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Original Article

Biochemistry Section

Is Xanthine Oxidase, a Marker in Pre-eclampsia? A Case-Control Study

VANISHREE BAMBRANA[1], C.D.DAYANAND[2], PUSHPA P KOTUR[3]

ABSTRACT

Introduction: Pre-eclampsia is an obstetrics problem that affects multiple systemic functions and leads to the increased maternal and fetal morbidity and mortality. The objective of the study was to evaluate the plasma levels of Xanthine oxidase (XO) activity, uric acid and Nitric oxide (NO) levels in women with pre-eclampsia and normal pregnancy during antenatal and postpartum period.

Materials and Methods: A case control study was conducted in women with normal pregnancy (n=50) and pre-eclampsia (n=50) before and after delivery. XO activity, uric acid and NO levels were determined from samples at 30-39 weeks of gestation. The current study was conducted in association with Obstetrics and Gynecology Department of R.L. Jalappa Hospital and Research Center. The blood samples were analysed for assay of XO, uric acid and NO. The results were analysed by using SPSS software version 2013. P-value < 0.05 was considered as statistically significant.

Results: The plasma XO activity was elevated (p<0.001) in the pre-eclampsia compared to normotensive pregnant women before delivery and decreased after delivery (p<0.001) significantly. Uric acid level showed a significant increase in pre-eclampsia when compared to the control before delivery (p<0.001) however values were non-significant after delivery.

Conclusion: Placenta plays a key role in the pathophysiology of pre-eclampsia. Placenta removal leads to decrease trend of xanthine oxidase activity, uric acid and elevation of Nitric oxide as reversible changes in pre-eclampsia patients within 48 hours after delivery.

Keywords: Antenatal, Nitric oxide, Postnatal, Uric acid

INTRODUCTION

Pre-eclampsia is a maternal syndrome characterized by hypertension, proteinuria, oedema after 20 weeks of pregnancy [1]. The symptoms of Pre-eclampsia can range from mild to severe due to slow or rapid progress of disease condition. They include persistent headache, blurred vision, Vomiting and abdominal pain [2]. The complications of pre-eclampsia is leading to fetal uterine growth restriction, preterm delivery, maternal and fetal morbidity and mortality [3,4]. It is a multisystem disorder, clearly shows the involvement of utero-placental blood flow, vascular resistance, endothelial integrity, endothelial damage, coagulation system in pre-eclampsia. Potential causes and mechanisms behind pre-eclampsia remain unknown, but the involvement of maternal, immune, genetic factors and placenta have been implicated.

Pre-eclampsia is a leading cause of hypertension results in complication up to 10% pregnancies. Pre-eclampsia and eclampsia accounts for 24% of all maternal deaths in India [5]. In the developing and developed countries, approximately 800 women die from pregnancy and child birth related complications around the world every day [6]. Pre-eclampsia is more common in first pregnancies [7] than the second pregnancies [8]. Early detection and management helps in reducing the complications of pre-eclampsia. Despite its prevalence, pathophysiology is poorly understood and aetiology has to be elucidated.

Xanthine oxidase (XO) is an iron, molybdenum containing flavoprotein which catalyzes the oxidation of xanthine/hypoxanthine into uric acid (2,6,8, trioxy Purine). XO levels are very less in healthy individuals but have shown to be increased in the pathological conditions [9]. Pregnancy induced hypertension is the leading cause of maternal and fetal morbidity and mortality [10]. Pre-eclampsia occurs only in the presence of placenta and resolves after placental delivery. The main hypothesis depends on the decreased placental perfusion due to impaired remodeling of spiral arteries. Inadequacy of placental perfusion might result in hypoxia. This hypoxic interface between maternal-fetus results in destruction of fetal tissue that can release OX, substrate like xanthine/hypoxanthine, cytokines etc [11]. Hypoxia stimulus generates reactive oxygen species like superoxide anion in living cells [12,13].

Reactive oxygen species (ROS) and nitric oxide (NO) interference changes the vascular function. Therefore in the present study, we hypothesized that increased ROS production may reduce availability of nitric oxide and might cause endothelial dysfunction which may play a role in the pathogenesis of pre-eclampsia.

