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Circulating biomarkers of oxidative stress in preeclampsia and efficacy of antioxidant Vitamin C supplementation.

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ABSTRACT

To detect circulating markers of oxidative stress in women with women with preeclampsia and to assess the effect of vitamin C. on the levels of markers of oxidative stress in all the groups. Single blind randomized clinical study with multiple group conducted in the Department of Pharmacology at Jawaharlal Nehru Medical College, Sawangi (Meghe) Wardha from Feb.2014 to Nov 2015. 60 women enrolled, Maximum numbers of pregnant women were between 19-23 years age group being 56.67%, 24-28 yrs 36.67% and 29-33 yrs 5.00%. in the groups respectively Mean MDA level was decrease in the group B, & H increase in group A & G . SOD level in units/gm Hb%. Increase in the group B, & H decrease in group A & G. This is due to antioxidant defence against oxidative stress in preeclampsia with resultant fall in plasma levels of SOD. Rising levels of MDA with the progression of gestation in preeclampsia and decline in the levels in those patients supplemented with antioxidant vitamins was observed. However there was no definite trend of significant rise or fall in the level of superoxide dismutase enzyme apart from preeclamptic women. Circulating biomarker, MDA levels were elevate significantly in women with preeclampsia. Superoxide dismutase, an antioxidant enzyme showed lower levels in preeclamptic patients. Following Vit. C supplementation marked improvement was observed oxidative stress in preeclampsia. Vitamin supplementation has definite role in reducing the oxidative stress & maternal & perinatal morbidity.

Keywords: Preeclampsia, Oxidative stress, Malondialdehyde (MDA), Superoxide dismutase (SOD)

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INTRODUCTION

Pregnancy is a physiological situation convoy by a high energy demand and an amplified oxygen requirement. a variety of compensatory adaptive changes, including augmented ventilation for enhanced oxygen demand, take place with advancing pregnancy to meet the mounting requirements for proper bodily functions of the mother to carry out the needs of the foetus. Such a condition might be responsible for raised oxidative stress in pregnancy [1].

Oxidative stress, defined as a interruption in the pro-oxidant antioxidant balance in favor of the former,causing potential cell damage [2,3].

In a healthy body, pro-oxidants (free radical species) & antioxidants remain in equilibrium. When the equilibrium is interrupted owards an overabundance of free radical species, oxidative stress (OS) take place. There is rising literature on the effects of oxidative stress in female reproduction through association in the pathophysiology of preeclampsia, gestational diabetes, hydatidiform mole, free radical persuade birth defects & other situation such as abortions & preterm labour [4,5].

MATERIAL AND METHOD

Single blind randomized clinical study with multiple group conducted in the Department of Pharmacology at Jawaharlal Nehru Medical College, Sawangi (Meghe) Wardha from Feb.2014 to Nov 2015. . The study was approved by Institutional Ethics committee. Total 60 pregnant women volunteered and gave written consent (consent was taken in vernacular language) for the study and comprised the study population after following inclusion criteria.

Sample size was estimated by using formula [n= (4 x p x q / L x L), Where P is the prevalence of preeclampsia (5-11%), q=1-p and L is Allowable Error (10-20% of P)].

Inclusion criteria

- Willing to give inform consent.
- Primigravidae 20 to 32 weeks.
- BP > 140/90 mm Hg & Proteinuria or edema. (Proteinuria to be detected by dipstix test)

Exclusion criteria

- Unwilling to sign inform consent form.
- Diseased Diabetes Mellitus, Tuberculosis, HIV.
- Patients with severe preeclampsia (BP > 160/100 mmhg)
- Patients with IUGR ,severe anaemia,Allergic to medication.
- Multigravidae
- Pregnancy more than 32 weeks of gestation [6].

Selected Patients will be randomly allocated into twelve groups as follows

- A – Methyldopa
- B - Methyldopa + vit C
- G - Nifedipine
- H - Nifedipine + Vit C

Specific markers [7,8]

- Serum malondialdehyde level (MDA): An end product of lipid peroxidation measured by thiobarbituric acid reactive substances assay (MDA-TBAR).
- Superoxide Dismutase (SOD): An antioxidant enzyme measured by pyrogallol oxidation method.

