

Study of free radicals (malondialdehyde) in pre-eclampsia and eclampsia in correlation with uric acid and total proteins

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Abstract

Introduction: Oxidative stress is a normal phenomenon in normotensive pregnancy; however, in preeclampsia, oxidative stress is exaggerated may result in a greater potential for endothelial oxidative damage. It has been reported that higher MDA (Malondialdehyde) /total antioxidant capacity (TAC) ratio is indicative of oxidative stress in women with pre-eclampsia.

Materials and Methods: Prospective study was done for duration of 2 years i.e, from January 2015 to December 2017 in the department of Biochemistry at Kakatiya Medical College, Hanmakonda, CKM hospital Warangal. A total number of 60 pregnant women were included in the study. Among these, 20 pregnant women were clinically diagnosed as pre-eclampsia and 20 are eclamptic cases and the remaining 20 were selected as control group and are normotensive antenatal women who are also in third trimester of pregnancy.

Results: The mean MDA, serum uric acid, 24 hr urinary protein were higher in pre-eclampsia and eclampsia compared to controls.

Conclusion: Increased lipid peroxidation products levels clearly shows that oxidation stress is more in pre-eclampsia and eclampsia than in control subjects and it plays a significant role in etiopathogenesis of PIH. Serum uric acid levels are significantly higher in cases than in controls and by estimating the uric acid levels we can assess the severity of the disease and we can avoid further progress of the disease process by early detection and prompt treatment. Total serum protein levels are drastically reduced in pre-eclampsia and eclampsia cases compared to controls.

Keywords: MDA, Serum uric acid, Proteins, Eclampsia, Pre-eclampsia.

Introduction

Pre-eclampsia, a syndrome peculiar to pregnancy characterized clinically by hypertension and proteinuria.¹ In India the incidence of preeclampsia is reported to be 8-10 per cent of the pregnancies.² Pregnancy-induced hypertension or preeclampsia (diastolic blood pressure >90 mm Hg) occurring after 20 weeks of gestation with proteinuria (either ≥ 300 mg protein per day or an urinary protein/ creatinine ratio ≥ 30 mg/ mmol).³

Hypertensive disorders are the most common medical complications of pregnancy, with a reported incidence ranging from 5-10%.⁴ Hypertension in pregnancy affects mostly after twentieth week of gestation and frequent occurrences are seen near term. This contributes significantly to the cause of maternal and perinatal mortality and morbidity.⁵⁻⁷

Oxidative stress is a normal phenomenon in normotensive pregnancy; however, in pre-eclampsia, oxidative stress is exaggerated may result in a greater potential for endothelial oxidative damage.^{8,9} It has been reported that higher MDA/ total antioxidant capacity (TAC) ratio is indicative of oxidative stress in women with pre-eclampsia.⁹

It has been suggested that uncontrolled lipid peroxidation may play a role in the etiology of the PIH. Elevated lipid peroxidation markers like urine (the isoprostane 8, 12-epi-iPF₂ α -VI) or plasma (8-epiprostaglandin F₂ α and MDA) are the markers of

oxidative stress.¹⁰ Recent reports suggest that free radical induced endothelial cell injury might be an etiologic factor.¹¹

This study was done to estimate certain biochemical parameters like serum free radicals levels, serum uric acid levels, serum total proteins, and 24 hours urinary proteins in pregnancy induced hypertensive patients. To assess the antepartum, severity and complication of preeclampsia/eclampsia in comparison with normotensive pregnant women by analyzing biochemical parameters to have early detection and better management of toxemia of pregnancy to avoid maternal and fetal mortality and morbidity

Materials and Methods

The Prospective study was done in the department of Biochemistry in collaboration with department of Obstetrics & Gynaecology at Kakatiya Medical College, Hanmakonda CKM hospital, Warangal over a period of 2 years i.e, from January 2015 to December 2017. Institute's Ethical committee approval was obtained. The pregnant women were selected randomly. A total of 60 pregnant women were included in the study. Among these, 20 pregnant women were clinically diagnosed as pre-eclampsia and 20 eclamptic cases. All the 40 antenatal women were in 3rd trimester of pregnancy and were admitted in Government maternity hospital. The remaining 20 were selected as

control group and were normotensive antenatal women who were also in third trimester of pregnancy.

