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Role of TGF-B1 in the Pathogenesis of Pre-Eclampsia.

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ABSTRACT

Background: Preeclampsia is one of the most frequently encountered medical complication of pregnancy and affects 3-5% of pregnancies worldwide. Classically, the condition presents with new onset hypertension and proteinuria after 20 weeks of gestation. In normal pregnancy, TGF-β decreases at nine weeks gestation, promoting cytotrophoblast invasion. TGF-β is increased in pre-eclamptic placentas thereby causing shallow cytotrophoblast invasion. It has been hypothesized that failure to downregulate TGF-β results in shallow trophoblast invasion and pre-eclampsia. Aim: To measure the level of serum TGF β-1 in pre-eclampsia patients and compare it with normal pregnant women. **Methods:** Serum TGF β-1 estimation was done with the help of Human TGF β-1 ELISA kit. Measurement of serum TGF β-1 levels was done by Elisareader. **Results:** Pre-eclamptics had a significantly increased serum TGF β-1 level as compared to normal pregnant women**.** We found significant decrease in mean TGF β-1 levels in pre-eclamptics with Gestational Age > 33 weeks as compared to those with Gestational Age 30-33 weeks. Mean TGF β-1 levels was increased significantly in pre-eclamptics with DBP>100 mmHg as compared with those having DBP 90-100mmHg. No significant difference was found in mean TGF β-1 levels of nullipaous and multiparous pre-eclamptics. **Conclusion:** We conclude from our study that increased TGF β-1 level may lead to pre-eclampsia.

Keywords: Pre-eclampsia, Pregnant, TGF β-1

INTRODUCTION

Preeclampsia is one of the most frequently encountered medical complication of pregnancy and affects 3-5% of pregnancies worldwide.[1] Classically, the condition presents with new onset hypertension and proteinuria after 20 weeks of gestation.[2] It is a major cause of maternal and perinatal mortality and morbidity worldwide. The World Health Organisation estimates that worldwide approximately 70,000 women die from pre-eclampsia each year.[3]

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Pre-eclampsia is defined by the International Society for the Study of Hypertension in Pregnancy as "Gestational hypertension of at least 140/90 mmHg on two separate occasions ≥4 hours apart accompanied by significant proteinuria of at least 300 mg in a 24-hour collection of urine, arising de novo after the 20th week of gestation in a previously normotensive woman and resolving completely by the 6th postpartum week".[3] Both hypertension and proteinuria implicate the endothelium as the target of the disease. The hypertension of preeclampsia is characterized by peripheral vasoconstriction and decreased arterial compliance.[4,5] The proteinuria of preeclampsia is associated with a pathognomonic renal lesion known as glomerular endotheliosis, in which the endothelial cells of the glomerulus swell and endothelial fenestrations are lost.[6,7]

Transforming growth factor beta 1 or TGF-β1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein from platelets and many leukocytes that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis.[8] Many studies have shown that elevated TGF β-1 is associated with increased risk of Preeclampsia.[9] It is proposed that trophoblasts shed from the placenta triggers the maternal symptoms of Preeclampsia. It is hypothesized that trophoblasts die by apoptosis in normal pregnancy but by necrosis in preeclampsia. Dead trophoblasts shed from placenta are deported to maternal pulmonary capillaries and here they are

phagocytosed by pulmonary capillary endothelial cells. Phagocytosis of necrotic trophoblasts activate endothelial cells to secrete TGF β-1. TGF β-1 induces IL-6 secretion which activates endothelial cells. IL-6 increases the amount of soluble endoglin (sEng) released from placental explants.[10] sEng impairs binding of TGF β-1 to its receptors and downstream signaling including inhibition of enzyme nitric oxide synthase (NOS) thereby inhibiting NO synthesis. Hence, it inhibits vasodilation of pregnancy thereby increasing the systemic blood pressure. Since TGF β-1 is unable to bind to its receptors therefore its concentration in plasma increases in pre-eclamptic females. We measured the level of TGF β-1 in the serum of pre-eclamptic females and compared it with that of normal pregnant females. Consequently, we hypothesized that increased serum TGF β-1 levels may be involved in the pathogenesis of pre-eclampsia.