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Initial blood samples collected at first visit (at entry of study, 20 weeks onwards) were taken as control. After the first blood samples were taken and analyzed for circulating biomarkers of oxidative stress, patients were assigned to receive study medication vitamin C 1000 mg daily [9,10] for 45 days and were called for follow up at every week interval for serial measurement of biomarkers for 45 days (study medication was provided in brown paper pack prepared by hospital staff not involved in research and clinical management and it was provided free of cost). Patients with preeclampsia were given antihypertensive treatment Alfa methyl dopa 250mg 8hrly or Nifedipine 10mg and were advised to come for follow up every 7 days, for BP monitoring and fetal growth monitoring and those patients who developed complications were hospitalised for active intervention.

Statistical Analysis

Statistical analysis was done by using descriptive and inferential statistics using chi-square test, one way ANOVA, Multiple Comparison-Tukey Test and Pearson's Correlation Coefficient. The software used in the analysis were SPSS (Statistical Package for Social Sciences) version 17.0 and GraphPad Prism version 5.0. All the results were tested at 5% level of significance.

OBSERVATIONS AND RESULTS

Table 1: Age wise distribution of patients

Age Group(yrs)	No of women	Percentage(%)
19-23 yrs	34	56.67
24-28 yrs	22	36.67
29-33 yrs	3	5.00
34-38 yrs	1	1.67
Total	60	100.00
Mean ±SD	23.36 ± 3.22(20-35 years)	

Graph 1: Age wise distribution of patients

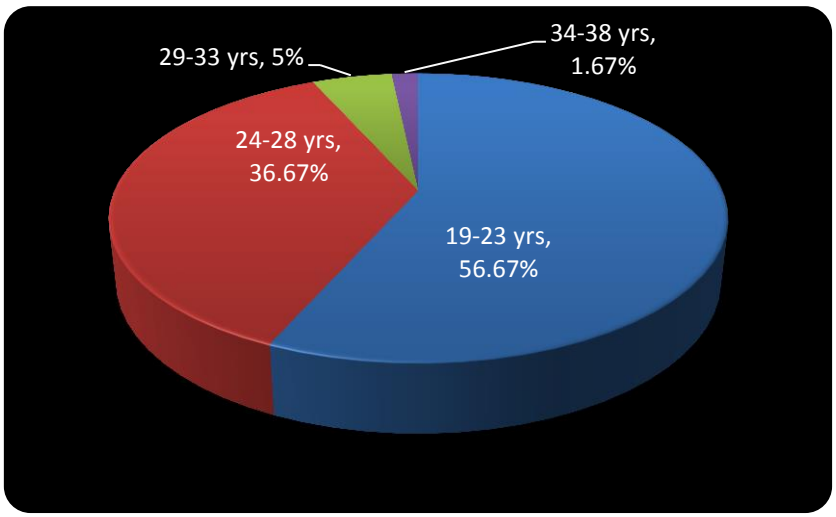

Table 2: Distribution of women according to weeks of gestation

Weeks of Gestation	No of women	Percentage(%)
20-22 weeks	3	5.00
22.1-24 weeks	8	13.33
24.1-26 weeks	18	30.00
26.1-29 weeks	31	51.67
Total	60	100.00
Mean ±SD	25.52 ± 2.05(20-28.20 weeks)	

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Graph 2: Distribution of women according to weeks of gestation

