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INFLUENCE OF BIOMARKERS IN PRE-ECLAMPSIA

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INTRODUCTION

Preeclampsia is a disorder of pregnancy that involves multiple organ system contributing largely to maternal and foetal morbidity across the globe. Worldwide, each year ten million women develop preeclampsia and about 76,000 pregnant women die from preeclampsia and related hypertensive disorders.[1] The disease complicates up to 10% of all pregnancies in the developing countries, where emergency care is often inadequate or lacking.

Preeclampsia, according to American College of Obstetrics and Gynaecology (ACOG) criteria is characterized by new onset of hypertension that develops after 20 weeks of gestation in previously normotensive women (systolic and diastolic blood pressure of ≥ 140 and 90 mmHg, respectively, on two occasions, at least 6 hours apart) associated with proteinuria (protein excretion of ≥ 300 mg/ 24 h urine collection, or a dipstick of ≥ 1+ or protein/creatinine ratio>0.3mg/dl) or one of the following criteria in absence of proteinuria: thrombocytopenia < 100000/mm3, deranged liver function (twice the normal concentration of liver transaminases), deranged renal function (serum creatinine>1.1mg/dl or a doubling serum creatinine in absence of any renal disease), pulmonary oedema or new onset neurological symptoms.[2] The disease initiating before 34 weeks of gestation is defined as early onset disease and that starting after is late onset preeclampsia. It is categorized into mild and severe forms. Sever preeclampsia is defined as systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg (on two occasions at least 4 hours apart while the patient is on bed rest) or any of the afore mentioned clinical findings. Preeclampsia not only affects maternal health but also compromise foetal wellbeing. Intrauterine growth restriction, prematurity, foetal distress, intrauterine demise or neonatal morbidity can be frequently seen associated with the disease.[3] Though multifactorial in origin, defective placentation is the principal pathology underlying the disease. It is associated with shallow decidual invasion of cytotrophoblasts and thus incomplete remodelling of placenta-supplying maternal uterine spiral arterioles results in hypoxic injury. The ensuing oxidative stress in the placenta causes release of endothelial deranging factors into maternal circulation.[4] These circulating factors such as matrix metalloproteinase (MMP), vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β1), interleukins (IL) and prostaglandins (PG) further lead to generalized endothelial dysfunction and an excessive systemic inflammatory response contributing to the maternal clinical features of preeclampsia.[5]

Since the onus of the disease lies over the activity of these intrinsic factors, gaining information about their differing levels in women with preeclampsia would answer many queries about the disease. Thus in the present study, we aimed to measure and compare the serum levels of the MMP2, VEGF and TGF-β1 in preclamptic and healthy women.

MATERIALS AND METHODS

A case control study was conducted in Departments of Biochemistry and Obstetrics and Gynaecology, Maulana Azad Medical College, Lok Nayak Hospital, New Delhi in 2015.

SAMPLE SIZE

1. Forty pregnant women diagnosed with pre-eclampsia (as defined by recent acog guidelines 2014) were selected as cases.
2. Forty healthy pregnant women were randomly selected as controls (age and gestational period matched).

Inclusion Criteria

1. Primigravida.
2. Singleton pregnancy.
3. Age 20-30 years.

Exclusion Criteria

1. Pre-existing hypertension,
2. Known cases of diabetes mellitus, acute infection or any malignancy.

3. Any medication, other than haematinics, folic acid, calcium that can influence the test parameters.

Written informed consent form signed by all subjects and research protocol was approved by the Local ethical committee. The study group was subjected to structured questionnaire regarding their demographic, medical lifestyle and reproductive information. A detailed history about the onset of disease and the treatment was taken. A detailed physical examination was carried out. Relevant biochemistry, pathology and radiological investigations if available with patients were also reviewed. Five millilitre of fasting venous blood sample was collected from cases and controls. Serum MMP-2, VEGF and TGF-b levels were estimated by ELISA method.

RESULTS

1. Age distribution

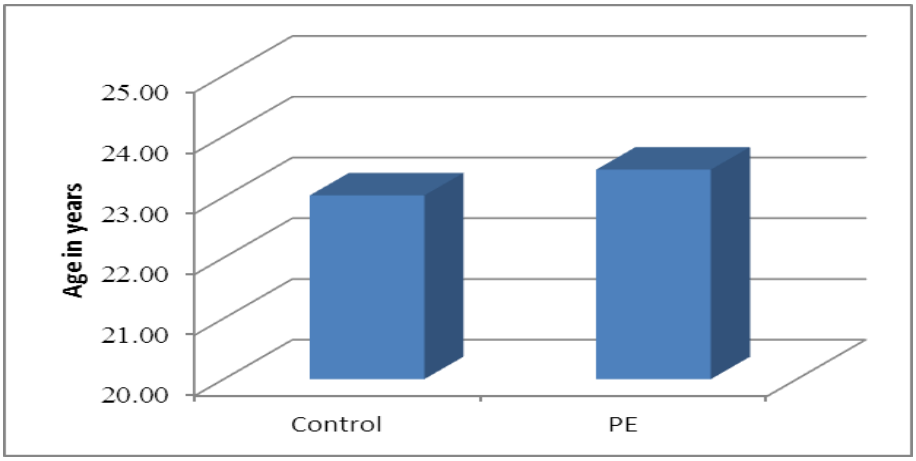
Majority of patients were in age group 18-30 yrs both in the cases and the controls.

