

## SERUM TUMOR NECROSIS FACTOR- $\alpha$ IN PRE ECLAMPSIA

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**Abstract** : Cytokines are major contributors in pathogenesis of pre eclampsia. Serum TNF- $\alpha$  was determined in 10 normal and 30 pre-eclamptic pregnant females with special reference to maternal age, parity and levels of mean arterial blood pressure. TNF- $\alpha$  was determined using sandwich ELISA technique. Serum TNF- $\alpha$  level in normal pregnant females was  $9.3 \pm 0.56$  pg/ml, while in pre-eclamptic pregnant females it was  $67.66 \pm 61.83$  pg/ml. This increase TNF- $\alpha$  was highly significant ( $P < 0.001$ ). There was no significant changes in serum TNF- $\alpha$  with respect to maternal age, parity and mean arterial pressure in both normal and pre-eclamptic pregnancies.

**Key words** : Serum TNF- $\alpha$

preeclampsia

### INTRODUCTION

Hypertensive disorders are the most common medical complications of pregnancy and are an important cause of maternal and perinatal morbidity and mortality. In spite of intensive efforts to delineate the pathophysiologic mechanisms of pre-eclampsia, neither a specific cause nor a pathogenesis has been identified. Previous studies suggested endothelial damage, vasoconstriction, placental ischemia and enhanced coagulation caused by a serum factor produced by trophoblasts (1, 2).

Endothelial cell dysfunction has been hypothesized to play a major role in

pathogenesis of pre-eclampsia. This hypertensive disease of pregnancy is characterized by changes in placenta, uteroplacental vasculature, kidney and liver consistent with endothelial damage. Neutrophil activation, and platelet activations, have been demonstrated in pre-eclampsia and are consistent with endothelial dysfunction (3, 4, 5).

Inflammatory cytokines are known to be potent activators of vascular endothelial and have been proposed as mediators of endothelial dysfunction during pre-eclampsia (4, 5). TNF- $\alpha$  is a cytokine derived from macrophages, lymphocytes, vascular endothelial cells, trophoblasts and Hofbauer

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cells in placenta; it induces functional alterations in endothelial cells (6).

TNF- $\alpha$  upregulates endothelial expression of platelet derived growth factor, endothelin-1 and plasminogen activator inhibitor-1, all of which are associated with vasoconstriction and are found to be elevated in pre-eclampsia (4). TNF- $\alpha$  also has been shown to cause microvascular protein leakage and hypertriglyceridemia which are associated with pre-eclampsia (7, 8).

Our purpose in this investigation was to compare TNF- $\alpha$  level in pre eclampsia patients with that in normotensive pregnant females.

## MATERIALS AND METHODS

The present study was conducted on 30 pre-eclamptic females (mean age  $25.53 \pm 3.90$  years) in their 3rd trimester of pregnancy selected from inpatient and outpatient department of Obstetrics & Gynaecology, JN Medical College, Aligarh. Pregnant females were considered to have pre-eclampsia if their blood pressure was persistently elevated to  $\geq 140/90$  mm Hg or if they had a mean arterial pressure (Diastolic pressure +  $1/3$  pulse pressure) of more than 110 mm Hg. The elevated blood pressure had to be present on at least two occasions 6 hours apart. In addition proteinuria of more than 300 mg in 24 hrs or 100 mg/dl had to be present. Exclusion criteria were previous history of (9, 10) :-

- Cardiac hepatic or renal disorder
- Diabetes mellitus
- Primary or secondary lipid disorders
- Concomitant severe complications of pregnancy

- Maternal age less than 20 years or more than 40 years
- Pregnancy less than 28 wks or more than 41 weeks
- Onset of labour
- Multiple pregnancy
- Any medication during pregnancy

Ten women (mean age  $25.00 \pm 3.71$  yrs) in 3rd trimester of pregnancy and apparently good health were selected as controls. Informed consent was taken from all subjects included in study. All the included patients were not exposed to any drugs or treatment. Diurnal variations of TNF- $\alpha$  was not included in the present study. Estimation of TNF- $\alpha$  was done with a commercially available kit (Diclone Research, France). Bioassay method was not adopted. Blood samples were collected between 9 to 10 AM.