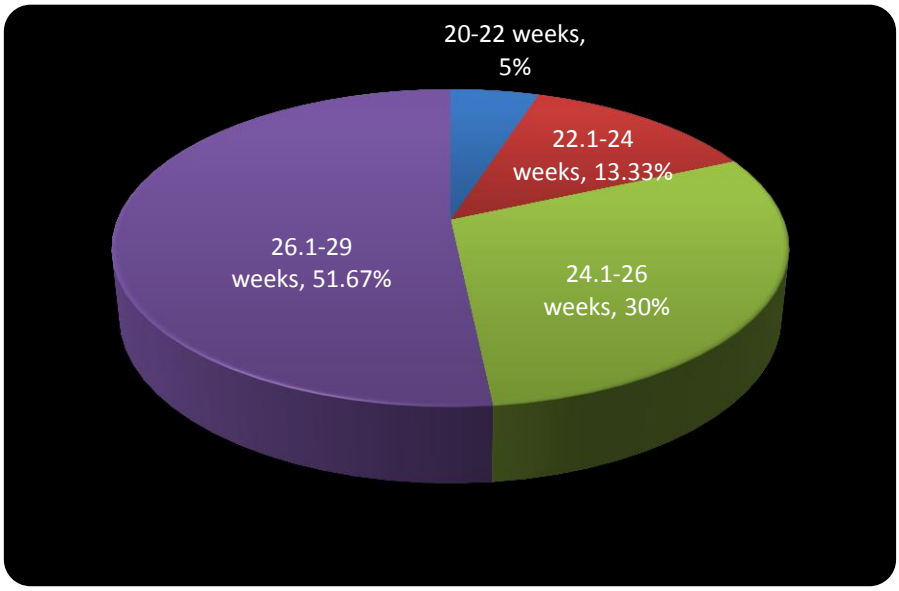

Table 3: Distribution of women according to Hb%

Hb%	No of women	Percentage(%)
8.1-10 gm%	23	38.33
>10 gm%	27	61.67
Total	60	100.0
Mean ±SD	10.33 ± 1.10(8.20-12.40 gm%)	

Graph 3: Distribution of women according to Hb%

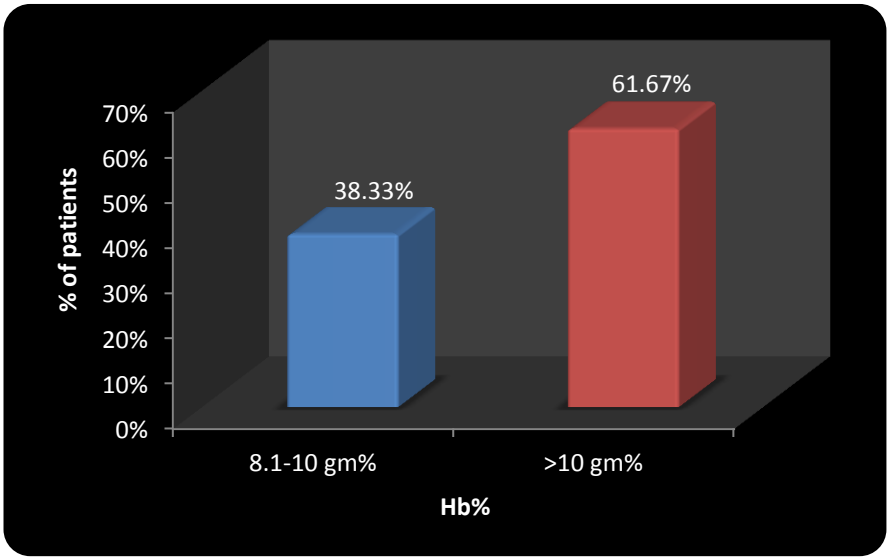

Table 4: Distribution of women according to Urine Albumin

Urine Albumin	No of women	Percentage(%)
Present	16	26.67
Absent	8	13.33
Trace	36	60.00
Total	60	100.00

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Graph 4: Distribution of women according to Urine Albumin

