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Biochemistry Section

Association of Xanthine Oxidase and Neutrophil Activation Marker Elastase in Preeclampsia: A Case-control Study

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ABSTRACT

Introduction: Preeclampsia is an obstetric emergency for both mother and the foetus with unknown aetiology. Delivery is the only effective way in the prompt management. Due to oxidative stress factors, there is an increased conversion of xanthine dehydrogenase to xanthine oxidase, so there is more production of hydrogen peroxide. Hydrogen peroxide affects the cell function of trophoblast. Therefore, oxidative stress is one of the causative factor for complications of preeclampsia.

Aim: To determine the association between xanthine oxidase and plasma elastase (Neutrophil activation marker) in preeclampsia.

Materials and Methods: The case-control study was conducted from March 2019 to December 2019 on normotensive pregnant females and preeclamptic patient categorised as group 1 (control) and group 2 (case) respectively. The level of xanthine oxidase and plasma elastase were estimated spectrophotometrically. To compare means between the two groups, Student's t-test was used and correlation between parameters were estimated through Pearson's correlation coefficient. A p-value of <0.05 was considered statistically significant.

Results: A total of 60 subjects were included and analysed in two groups 1 and 2 respectively. Neutrophil activation marker (elastase) was elevated 4.5-fold in group 2 (26.81±7.9) but, it was non significant when compared to group 1 (6.02±3.4). Xanthine oxidase levels amongst group 1 was 34.01±38.26 U/L which was significantly elevated in pregnant group 2 patients as 218.78±220.42 U/L with probability p-value <0.05 and positive correlation of r=0.320.

Conclusion: Elevated levels of xanthine oxidase adds to oxidative stress and may result in trophoblastic dysfunction in preeclampsia. The situation is convoyed by increased concentration of pro-inflammatory signaling molecules like cytokines such as Tumour Necrosis Factor-α (TNF-α), activated neutrophils and positive acute phase plasma proteins. Elastase as neutrophil activation marker showed 4.5-fold increase in preeclampsia which shows aggravated inflammatory condition.

Keywords: Acute phase plasma proteins, Neutrophils, Reactive oxygen species, Tumour necrosis factor

INTRODUCTION

Preeclampsia is one of the major cause of maternal and foetal morbidity and mortality. It is a hypertensive disorder of pregnancy characterised by proteinuria occurring after 20 weeks of gestation which complicates 10% of the pregnancies [1,2]. In India, preeclampsia accounts for 24% of all maternal deaths [3]. Exact aetiology of preeclampsia is still unknown. The pathophysiology involves impaired placentation, inflammation and vascular endothelial damage [4]. Before the clinical onset of preeclampsia trophoblastic invasion is impaired and placental perfusion is disturbed [5].

It occurs in presence of placenta and automatically resolves after delivery. Impaired remodeling of spiral arteries leads to the decreased placental perfusion which will result in hypoxia [6]. Hypoxia causes destruction of trophoblastic tissue, thus may result in increased xanthine, hypoxanthine and proinflammatory substances such as cytokines, activated neutrophils which may lead to endothelial dysfunction and systematic inflammatory response [7].

Xanthine Oxidase (XO) is a flavoprotein which contains iron and molybdenum. Xanthine oxidase catalyses oxidation of hypoxanthine to uric acid. It is found in less concentration in normal individuals but in pathological conditions due to limited proteolysis and oxidation of sulfhydryl groups; xanthine dehydrogenase is converted to xanthine oxidase [8].

Elastase is a serine protease. Once neutrophil is activated, variety of substances are released. One such substance is elastase, which is established marker for neutrophil activation [9]. It degrades extracellular matrix protein collagen IV and elastin. This may damage the vascular basement membrane causing proteinuria and oedema [10].

Increased uric acid in preeclampsia may be due to increased XO activity not only due to decreased renal excretion. In-vitro studies on endothelial cell culture have shown activated neutrophils induce conversion of xanthine dehydrogenase to XO by secretion of elastase [11,12]. But the association between XO and neutrophil activation in terms of plasma elastase in preeclampsia has not been established. According to our knowledge, this is the first study to investigate the relationship between XO and plasma elastase in preeclampsia in Indian population.