Mean age of patients in cases was 23.45 ± 2.46 yrs and in the controls was 23.03 ±.2.33 yrs.

p value was non-significant (0.215).

Table 1: Age distribution.

	CONTROL (40)		CASES (40)		p- VALUE
AGE	MEAN	± SD	MEAN	± SD	
	23.03	2.33	23.45	2.46	0.215



Graph 1: Age distribution.

2. Gestational Age

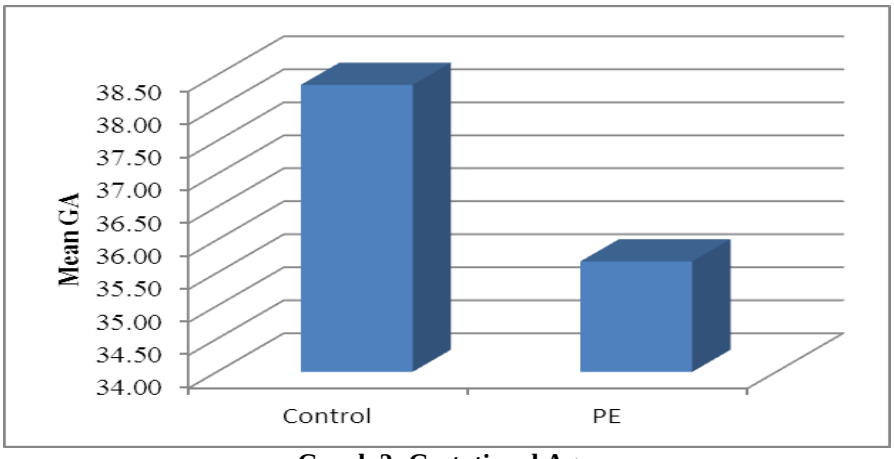
The mean gestation age among cases was 35.68 ± 2.35 weeks.

In the control group the mean gestation age was 38.35 ± 1.56 weeks.

The p value was significant (<0.001).

Table 2: Gestational Age.

	CONTROL (40)		CASES (40)		p- VALUE
GESTATION AGE	MEAN	± SD	MEAN	± SD	
	38.35	1.56	35.68	2.35	<0.001



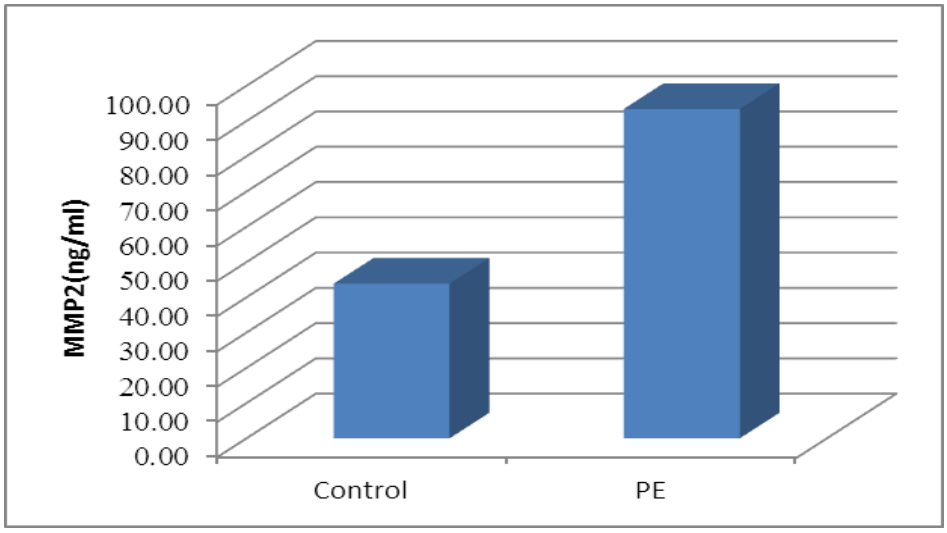
Graph 2: Gestational Age.