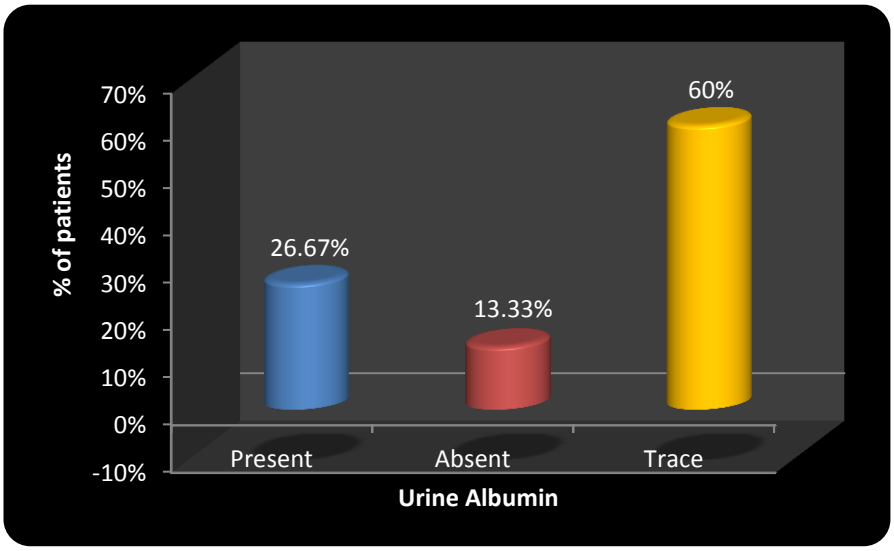

Table 5: Progressive changes in MDA

Days	Group A	Group B	Group G	Group H
1st Day	29.500±4.01	31.212±1.36	32.098±1.31	31.346±1.46
15th Day	33.207±1.85	30.026±1.60	33.223±1.85	33.428±0.86
30th Day	34.290±1.50	29.128±1.53	35.560±1.39	29.127±1.53
45th Day	36.487±1.81	28.062±1.64	37.145±1.37	27.504±2.43

Graph5: Progressive changes in MDA for group A,B,G,H

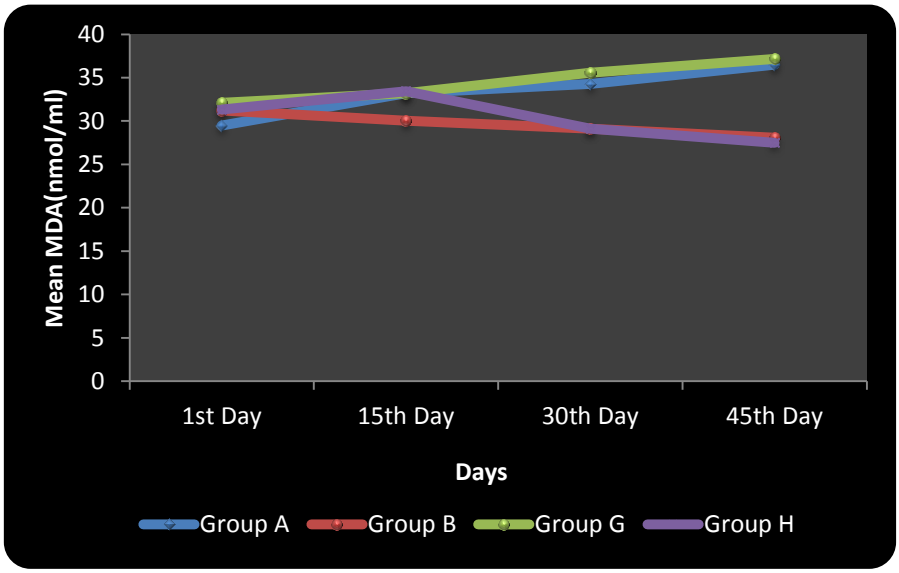

Table 6: Progressive changes in SOD

Days	Group A	Group B	Group G	Group H
1st Day	0.158±0.005	0.122±0.006	0.158±0.005	0.158±0.005
15th Day	0.156±0.008	0.132±0.006	0.154±0.006	0.149±0.008
30th Day	0.150±0.010	0.138±0.006	0.109±0.029	0.169±0.006
45th Day	0.143±0.007	0.155±0.007	0.100±0.026	0.178±0.007

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Graph 6: Progressive changes in SOD for group A,B,G,H