The data of various biochemical parameters is compared between the two groups and students "t" test was used as statistical method.

Inclusion Criteria

1. Cases of pre-eclampsia and eclampsia primi patients in the age group of 18 to 30 years and with gestation age more than 20 weeks.
2. Controls of normotensive primi pregnant women in the age group of 18 to 30 years and more than 20 weeks of gestation

Exclusion Criteria

1. Elderly primi gravida subjects,
2. gestational diabetes,
3. chronic hypertension
4. multiple gestation
5. cardiovascular disease
6. renal disease
7. liver disease
8. endocrine disorders
9. chronic infections

Sample Collection: Blood samples were obtained by venous puncture from the study group immediately after admission and before the commencement of treatment.

Serum was separated after one hour centrifugation and clear serum is transferred to separate aliquots and analysis was done on the same day. Urine samples also collected to assess the 24 hours total urinary proteins.

5 ml of venous blood samples were collected, the serum was separated and analysed for Malondialdehyde (MDA), a lipid peroxidation product, by thiobarbituric

acid reactive substances (TBARS) method and serum uric acid by automated Chemistry Analyser [HUMASTAR 300 (Human Gm BH Germany)] using available commercial kit.

Principle: Malondialdehyde (MDA), a reactive aldehyde is a product of lipid peroxidation. It reacts with thiobarbituric acid (TBA) to form pink colored complex of TBA-MDA adduct and this color is measured at 532 nm. The formation of the MDA-TBA adduct is initiated by a nucleophilic attack involving carbon-5 of TBA onto carbon-1 of MDA, followed by dehydration and a similar reaction of the intermediate MDA-TBA adduct with a second molecule of TBA.

Reagents: TBA (0.67% w/v) was prepared by dissolving 335 mg TBA in 50 ml of water. TCA (40% w/v) was prepared by dissolving 20g TCA in 50 ml of water.

Procedure: One ml of serum was mixed with each 1 ml of TCA and TBA. For blank, 1 ml of distilled water was mixed with 1 ml of TCA and TBA. Both the test tubes were kept in boiling water bath and cooled with ice-cold water and centrifuged at 3000 rpm for 10 minutes. The upper clear supernatant fluid was transferred to a cuvette and the absorbance was measured at 530 nm with a spectrophotometer after adjusting to zero with blank.

Calculation: The MDA level (nmol/l) of serum was calculated based on the molar absorption coefficient of MDA. The molar 5 absorption coefficient for 1 mol/L of MDA is 1.56×10^5 .

Results

Table 1: Mean and standard deviation of various biochemical parameters in controls and total cases (preeclampsia & eclampsia)

S. No	Biochemical Parameters	Mean & SD of Controls	Mean & SD of Cases	t value	P value	Remarks
1	MDA	1.6±0.68	5.11±1.78	9.5	<0.001	Significant
2	Serum Uric Acid	3.5±0.74	7.2±1.61	9.0	<0.001	Significant
3	Total Serum Proteins	5.8±0.74	4.68±0.73	4.86	<0.01	Significant
4	24hr Urine Proteins	5.8±0.70	879.31±518.27	7.45	<0.001	Significant

Table 2: Mean and standard deviation of various biochemical parameters in preeclampsia & eclampsia

S. No	Biochemical Parameters	Mean & SD of Preeclampsia	Mean & SD of eclampsia	t value	P value
1	MDA	4.16±1.27	5.82±1.99	3.19	0.05
2	Serum Uric Acid	7.0±1.58	7.85±1.86	1.3	Not significant
3	Total Serum Proteins	5.03±0.9	4.22±0.77	3.1	0.05
4	24hr Urine Proteins	754.1±391.82	1038.45±558.41	1.86	Not significant

The mean MDA level in the study group of total 40 cases (preeclampsia and eclampsia) is 5.11 ± 1.78 and

the control group was 1.6 ± 0.68 . The difference between the means of the two groups was statistically

significant ($P < 0.001$). So, we can conclude that there is increase in the oxidative stress in preeclampsia and eclampsia than in controls and we can also compare the mean MDA levels in preeclampsia 4.26 ± 1.27 , with eclampsia cases that is 5.82 ± 1.99 . So, the oxidative stress is still high in eclampsia cases than preeclampsia according to our study.