MATERIALS AND METHODS

A. Selection Of Cases And Controls

In this study the work was conducted on the newly diagnosed cases of pre-eclampsia. Thirty pre-eclamptic women in third trimester of pregnancy were selected from Obstetrics and Gynaecology Inpatient Department of J.N. Medical College Hospital. A.M.U., Aligarh between October 2013 and March 2014. Thirty women with normal pregnancy in their third trimester and apparently good health were selected as controls. They were selected from Antenatal Clinic of Obstetrics and Gynaecology Department, J.N. Medical College Hospital, Aligarh.

Informed consent (in accordance with the Helinski Declaration of 1975, revised in 1983) was taken from the cases and controls for participation in the study with approval of institutional Ethical Committee, J.N. Medical College Hospital, Aligarh.

B. Exclusion Criteria

1. Maternal age less than 20 years and more than 30 years.
2. Pre-eclamptic patients who were suffering from such a disease in which oxidative stress was implicated in the pathophysiology, for e.g, diabetes, hypertension etc.
3. Patients of Gestational Hypertension.
4. Patients with history of smoking and alcohol intake.
5. Duration of pregnancy less than 30 weeks.
6. Patients taking antioxidants.

C. Biochemical Analysis

Serum TGF β-1 estimation was done with the help of Human TGF β-1 ELISA kit as described in the user manual (Revised Mar 1, 2012) by RayBiotech, Inc.[14] It was measured in nanograms/ml (ng/ml) of serum. The Ray Bio® Human TGF β-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human TGF β-1 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human TGF β-1 coated on a 96-well plate. Standards and samples were pipetted into the wells and TGF β-1 present in a sample got bound to the wells by the immobilized antibody. The wells were washed and biotinylated antihuman TGF β-1 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color developed in proportion to the amount of TGF β-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color was measured at 450 nm by the Elisareader.

D. Statistical Analysis of Data

Results were analysed using appropriate statistical tests with the help of GraphPad Prism-5.0 software and t-test was applied for analysis. A p-value of < 0.05 was taken as significant.

Observations and Results

1. TGF β-1 levels were observed in normal pregnant women and pre-eclampsia patients. Mean TGF β-1 levels in normal pregnant women were found to be 34.52 ± 2.61ng/ml (N=30) and in pre-eclamptic patients were found to be 48.50 ± 1.54 ng/ml (N=30). This increase in pre-eclamptic patients was found to be significant (P value < 0.01). Refer [Table 1].
2. Pre-eclamptic patients were divided into two groups on the basis of gestational age. Mean TGF β-1 levels in pre-eclamptics having gestational age 30-33 weeks was 49.82 ± 1.72 ng/ml and in those with gestational age > 33 weeks was found to be 47.84 ± 0.92 ng/ml. This decrease was also significant (P value < 0.01). Refer [Table 2].
3. Pre-eclamptic patients were divided into two groups according to diastolic blood pressure; (i) DBP = 90-100 mm Hg (ii) DBP > 100 mm Hg. Mean serum TGF β- 1 level in group (i) was found to be 47.33 ± 0.87 ng/ml and in group (ii) was found to be 49.28 ± 1.40 ng/ml. This increase in mean TGF β-1 level was statistically significant (P value < 0.01). Refer [Table 3].
4. Pre-eclampsia patients were also divided according to nulliparity and multiparity and mean levels of serum TGF β-1 were calculated in both the groups. Mean serum TGF β-1 level in nulliparous pre-eclamptics was found to be 48.54 ± 1.52 ng/ml (N= 18); and that in multiparous pre-eclamptics was 48.43 ± 1.64 ng/ml (N=12). This difference in mean TGF β-1 levels was insignificant (P value > 0.05). Refer [Table 4].