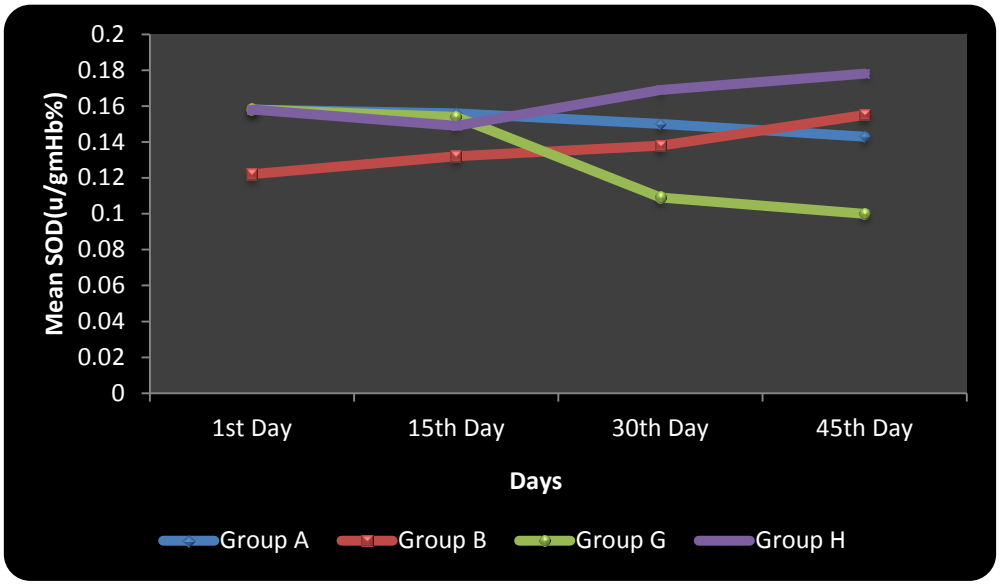

Table 7: Progressive changes in protective index(SOD/MDA)

Stress Parameter: SOD/MDA				
Days	**Group A**	**Group B**	**Group G**	**Group H**
1st Day	0.005±0.0009	0.003±0.0002	0.004±0.0002	0.005±0.0003
15th Day	0.004±0.0003	0.004±0.0002	0.004±0.0003	0.004±0.0003
30th Day	0.004±0.0003	0.004±0.0002	0.003±0.0002	0.005±0.0002
45th Day	0.003±0.0002	0.005±0.0003	0.002±0.0001	0.006±0.0007

Graph 7: Progressive changes in protective index(SOD/MDA) for group A-F

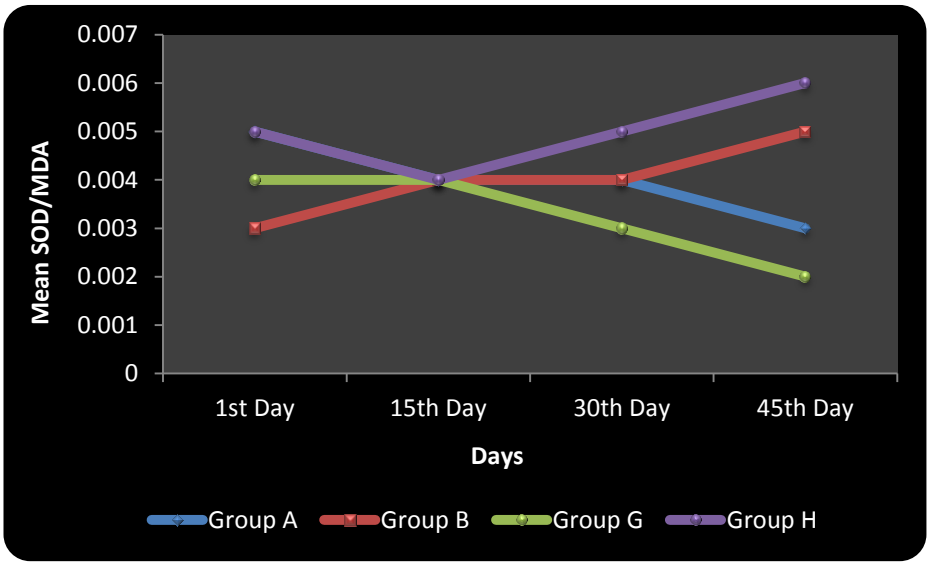

Table 8: Progressive changes in stress index(MDA/SOD)