3. Serum levels of MMP-2

Mean value of Serum MMP-2 in cases was 93.45 ± 15.49 ng/ml and in the controls was 43.86 ± 5.72 ng/ml p- value was significant (<0.001).

Table 3: Serum levels of MMP-2.

	CONTROL (40)		CASES (40)		p- VALUE
	MEAN	± SD	MEAN	± SD	
MMP-2	43.86	5.72	93.45	15.49	<0.001



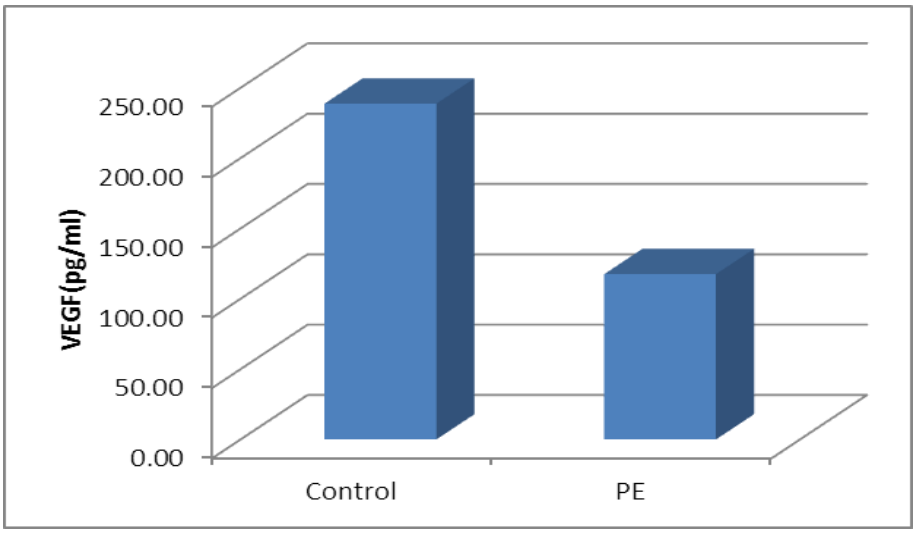
Graph 3: Serum levels of MMP-2.

4. Serum levels of VEGF

Mean value of VEGF in cases was 117.47 ± 37.93 pg/ml and in the controls was 238.70 ± 40.82 pg/ml. p value was significant (<0.001).

Table 4: Serum levels of VEGF.

	CONTROL (40)		CASES (40)		p- VALUE
	MEAN	± SD	MEAN	± SD	
VEGF	238.70	40.82	117.47	37.93	<0.001



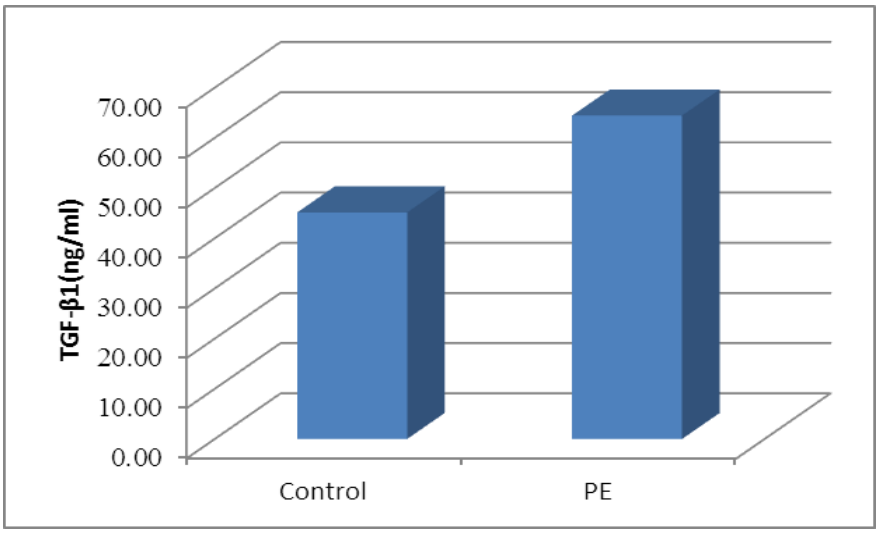
Graph 4: Serum levels of VEGF.

5. Serum levels of TGF-β1

Mean value of TGF-β1 in cases was 64.44 ± 26.18 pg/ml and in the controls was 45.14 ± 21.62 pg/ml p value was significant (<0.001).

Table 5: Serum levels of TGF-β1.

	CONTROL (40)		CASES (40)		p- VALUE
	MEAN	± SD	MEAN	± SD	
TGF-β1	45.14	21.62	64.44	26.18	<0.001



Graph 5: Serum levels of TGF-β1.