Venous blood samples were collected in endotoxin free tubes. Blood samples were centrifuged at 1500 rpm for 10 minutes and serum was stored in polypropylene tubes at  $-20$  degree centigrade and TNF- $\alpha$  was assayed by Enzyme Linked Immuno sorbent Assay (ELISA) technique (Mois Junior Microplate Reader Model 2104, Merck, Germany) which is based on reaction between antibodies and antigen which are linked to a solid phase. A monoclonal antibody specific for TNF- $\alpha$  had been coated on to the wells of plate by microtitre strips provided by Diclone Research, France. Samples including standards of known TNF- $\alpha$  concentration, control and of patients were pipetted into those wells. During first incubation, TNF- $\alpha$  antigen and biotinylated monoclonal antibody specific for TNF- $\alpha$  were simultaneously incubated and, after

washing, enzyme (streptavidinperoxydase) was added. After incubation all unbound enzyme was removed by washing, then a substrate solution was added to induce a coloured reaction product. The intensity of coloured product was directly proportional to concentration of TNF- $\alpha$  in samples.

#### Statistical analysis

All TNF- $\alpha$  values are expressed as mean  $\pm$  SD and statistical analysis was done using the Student 't' test. Results were considered significant if 'P' value was  $<0.05$ . Correlation between TNF- $\alpha$  and mean arterial blood pressure was determined by co-efficient of correlation (r).

### RESULTS

In normal pregnancy, the concentration of TNF- $\alpha$  was  $9.3 \pm 0.56$  pg/ml, while in preeclampsia the level was  $67.66 \pm 61.83$  pg/ml ( $P < 0.001$ ), (Table I, Fig. 1).

Both the normal and preeclampsia subjects were divided in two groups according to age, parity and mean arterial pressure. In preeclampsia 43.33% (n=13) patients were below 25 years of age and

56.33% (n=17) were above 25 years of age, while in control group there were five subjects in each group. There was no significant change in TNF- $\alpha$  levels in patients and control according to age. TNF- $\alpha$  level in normal pregnant females below 25 years was  $9.6 \pm 7.12$  pg/ml and above 25 years of age was  $9.0 \pm 3.3$  pg/ml. TNF- $\alpha$  levels in pre-eclamptic subjects below 25 years was  $66.53 \pm 49.4$  pg/ml and in those above 25 years of age the mean level was  $68.52 \pm 71.38$  pg/ml (Table I).

In preeclampsia 50% patients were primiparous and 50% were multiparous. Control group was having 5 primiparous and five multiparous. Serum TNF- $\alpha$  in normal primiparous was  $9.6 \pm 7.12$  pg/ml and in multiparous was  $9.0 \pm 12.44$  pg/ml. TNF- $\alpha$  in pre-eclamptic primiparous was  $59.33 \pm 59.48$  pg/ml and in multiparous was  $76.0 \pm 65.06$  pg/ml. The change was not significant according to parity (Table I).

The pre-eclamptics were divided into 2 groups according to mean arterial pressure. Group 1 (n=18) with mean arterial pressure between 110–115 mm Hg and group II with MAP more than 115 mm Hg (n=12). The serum TNF- $\alpha$  did not change significantly

TABLE I: TNF-alpha (pg/ml) in normal and pre-eclamptic pregnancies divided on the basis of age, parity and mean arterial pressure (MAP).

	Normal pregnancy		Pre-eclampsia	
	No. of subjects	pg/ml	No. of subjects	pg/ml
All subjects	10	$9.3 \pm 0.56$	30	$67.66 \pm 61.83^*$
< 25 yrs age	5	$9.6 \pm 7.12$	13	$66.53 \pm 49.4^*$
$\geq 25$ yrs	5	$9.0 \pm 3.3$	17	$68.52 \pm 71.38^*$
Primipara	5	$9.6 \pm 7.12$	15	$59.33 \pm 59.48^*$
Multipara	5	$9.0 \pm 12.44$	15	$76.0 \pm 65.06$
MAP 110–115 mm Hg	–	–	18	$69.72 \pm 58.1$
MAP $\geq 115$ mm Hg	–	–	12	$64.5 \pm 69.5$

\*Significantly increase from control values.

