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Research Article

PHOSPHOLIPASE A_2, PLASMA ELASTASE ACTIVITY IN PRE- AND POST-PARTUM OF PRE-ECLAMPTIC WOMEN

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ABSTRACT

Objective: The objectives of the present study were to evaluate the activity of phospholipase A_2, plasma elastase enzymes and to assess relation with an inflammatory marker high sensitive C-reactive protein (hs-CRP) in nonpregnant before and after delivery of normotensive pregnant and pre-eclamptic women.

Methods: The study population consists of three groups: Nonpregnant (Group 1, n=57), normotensive pregnant (Group 2, n=57), and pre-eclamptic women (Group 3, n=57). Groups 2 and 3 were followed after delivery within 48 hrs. Phospholipase A_2, plasma elastase, and hs-CRP levels were determined spectrophotometrically.

Results: The plasma elastase, phospholipase A_2 activity, and hs-CRP were elevated in pre-eclampsia significantly ($p<0.05$), nonsignificant rise in normotensive pregnant before delivery condition compared to nonpregnant women. However, plasma elastase in normal pregnancy and pre-eclampsia were decreased by 1.2- and 2.07-fold, respectively, after delivery. Whereas phospholipase A_2 and hs-CRP found to be nonsignificantly decreased in the postdelivery status of the both the groups. Receiver operating characteristics curve analysis showed that elastase enzyme has diagnostic importance to assess inflammation on the basis of area under curve (0.758).

Conclusion: Our research findings generated knowledge about raised level of plasma elastase enzyme by neutrophil degranulation represents inflammation in pre-eclampsia. Elevated elastase, phospholipase A_2 with hs-CRP in pre-eclampsia serves as indicators of inflammation.

Keywords: Elastase, High sensitive C - reactive protein, Phospholipase A_2, Pre-eclampsia.

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INTRODUCTION

Pre-eclampsia is a multisystem obstetric problem associated with hypertension, proteinuria, and edema after 20 weeks of pregnancy [1]. The symptoms are persistent headache, blurred vision, vomiting, and abdominal pain [2]. The pre-eclampsia complications may result in fetal uterine growth restriction, preterm delivery, maternal and fetal morbidity and mortality [3,4]. Research reports are available on inflammatory response [5], leukocyte activation [6], dyslipidemia [7], oxidative stress, and intrauterine hypoxia [8] as a potential cause for generation of free radicals and inflammatory mediators.

Neutrophil elastase gene located on chromosome 19 at p13.3. Protein expressed is a serine protease (EC No. 3.4.21.37) consisting of 218 amino acid residues with a molecular weight 29.5 kDa. It exerts biological role in the degradation of collagen IV and elastin in causing inflammation [9]. Phospholipase A_2 gene located on chromosome number 1 at q31.1. The expressed protein is heat stable, calcium-dependent enzyme (EC No. 3.1.1.4) with molecular weight 85 kDa. Phospholipase A_2 enzyme cleaves arachidonic acid at Sn2 position in phospholipid; the released product serves as precursor for leukotriene's synthesis which acts as inflammatory mediators [10].

High sensitive C-reactive protein (hs-CRP) is a plasma globulin fraction synthesized in the liver during acute inflammation phase in response to pneumococci infection where it binds to carbohydrate moiety of the capsule of bacteria. Circulating level of CRP in the range of 0.5-10 mg/L considered as hs-CRP, whereas the concentration in the range of 10-1000 mg/L is a nonspecific indicator of inflammation [11].

In our earlier research reports, we presented increased concentration of oxidative stress and decreased total antioxidant status [12] and also increased xanthine oxidase activity with the inverse relation of nitric oxide in pre-eclampsia [13,14]. There is seldom information available with respect to phospholipase A_2 and plasma elastase in pre-eclampsia. Therefore, in the present study, an attempt was made to estimate the phospholipase A_2 and plasma elastase activities in comparison with hs-CRP to assess inflammation in pre-eclampsia and normotensive pregnant women. These parameters were also measured in both the groups after delivery within 48 hrs made the study distinct.

METHODS

The study was conducted between December 2014 and May 2016 by the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology, R. L. Jalappa Hospital and Research Center, Kolar, Karnataka, India. It was conducted after obtaining the Institutional Ethics Committee approval from Sri Devaraj Urs Medical College. The participants of the study population were enrolled after obtaining individual patient written informed consent.

The study population was allocated into three groups. Group 1, nonpregnant (n=57); Group 2, normotensive pregnant (n=57); Group 3 pre-eclamptic women (n=57). G_2 and G_3 were in 30-39 weeks of gestation before delivery and were followed after delivery within 48 hrs. G_2 and G_3 subjects were clinically diagnosed from obstetrics and gynecology. The age-matched control populations (G_1) were randomly recruited from the healthy volunteers of the Sri Devaraj Urs Medical College and were in age group of 20-30 years.

