

Correlation of Plasma Neutrophil Elastase Activity and Endogenous Protease Inhibitor Levels with the Severity of Pre-eclampsia

MAMATHA KUNDER¹, AV MOIDEEN KUTTY², V LAKSHMAIAH³, SR SHEELA⁴

ABSTRACT

Introduction: Pre-eclampsia (PE) is a common maternal syndrome characterized by severe systemic inflammatory response including neutrophil activation leading to uncontrolled activity of elastase. The excessive activity of elastase would lead to destruction of the integrity of endothelial cells and could exacerbate the pathophysiological symptoms in PE. Thus, assessment of NE activity and its control mechanisms would be of relevance in the determination of severity of PE.

Aim: To correlate the activity of plasma NE and its endogenous inhibitors α_1 -antitrypsin (α_1 -AT) and α_2 -macroglobulin (α_2 -MG) with severity of PE.

Materials and Methods: A comparative study was conducted between normotensive pregnant (n=50) and pre-eclamptic (n=50) women. Serum C-Reactive Protein (CRP) was estimated by rapid latex slide and uric acid by uricase method. Plasma elastase was estimated using succinyl tri-L-alanyl-p-nitroanilide as substrate. Plasma α_1 -AT, α_2 -MG and NE- α_1 -AT complex

were quantified by ELISA. ANOVA and Pearson's correlation tests were used to analyze the data. The results were expressed as mean \pm SD and p-value <0.001 was considered statistically highly significant.

Results: The activity of elastase was increased significantly in severe PE (0.62 \pm 0.08) in comparison to normal (0.35 \pm 0.10) and mild pre-eclamptic subjects (0.37 \pm 0.03). The values of α_1 -AT were significantly less in mild (83.94 \pm 25.08) and severe PE (68.58 \pm 26.39) in comparison to normal (110.26 \pm 42.39). There was a significant rise in the levels of α_2 -MG in severe PE. However, the complex estimation did not evince any significant changes.

Conclusion: The results of the present study indicate a significantly elevated elastase activity, α_2 -MG levels and decreased α_1 -AT in severe PE patients. The correlation analyses of PE severity parameters with NE, α_1 -AT and α_2 -MG further support the roles of these molecules in the assessment of severity of PE.

Keywords: α_1 -antitrypsin, α_2 -macroglobulin, NE- α_1 -AT complex

INTRODUCTION

PE is a major cause of maternal and neonatal morbidity and mortality. It is a multisystem disorder which is characterized by vasoconstriction [1], leukocyte activation [2], enhanced inflammatory response [3] and oxidative stress [4]. The causes for the development of PE are still unclear and are a topic of active investigation. The pathological lesions of decidual vessels in PE have similarity to atherosclerotic lesions of arteries [5]. Neutrophils have been implicated in the pathogenesis of atherosclerotic changes and endothelial dysfunction through release of variety of substances. Elastase is one of such molecules released from neutrophils and is an established marker for neutrophil activation [6-8].

Neutrophil Elastase (NE), a serine protease stored in the primary granules of neutrophils, is capable of degrading various extracellular matrix proteins such as elastin, collagen, fibrinogen and proteoglycans [9]. Therefore, it can cause vascular basement membrane damage and can facilitate tissue infiltration of neutrophils. Activation of neutrophils is implicated in PE and consequently contributes to vascular basement membrane damage leading to oedema and proteinuria [10], a usual observation in PE. A positive correlation has been demonstrated between von Willebrand Factor (a marker of endothelial damage) and NE by Greer IA et al., indicating that neutrophil activation could contribute to endothelial damage and dysfunction in PE [11].

Thus, uncontrolled neutrophil activation can lead to destruction of the integrity of endothelial cells and could exacerbate the pathophysiological symptoms in PE. It is well established that PE is

manifested as mild, moderate and severe forms in pregnant women but it is unclear what exaggerates the symptoms and the severity. This study is an attempt in this direction to correlate the activity of neutrophil elastase and its endogenous inhibitors α_1 -antitrypsin (α_1 -AT) and α_2 -macroglobulin (α_2 -MG) with severity of PE.