MATERIALS AND METHODS

The case-control study was conducted from March 2019 to December 2019 in M.V.J Medical College and Research Hospital, Bangalore, Karnataka, India, in the Department of Obstetrics and Gynecology after obtaining the Institutional Ethical Committee Clearance (IEC NO. MVJ&RH/IEC/2019/004) and informed consent from the included subjects. The sample was collected from females admitted to or attended the Outpatient Department of Obstetrics and Gynaecology. Normotensive pregnant females as control group (group 1, n=30) and clinically diagnosed preeclamptic females as case group (group 2, n=30).

Sample size calculation: The study parameters such as XO and elastase were based on the mean difference and standard deviation levels of the research reports [11,12] at 90% power (Z_{β}).

Inclusion criteria

For control: Pregnant females admitted with singleton pregnancy after 20 weeks of gestation with no foetal anomalies and those who were non smokers were included in the study as controls.

For case: Those pregnant females with the Blood Pressure (BP) of ≥140/90 mmHg on two or more than two occasions within four hours apart after 20 weeks of gestation and proteinuria of ≥300 mg/24 hours or ≥1+ detected by dipstick method in a random urine sample were included in the study as case [13].

Exclusion criteria: Those patients with history of chronic hypertension, cardiovascular disease, hypertensive encephalopathy, thyroid disorder and renal disease were excluded from the study [14].

Procedure

A 4 mL of venous blood was collected in a heparinized tube from both normotensive and preeclamptic females. The sample was centrifuged for 10 mins at 3000 rounds per minute (rpm) to obtain clear plasma and it was stored at -80°C until analysis. The levels were estimated using spectrophotometer [15]. For estimating xanthine oxidase levels, spectrophotometer continuous rate determination assay was done where uric acid formation from xanthine was recorded at 290 nanometre (nm) using Bergmeyer's method. Whereas for estimating elastase, p-nitro anilidine was used since elastase hydrolyses succinyl trialanine p-nitroaniline to release N-succinyl trialanine and p-nitroanilide. The absorbance of p-nitroanilide was measured in a spectrophotometer at 410 nm wavelength as described by Bieth J et al., [16].

STATISTICAL ANALYSIS

Results were expressed in terms of mean±standard deviation. If p-value was found to be <0.05, it was considered as statistically significant. Student's t-test was used to compare the mean between the two groups. To find the correlation between parameters, Pearson's correlation coefficient was used.

RESULTS

The mean±standard deviation of age, systolic and diastolic BP and gestational age of normotensive pregnant and preeclamptic pregnant females depicted in [Table/Fig-1]. There was no significant difference in age between the groups. But gestational age showed significance difference between the groups. Moreover, systolic and diastolic blood pressure were significantly increased in preeclampsia when compared to normotensive pregnant women.

Parameters	Normotensive Group 1 (Mean±SD)	Preeclampsia Group 2 (Mean±SD)	p-value
Age (years)	23.43±4.26	23.46±3.49	>0.05
Gestational age (weeks)	39±0.98	35.60±3.29	**<0.001**
Systolic blood pressure (mm/Hg)	115.66±6.78	156.00±16.10	**<0.001**
Diastolic blood pressure (mm/Hg)	74.33±5.04	102.66±11.12	**<0.001**

[Table/Fig-1]: Comparison of the normotensive and preeclamptic pregnant females (n=30).
A p-value of <0.05 was considered statistically significant

[Table/Fig-2] showing the comparison of XO and elastase between normotensive pregnant and preeclamptic females. XO levels in normal pregnant patients was 34.01±38.26 U/L which was significantly elevated in preeclampsia (218.78±220.42 U/L) with probability p-value <0.001. Elastase levels in normotensive pregnant females was 6.02±3.43 Unit/mL which was non significantly elevated (4.5-fold) in preeclampsia (26.81±77.95 Unit/mL).

