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Oxidative Stress Parameters - A Predictive Tool for the Development of Preeclampsia

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ABSTRACT

Introduction: Preeclampsia, a pregnancy induced hypertensive disorder is known to be responsible for innumerable adverse fetal outcomes. This condition is known to alter the level of thiols in preeclamptic patients. Hence the present study was conducted to assess if the alteration in the levels of thiols could be used as a predictor for detecting preeclampsia and to check for their levels after the administration of L-Arginine.

Material and methods: This study recruited 186 Primigravidae women of age group 18 to 35 years. Doppler ultrasound was performed to categorize the participants into 4 groups. The recruited participants were subjected to blood tests to analyze the level of plasma thiols. Further the level of thiols was evaluated after the administration of L-Arginine.

Results: The plasma level of thiols was assessed in all the four categorized groups. Mean Cysteine levels (54.5) was lower in the subjects with Normal doppler (Group 1) than in subjects with abnormal doppler (Group 2) (114.2) which decreased back to normal after supplementation with Arginine. Similar trends were seen with respect to homocysteine and cysteinyl glycine. On the contrary mean glutathione levels (4.6) were low in subjects with abnormal doppler group 2 than the normal doppler group (6.6).

Conclusion: Altered level, i.e. increased levels of glycine, cysteinyl glycine, homocysteine and decreased levels of glutathione could be used as a marker for preeclampsia.

Keywords: Preeclampsia; L-Arginine; Doppler ultrasound; Glycine; Cysteinyl Glycine; Homocysteine; Nitric oxide

INTRODUCTION

Hypertensive disorders of pregnancy consist of a broad spectrum of conditions associated with significant maternal and fetal mortality and morbidity, preeclampsia being the most common one affecting 5-8 % of the pregnancies. [(1-2)] According to the World Health Organization (WHO), the incidence is higher in developing countries (2.8% of live births) than in developed countries (0.4%). [(3)] Preeclampsia is defined as pregnancy induced hypertension associated with proteinuria with the onset occurring following the 20th week of pregnancy. [(4)] It is associated with adverse fetal outcomes, like fetal growth restriction, premature births, fetal hypoxemia etc. [(5)] It is a major contributing factor accounting to intensive care unit admissions during pregnancy. [(1)]

There are four major factors implicated in the Etiopathogenesis of Pre eclampsia

1. Placental implantation with abnormal trophoblastic invasion of uterine arteries
2. Immunological maladaptive tolerance between maternal/fetal tissues
3. Maternal mal adaptation to cardio vascular system or inflammatory changes of normal

Pregnancy leading to endothelial cell activation

4. Genetic factors [(6)]

More recently, evidence has been found for the role of oxidative stress in the development of preeclampsia. Oxidative stress can be combated both by enzymatic as well as non enzymatic antioxidants. [(7)]

Thiols constitute the major part of the total body of antioxidants and play a prominent role in defense against the reactive oxygen free radicals. [8]

Cysteine, homocysteine, cysteinlyglycine and glutathione are the biologically low molecular mass thiols found in the plasma which help in combating this oxidative stress. [9] According to a well known theory, excess free radicals triggers off preeclampsia in those women who have lowered level of glutathione and higher levels of cysteine, homocysteine and cysteinyl Glycine. [10] It is also a well known fact that there is a dysfunction of the L-Arginine–Nitric oxide system in preeclampsia. Recent studies have shown that supplementation with L-Arginine has improved both endothelial function and oxidant stress [11] Hence this study was conducted to analyze the levels of thiols in pre eclampsia patients and to check for any change in the levels of thiols after the administration of L-Arginine. The main objective of the study was to analyze the level of oxidative stress parameters like cysteine, homocysteine, cysteinylglycine and glutathione in subjects with normal and abnormal doppler and to analyze their levels after the administration with L-Arginine.

METHODOLOGY

Women who are prone to develop preeclampsia can be recognized by abnormal wave patterns in doppler ultrasound between 17-20 weeks of gestation and as early as the 1st trimester (11-14 weeks) of pregnancy. [12] This is based on the principle that there is reduced uteroplacental perfusion as a result of abnormal trophoblastic invasion of the spiral arterioles and in addition there are reduced antioxidants like superoxide dismutase glutathione peroxidase / reductase and an increase of oxidative stress parameters like Cysteine, Homocysteine, cysteinylglycine. Therefore a uterine artery doppler done in the 1st trimester, in the 17-20 week of gestation and a blood estimates of these thiols would help recruit pregnant women who are prone to develop Preeclampsia.