Stress Parameter: SOD/MDA				
Days	**Group A**	**Group B**	**Group G**	**Group H**
1st Day	186.85±28.97	255.01±16.04	203.02±12.40	198.27±12.84
15th Day	212.32±17.02	227.39±14.07	215.81±16.96	224.17±14.71
30th Day	228.48±18.65	210.65±13.09	241.54±15.22	171.65±9.34
45th Day	255.76±17.72	181.32±12.30	250.79±17.93	154.40±15.21

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Graph 8: Progressive changes in stress index(MDA/SOD) for group A-F

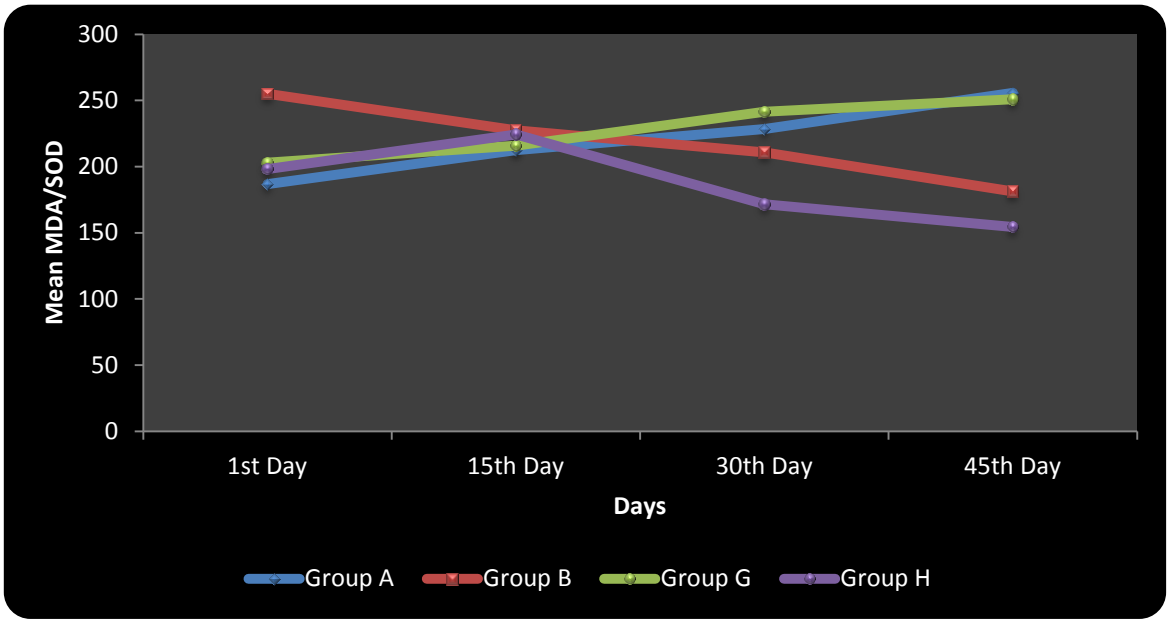

Table 9: Comparison of treated with untreated groups for Protective Index(SOD/MDA) Student's Unpaired t test

	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
I	Group A	15	0.004	0.0004	0.0001	0.25	0.79 NS,p>0.05
	Group B	15	0.004	0.0002	0.00006		
II	Group G	15	0.003	0.0001	0.00004	17.13	0.0001 S,p<0.05
	Group H	15	0.005	0.0002	0.00007		

Table 10: Comparison of treated with untreated groups for Stress Index(MDA/SOD) Student's Unpaired t test

	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
I	Group A	15	220.85	17.38	4.48	0.42	0.67 NS,p>0.05
	Group B	15	218.59	10.71	2.76		
II	Group G	15	266.84	10.89	2.81	22.80	0.0001 S,p<0.05
	Group H	15	187.12	8.043	2.07		

DISCUSSION

In this experimental study, table 1,2,3 shows distribution of patients in . different groups according to Age They were further divided based on weeks of gestation, Hb%, Albumin in urine.