Uric acid is the end product of purine catabolism, its production increased in condition of ischemia reperfusion [14]. Evidence suggests that uric acid has action of antioxidant but under certain conditions the formation of uric acid connected to the production of free radicals [15]. Therefore, present study aims to evaluate the activity of Xanthine oxidase as an enzyme marker in pre-eclampsia. The objectives were to estimate the plasma levels of Xanthine oxidase (XO) activity, uric acid and Nitric oxide (NO) levels in women with the pre-eclampsia and normal pregnancy during before and after delivery.

MATERIALS AND METHODS

Group 1 (n=50) normal pregnant women and Group 2 (n=50) pre-eclampsia in 30-39 weeks of gestation before delivery and same subjects after delivery were recruited in the study from Obstetrics and Gynecology Department of R.L. Jalappa Hospital and Research Center, Kolar, India with the approval of Institutional Ethics Committee. Pre-eclampsia was defined as blood pressure constantly greater than 140/90mm Hg and proteinuria above 0.3 g/24 hours after 20 weeks of gestation. Eligibility for the study was defined as per the Classification of National High Blood Pressure Education Programme working group (NHBPEP). Pregnant women beyond 28 weeks of gestation with pre-eclampsia diagnosed included in the study group as cases and age, gestation matched normotensive pregnant women were included in the controls after obtaining Informed Consent from the patient.

Inclusion criteria were Singleton pregnancy, no fetal anatomical anomaly; nonsmokers were included in the study. Exclusion criteria were chronic hypertension, molar pregnancy, gestational diabetes and multiple gestations.

Three ml of venous blood sample was collected into heparinized tubes and plasma separated by centrifugation at 3000rpm for 15 minutes. OX estimation is done within 2 hours of sample collection. Aliquots were stored at -80ºC until further analysis.

Spectrophotometric continuous rate determination assay of XO through uric acid formation from substrate xanthine recorded at 290nm using a method described by Bergmeyer [16]. Plasma nitric oxide measured in terms of reduction of nitrate into nitrite by reducing agent copper coated cadmium granules using sodium nitrite as standard ($NaNO_2$). The nitrite produced is determined by diazotization of Sulfanilamide in acidic medium and then coupling with Napthyl ethylene Diamine to produce pink colored compound which was measured spectrophotometrically at 540nm [17]. Plasma uric acid oxidized by uricase to produce hydrogen peroxide and allontoin. Peroxidase acts on hydrogen peroxide and oxidizes 3, 5-dichloro-2 –hydro benzenesulfonic acid and 4-aminophenazone to form a red–violet quinoneimine compound which was measured at 505 nm [18].

STATISTICAL ANALYSIS

The unpaired t-test was used to assess the statistical significance of difference between the study groups. A probability level of $p<0.05$ considered as statistically significant. Results were presented as mean±SD. Pearson correlation coefficient was used to measure the correlation between NO and XO.

Parameters	Group (1) Healthy pregnant women Before Delivery (Mean±SD)	Group(2) Pre-eclampsia Before Delivery (Mean±SD)	p-value
Nitric oxide (µmoles/L)	7.29±3.38	6.24±3.8	>0.05
Xanthine Oxidase (Units/L enzyme)	39.10±54.04	205±197.02	<0.001**
Uric Acid (mg/dl)	4.20±1.54	6.44±2.21	<0.001**

[Table/Fig-1]: Plasma Xanthine oxidase, Nitric oxide and Uric acid levels of normal pregnant and pre-eclamptic cases before delivery

**p-value <0.001 is considered as highly significant

Parameters	Group (1) Healthy pregnant women After Delivery (Mean±SD)	Group (2) Pre-eclampsia After Delivery (Mean±SD)	p-value
Nitric oxide (µmoles/L)	6.12±3.031	7.12±9.9	>0.05
Xanthine Oxidase (Units/L enzyme)	17.9±14.2	96.6±141.3	<0.001**
Uric Acid (mg/dl)	4.27±2.41	4.5±1.9	>0.05