MATERIALS AND METHODS

The present study is a comparative study conducted during the period of October 2015 to April 2016. The subjects of this study were the pregnant women attending or admitted in the Department of Obstetrics and Gynecology, RL Jalappa Hospital and Research Center, the teaching hospital of Sri Devaraj Urs Medical College (SDUMC) and the biochemical evaluation was carried out in the Department of Biochemistry of SDUMC, a constituent college of Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India. Every enrolled pregnant woman gave their informed written consent to participate in the study. This study was performed after obtaining Institutional Ethical Committee approval and the study complied with the Helsinki Declaration.

A total of 50 pregnant normotensive women and 50 pre-eclamptic pregnant women (27 mild and 23 severe cases), were included in the study. All the women were in the age group of 19-36 years and were over 20 weeks of gestation. Normal pregnancy was diagnosed on the basis of clinical and ultrasound evaluation and all of them presented a normal course and outcome of pregnancy. The pre-eclamptic patients were diagnosed by the presence of hypertension (\geq 140 mmHg systolic BP and \geq 90 mmHg diastolic BP) on two occasions

with 4-6 hours apart, proteinuria ($\geq 1+$ by urine dipstick method) with or without pathological oedema. PE was considered as severe, if the subjects had at least two of the following: ≥ 160 mmHg systolic BP; ≥ 110 mmHg diastolic BP; dipstick proteinuria of 3+ or more. All the other cases were considered as mild PE. All patients with any infection, twins, history of pregestational diabetes, gestational diabetes mellitus, renal disease, liver disease, cardiovascular disease and hypertension were excluded from the study.

Almost 6 ml of blood was collected from an antecubital vein from all the subjects in tubes containing EDTA (for haematologic studies); Sodium heparin (for NE, α_1 -AT, α_2 -MG and NE- α_1 -AT complex estimation) and in tubes without anticoagulant (for CRP estimation). Blood samples were centrifuged within two hours of collection. After centrifugation, serum and plasma were separated and aliquots were stored at -70°C until assayed. Samples were thawed at room temperature, vortexed and centrifuged before analysis.

Complete blood count was performed by Beckman- Coulter, an automatic blood cell counter. Serum C - Reactive Protein (CRP) estimation was done by rapid latex slide tests. Serum uric acid was estimated by uricase method [12] using Dry Chemistry Vitros 250 Johnson and Johnson analyzer. Estimation of plasma elastase was done using succinyl tri- L-alanyl-p-nitroanilide (STANA, from SIGMA) as substrate at 410 nm as per the procedure described by Beith J et al., [13]. Plasma α_1 -AT and α_2 -MG were analyzed using ELISA kit purchased from Immunology Consultants laboratory, Inc, USA. NE- α_1 -AT complex was quantified by ELISA (Calbiochem).

STATISTICAL ANALYSIS

The data were statistically analyzed by SPSS software version 22.0. The results were expressed as mean \pm SD. For statistical differences in means between the groups ANOVA (Analysis of variance) was used. Pearson's correlation coefficient test was used to analyze the correlation of severity parameters (BP and proteinuria) with elastase and its inhibitors. A p-value <0.001 was considered highly significant.

RESULTS

The baseline physical and biochemical characteristics of the normal, mild and severe pre-eclamptic subjects are depicted in [Table/Fig-1]. The gestational age was in the range of 34 to 37 weeks for normal, and 31 to 36 weeks for mild to severe PE subjects. The blood pressure was elevated significantly in the case of mild and severe cases of PE in comparison to normal. The blood pressure was also significantly higher in severe PE compared to mild PE.