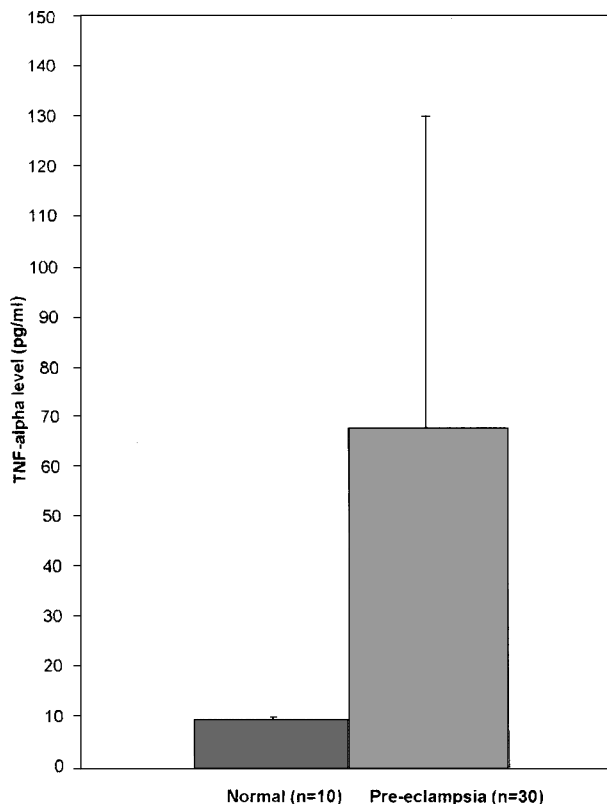


Fig. 1 : Serum TNF-alpha levels in normal and Pre-eclamptic pregnancies.

with increase in mean arterial pressure (Table I). When coefficient of correlation ( $r$ ) was determined the TNF- $\alpha$  showed no significant correlation with mean arterial pressure ( $P > 0.05$ ).

## DISCUSSION

Immunologic stimuli occurring as a part of adaptation of immune system to pregnancy may account for detection of TNF- $\alpha$  in plasma and amniotic fluid of some normotensive pregnant females. Maternal plasma TNF- $\alpha$  may originate from local release into systemic circulation or from systemic production by peripheral macrophages and lymphocytes in response to pregnancy. On the contrary increased

concentration of TNF- $\alpha$  in pre-eclampsia involves activation of immunologic system against foetal allograft (11). An altered immune response to foetal allograft might also play a role in impaired trophoblastic invasion into maternal spiral arterioles in pre-eclampsia by 16–20 weeks of gestation (12). The elevation of TNF- $\alpha$  in maternal blood and amniotic fluid in severe pre-eclampsia may be either an effect or a cause. TNF- $\alpha$  may be related to pathogenesis of pre-eclampsia and to exaggeration of normal immune response in pre-eclampsia.

In the present study concentration of TNF- $\alpha$  was higher in pre-eclamptic patients compared to normal pregnancy. TNF- $\alpha$  is detected more frequently and at higher concentrations in pre-eclampsia than in normal pregnancy. Because the half life of TNF- $\alpha$  is only a few minutes in circulation, a single blood sample might fail to detect a periodic elevation or decline in TNF- $\alpha$  levels. In our present study only a single sample could be drawn due to budgetary constraints. Further, an unknown portion of TNF- $\alpha$  bound to its soluble receptor might not be detected by bio reactive or immuno reactive assays.

Elevated serum TNF- $\alpha$  levels in pre-eclamptic female could explain increase blood pressure as TNF- $\alpha$  has an inhibiting effect on nitric oxide and stimulatory action on endothelin-1 and prostaglandins (13, 14). Yang et al showed that TNF- $\alpha$  can induce endothelial dysfunction and injury to ultra structure of placenta and umbilical vascular endothelium. This injury may play a role in the pathogenesis of pregnancy induced hypertension (14).

The important findings in literature of TNF- $\alpha$  role in preeclampsia can be

understood by evaluating the report of Vercruysse et al who localized TNF- $\alpha$  in the placental bed by immuno localization method using placental bed biopsies. They found that in early pregnancy TNF staining was detected in proliferative tip of microvilli of cytotrophoblast, and in endovasculature, but in term pregnancy TNF staining was seen in trophoblastic microvilli and foam cells. This showed the role of cytokines in pathogenesis of preeclampsia (12). There have many reports in literature about the congenital risk in pre eclampsia on the basis of cytokines and TNF- $\alpha$  during pregnancy,

and labour at term and preterm (15, 16). The present study supports the previous report that TNF- $\alpha$  played role in pathogenesis of preeclampsia.

In the present study the small sample size and single blood sample prevent us from reaching a conclusion on the exact relationship between preeclampsia and serum - TNF- $\alpha$  levels. This is specially so in view of the fact that three preeclamptic did not have elevated TNF-alpha. A larger study including more subjects may be necessary.

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