[Table/Fig-2]: Plasma Xanthine oxidase, Nitric oxide and Uric acid levels of normal pregnant and pre-eclamptic cases after delivery

**p-value <0.001 is considered as highly significant

RESULTS

The mean and standard deviation values of XO and uric acid (39.10 U/L ±54.04, 205U/L ±197.02 p<0.001), (4.20mg/dl±1.54, 6.44 mg/dl±2.21 p<0.001) were higher in pre-eclamptic group compared to the control. There was decreased Nitric oxide 7.29µ moles/L ±3.38, 6.24µmoles/L ±3.8 (p>0.05) in pre-eclampsia when compared to healthy pregnant before delivery but it was not significant. Similarly Xanthine oxidase 17.9U/L±14.2, 96.6 U/L±141.3 (p<0.001), Uric acid 4.27mg/dl±2.4 4.5mg/dl±1.9 (p>0.05), and Nitric oxide 6.12µmoles/L±3.031, 7.12µmoles/L±9.9 (p>0.05), in healthy pregnant and pre-eclampsia after delivery within 48 hours were presented in [Table/Fig-1,2] respectively. Maternal plasma xanthine oxidase activity, nitric oxide and uric acid are measured before delivery and after delivery from Group (1) and Group (2) are presented in [Table/Fig-3-5] respectively.

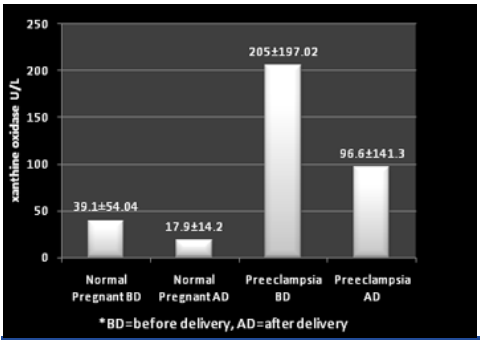
DISCUSSION

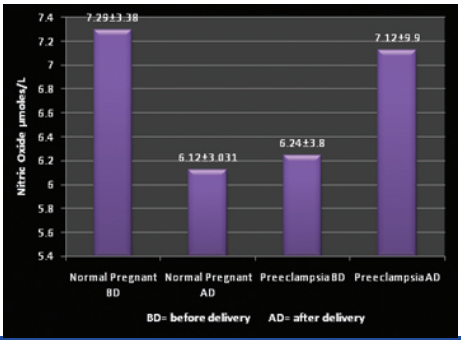
Pre-eclampsia is characterized by increased production of ROS and decreased levels of antioxidant status and hyperuricemia [19]. Pre-eclampsia is influenced by various factors such as increased oxidative stress, hypoxicated trophoblastic tissue destruction that produces xanthine, hypoxanthine and cytokines leading to inflammatory process [20]. Current study results indicated that increased activity of XO, non-significantly decreased nitric oxide level and marginally elevated uric acid levels observed.

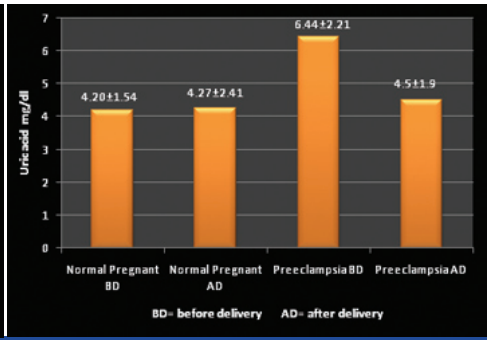
Improper spiral arteries implantation leads to hypoxia and increased turnover of trophoblastic tissue which might result in increased xanthine and hypoxanthine that served as substrate for XO that might lead to increased uric acid [21]. However, there is no data available on measurement of XO that can be considered as enzyme marker in pre-eclampsia before and after delivery status. Even though in a research report of Karabulut et al., evinced the increased XO activity in pre-eclampsia, but data on XO activity is not available after delivery condition of pre-eclampsia [11].