Variables	Normal pregnancy (n=50)	Mild pre-eclampsia (n=27)	Severe pre-eclampsia (n=23)	p-value
Maternal age (years)	23.62 \pm 2.98	25.11 \pm 4.20	25.70 \pm 4.35	
Gestational age (weeks)	37.18 \pm 3.05	34.70 \pm 3.37	34.96 \pm 3.11	
Blood Pressure (mmHg)				
Systolic	120.28 \pm 8.70	147.11 \pm 9.35	170.87 \pm 14.11	*p<0.001
Diastolic	80.20 \pm 5.88	100.22 \pm 8.84	106.52 \pm 11.12	
Cases with proteinuria n (%)	-			
• Traces		4 (14.8)	1(4.3)	
• 1+		16 (59.3)	3(13.0)	
• 2+		7 (25.9)	5(21.7)	
• 3+		-	14(60.9)	
Serum uric acid (mg/dl)	4.53 \pm 1.30	7.53 \pm 1.35	8.16 \pm 1.57	*p<0.001
Serum CRP (ug/ml)	0	12.44 \pm 11.40	14.35 \pm 13.98	*p<0.001

[Table/Fig-1]: Baseline characteristics of study groups.

ANOVA with Bonferroni test was used. Results represented as mean \pm SD.*p<0.001 statistically highly significant.

The data on proteinuria was suggestive of PE as per the criteria defined. Serum uric acid showed significant rise in PE group (mild 7.53 \pm 1.35; severe 8.16 \pm 1.57) compared to controls (4.53 \pm 1.30). When serum CRP was compared, mild (12.44 \pm 11.40) and severe (14.35 \pm 13.98) pre-eclamptic women presented significantly higher CRP levels as compared to normotensive pregnant women.

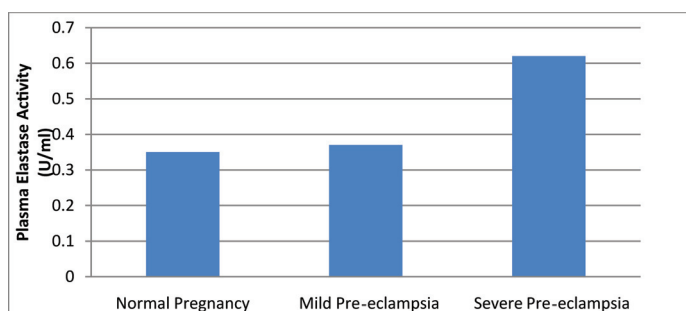
The data on NE, α_1 -AT, α_2 -MG and NE- α_1 -AT complex are presented in [Table/Fig-2]. The activity of neutrophil elastase was increased two fold in severe PE (0.62 \pm 0.08) in comparison to normal (0.35 \pm 0.10) and mild preeclamptic subjects (0.37 \pm 0.03) and was statistically highly significant. The values of α_1 -AT have been on the decline and were significantly less in mild and severe PE in comparison to normal; a 60% reduction in severe and 40% reduction in mild. There was a significant rise in the levels of α_2 -MG in severe pre-eclamptic women. However, the complex estimation did not evince any significant changes indicating normal balance and did not contribute to analytic value. Significant association between elastase activity and disease severity is depicted in [Table/Fig-3].

Correlation studies of the severity parameter; proteinuria with the levels of NE and α_1 -AT indicated a positive and negative picture respectively in severe form of PE [Table/Fig-4]. On the other hand,

Parameters	Normal pregnancy (n=50)	Mild pre-eclampsia (n=27)	Severe pre-eclampsia (n=23)	p-value
Plasma Elastase Activity (U/ml)	0.35 \pm 0.10	0.37 \pm 0.03	0.62 \pm 0.08	*p<0.001
Plasma α_1 -AT (mg/dl)	110.26 \pm 42.39	83.94 \pm 25.08	68.58 \pm 26.39	*p<0.001
Plasma α_2 -MG (mg/dl)	265.37 \pm 66.91	201.06 \pm 38.23	298.79 \pm 32.52	*p<0.001
Plasma NE- α_1 -AT complex (ng/ml)	171.08 \pm 23.81	176.19 \pm 9.27	164.31 \pm 11.63	p=0.285

[Table/Fig-2]: Plasma levels of elastase activity, α_1 -AT, α_2 -MG and NE- α_1 -AT complex in the study groups.