Ethical Approval

Ethical approval was obtained from the Ethical committee of Osmania medical college as the study involved administering a drug. Written informed consent was taken from the enrolled participants in their own language prior to the enrolment of cases.

Study design

An interventional cohort study among pregnant women was conducted at the Institute of Obstetrics and Gynecology, Osmania Medical College, Government Maternity Hospital, Hyderabad and some referrals from the local practitioners in the old City of Hyderabad from August 2008 to December 2012. The participants included in the study was, Primigravidae women with the age group of 18-35 years. All the participants were explained about the study and written consent was taken from them. All the participants were grouped into three categories:

- Group 1: Normal Doppler
- Group 2: Abnormal Doppler with no L-Arginine supplementation)
- Group 3: Abnormal Doppler with supplementation of L-Arginine at around the 17th week of pregnancy

All women in the Group 3 were given L-Arginine sachets of 3 gms/twice a day until delivery. All the cases received their regular supplementation of Iron, Calcium and Folic acid.

Doppler Ultrasound Test

All the patients underwent the doppler ultrasound of the uterine arteries. The equipment used was a GE Voluson 730 Expert doppler unit having a pulsed wave, continuous wave and HPRF Doppler with dual convex sector transducer. Doppler as a predictor for the development of preeclampsia was as high as 86.8 %. [13] Doppler Ultrasound of the uterine arteries was analyzed in all women at the 1st trimester of pregnancy (11-14 weeks) and between 17-22 weeks of pregnancy.

Thiols Estimation

All the participants were subjected to blood tests to analyze the level of plasma thiols i.e. Plasma Cysteine, Plasma Homocysteine, Plasma Cysteinyl Glycine, and Plasma Glutathione. Collected plasma samples were treated with N-butyl phosphine and incubated at 40° C/60 mins before precipitation with trichoroacetic acid and centrifuged for 10 mins to separate the proteins. The supernatant was incubated with borate, EDTA –sodium hydrochloride buffer and fluorescent 7-benzo-2-oxa-1,3-diazole-4 sulfonic acid solution at 60° C/60 mins. The solution was cooled at room temperature, filtered and injected into HPLC, Agilent 1100 series and fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm. [14,15] Agilent Chemstation software was used for the quantitation. Supel cosil 7m LC-18-DB HPLC column (150 mm x 4.6 mm) was used for the separation and an isocratic mobile phase of potassium dihydrogen phosphate buffer (10 mM, pH 2.1) containg 4% acetonitrite was used with a flow rate of 2.0 ml/min. This technique provides a reliable method for determination of plasma thiols where a simple chemical reduction transformed all the disulfides to thiols which are then derivatized and measured using HPLC flourescence.

The blood levels were repeated in Group 3 after the 32nd week of gestation to form a Group 3b in order to see whether there was any statistically significant improvement in the blood levels. In addition to the tests all cases had a routine obstetric profile which includes complete blood picture, urine examination, Blood sugar, Thyroid profile, HIV and HBSAg. In cases with Preeclampsia, platelet count, renal and liver function tests with clotting profile were obtained. Women with severe preeclampsia were hospitalized and closely monitored.

Statistical Analysis

Descriptive statistics were calculated for all variables. Mean values across the groups were compared using ANOVA followed by post hoc test. Pre and post values were compared by paired "t"-test. Further to study the associations with categorical variables, Chi-square test was done. Level of significance was set as 0.05. SPSS version 19.0 was used for statistical analysis.