Pre-eclampsia was diagnosed with blood pressure ≥140/90 mm Hg, proteinuria ≥300 mg/24 hrs after 20 weeks of gestation, were included as per national high blood pressure education program working group. The subjects with any history of renal disease, thyroid disorder, chronic hypertension, gestational diabetes, epilepsy, hypertensive encephalopathy, and cardiovascular disease were excluded from the study.

About 4 ml of blood sample was collected from each subject under the study groups using appropriate vacutainer under aseptic conditions to obtain serum and plasma. These samples were stored at −80°C until further analysis.

Fine chemicals such as N-Succinyl-ala-ala-ala-p-Nitroanilide (SAAANA), leukocyte elastase, phospholipid, and phospholipase A_2 were obtained from Sigma-Aldrich, USA. hs-CRP immunoturbidimetric kit was commercially obtained from Euro-Diagnostic System, Chennai, India. All other chemicals and reagents used were of analytical grade.

Elastase hydrolyses synthetic substrate SAAANA to give rise to products N-Succinyl-ala-ala-ala and p-nitroanilide. The absorbance of p-nitroanilide was measured in a spectrophotometer at 410 nm wavelength [15].

The CRP-ultrasensitive is a quantitative turbidimetric test for the measurement of low levels of CRP in human serum or plasma. Latex particles coated with specific antihuman CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent on the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

Phospholipase A_2 hydrolyzes the Sn2-fatty-acyl ester bond of phosphorglyceride liberating free fatty acid and lysophospholipid. Based on the absorbance change of bromothymol blue, indicators with the concentration of hydrogen ion released from enzyme catalyzed reaction were measured at 620 nm [16].

The results were tabulated as mean±standard deviation (SD) and analyzed using one-way ANOVA test with *post-hoc*-Dunnett analysis to compare the values between the three groups. Paired *t*-test was used to compare the changes before and after delivery in normal pregnancy and pre-eclampsia. A probability value of $p<0.05$ was considered as statistically significant. Receiver operating characteristics (ROC) curve was plotted to find out the diagnostic importance of the parameters measured under study. Statistical analysis was performed with licensed version of SPSS 20.

RESULTS

Table 1 depicted the mean±SD of the elastase, phospholipase A_2, and hs-CRP as an inflammatory indicator. Considerable increase in elastase (5.8 U/ml±3.01), phospholipase A_2 (70.7 U/ml±18.4), and hs-CRP (8.6 mg/L±14.1) in normal pregnancy when compared to nonpregnant and further increase of these parameters (26.85±79.31, 79.02±27.6, 11.5±14.2), respectively, in pre-eclampsia when compared to normal pregnancy. Analysis of the results showed that the marked rise in the elastase, phospholipase A_2 activity in pre-eclampsia noticed when compared with the well-known inflammatory marker hs-CRP. In pre-eclampsia, there was 6.7-fold increase of elastase enzyme and 4.0-fold increase of hs-CRP observed when compared to nonpregnant women.

Table 2 shows the comparison of study parameters among nonpregnant, normotensive pregnant, and pre-eclampsia. Phospholipase A_2 and elastase were significantly increased ($p<0.05$) in pre-eclampsia, and the same parameters were statistically nonsignificant in normotensive pregnant when compared to nonpregnant women. Whereas hs-CRP level significantly increased in normal pregnancy ($p<0.05$) and pre-eclampsia ($p<0.001$) when compared with that of nonpregnant.

Table 3 depicts the elastase, phospholipase A_2, and hs-CRP levels during pre- and post-delivery of normal pregnant and pre-eclampsia cases. These parameters were increased in pre-eclampsia prominently compared to normal pregnant during predelivery. However, elastase activity decreased by 1.2 and 2.07-fold, respectively, in normal pregnancy and pre-eclampsia during after delivery, whereas phospholipase A_2 and hs-CRP were found to be nonsignificantly decreased ($p>0.05$) during postdelivery.

Table 4 illustrates the ROC curve features such as sensitivity, specificity, and area under curve for hs-CRP, phospholipase A_2, and plasma elastase in pre-eclampsia. ROC analysis showed that plasma elastase had area under the curve of 0.758. Phospholipase A_2 did not show any appreciable diagnostic importance. Figs. 1-3 shows the levels of phospholipase A_2, plasma elastase, and hs-CRP in nonpregnant before and after delivery of normal pregnant and pre-eclamptic cases.