ANOVA with Bonferroni test was used. Results represented as mean \pm SD. *p<0.001 statistically highly significant.



[Table/Fig-3]: Elastase activity expressed in U/ml of plasma in study groups.

the correlation analysis of α_2 -MG with proteinuria presented a negative correlation in mild and a positive correlation in severe group. However, complex correlation with the severity parameters did not present any definite correlations.

DISCUSSION

PE exhibits characteristics of an inflammatory disease including neutrophil activation [2,14,15]. The activation of neutrophils in PE may be due to some pro-inflammatory cytokines and chemottractants released during an inflammatory response (i.e., TNF- α , IL-6 and IL-8) [16]. Elastase activity is measured as marker of neutrophil activation in several inflammatory conditions including PE [7,8,14,15]. The complications induced by PE state are detrimental to both the mother and the foetus and have been a serious subject of investigation. Research often focuses on the changes in the biochemical parameters with no data on its onset and progress.

Parameters	Plasma Elastase Activity (U/ml)		Plasma α_1 -AT (mg/dl)		Plasma α_2 -MG (mg/dl)		Plasma NE- α_1 -AT complex (ng/ml)	
	r	P	r	P	r	P	r	P
Mild PE								
Systolic BP (mmHg)	-0.178	0.373	0.361	0.064	0.131	0.156	-0.130	0.673
Diastolic BP (mmHg)	-0.004	0.983	-0.201	0.314	0.005	0.978	0.280	0.354
Proteinuria –traces	0.899	0.101	-0.690	0.310	-0.856	0.144	1.000	-
Proteinuria -1+	0.014	0.960	0.266	0.338	-0.436	0.104	-0.338	0.412
Proteinuria -2+	0.090	0.832	0.046	0.914	-0.076	0.857	-0.987	0.104
Severe PE								
Systolic BP (mmHg)	-0.015	0.945	-0.075	0.733	-0.263	0.224	-0.258	0.419
Diastolic BP (mmHg)	-0.376	0.077	0.153	0.486	0.054	0.808	0.182	0.572
Proteinuria -1+	0.064	0.959	-0.247	0.841	0.933	0.234	0.920	0.256
Proteinuria -2+	0.366	0.544	-0.282	0.645	0.692	0.195	-0.331	0.586
Proteinuria -3+	0.169	0.565	-0.171	0.558	0.290	0.315	0.329	0.589

[Table/Fig-4]: Correlation of plasma levels of elastase activity, α_1 -AT, α_2 -MG and NE- α_1 -AT complex with severity markers in mild and severe groups. Pearson's correlation coefficient test was used.

In the present study we have measured and compared the plasma levels of NE, α_1 -AT, α_2 -MG and NE- α_1 -AT complex in patients suffering from mild and severe PE with normal pregnant women [Table/Fig-2]. The analysis of NE activity indicated a significant increase in the severe PE which is in agreement with previous studies [7,8,11,14] and it could contribute to progressive inflammation.

α_1 -antitrypsin inhibits several serine proteases (mainly NE), and adequate activity of this inhibitor is critical for the maintenance of protease-antiprotease homeostasis and the prevention against proteolytic tissue damage [17]. Determination of the plasma α_1 -AT level demonstrates the available level of the inhibitor capable of inhibiting intravascular proteases. Contrary to expectation of an increased level of α_1 -AT in inflammation, a decreased level was observed in the study groups compared to the normal and it was highly significant paving ways to overpowering role of elastase in the complications of PE.

It is also pertinent to note that, there was no increase in the levels of NE- α_1 -AT complex in PE group consequent to the increased elastase activity. This observation could be an indication of decreased synthesis of α_1 -AT rather than its involvement in complex formation to control elastase activity. The reason for decreased α_1 -AT is an area of concern and is suggestive that supplementation of α_1 -AT would be able to minimize the destructive effects of NE on vascular tissues.