RESULTS

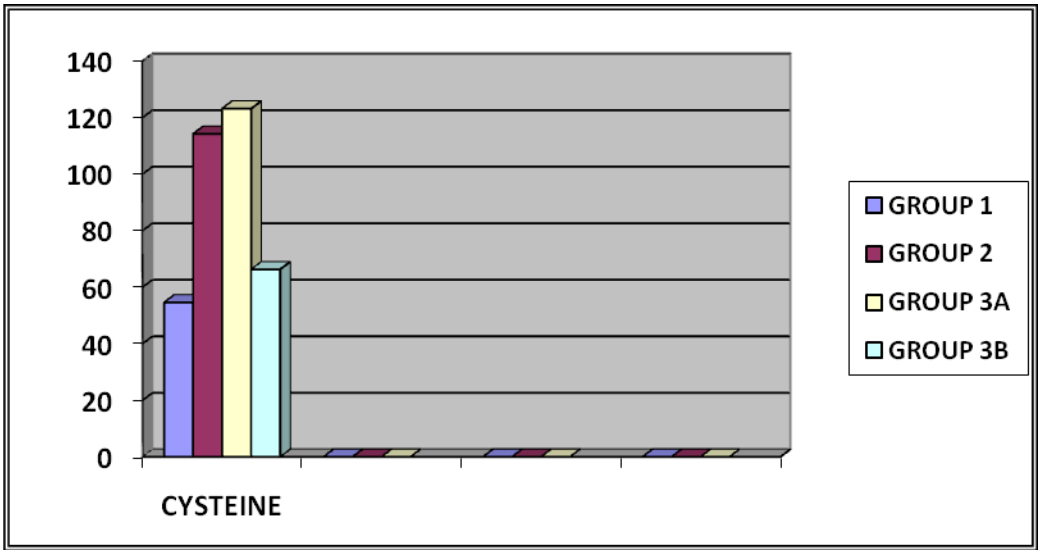
A total of 186 Primigravidae were recruited after scanning approximately 3800 women. All cases were monitored clinically throughout their pregnancy every 4 weeks up to 28th week and once in 2 weeks until 36th week and weekly thereafter.

The Thiol values were analyzed and there was a statistically significant abnormal level in those cases that developed preeclampsia.

Table 1: Mean values of Cysteine, Homocysteine, Cysteinyl glycine and glutathione in all the categorized groups (Group 1, 2, 3a, 3b).

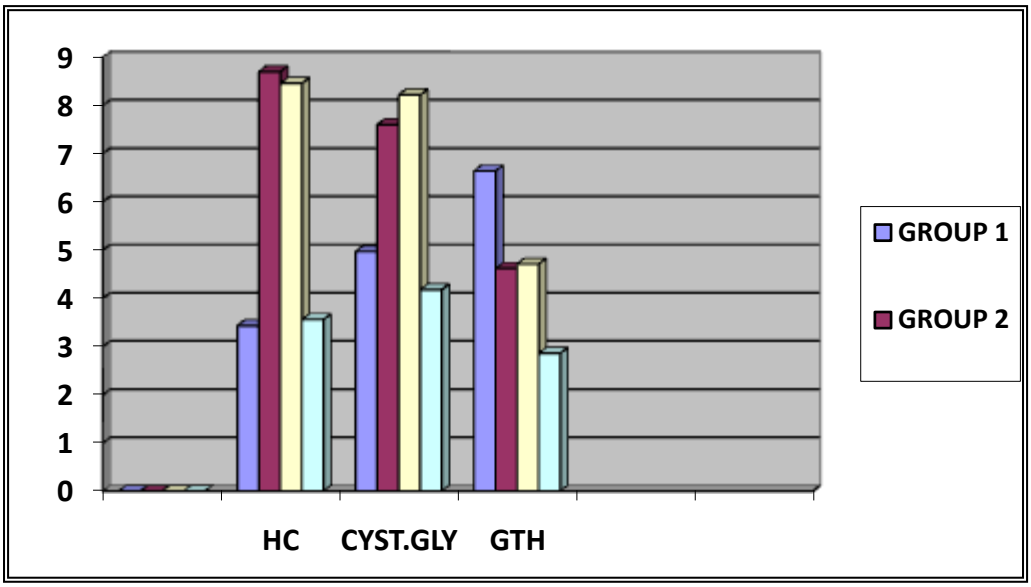
	Number	Mean	Std deviation	Std error	P- value
CYSTEINE					
GROUP 1	50	54.5	15.4	2.1	< 0.001
2	50	114.2	24.1	3.4	"
3a	84	123.1	34.9	3.8	"
3b	60	93.3	20.8	2.6	"
HOMOCYST.					
GROUP 1	50	3.4	1.1	0.1	< 0.001
2	50	8.7	1.6	0.2	"
3a	84	8.4	2.2	0.2	"
3b	60	3.5	0.8	0.1	"
CYST gly.					
GROUP 1	50	4.9	8.1	1.1	< 0.001
2	50	7.6	2.3	0.3	"
3a	84	8.2	1.9	0.2	"
3b	60	4.1	0.7	0.1	"
GTH					
GROUP 1	50	6.6	3.5	0.4	< 0.001
2	50	4.6	3.2	0.4	"
3a	84	4.7	7.9	0.8	"
3b	60	2.8	1.0	0.1	"

