Original Research Article

"Free radical induced oxidative stress" (frios) parameters: key to reduce feto -maternal mortality in high risk pregnancies

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ABSTRACT

Background: ROS plays role during pregnancy and normal parturition and in recurrent pregnancy loss, initiation of preterm labor, anaemia, preeclampsia, eclampsia, Intrauterine growth retardation. Elevated oxidative stress is found in term infants with fetal distress and in preterm infants. With this background this study was conducted to evaluate the role of FRIOS (free radical induced oxidative stress) in reducing feto -maternal mortality in high risk pregnancies in District Kanpur.

Methods: This prospective study was conducted on pregnant women at high risk attending department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur after taking permission from the institutional ethical committee. Informed consent was obtained from all patients. Investigations were carried out to measure oxidant level of Malonaldialdehyde (MDA) enzyme & to measure anti oxidant Super Oxide Dismutase (SOD) enzyme. Levels of these enzymes were compared between normal & each high risk sub groups separately. SPSS software was used for statistical analysis and suitable tests were applied.

Results: The mean value in study group was highest in severely anaemic patients (8.53±1.398 Nmoles/ml of plasma) followed by pre eclamptic & eclamptic patients (8.33±1.355 Nmoles/ml of plasma). The mean levels of in study group was lowest in pre eclamptic & eclamptic patients (0.394±.191 u/mg of protein) followed by pre term patients (0.413±0.141 u/mg of protein). Significant difference between MDA enzyme levels in control group and all sub groups of study group was found.

Conclusions: Measurement of Malonaldialdehyde (MDA) & Super Oxide Dismutase (SOD) enzymes at an earlier stage can be a valuable tool for early diagnosis, so that we can timely intervene & improve the maternal outcome.

Keywords: Free radical induced oxidative stress, High risk pregnancies, Maternal mortality

INTRODUCTION

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.[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 (OS) occurs. Free radical species are unstable and highly reactive. They become stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease.

There are two major types of free radical species: Reactive oxygen species (ROS) - superoxide (0_2^-) hydrogen peroxide (H_2O_2) hydroxyl ($^\bullet OH$).

They have physiological and pathological role in the female reproductive functions such as oocyte maturation, ovarian steroidogenesis, corpus luteal function and leuteolysis. ROS may also originate from embryo metabolism and from its surroundings. Reactive nitrogen species (NOS) - Nitric oxide (NO). With an unpaired electron, NO, which is a highly reactive free radical results in cell and tissue damage, low grade, sterile inflammation and adhesions

There are two types of antioxidants in the human body. Enzymatic antioxidants which neutralize excessive ROS and prevent it from damaging the cellular structure. e.g. Superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase and non-enzymatic antioxidants. The body's complex antioxidant system is influenced by dietary intake of antioxidant vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, taurine, hypotaurine, glutathione, beta carotene, and carotene

ROS plays role during pregnancy and normal parturition and in recurrent pregnancy loss, initiation of preterm labor, anaemia, preeclampsia, eclampsia, intrauterine growth retardation. Elevated oxidative stress is found in term infants with fetal distress and in preterm infants. With this background this study was conducted to evaluate the role of FRIOS (free radical induced oxidative stress) in reducing feto -maternal mortality in high risk pregnancies in district Kanpur.

METHODS

This prospective study was conducted on pregnant women attending OPD & admitted in UISEMH, Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur in collaboration with department of pathology after taking permission from the institutional ethical committee. Considering the inflow of patients in our hospital, sample size was taken as 140.

For a valid statistical comparison, a control group of 30 women was formed with normal natural conception with healthy pregnancy and healthy outcome. Study group comprised of 110 patients, out of which there were 24 pregnant women with anaemia, 20 with IUGR, 22 with pre eclampsia, 10 with eclampsia, 24 with pre term labour & 10 with recurrent pregnancy loss. Informed consent was obtained from all patients.