DISCUSSION

Pre-eclampsia is a multifactorial pregnancy disorder involving various types of system attributing to the pathophysiology of placenta. Feto-maternal immune reactions in the 1st week of pregnancy, impaired arterial invasion by trophoblast, and transformation of spiral arteries followed by altered placental perfusion. It results in chronic hypoxia

Table 1: Comparison of the biochemical parameters between groups in the study population

Parameters	Mean±SD		
	G_1 (n=57)	G_2 (n=57)	G_3 (n=57)
Elastase (U/ml)	4.01±1.31	5.8±3.01	26.85±79.31
Phospholipase A_2 (U/ml)	68.7±22.85	70.7±18.4	79.02±27.6
hs-CRP (mg/l)	2.9±1.54	8.6±14.1	11.5±14.2

G_1: Nonpregnant, G_2: Normotensive pregnant, G_3: Pre-eclampsia, SD: Standard deviation, hs-CRP: High sensitive C-reactive protein

Table 2: Comparisons of biochemical parameters showing significance between groups in study population by *post-hoc*-Dunnett multiple comparisons

Parameters	G_1 versus G_2	G_1 versus G_3
Elastase (U/ml)	NS	<0.05*
Phospholipase A_2 (U/ml)	NS	<0.05*
hs-CRP (mg/l)	<0.05*	<0.001**

G_1: Nonpregnant, G_2: Normotensive pregnant, G_3: Pre-eclampsia. *Statistically significant, **Highly significant, NS: Nonsignificant, hs-CRP: High sensitive C-reactive protein

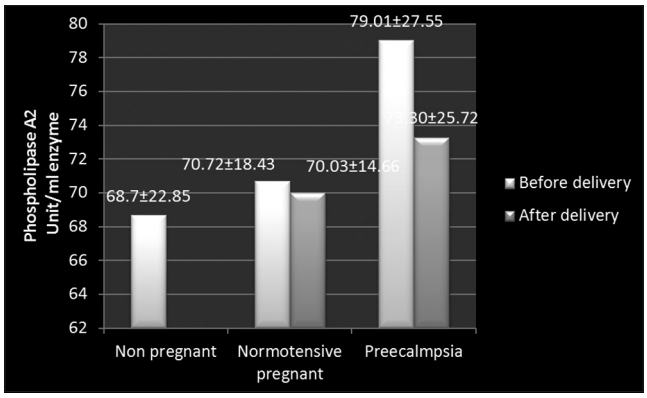

Fig. 1: It shows the phospholipase A_2 activity in nonpregnant before and after delivery of normal pregnant and pre-eclamptic women

Table 3: Elastase, phospholipase A_2, and hs-CRP parameters during before and after delivery in normotensive pregnant and pre-eclamptic women

Parameters	Mean±SD					
	Normotensive pregnant (n=57)			Pre-eclampsia (n=57)		
	Predelivery	Postdelivery	p	Predelivery	Postdelivery	p
Elastase (U/ml)	5.80±3.019	4.6±1.64	<0.05*	26.85±79.30	12.91±7.72	>0.05
Phospholipase A_2 (U/ml)	70.72±18.43	70.03±14.66	>0.05	79.01±27.55	73.30±25.72	>0.05
hs-CRP (mg/L)	8.6±14.15	6.6±15.2	>0.05	11.5±14.24	10.3±20.4	>0.05

*Statistically significant. SD: Standard deviation, hs-CRP: High sensitive C-reactive protein

Table 4: ROC curve analysis of the parameters in pre-eclampsia

Parameters	Sensitivity (%)	Specificity (%)	AUC	95% CI
hs-CRP	57.1	73.7	0.708	0.615-0.790
Phospholipase A_2	35.7	86	0.580	0.484-0.672
Plasma elastase	74.3	86	0.758	0.669-0.834

AUC: Area under curve, hs-CRP: High sensitive C-reactive protein, CI: Confidence interval, ROC: Receiver operating characteristics

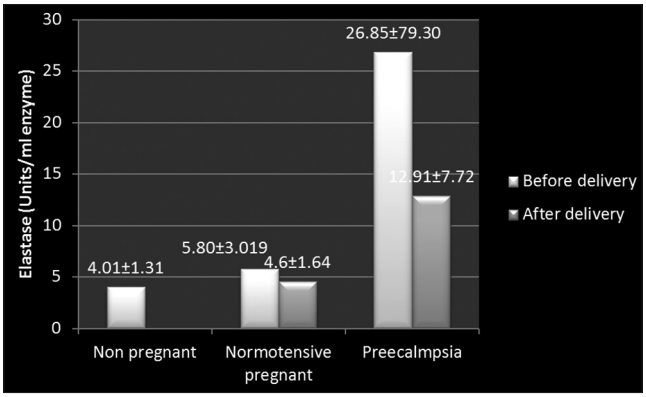

Fig. 2: It Illustrates the plasma elastase activity in nonpregnant before and after delivery of normal pregnant and pre-eclamptic cases