XO (E.C 1.17.3.2) catalyzes the conversion of substrates hypoxanthine/xanthine in to Uric acid and hydrogen peroxide. Xanthine dehydrogenase (E.C.1.17.1.4) is NAD+ dependent and xanthine oxidase (E.C.1.17.3.2) uses oxygen exists during purification protocol [22]. However, limited proteolysis and oxidation of sulfhydryl groups converts irreversibly Xanthine dehydrogenase in to XO [23]. Placental incompatibility in ischemic condition due to free radical formation and increased oxidative stress makes endothelial damage and cell death the cause for elevation of XO in circulation [11].

Activated leukocytes produce cytokines that in turn increases the XO activity and also ROS from endothelium and increases production of uric acid [24,25]. In our research work, an observation is significantly recorded in terms of elevation of XO in pre-eclampsia in comparison with normal pregnant women. In the same way uric acid level also increased before delivery. Two fold decrease of XO activity noticed after delivery condition in pre-eclampsia. XO activity in pre-eclampsia increased by 5.26 fold compared to normal pregnant group before delivery. However, after delivery reverts to







[Table/Fig-3]: Showing the levels of Xanthine oxidase normal pregnant and pre-eclamptic cases before delivery. **[Table/Fig-4]:** Showing the levels of Nitric oxide between normal pregnant and pre-eclamptic cases before delivery. **[Table/Fig-5]:** Showing the levels of Uric acid between normal pregnant and pre-eclamptic cases before delivery

2.1 fold in pre-eclampsia. The level was persistently high compared to healthy pregnant whereas the same enzyme reverts to 2.1 fold after delivery in healthy pregnant women. These results indicated that the rise of XO level in pre-eclampsia before delivery proves that measurement of XO in pre-eclampsia is appropriate to consider as a marker since the expression of XO in pre-eclampsia is evident. However, elevation of uric acid observed in pre-eclampsia compared to normal pregnant before delivery but the level return to normal in both the groups after delivery.

Nitric oxide has not shown significant changes in both the groups before and after delivery. It has been proposed that reduced production of vasodilatory agent nitric oxide [26] might cause pre-eclampsia but there are studies showing increased nitric oxide production in pre-eclampsia suggested to overcome the adverse placental effect [27]. In our study nitric oxide level was increased in normal pregnancy before delivery as an indication of adaptive haemodynamic changes [28] and decreased after delivery in normal pregnant women may be due to down-regulation of maternal NO synthesis [29]. NO level was elevated after delivery in pre-eclampsia within 48 hours may be an indication of reversible changes of pre-eclampsia. Elevated ROS production may suppress the expression of endothelial nitric oxide synthase (Enos). Nitric oxide (NO) combines rapidly with superoxide (O2-) to form peroxynitrite ion (ONOO-). Peroxynitrite oxidizes the DNA, lipids, Proteins and also interferes with the vascular signaling pathways [10].

However in an attempt to evaluate the correlation between xanthine oxidase activity and nitric oxide level a non-significant negative correlation is observed before (r= -0.260) and after delivery (r= -0.224).

LIMITATION

However, limitations of the study were measurement of XO level from time of pregnancy to at all level of trimesters to understand whether or not gradual increase of XO activity as a marker to denote the number of chances translated into pre-eclampsia. Further study can be designed by culturing trophoblastic cells and expose them to free radical stress environment to measure XO activity. Understanding of pre-eclampsia at early stage is a good indication to decide suitable treatment strategies to prevent its onset and pathological changes.

CONCLUSION

Our research findings generated knowledge about increased XO activity and uric acid in pre-eclampsia in comparison with normal pregnant women. The inverse relation between XO and NO found in our study may be an indication of trophoblastic cell destruction and endothelial dysfunction.

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PARTICULARS OF CONTRIBUTORS:
1. Ph.D. Scholar, Department of Biochemistry, Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka, India.
2. Professor, Department of Biochemistry, Head of Allied Health Sciences, Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka, India.
3. Professor, Department of Obstetrics and Gynecology, Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. C D Dayanand,
Professor, Department of Biochemistry, Head of Allied Health Sciences, Sri Devaraj Urs Medical College, SDUAHER,
Kolar, Karnataka -563 101, India.
E-mail: cd8905@yahoo.co.in

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