Detailed history and thorough examination was done and investigations were carried out to measure oxidant level. Malonaldialdehyde (MDA)enzyme & to measure anti oxidant Super Oxide Dismutase (SOD) enzyme was taken. Levels of these enzymes were compared between normal & each high risk sub groups separately. SPSS software was used for statistical analysis and suitable tests were applied.

RESULTS

The mean age of control was 25.3±3.51 yrs and in the study group was 25.04±3.36 yrs.

Table 1: Mean levels of MDA and SOD in control and study patients.

Patients	No.	Range of MDA (n moles/ ml of plasma)	Mean MDA Level (nmoles/ml of plasma)	95%CI of Mean (Nmoles/ml of plasma)	Range of SOD (unit/mg of protein)	Mean SOD level (unit/mg of protein)	95%CL of mean (unit/mg of protein)
Control	20	3.45 – 6.66	5±0.878	4.67-5.32	0.506-0.841	0.706±0.094	0.671-0.742
Anaemia	24	6.5-9.92	8.53±1.398	7.48-9.647	0.210-0.639	0.428±0.140	0.368-0.487
Pre term labour	24	5.04-9.20	7.586±1.304	7.035-8.137	0.221-0.670	0.414±0.141	0.354-0.473
Pre eclampsia Eclampsia	32	5.79-10.01	8.33±1.355	7.842-8.82	0.118-0.704	0.394±0.191	0.276-0.501
IUGR	20	5.55-9.26	7.353±1.358	6.628-8.412	0.210-0.66	0.461±0.112	0.407-0.514
RPL	10	6.62-9.1	7.608±1.101	6.82-8.395	0.301-0.652	0.451±0.113	0.369-0.531

The mean value of MDA in control group was found to be 5±0.878 Nmoles/ml of plasma and mean value of MDA in study group was much higher , highest being in severely anaemic patients (8.53±1.398 Nmoles/ml of plasma)followed by pre eclamptic & eclamptic patients(8.33±1.355 Nmoles/ml of plasma).

The mean level of SOD is much less in study group as compared to control group (.7069±.095 u/mg of protein). In study group lowest value is found in pre eclamptic & eclamptic patients (.394±.191 u/mg of protein) followed by pre term patients (.413±.141 u/mg of protein) (Table 1).
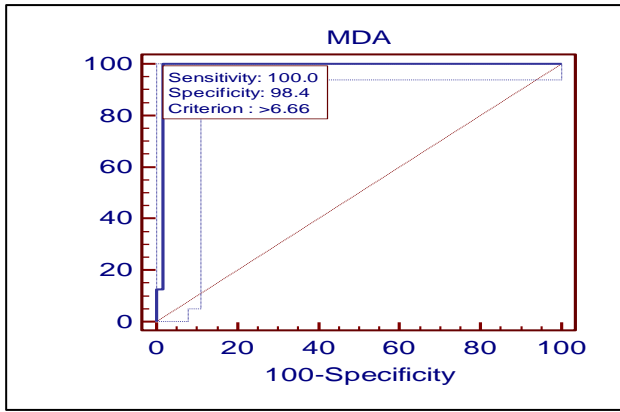
Figure 1: ROC curve analysis of MDA enzyme.

Figure 1 shows that when cut off value of MDA is taken as 6.66 nmoles/ml of plasma, sensitivity of the test is 100% & specificity is 98.4%. ROC analysis of MDA enzyme shows that AUC is 0.986 (95 % CI .958-1.0) being statistically highly significant. (Z=33.889 p=0.001).It also shows that the values of sensitivity & specificity are very close 100% (CI 95.4-100) & 98.6% (91.2-100) respectively when cut off value of MDA is taken as >6.66 Nmoles/ml of plasma (Figure 1).