DISCUSSION

In the present study, both the cases and controls were taken in the similar age group ranging from 18 years to 30 years with the mean age of 23.45 years in cases and 23.03 years in controls. Comparing the gestational age at delivery between cases and controls, it was observed that mean gestational age amongst the controls was 38.35 weeks and cases was 35.68 weeks. The gestational age distribution between the cases and controls was not statistically different. Similar to our study, Sarkar et al observed mean gestational age of 38.58 weeks in controls and 33.64 weeks in cases.[6] In the study by Pasupathi et al mean gestational age in controls was 36 weeks and in cases was 35 weeks.[7]

Matrix metalloproteinases and vascular endothelial growth factor (VEGF) together play a crucial role during pregnancy. These markers aid in the process of implantation, trophoblastic invasion and placentation. Matrix metalloproteinases mainly target extracellular matrix components during development and morphogenesis.[8] MMP-2 is one of such enzymes that is involved in remodelling of placental and uterine arteries through foeto-maternal membrane lysis.[9] In normal pregnancy, MMP-2 levels are finely regulated throughout the gestational period, but they are significantly higher in women with preeclampsia, as is observed in our study, indicating their contribution towards pathophysiology of preeclampsia. We found serum MMP-2 concentration to be 93.45 ng/ml in cases and 43.86 ng/ml in controls with p-value<0.001. In a similar study conducted by Montagnana et al, the serum MMP-2 level in cases was 834 ng/ml and in controls was 669 ng/ml (p-value 0.003).[10] Many others too found significantly raised serum levels of MMP-2 in preclamptic women as compared to the lower levels in normal pregnant women[11], thus attributing raised mmp2 levels to defective placentation.[12-14] Lavee et al in their study measured levels of MMP-2 in amniotic fluid of women with hypertensive disorders and compared it to normotensive women. They concluded that higher amniotic fluid MMP-2 levels were found in women who eventually developed preeclampsia. The MMP-2 levels in the amniotic compartment contains MMP-2 which is most reliably foetal in origin, whereas maternal serum MMP-2 could be of placental origin or influenced by non-pregnancy related causes.[15]

VEGF is an endothelial cell-specific growth factor that stimulates angiogenesis and increases microvascular permeability along with promoting coagulation.[16] Several studies have demonstrated significantly raised serum VEGF levels in preclamptic women, thus defining their role in shallow placentation. However, some recent studies have shown that the levels of serum free VEGF decrease in patients with preeclampsia. This discrepancy could be explained by the fact that VEGF protein complexes are undetectable by the sandwich-type ELISA because there is a substantial increase in circulating VEGF binding proteins during pregnancy.[17, 18] All those studies reporting on decreased VEGF, as in our study, have used an ELISA kit, which measures free (unbound) VEGF[18], whereas all studies reporting on an increased VEGF in pre-eclampsia used either a radioimmunoassay or an ELISA system measuring total (bound and unbound) VEGF.[18-20] In our study mean free VEGF concentration in cases was 117.47pg/ml and in controls it was 238.70pg/ml which is significantly higher.

Rise in TGF-β1 is also associated with endothelial injury.[21] Feinberg et al. reported TGF-β1 to be a significant stimulator of trophoblast onco-foetal

fibronectin production which contributes to trophoblast adhesion. Thus, the increase in TGF-β1, in preeclampsia may affect several processes in the pathogenesis of this disorder. In our study mean TGF-β1 concentration was observed to be 64.44ng/ml in cases and 45.14ng/ml in controls which is significantly lower. Similarly in a case-control study of Norwegian women, Djurovic et al[22] TGF-β1 concentrations were elevated in women with severe and mild preeclampsia in late gestation phase (mean gestational age, 40 weeks) compared with normotensive pregnant women. Plasma concentrations of the active form of TGF-β1 were increased in all preeclampsia subgroups as well as in the total group (5.63ng/ml) compared to controls (4.67ng/ml). This increase in TGF-β1 was highly significant. Also in study by Benian et al, elevated TGF-β1 levels were observed among preeclampsia patients compared with normotensive pregnant women (41.27 pg/ml vs 18.62 pg/ml).[23]

CONCLUSION

Preeclampsia is a systemic disorder that largely contributes to foeto-maternal morbidity during pregnancy. Though many theories have been proposed regarding its pathogenesis, the inexplicit reasons for its origin and inadequacy of definite preventive measures warrants more research work. The association of preeclampsia with altered levels of VEGF, TGF-B, and MMP2 proteins, as observed in our study, provide us an opportunity for further exploration of the disease. These makers serve as a probe to predict the onset of clinical disease and evolve specific preventive measures along-with more definite therapeutic drugs. Further, such measures will aid in introducing reforms in maternal and perinatal healthcare systems thus reducing the burden of the disease.

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