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RESEARCH ARTICLE

USEFULNESS OF NEUTROPHIL ELASTASE AND ENDOGENEOUS PROTEASE INHIBITOR IN ASSESSMENT OF SEVERITY OF PRE-ECLAMPSIA.

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Abstract

Background : (PE) is one of the main causes of maternal and fetal mortality and morbidity. PE is associated with an inflammatory state and with oxidative stress, in maternal circulation. Our aim was to evaluate and compare the levels of oxidative stress and inflammatory markers**.** Neutrophil Elastase (NE), a serine protease stored in the primary granules of neutrophils, is capable of degrading various extracellular matrix proteins and thus uncontrolled elastase activity can lead to destroyal of the integrity of endothelial cells and could lead to exacerbate the symptoms in preeclampsia.

Aim: The aim of this study was to study usefulness of the activity ,of neutrophil elastase and its endogenous inhibitors α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG) with severity of preeclampsia.

Methodology: The present was conducted on 100 subjects, of various age groups. Study included diagnosed pre-eclamptic patients (50) attending Medical OPD, patients admitted in medical and obsetritic & Gynecological department and patients coming in Radioimmunoassay (RIA) laboratory, Biochemistry Department J.L.N. Medical College & Hospital, Ajmer. The results of patients were compared with the fifty healthy subjects. Serum 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 α1-AT, α2-MG and PMN elastase/ α1-PI complex were quantified by ELISA.

Results: The activity of elastase was increased significantly in severe preeclampsia (0.72+0.08) in comparison to normal (0.40+0.10) and mild preeclamptic subjects (0.37+0.03). The values of α1-AT were significantly less in mild (83.94+25.08) and severe preeclampsia (68.58+26.39) in comparison to normal (110.26¬+42.39). There was a significant rise in the levels of α2-MG in severe preeclampsia. However, the complex estimation did not evince any significant changes. Serum uric acid and CRP levels significantly elevated in

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preeclampsia compared to controls. **Conclusion:** Present study indicates that monitoring elevated NE activity is a characteristic marker for severity of preeclampsia. Also, the reduced level of α1-AT is an indication of a possible role of this inhibitor supplementation for the control of complications of preeclampsia ultimately reducing neonatal mortality.

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Introduction:-

Preeclampsia is a condition during pregnancy where there is a sudden rise in blood pressure and swelling, mostly in the face, hands, and feet.Preeclampsia is the most common complication to occur during pregnancy. It generally develops during the third trimester and affects about 1 in 20 pregnancies.If the preeclampsia remains untreated, it can develop to eclampsia, in which the mother can experience convulsions, coma, and can even die. However, complications from preeclampsia are extremely rare if the mother attends her prenatal appointments.

Preeclampsia (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 is still unclear and is a topic of active investigation. The pathological lesions of decidual vessels in PE have similarity to atherotic lesions of arteries (5). Neutrophils have been implicated in the pathogenesis of atherotic 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, 7, 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 edema and proteinuria (10), a usual observation in PE. A positive correlation have been demonstrated between von Willebrand Factor (a marker of endothelial damage) and NE by Greer et al indicating that neutrophil activation could contribute to endothelial damage and dysfunction in preeclampsia (11).

Thus uncontrolled neutrophil activation can lead to destroyal 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 α2-macroglobulin (α_2-MG) with severity of PE.

Methodology:-

The present was conducted on 100 subjects, of various age groups. Study included diagnosed pre-eclamptic patients (50) attending Medical OPD, patients admitted obsetritic & Gynecological department and patients coming in Radioimmunoassay (RIA) laboratory, Biochemistry Department J.L.N. Medical College & Hospital, Ajmer. The results of patients were compared with the fifty healthy subjects. Serum 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 α1-AT, α2-MG and PMN elastase/ α1-PI complex were quantified by ELISA.

50 pregnant normotensive women and 50 preeclamptic 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 preeclamptic patients were diagnosed by the presence of hypertension (>140mmHg systolic BP and >90mmHg diastolic BP) on two occasions with 4-6hours apart, proteinuria (>1+ by urine dipstick method) with or without pathological edema. PE was considered as severe, if the subjects had at least two of the following: >160mmHg systolic BP; >110mmHg 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.

6ml of blood was collected from an antecubital vein from all the subjects in tubes containing EDTA (for hematologic studies); Sodium Heparin (for NE, α_1-AT, α_2-MG and Polymorphonuclear Elastase/ α1-Proteinase inhibitor complexestimation) and in tubes without anticoagulant (for CRP estimation). Blood samples were centrifuged within 2 hours of collection. After centrifugation, serum and plasma were separated and aliquots were stored at -70^{o}C until assayed. Samples were thawed at room temperature, vortexed and centrifuged before analysis.

Methods:-

Complete Blood Count was performed by Beckman- Coulter, an automatic blood cell counter.SerumC - reactive protein (CRP) estimation was done by rapid latex slide tests. Serum uric acid was estimated by uricase method (12) . Estimation of plasma elastase was done using Succinyl tri- L-alanyl-p-nitroanilide as substrate at 410nm as per the procedure described by Beith.J, et al (13).Plasma α_1-AT and α_2-MGwere analyzed using Enzyme Linked Immunosorbent Assay kit purchased from Immunology Consultants laboratory, Inc, USA.PMN elastase/ α1-PI complex (NE-α_1-ATcomplex)was quantified by Enzyme Linked Immunosorbent Assay (Calbiochem).