Figure 2 shows that when cut off value of SOD enzyme is taken as 0.503 unit/mg of protein, both sensitivity and specificity of the test. ROC analysis of SOD shows that AUC is 1.0 (95 % CI 1.0-1.0) being statistically highly significant (p=0.000). It also shows that the values of sensitivity & specificity are 100% (95% CI 95.4-100) and 100% (95%CI 94.2-100) respectively when cut off value of SOD is taken as ≤0.503 U/mg of protein (Figure 2).
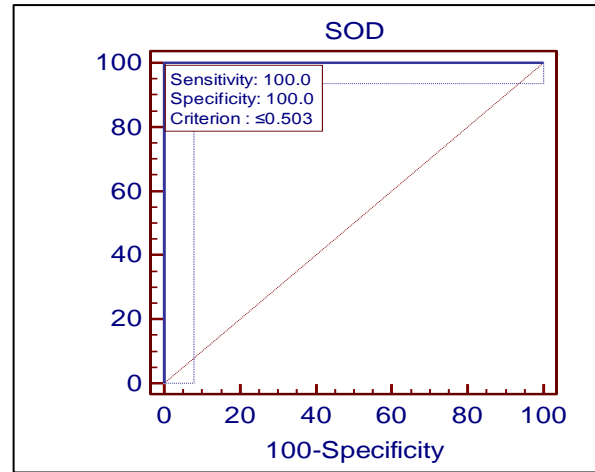

Figure 2: ROC curve analysis of SOD enzyme.

Analysis of variance technique was used to compare the MDA levels and for pair wise comparisons Tukey's post hoc test was used. Significant difference between MDA enzyme levels in control group and all sub groups of study group was found. Among study group, there is significant difference in MDA levels between anaemic and IUGR patients while in other sub groups difference in MDA levels is not statistically significant (Table 2).

In Table 3, for pair wise comparison amongst groups Tukey's post hoc test was used for analysis. This shows that there is statistically significant difference between SOD enzyme levels in control group and all sub group of study group. Among study group there is no significant difference in any of the sub groups.

Table 2: Comparison of the MDA ENZYME levels using ANNOVA and pair wise comparisons using Tukey's post hoc test.

MDA enzyme	(I) Category	(J) Category	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
						Lower bound	Upper bound
Tukey HSD	0.00	1.00	-3.33056*	0.31770	0.000	-4.2492	-2.4120
		2.00	-3.53358*	0.34236	0.000	-4.5235	-2.5437
		3.00	-2.35250*	0.36088	0.000	-3.3959	-1.3091
		4.00	-2.58567*	0.34236	0.000	-3.5756	-1.5958
		5.00	-2.60700*	0.45648	0.000	-3.9269	-1.2871
	1.00	2.00	-.20302	0.33757	0.991	-1.1791	.7730
		3.00	.97806	0.35634	0.073	-.0523	2.0084
		4.00	.74490	0.33757	0.242	-.2312	1.7210
		5.00	.72356	0.45290	0.602	-.5860	2.0331
	2.00	3.00	1.18108*	0.37850	0.026	.0867	2.2755
		4.00	.94792	0.36088	0.098	-.0955	1.9914
		5.00	.92658	0.47053	0.365	-.4339	2.2871
	3.00	4.00	-.23317	0.37850	0.990	-1.3275	.8612
		5.00	-.25450	0.48417	0.995	-1.6544	1.1454
	4.00	5.00	-.02133	.47053	1.000	-1.3818	1.3392

Table 3: Comparison of the SOD ENZYME levels using ANNOVA and pair wise comparisons using Tukey's post hoc test.