The mean serum uric acid level in the study group was 7.2 ± 1.61 and in the control group was 3.5 ± 0.74 . The difference between means of two groups was statistically significant ($P < 0.001$). The mean in preeclampsia cases is 7.0 ± 1.58 and mean in eclampsia cases is 7.85 ± 1.86 .

The mean 24 hour urinary protein in study group 876.31 ± 518.27 and in control group is 8.55 ± 43.74 . The difference between the means of two groups was statistically significant ($P < 0.001$). The mean in preeclampsia cases is $754.1 + 392.82$ and mean in eclampsia cases is 1038.45 ± 558.41 .

Discussion

A total of 20 controls have been studied. All the subjects were normotensive and healthy pregnant women and in 3rd trimester of pregnancy. We found that serum MDA levels, uric acid and total proteins (including 24 hour urinary proteins) in all the control individuals were within normal limits.

A total number of 20 cases of preeclampsia and 20 cases of eclampsia also in 3rd trimester have been studied. All preeclampsia subjects show classical triad of hypertension, proteinuria and edema.

All the subjects showed an increased levels in all above investigations when compared with control subjects.

The mean MDA level in the study group of total 40 cases (preeclampsia and eclampsia) is 5.11 ± 1.78 and the control group was 1.6 ± 0.68 . The difference between the means of the two groups was statistically significant ($P < 0.001$). So, we can conclude that there is increase in the oxidative stress in preeclampsia and eclampsia than in controls and we can also compare the mean MDA levels in preeclampsia 4.26 ± 1.27 , with eclampsia cases that is 5.82 ± 1.99 so, the oxidative stress is still high in eclampsia cases than preeclampsia according to our study.

The mean serum uric acid level in the study group was 7.2 ± 1.61 and in the control group was 3.5 ± 0.74 . The difference between means of two groups was statistically significant ($P < 0.001$). The mean in preeclampsia cases is 7.0 ± 1.58 and mean in eclampsia cases is 7.85 ± 1.86 . All the preeclampsia and eclampsia cases are having hyperuricemia.

Uric acid is probably the most important investigation for assessing the severity of pre-eclampsia of all the investigations carried out. It has generally been found to be useful when differentiating the preeclampsia from chronic hypertension, in the latter uric acid level is usually within normal range. It is also

useful prognostically i.e, the higher is the level of urate the greater is the perinatal mortality.¹²

A raised urate in the maternal blood results from tissue ischemia and reduced renal clearance, urate is actively secreted by distal convoluted tubules. Damage to distal convoluted tubules in preeclampsia results in decreased urate secretion which results in its increase in the circulation.

Mainly uric acid levels were estimated to indicate the degree of severity of toxemia of pregnancy. A correlation between high serum uric acid, with the severity of disease and perinatal mortality has been suggested by several authors.¹² An increase in uric acid is a fore runner for proteinuria.

All the preeclampsia and eclampsia patients showed significant proteinuria more than 300 mg/24 hours.

The mean 24 hour urinary protein in study group 876.31 ± 518.27 and in control group is 8.55 ± 43.74 . The difference between the means of two groups was statistically significant ($P < 0.001$). The mean in preeclampsia cases is $754.1 + 392.82$ and mean in eclampsia cases is 1038.45 ± 558.41 .

The definition of pre-eclampsia can be made if the pregnant women excretes more than 300 mg of protein in 24 hrs urine. These women were at a significantly increased risk of poor perinatal outcome.

Glomerular endotheliosis causes increased leakage of protein in urine. The amount of leakage of protein results in hypoproteinaemia and hypoalbuminemia. According to this study, the mean total serum proteins in control group is 5.8 ± 0.74 and in total cases 4.68 ± 0.73 . The difference between the means of two groups was significant ($P < 0.001$). The mean in preeclampsia cases 5.03 ± 0.9 and the mean in eclampsia cases is 4.22 ± 0.77 . Hypoproteinemia alter intravascular oncotic pressure and causes the patients to suffer from generalised edema.

