regression analysis was applied to above data and was seen that P < 0.0001 and 95% CI 1.99 - 10.18% which is statistically highly significant. Regression coefficient: 1.504. Odds Ratio: 4.5. Which showed that Vit C 1000 mg IOD when given in patients with already reduced SOD status has a role in prevention of PIH and decreasing severity of PIH. This is in accordance with the study of Rumiris D, Purwosumu Y, Wibowo N, Farina A, Seikizawa A. Compares mode of delivery in patients of PIH between study group and control group. There is statistically significant difference in mode of delivery by LSCS in study group A as compared to study group B and control in PIH patients. P value is 0.0008 which is statistically significant. Compares incidence of preterm birth in control and study group in patients of PIH. There is statistically significant difference in the incidence of preterm birth in study group A as well as study Group B as compared to control group, However, there was no statistically significant difference in the incidence in the of preterm birth after giving intervention to the study group.[18]

ROC curve analysis of SOD in prediction of PIH. It shows that Area under curve is 0.618, 95% C.I. 0.563-0.671. 'P' value 0.0034 which is statistically not significant. This shows that SOD enzyme is a good but not an excellent discriminator between control and case (PIH) and can be successfully used for predicting high risk pregnancies. This is in agreement with studies showing highly decreased levels of SOD enzymes in placental tissue of PET. Further ROC analysis shows that the values of sensitivity and specificity is 66.07 and 51.85% respectively when cut off value of SOD is taken as <0.578 /mg of protein. Thus, according to evidence of our sample, cut off value of SOD should be taken as without < 0.578 U/mg of protein for prediction of PIH case Mahadikkv, Sina SA.[19]

CONCLUSION

Roc analysis of SOD shows that AUC is 0.618 which is not statistically significant. This shows that SOD is not excellent discriminator of PIH. i. e. not every case of PIH is associated with decreased SOD activity. Intervention with vitamin c 1000 mi OD when given to Pt with already reduced SOD status shows marked improvement in SOD activity at 32 weeks as compared to SOD level at 20 weeks (statistically significant). Hence-vitamin-C supplementation will only prevent PIT in PIH with already reduced antioxidant status. When given from early weeks as evident from the present study.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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