As interpreted in the table 1, mean levels of cysteine were higher in the group 2 (114.2) and Group 3a (123.1), those groups may prone to preeclampsia. The mean values decreased after supplementation with L-Arginine in the group 3b (93.3) ($p<0.001$). Similar findings were seen with respect to homocysteine and cystenyl glycine. On the contrary the mean values of glutathione were lower in the Group 2 (4.6) and Group 3a (4.7) when compared to subjects with normal doppler (6.6) ($p<0.001$).



Graph 1: Plasma serum cysteine levels in the categorized groups.

The levels of serum cysteine in graph 1 showed a mean of 54.58 µmol/L in Group 1 but elevated levels in Group 2 (114.25umol/L) & Group 3a (123.17umol/L) and a fall in Group 3b women (p value <0.001)



Graph 2: Plasma homocysteine, cysteinyl glycine and glutathione levels in all the three groups.

As shown in the graph 2, the levels of homocysteine values in Group 1 (3.43 µmol/L) and Group 3b (3.56 µmol/L) were comparable and elevated levels of 8.70 µmol/L and 8.46 µmol/L in Group 2 and Group 3a respectively with the p value < 0.001). The levels of cystenyl glycine showed lower value of 4.97 µmol/L in Group 1 and 4.18 µmol/L in group 3b and elevated levels of 7.60 µmol/L and 8.22 µmol/L in Groups 2 and 3a respectively (p value <0.001). The mean values of

glutathione was 6.64 µmol/L in Group 1 and lowered levels of 4.62 µmol/L and 4.71 µmol/L in Group 2 and 3a and a mean of 2.61 µmol/L in Group 3b (p value <0.01).

The levels of cysteine, homocysteine, and cysteinyl glycine were increased in subjects with abnormal doppler (prone to preeclampsia) which further decreased to the normal level after the administration of L-Arginine (p<0.01). On the other hand glutathione levels dropped down in subjects with abnormal doppler (p<0.01).

Table 2: Levels of Thiols Before and After Supplementation

	Group 1 Normal Doppler	Group 2 Abnormal Doppler	Group 3 (Abnormal Doppler before supplementation)	Group 4 (After supplementation with Arginine)
Cysteine (Normal level <110 µmol/l)	100% (<110 µmol/l)	66% (>110 µmol/l)	71.4% (>110 µmol/l)	5% (<110 µmol/l)
Homocysteine (Normal level <7 µmol/l)	100% (<7 µmol/l)	86% (>7 µmol/l)	84.5% (>7 µmol/l)	100% (<7 µmol/l)
Cysteinyl Glycine (Normal levels < 5 µmol/l)	80% (< 5 µmol/l)	84% (> 5 µmol/l)	94% (> 5 µmol/l)	83.3% (< 5 µmol/l)
Glutathione (Normal levels > 4 µmol/l)	72% (> 4 µmol/l)	60% (<4 µmol/l)	71.4% (< 4 µmol/l)	25% (>4 µmol/l)

Levels of cysteine, homocysteine, cysteinyl glycine and glutathione were all within the normal range in subjects with normal doppler (Table 2). Increased levels were found in subjects with abnormal doppler which reverted back to normal after the administration with L- Arginine (p<0.01). With respect to glutathione the decreased values were found in subjects with abnormal doppler which increased back to normal range in about 25% of the subjects.

Routine and other blood tests were within the normal limits in Group 1, with mild abnormality in liver function test seen in 6 cases of Group 2 and Group 3 showed 4 cases of mild altered renal function tests.