	(I) Category	(J) Category	Mean difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower bound	Upper bound
Tukey HSD	0.00	1.00	0.31290*	0.03583	0.000	0.2093	0.4165
		2.00	0.27889*	0.03861	0.000	0.1673	0.3905
		3.00	0.24613*	0.04070	0.000	0.1285	0.3638
		4.00	0.29302*	0.03861	0.000	0.1814	0.4047
		5.00	0.25633*	0.05148	0.000	0.1075	0.4052
	1.00	2.00	-0.03401	0.03807	0.948	-0.1441	0.0761
		3.00	-0.06677	0.04019	0.560	-0.1830	0.0494
		4.00	-0.01989	0.03807	0.995	-0.1300	0.0902
		5.00	-0.05657	0.05108	0.878	-0.2043	0.0911
	2.00	3.00	-0.03276	0.04269	0.972	-0.1562	0.0907
		4.00	0.01412	0.04070	0.999	-0.1036	0.1318
		5.00	-0.02256	0.05307	0.998	-0.1760	0.1309
	3.00	4.00	0.04688	0.04269	0.881	-0.0765	0.1703
		5.00	0.01020	0.05460	1.000	-0.1477	0.1681
	4.00	5.00	-0.03668	0.05307	0.983	-0.1901	0.1168

DISCUSSION

In our study among anemic patients 70.8% patients were severely anemic which adversely affected perinatal outcome. This indicates that severe anemia is highly prevalent condition in pregnant women especially in our state U.P. This is in accordance with the study of Jallel et al who also reported that severe anemia and its association with adverse pregnancy outcome.[3] The levels of antioxidant enzymes namely catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and reduced glutathione were significantly reduced in all IDA groups in the study by Tiwari et al.[4] In this study, the mean value of MDA in control group was found to be 5±0.878 Nmoles/ml of plasma and mean value of MDA in study group was much higher, highest being in severely anaemic patients (8.53±1.398 Nmoles/ml of plasma) followed by pre eclamptic & eclamptic patients (8.33±1.355 Nmoles/ml of plasma). This is in accordance with studies showing increased lipid peroxidation in PET patients [5] and alterations in antioxidant enzymes & MDA status in pre eclampsia.[6] The mean levels of SOD is much less in study group as compared to control group (0.7069±0.095 u/mg of protein) in our study. In study group lowest value is found in pre eclamptic & eclamptic patients (0.394±0.191 u/mg of protein) followed by pre term patients (0.413±0.141 u/mg of protein). Our results are in accordance with the prior studies done by Johnkenndy N et al.[7] Pre-eclampsia was also significantly associated with free radical induced oxidative stress in a study by Sharma et al.[8] They found significant increase in MDA levels, an indicator of lipid peroxidation, in preeclampsia and eclampsia as compared to healthy pregnancy.

According to evidence of our sample, cut off value of MDA should be taken as >6.66 Nmoles/ml of plasma for predicting high risk pregnancies. This is in accordance with the study of Rani N et al which showed cut off value of MDA as 6.2 nmol/g of placental tissue with 87% sensitivity and 87% specificity.[4] According to evidence of our sample, cut off value of SOD should be taken as ≤0.503 U/mg of protein for predicting high risk pregnancies. This is again in agreement with studies showing highly decreased levels of SOD enzyme in placental tissue of PET patients estimated by ROC analysis Rani N et al, but they did not calculate the cut off value of SOD enzyme in placental tissue.[4] Choudhary et al also observed ROC curve analysis of SOD in prediction of PIH and reported area under curve was 0.618, 95% C.I. 0.563- 0.671. 'P' value 0.0034. They concluded 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.[9]

Prenatal hypoxia, nutritional deficiency/excess, and glucocorticoid exposure are each capable of generating excessive ROS levels by differing mechanisms. Organ-specific responses are dependent on the relative balance between ROS generation and the antioxidant capacity of the cell.[10]

CONCLUSION

The results of our study brings out the fact that, in high risk pregnancies free radical induced oxidative stress increases which is evident by high levels of oxidant (MDA) & low levels of antioxidant (SOD). By applying ROC analysis we got cut off values for all the enzymes which are good discriminator between control & high risk pregnancy cases & can be successfully used for predicting high risk pregnancies & thus make it possible to manage the cases of high risk pregnancy from early

stages ensuring better prognosis. Thus measurement of these enzymes at an earlier stage can be a valuable tool for early diagnosis, so that we can timely intervene & improve the maternal outcome.

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