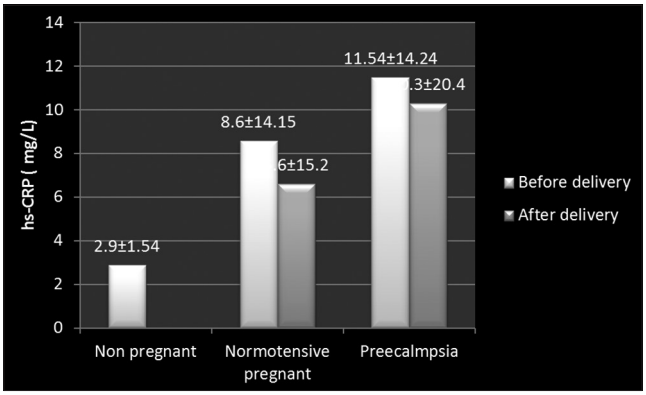

Fig. 3: It presents the high sensitive C-reactive protein levels in nonpregnant before and after delivery of normal pregnant and pre-eclamptic cases

that triggers intensity of oxidative stress that has an impact on placental syncytiotrophoblast cell apoptosis and necrosis [17]. The rate of formation of placental debris has pro-inflammatory substances, angiogenic, and antiangiogenic factors, etc., which lead to endothelial dysfunction and systemic inflammatory response, thus placenta play a central role in inflammatory process [18].

An inflammatory response in pre-eclampsia is usually accompanied by increased concentration of pro-inflammatory signaling molecules such as cytokines, activated neutrophils, and positive acute phase plasma proteins. Neutrophil activation may occur in the presence of cytokines such as tumor necrosis factor-α during an inflammatory process. Degranulation of neutrophils releases elastase enzyme that prolongs the inflammation by modification of pro-inflammatory cytokines and degrading proteins involved in the inflammation. In addition to this, myeloperoxidase also present in neutrophil granules increases oxidative stress by the additional production of hydroxyl radical and hypochlorous acid [19]. Hence, neutrophil activation results in vascular damage and dysfunction. Therefore, plasma elastase can be used to assess *in vivo* neutrophil activation. CRP is increased rapidly in response to inflammatory stimuli along with elastase in pre-eclampsia [20]. So that, increased elastase in plasma serves as a predictive marker of pregnancy induced inflammation [21].

A systemic inflammatory response involves leukocyte activation by elastase, acute phase response by hs-CRP, and metabolic features of systemic inflammation by phospholipase A_2 were presented in the current study. Elastase and phospholipase A_2 activity were compared with reliable inflammatory marker hs-CRP. In our study, these biochemical parameters were elevated in normal pregnancy and further accentuated in pre-eclampsia.

We observed the systemic inflammatory response in pre-eclampsia through hs-CRP which is similar to the research reports of von Versen-Hoeynck 2009 [22], Belo 2009 [23]. The increase in plasma elastase in early onset of pre-eclampsia has been reported by Gupta 2006 [24]. Elastase released from polymorphonuclear lymphocytes during inflammatory condition; hence, the plasma levels were increased considerably [25]. Therefore, in the current study, plasma elastase was compared with the hs-CRP.

Phospholipase A_2 by virtue of hydrolysis of phospholipid releases arachidonic acid that serves as precursor for the synthesis of eicosanoids which participates in the inflammatory process. Phospholipase A_2 enzyme activity was measured to know whether this enzyme can be included under inflammatory marker for consideration. Statistical significance of phospholipase A_2 activity was seen in nonpregnant and pre-eclampsia cases.

Even though, the importance of phospholipase A_2 in pre-eclampsia found to be contradictory [26-28], but our results highlighted increased phospholipase A_2 activity in pre-eclampsia compared to nonpregnant women. However, phospholipase A_2 did not show diagnostic importance as per ROC analysis. Limitation of the study confines to the determination of plasma elastase, phospholipase A_2, and hs-CRP from the time of pregnancy to all trimesters to denote the number of chances of pregnancy translated into pre-eclampsia.

CONCLUSION

Our research findings generated a new knowledge about plasma elastase and phospholipase A_2 increase in normal pregnancy and further rise in pre-eclampsia. Elevation of these enzymes evidences inflammation

in pre-eclampsia when tested along with hs-CRP. Phospholipase A_2 activity increased but did not show diagnostic importance, whereas plasma elastase can be considered for diagnostic utility. Elevated elastase activity represents enhanced maternal inflammatory process by neutrophil activation and degranulation in pre-eclampsia.

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