We have observed a significantly higher α_2 -MG level in severe PE patients compared with normal or mild PE patients against an expected reduced α_2 -MG concentration in severe PE as it is supposed to bind to elastase and get rapidly cleared from the plasma through macrophage receptors [18]. Raised levels observed in this study could be attributed to renal insufficiency, a common feature in PE patients. Moreover, increased levels of this inhibitor in severe PE possibly contribute to the intravascular coagulation as α_2 -MG has antiplasmin activity [19] adding to further severe complications. Horne CHW et al., also found high α_2 -MG levels in PE with proteinuria as compared to normal pregnant women [19].

In order to understand the relation of PE severity parameters with the levels of NE, α_1 -AT, α_2 -MG and NE- α_1 -AT complex correlation analyses were carried out. Though we have correlated these molecules with both proteinuria and BP, the most meaningful and relevant correlation was observed only with proteinuria and not with BP. The observation of increase in the activity of NE in severe PE and its positive correlation with severity marker proteinuria makes the measurement of NE a dependable parameter in the determination of severity of PE. Similar picture but in a negative direction was observed in case of α_1 -AT suggesting that measurement of α_1 -AT along with the activity of NE could strengthen the assessment of severity of PE.

As reported by earlier studies [20-22], a significant increase in the levels of serum uric acid and CRP in PE compared to controls was observed and points to generalised inflammation in these patients. However, the levels did not yield any information on the severity of the PE.

LIMITATION

Since PE is a progressive disease, a follow up of mild preeclamptic women to assess the progression of these women to severe form would have been the better study design to emphatically conclude the role of elastase enzyme and its inhibitors in severity of PE. Moreover, urinary estimation of α_1 -AT would have explained the reduced levels of α_1 -AT in PE group. We do agree a larger sample size definitely would have supported the results obtained.

CONCLUSION

The present study clearly indicates an association between increased levels of NE and decreased α_1 -AT with the severity of PE suggesting that both would be relevant markers for assessment of severity. Monitoring the plasma levels of these molecules thus could be of use for evaluation of the status of PE.

REFERENCES

- [1] Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol.* 1989;161(5):1200-04.
- [2] Lok CA, Jebbink J, Nieuwland R, Faas MM, Boer K, Sturk A, et al. Leukocyte Activation and circulating leukocyte-derived micro particles in preeclampsia. *Am J Reprod Immunol.* 2009;61(5):346-59.
- [3] Borzychowski AM, Sargent IL, Redman CW. Inflammation and preeclampsia. *Semin Fetal Neonatal Med.* 2006;11(5):309-16.
- [4] Bernardi F, Guolo F, Bortolin T, Dal-Pizzol F. Oxidative stress and inflammatory markers in normal pregnancy and preeclampsia. *J Obstet Gynaecol Res.* 2009;34(6):948-51.
- [5] Desai P. Oxidative stress and obstetric vasculopathies. In: Pankaj Desai. *Obstetric Vasculopathies.* New Delhi: Jaypee Brothers Medical Publishers (P) Ltd, 2013: 69.
- [6] Belo L, Santos-Silva A, Rocha S, Caslake M, Cooney J, Pereira-Leite L, et al. Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. *Eur J Obstet Gynecol Reprod Biol.* 2005;123(1):46-51.
- [7] Catarino C, Santos-Silva A, Belo L, Rocha-Pereira P, Rocha S, Patricio B, et al. Inflammatory disturbances in preeclampsia: Relationship between maternal and umbilical cord blood. *Journal of Pregnancy.* 2012;2012:684384.
- [8] Gupta AK, Gebhardt S, Hillermann R, Holzgreve W, Hahn S. Analysis of plasma elastase levels in early and late onset preeclampsia. *Arch Gynecol Obstet.* 2006;273(4):239-42.
- [9] Chua F, Laurent GJ. Neutrophil Elastase: Mediator of Extracellular Matrix Destruction and Accumulation. *Proc Am Thorac Soc.* 2006;3(5):424-27.
- [10] Janoff A. Elastase in tissue injury. *Annu Rev Med.* 1985;36:207-16.
- [11] Greer IA, Leask R, Hodson BA, Dawhs J, Kilpatrick DC, Liston WA. Endothelin, elastase and endothelial dysfunction in preeclampsia. *Lancet.* 1991;337(8740):558.
- [12] Lamb E, Newman DJ, Price CP. Estimation of serum uric acid. In: Carl A Burtis, Edward R Ashwood and David E Bruns, ed. *Teitz Textbook of Clinical Chemistry and Molecular Diagnostics.* New Delhi: Elsevier Co, 2006:807.