Sociodemographic features of the patients were comparable, no significant difference was found between clinical features of preeclampsia (at the entry of study) in hypertension, proteinuria and oedema.

End product of lipid peroxidation, marker of oxidative stress was measured by MDA- TBAR assay.

In this study serum MDA levels compared between A,G & B, H groups and levels were raised in women with preeclampsia and showed significant difference with antioxidant supplemented groups B, H .

women at risk of preeclampsia ($p<0.05$). Many studies confirm that levels of lipid peroxidation products such as MDA and lipid peroxides are increased in pregnancy and show significant rise in the levels in women with preeclampsia.

JJ Wu (1996) [11] found raised MDA levels in pregnant women with preeclampsia. Serum MDA levels were raised in women with preeclampsia or eclampsia pregnancies compared with uncomplicated pregnancy. suggesting that lipid peroxidation is an important factor in the pathogenesis of preeclampsia. Above table shows comparative values of MDA in preeclampsia and normal pregnancy reported by various authors. Present study is comparable with the studies by Mohanty S.et al (2006) [12] and J.B. Sharma et al (2009) [13].

Superoxide dismutase (SOD)

Table 6 shows Mean ± SD plasma superoxide dismutase (SOD) levels of preeclampsia group were significantly increase in groups B,&H and decrease in A,G groups . Further the magnitude of oxidative stress and antioxidant SOD level changes correlated well with diastolic blood pressure.

Necipllhana et al, (2002) [14] found significantly increased levels of malondialdehyde and Cu and decreased SOD and Zn in women with preeclampsia compared to normal group. Our findings give support that radical scavenger SOD is consumed by the increased lipid peroxidation in preeclampsia. This may indicate an involvement of free radicals in the pathophysiology of preeclampsia. Mahadik KV (2003) et al [15] conducted a study of serum levels of superoxide dismutase in preeclampsia and eclampsia to test the predictive value and found low levels of SOD, less than 0.52U/ml being the predecessor of fulminating eclampsia. Thus they concluded that low serum SOD levels are important in deciding the time of intervention as termination of pregnancy.

Table 7,8 shows comparison between protective index (SOD/MDA) was found to increase in treated groups as reduced in control groups & stress index (MDA/SOD) was lower in treated group & Higher in control groups. Chappell et al, (1999) [16] tested the efficacy of Antioxidant supplementation (before clinically evident disease) in high risk women and found, supplementation with Vitamin C and E was associated with a 21% decrease in the PAI-1/PAI-2 ratio during gestation (95% CI 4-35; $p=0.015$). They thus concluded that antioxidant supplementation in women who were at risk of preeclampsia was associated with improvement in biochemical indices of the disease.

Comparison of vitamins showed better results with vitamin C, as vitamin C is scavenger of superoxide radicals and may help to preserve nitric oxide, also it is a chain breaking antioxidant and helps to maintain intracellular glutathione concentration. It was seen that there were rising levels of MDA with the progression of gestation in placebo subgroups of all the pregnant women i.e. normotensive, at risk of preeclampsia and preeclampsia and decline in the levels in those patients supplemented with antioxidant vitamins. Ghate J et al (2011) [17] found rise in oxidative stress with progression of gestation.

No major side effects were observed except for nausea and vomiting in few patients and abdominal pain and belching in those patients supplemented with combination of vitamin C 1000 mg.

CONCLUSION

Circulating biomarker, MDA levels were raised significantly in women with preeclampsia.

Superoxide dismutase, an antioxidant enzyme showed lower levels in preeclamptic patients.

Protective Index is found to be significant increase in treatment group simultaneously Stress index get reduced with antioxidant treated groups.

Superoxide dismutase, an antioxidant enzyme showed lower levels in preeclamptic patients.

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