Statistical Analysis:

The data were statistically analyzed by SPSS software version 22. The results are expressed as Mean+SD. For statistical differences in means between the groups ANOVA (Analysis of variance) was used. P value <0.001 was considered highly significant.

Results:-

The baseline physical and chemical characteristics of the normal, mild and severe preeclamptic subjects are depicted in Table – 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. The data on proteinuria was suggestive of PE as per the criteria defined. Serum uric acid showed significant rise in preeclampsia group (Mild 7.53+1.35; Severe 8.16+1.57) compared to controls (4.53+1.30). When serum CRP was compared, mild (12.44+11.40) and severe (14.35+13.98) preeclamptic 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 2. The activity of neutrophil elastase was increased two fold in severe preeclampsia (0.72+0.08) in comparison to normal (0.40+0.10) and mild preeclamptic subjects (0.37+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 preeclamptic women. However, the complex estimation did not evince any significant changes indicating normal balance and did not contribute to analytic value.

Discussion:-

Preeclampsia 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 chemoattractants 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 fetus 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.

The present study was aimed to measure the plasma activity of NE to obtain an insight into the association of this critical parameter with the severity of PE. As anticipated there was a significant association between elastase activity and severity (Fig 1). Our result is in agreement with previous studies conducted (7, 8, 11, 14). This study is supplemented with the measurement of endogenous inhibitors of elastase: α_1-AT, α_2-MG as well as α_1-AT - elastase complex.

Alpha$_1$-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 antiproteolytic activity demonstrates the available level of the inhibitor capable of inhibiting intravascular proteases. Contrary to expectation of an increased levels as response to elevated elastase activity, the levels of α_1-AT was decreased significantly in preeclamptic cases suggestive of overpowering role of elastase in the complications of PE.

It is also pertinent to note that, there was no increase in the levels of complex in preeclampsia group consequent to the increased elastase activity. This observation indicates decreased synthesis of α1-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 levels in severe PE patients compared with normal or mild PE patients. We expected a reduced α_2-MG concentration in severe PE as it is expected to bind to elastase and get rapidly cleared from the plasma through macrophage receptors (18). Raised levels is of concern as it opines a decreased receptor-mediated clearance in preeclampsia 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 et al also found high α_2-MG levels in PE with proteinuria as compared to normal pregnant women (19).

As expected and reported by earlier studies (20, 21, 22), a significant increase in the levels of serum uric acid and CRP in preeclampsia compared to controls was observed and points to generalized inflammation in these patients. However, the levels did not yield any information on the severity of the preeclampsia.

Conclusion:-

Our study concluded that there is significant importance of neutrophil elastase and α2MG to asses the severity of pre-eclampsia The overall result is an indication of a possible role of α1-AT supplementation for the control of complications of preeclampsia.

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Table 1:-Baseline characteristics of study groups

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

Table 2:-Plasma levels of elastase activity, α_1-AT, α_2-MG and NE-α_1-ATcomplex in the study groups

Parameters	Normal Pregnancy (n=50)	Mild Preeclampsia (n=27)	Severe Preeclampsia (n=23)	P value
Plasma Elastase Activity (U/ml)	0.40+0.10	0.37+0.03	0.70+0.08	*p<0.001
Plasma α_1-AT (mg/dl)	110.26+42.39	83.94+25.08	68.58+26.39	*p<0.001
Plasma α_2-MG (mg/dl)	265.37+66.91	201.06+38.23	298.79+32.52	*p<0.001
**Plasma NE-α_1-ATcomplex (ng/ml)	171.08+23.81	176.19+9.27	164.31+11.63	p=0.285

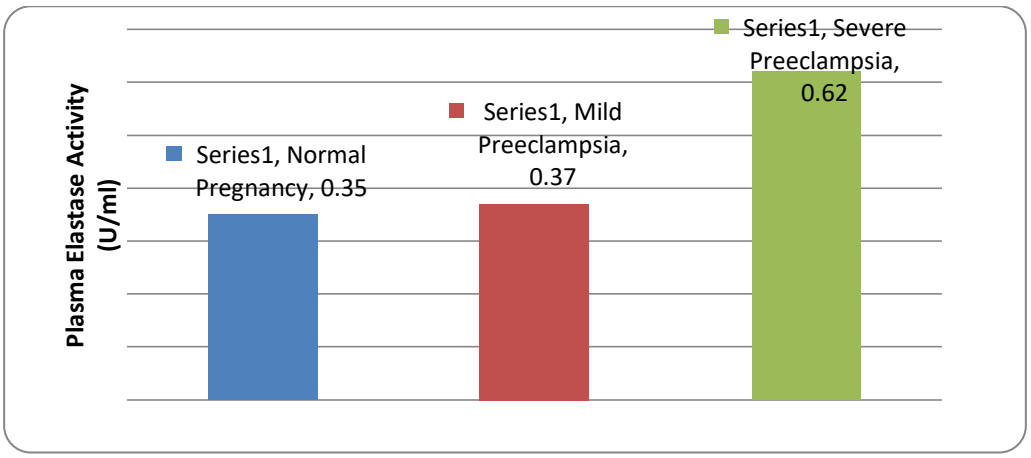

Fig 1:-Plasma elastase activity expressed in U/ml of plasma in study groups.

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