Parameters	Normotensive Group 1 (Mean±SD)	Preeclampsia Group 2 (Mean±SD)	p-value (unpaired t-test; significance p<0.05)
Xanthine oxidase (U/L)	34.01±38.26	218.78±220.4	**<0.001**
Elastase (Unit/mL enzyme)	6.02±3.4	26.81±77.95	>0.05

[Table/Fig-2]: Comparison of the biochemical parameters between normotensive and preeclamptic pregnant females (n=30).
A p-value of <0.05 was considered statistically significant

[Table/Fig-3] depicting the correlation between XO and elastase in preeclampsia using Pearson's correlation. XO was positively correlated (+0.320) with elastase which showed significance of p<0.05.

Parameters	Correlation coefficient (r)	p-value
Xanthine oxidase vs Elastase	0.320	**<0.05**

[Table/Fig-3]: Correlation between the xanthine oxidase and elastase in preeclampsia.
A p-value of <0.05 was considered statistically significant

DISCUSSION

According to our research findings, the demographic data showed no significant difference between the age of normotensive pregnant females and preeclamptic females. However, study conducted by Liu X and Zhang W; showed the risk of preeclampsia increases with increased maternal age and teenage pregnancy [17].

Increased production of reactive oxygen species, uric acid and decline in the antioxidant levels [18] causes tissue destruction which produces xanthine, hypoxanthine and cytokines ultimately contributing to inflammatory process [19,20]. In this study, increased activity of XO was observed which signifies the high levels of xanthine and hypoxanthine in preeclampsia due to tissue destruction.

Inappropriate spiral arteries implantation leads to decreased oxygen supply to the trophoblastic tissue which may lead to the destruction of trophoblastic tissues. This will result in increased turnover of trophoblastic tissue which might cause an increased xanthine and hypoxanthine that serves as substrate for XO which in turn result in increased uric acid [7]. According to research conducted by Karabulut AB et al; increased activity of xanthine oxidase was observed in preeclampsia. But the association between plasma elastase and xanthine oxidase was not available [6].

A study conducted by Bambrana.V showed that there is elevation of xanthine oxidase levels in preeclampsia when compared to normal pregnant females [18]. This indicates that measurement of XO can be considered as a marker, because there is rise of XO levels in preeclampsia before delivery. Once the neutrophil is activated,cytokine is produced which stimulates xanthine oxidase activity and liberation of reactive oxygen species from endothelium [6,21,22]. Due to oxidative stress factors, there is increased conversion of xanthine dehydrogenase to xanthine oxidase adding to increased production of hydrogen peroxide which affects the trophoblast cell function [11]. Oxidative stress is one of the causative factor for complications of preeclampsia.

Inflammatory response is found along with increased concentration of proinflammatory signaling molecules such as cytokines,acute phase plasma proteins, and TNF-α, etc [23]. Degranulation of mast cells releases an elastase enzyme which prolongs the inflammatory process causing vascular damage and dysfunction. Therefore, elastase is used to assess neutrophil activation [24,25].

In early onset of preeclampsia, increase in plasma elastase were reported by Gupta AK. During an inflammatory condition,elastase is released from polymorph nuclear lymphocytes, therefore plasma elastase level is increased considerably [26].

In the present study, increase in elastase had positive correlation with xanthine oxidase, but was non significant in preeclampsia when compared to normal pregnant females. But, there was positive correlation between xanthine oxidase and plasma elastase. However, the present study reported positive correlation between xanthine oxidase and elastase in preeclampsia.

Limitation(s)

Determination of plasma elastase and xanthine oxidase in larger sample size would have confirmed the reliability of results. The present study sample was small and requires larger sample size in future studies.

CONCLUSION(S)

This study concluded that there is a positive correlation between XO and elastase in preeclampsia. In our study, we observed elevated XO which leads to increased hydrogen peroxide which further adds to oxidative stress. Elastase as neutrophil activation marker showed 4.5 fold increases in preeclampsia which shows aggravated inflammatory condition in preeclampsia. Future studies are recommended with larger sample size to further validate the results.

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