- [13] Bieth J, Spiess B, Wermuth CG. The synthesis and analytical use of a highly sensitive and convenient substrate of elastase. *Biochemical Medicine*. 1974;11(4):350-57.
- [14] Belo L, Santos-Silva A, Caslake M, Cooney J, Pereira-Leite L, Quintanilha A, et al. Neutrophil activation and C-reactive protein concentration in preeclampsia. *Hypertens Pregnancy*. 2003;22(2):129-41.
- [15] Halim A, Kanayama N, El Maradny E, Maehara K, Bhuiyan AB, Terao T. Correlated plasma elastase and sera cytotoxicity in preeclampsia. A possible role of endothelin -1 induced neutrophil activation in preeclampsia-eclampsia. *Am J Hypertens*. 1996;9(1):33-38.
- [16] Sharma A, Satyam A, Sharma JB. Leptin, IL-10 and inflammatory markers (TNF- α , IL-6 and IL-8) in preeclamptic, normotensive pregnant and healthy non-pregnant women. *American Journal of Reproductive Immunology*. 2007;58(1):21-30.
- [17] Fujie K, Shinguh Y, Inamura N, Yasumitsu R, Okamoto M, Okuhara M. Release of neutrophil elastase and its role in tissue injury in acute inflammation: effect of the elastase inhibitor, FR134043. *Eur J Pharmacol*. 1999;374(1):117-25.
- [18] Petersen CM. Alpha 2-macroglobulin and pregnancy zone protein. Serum levels, alpha 2-macroglobulin receptors, cellular synthesis and aspects of function in relation to immunology. *Dan Med Bull*. 1993;40(4):409-46.
- [19] Horne CHW, Howie PW, Goudie RB. Serum alpha2-macroglobulin, transferrin, albumin and IgG levels in preeclampsia. *J Clin Path*. 1970;23(6):514-16.
- [20] Sunitha T, Sameera K, Umaramani G. Study of biochemical changes in preeclamptic women. *Int J Biol Med Res*. 2012;3(3):2025-28.
- [21] Mirzaie F, Rahimi-Shorbai F, Kazeronie AH. Association of maternal serum C-reactive protein levels with severity of Preeclampsia. *Acta Medica Iranica*. 2009;47(4):293-96.
- [22] Omkaramurthy K, Rathodvasantkumar R, Dhanajaya BS, Sameena Sultana, Shobha UN. Pattern of C-reactive protein in preeclampsia and normal pregnancy. *Int J Biol Med Res*. 2012;3(4):2616-21.

PARTICULARS OF CONTRIBUTORS:

1. Lecturer, Department of Biochemistry, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.
2. Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.
3. Professor, Department of Medicine, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.
4. Professor, Department of Obstetrics and Gynaecology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. AV Moideen Kutty,
Registrar, Professor, Sri Devaraj Urs Academy of Higher Education and Research,
Tamaka, Kolar-563103, Karnataka, India.
E-mail: kuttyavm@gmail.com

Date of Submission: **Sep 15, 2016**Date of Peer Review: **Nov 05, 2016**Date of Acceptance: **Dec 01, 2016**Date of Publishing: **Mar 01, 2017****FINANCIAL OR OTHER COMPETING INTERESTS:** None.