RESULTS & DISCUSSION

We measured serum TGF β-1 levels in pre-eclampsia patients. Patients had an increased serum TGF β-1 level as compared to normal pregnant women. This increase was highly significant (P value = 0.0001). This result is consistent with previous studies done by many investigators.[12] The presence of transforming growth factor beta (TGF-β) is inversely correlated with cytotrophoblast invasion. In normal pregnancy, TGF-β decreases at nine weeks gestation, promoting cytotrophoblast invasion. TGF-β is increased in pre-eclamptic placentas.[11] Inhibiting TGF-β activity with antibodies enhances the invasive properties of trophoblasts ex vivo. Caniggia et al. (1997) hypothesized that failure to downregulate TGF-β results in shallow trophoblast invasion and pre-eclampsia.[11] So we can say that shallow trophoblast invasion is responsible for the development of pre-eclampsia.

Table 1: Mean levels of serum TGF β-1 in normal pregnant women and pre-eclampsia patients

Mean level of serum TGF β-1(ng/ml)	Normal Pregnant (N = 30)	Pre-eclampsia (N = 30)
	34.52 ± 2.61	48.50 ± 1.54*

*P value < 0.01

Table 2: Mean levels of serum TGF β-1 in pre-eclampsia patients divided into two groups according to gestational age

Mean levels of TGF β-1 (ng/ml)	Pre-eclampsia patients	
	GA= 30-33 weeks	GA > 33 weeks
	49.82 ± 1.72	47.84 ± 0.92*

*P value < 0.01 (GA = gestational age)

Table 3: Mean serum TGF β-1 levels in pre-eclampsia patients divided on the basis of Diastolic Blood Pressure (DBP)

Mean levels of serum TGF β-1 (ng/ml)	Pre-eclampsia patients	
	DBP = 90-100 mm Hg	DBP > 100 mm Hg
	47.33 ± 0.87	49.28 ± 1.40*

*P value < 0.01

Table 4: Mean levels of serum TGF β-1 in nulliparous and multiparous pre-eclamptics

Mean levels of serum TGF β-1 (ng/ml)	Pre-eclampsia patients	
	Nullipara (N=18)	Multipara (N=12)
	48.54 ± 1.52	48.43 ± 1.64*

*P value > 0.05

Pre-eclampsia patients were grouped into two according to gestational age; Group I with GA 30-33 weeks and Group II with GA > 33 weeks. We found significant decrease in mean TGF β-1 levels in pre-eclamptics with GA > 33 weeks as compared to those with GA 30-33 weeks (P value = 0.0003). It has been proved by previous studies done by Mandeep singh et al (2013) that TGF β-1 level decrease with increase in gestational age of pregnant women.[16]

We also divided the pre-eclamptics on the basis of diastolic blood pressure (DBP) into two groups; Group I- DBP = 90-100 mmHg and Group II- DBP > 100 mmHg. Mean TGF β-1 levels was increased significantly in Group II as compared to Group I (P value < 0.01). This increase in mean TGF β-1 level with diastolic blood pressure is in consistence with a previous study carried out by Benian and Madazil (2002).[15] They hypothesized that increase in TGF β-1 is correlated with the severity of Pre-eclampsia. Mechanism for developement of hypertension is as follows. Placental ischemia is thought to lead to widespread dysfunction of the maternal vascular endothelium that results in enhanced formation of endothelin and thromboxane, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as NO and prostacyclin. These endothelial abnormalities, in turn, cause hypertension by impairing renal-pressure natriuresis and increasing total peripheral resistance.

We also grouped pre-eclampsia patients into nulliparous and multiparous women. We do not found any significant difference in mean serum TGF β-1 levels of nullipaous and multiparous pre-eclamptics (P value > 0.05). Although nulliparity is a risk factor for pre-eclampsia, which is more important than multiparity. But the relation of TGF β-1 levels to nulliparity or multiparity is not clearly defined by the previous studies. We do not found any study in support of this correlation. Therefore, further studies are required to find out the level of TGF β-1 in nulliparous and multiparous pre-eclamptics.

CONCLUSION

We conclude from our study that increased TGF β-1 level inhibits trophoblast invasion which may lead to pre-eclampsia. Thus, TGF β-1 may be involved in the pathogenesis of pre-eclampsia.

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