Awareness, early detection, effective antenatal services, prompt and proper management will definitely decrease the maternal mortality, morbidity and also perinatal mortality.

In Shikha Saxena et al study Serum MDA level (mmols/L) in PIH subjects was higher (1.071 ± 0.26) as compared to the control subjects (0.42 ± 0.11) and this difference was highly significant ($p < 0.001$).¹³

Shikha Saxena et al study a similar significant difference ($p < 0.001$) was noted for serum uric acid level between the PIH subjects (6.65 ± 1.36) and the control subjects (4.72 ± 0.85). The comparison of serum MDA and uric acid levels within control and different PIH sub-groups were found significantly higher ($p < 0.001$) in PIH I, II and III sub-groups when compared with Group IV (normotensive pregnant women). Similarly MDA level in PIH group III was also found significantly higher ($p < 0.001$) when compared with the other two PIH groups. Uric acid level was also found significantly higher in group III

when compared with group I ($p < 0.01$) and group II ($p < 0.05$).¹³ Uric acid level was also found significantly higher in eclamptic women when compared with gestational hypertension (GH) ($p < 0.01$) and pre-eclampsia (PE) ($p < 0.05$) subjects.¹³

MDA level in eclamptic women was also found significantly higher ($p < 0.001$) when compared with other two groups.

Other studies conducted by Hubel et al and Freud et al have also shown that lipid peroxides like MDA were significantly elevated in mild and severe PIH.^{14,15} The increased MDA level in PIH is known to be due to increased generation of reactive oxygen species and reduction in anti-oxidants activity. Reactive oxygen species thus produced can cause enhanced lipid peroxidation in PIH which play a significant role in pathophysiology of PIH.¹⁶

Sharmila Krishna et al study, the serum MDA (Mean \pm SEM 24.4 \pm 2.38 vs 7.9 \pm 0.28 nmol/ml) and serum uric acid levels (7.2 \pm 0.25 vs 3.9 \pm 0.14 mg/dl) were significantly increased in preeclampsia cases compared to that of normotensive pregnant women respectively. They observed a weak positive correlation between Uric acid and MDA in preeclampsia cases. ($r = 0.065$, $p < 0.734$).¹⁷

Vanitha Gowda MN et al Serum MDA levels were moderately increased in pregnant controls (1.3nM/mL \pm 0.15 $p = < 0.001$ for non-pregnant controls vs pregnant controls). In preeclamptic women (2.52 nM/mL \pm 0.22), the rise in MDA levels was more marked.¹⁸

Conclusion

Increased lipid peroxidation products levels clearly shows that oxidation stress is more in pre-eclampsia and eclampsia than in control subjects and it plays a significant role in etiopathogenesis of PIH.

Serum uric acid levels are significantly higher on cases than in controls and by estimating the uric acid levels we can assess the severity of the disease and we can avoid further progress of the disease process by early detection and prompt treatment.

Total serum protein levels are drastically reduced in pre-eclampsia and eclampsia cases, compared to controls.

It reflects hyper proteinuria. This test also helpful in assessment of severity of disease, which facilitates initiation of proper treatment and prevents development of fatal complications.

The 24 hours urinary protein levels show much higher level i.e, more than 300 mg/24 hrs in cases than in controls. This is also good indicator to have awareness about the trait of preeclampsia so that we can guide the patient to have better antenatal care. It can reduce perinatal morbidity and mortality by supplementing iron and protein rich diet.

The comparative study of all the above parameters between the controls and total cases of pre-eclampsia and eclampsia conclude that the parameters are

significantly raised in cases than in controls due to various pathological changes in the disease process. So we can conclude that the eclampsia is the more serious and severe form of pre-eclampsia associated with convulsions.

Finally my study shows that free radicals play an important role in the pre-eclampsia and eclampsia. However, further extensive human studies are also needed to confirm the role of oxidative stress.

In this context our results suggest that definite antioxidant supplementation, early diagnosis, prompt treatment, better antenatal services reduce the severity of complications.

Conflict of Interest: None

Acknowledgement: Nil

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