DISCUSSION

This study assessed the level of plasma thiols in 186 pregnant women with normal and abnormal doppler. Further evaluation of the plasma level of thiols was done after the administration of L-Arginine in patients with abnormal doppler.

S Urmila et al [(16)] conducted a study on homocysteine association with preeclampsia and normotensive pregnancy. It was seen that normotensive pregnant women had a lower mean of 11.5 when compared to women with preeclampsia (14.5). This was in accordance with our study in which a lower mean of 3.4 was seen in normal doppler (normal pregnancy) versus abnormal doppler (preeclampsia) (8.7).

In the study conducted by Raijmakers et al [(7)] the preeclamptic women showed higher mean (13.1) concentrations of homocysteine than the women without preeclampsia (11.5). Similar trends were seen in the present study wherein women with abnormal doppler had a higher mean homocysteine concentration (8.7) than normal doppler's (3.4).

The mean cysteinyl glycine values in women with preeclampsia (34) were higher than the normotensive women (37.5) in the study by Raijmakers et al. [(7)] The present study showed similar results with a higher mean of (7.6) in subjects with abnormal doppler to normal doppler (4.9) was seen.

The high concentration of homocysteine observed in preeclampsia could be attributed to decreased activity of one of the enzymes responsible for the remethylation of homocysteine or pathological process of haemoconcentration. [(17)]

These elevated levels of homocysteine would have lead to endothelial dysfunction, which eventually would have resulted in preeclampsia. [(18,19)]

The mean level of plasma glutathione was lower in subjects with abnormal doppler (4.6) than in normal doppler subjects (6.6) in this study. This significantly ($p<0.001$) low level of glutathione observed in subjects with abnormal doppler is in agreement with previous study done by Modupe Fisayo ASAOLO et al [(15)] where a higher mean glutathione level of (10.2) was seen in a normotensive pregnant women when compared to a hypertensive pregnant women (6.7).

HD Mistry et al [(20)] conducted a study to assess the glutathione activity in preeclamptic pregnancies. The results of his study revealed a highly significant reduction in plasma glutathione peroxidase activity in preeclamptic women when compared to controls which was very much in accordance with our study.

Previous reports given by JB Sharma et al [(21)] and S Kharb [(22)] found a similar trend of reduced glutathione level in pre eclamptic women which was very much in line with our study.

Depletion in the levels of glutathione has also found to be linked with lipid peroxidation, which occurs as a result of hypertension and thus the production of free radicals that cause endothelial damage. [(17)] The roles of glutathione in neutralizing free radicals is clearly evident from our findings since levels of glutathione were significantly higher in subjects with normal doppler than subjects with abnormal doppler. The decreased glutathione levels in subjects with abnormal doppler might be due to disturbances in the synthesis of glutathione in this condition. This indicates that glutathione might be a one of the contributory factor to hypertension in pregnancy. [(17)]

This study also evaluated the levels of thiols after the administration of L-Arginine a Nitric oxide donor. It was seen that the increased levels of thiols in subjects with abnormal doppler dropped down after the administration of L-Arginine which was statistically significant at $p<0.05$.

This study could not be compared with any other previous findings as this was one of the kinds of study that checked for the plasma levels of thiols after the administration of L-Arginine.

Jean Luc Balligand et al [(23)] in his paper discussion on Vitamin B or L-Arginine supplementation in hyperhomocysteinaemia elaborated that this could have been the possible reason. With respect to metabolism of homocysteine it can be re-methylated to methionine involving adenosyl intermediates. One of these methylation reactions involves the formation of asymmetric dimethyl-arginine (ADMA), an inhibitor of nitric oxide synthase. The inhibitory effect of ADMA can be competed away with excess L-Arginine, the natural substrate of nitric oxide synthase.

CONCLUSION

Present study revealed that subjects with normal doppler had all the oxidative stress parameters in the statistically normal range. Participants with abnormal uterine artery doppler have had abnormal level of oxidative stress parameters. Hence this altered level of oxidative stress parameters could be used as a marker to predict preeclampsia in pregnant women. Additionally L-Arginine supplementation in those with doppler abnormality showed up a reversal of the thiol values back to normal. This study gives further scope to research where L-Arginine supplements could be used to reduce the markers of oxidant stress and improve endothelial function.

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Conflict of Interest

All the authors declared that they have no conflict of interest.

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