

# Comparison of maternal serum Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) in severe and mild preeclampsia versus normal pregnancy

Fatemeh Vahid Roudsari<sup>1</sup> M.D., Sedigheh Ayati<sup>1</sup> M.D., Hossein Ayatollahi<sup>2</sup> M.D., Habibollah Esmaeily<sup>3</sup> Ph.D., Maliheh Hasanzadeh<sup>1</sup> M.D., Masoud Shahabian<sup>1</sup> M.D., Leila Pour Ali<sup>1</sup> M.D.

- 1 Department of Obstetrics and Gynecology, Ghaem Hospital, Women's Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
- 2 Department of Hematology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.
- 3 Department of Medicosocial, Ghaem Hospital, Women's Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

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## Abstract

**Background:** Preeclampsia is a disorder unique to pregnancy and has long been recognized as an important contributor of maternal and fetal morbidity and mortality. It is suggested that cytokines such as Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) have an important role in the pathogenesis of preeclampsia and may cause generalized endothelial dysfunction.

**Objective:** The aim of this study was comparison of maternal serum TNF- $\alpha$  in severe and mild preeclampsia versus normal pregnancy.

**Materials and Methods:** This study was performed on 37 women with preeclampsia (17 mild and 20 severe preeclampsia) and 41 normotensive pregnant women with similar gestational age at third trimester of pregnancy. All the preeclamptic cases had blood pressure  $\geq 140/90$  mmHg, and proteinuria  $\geq 300$  mg in a 24-h urine sample. Maternal serum TNF- $\alpha$  concentration was compared in all of them.

**Results:** The level of TNF- $\alpha$  concentration was not statistically different between the studied groups. No significant correlation was found between preeclampsia and control group as they were compared in the view of maternal serum TNF- $\alpha$  concentration.

**Conclusion:** These findings suggest that serum TNF- $\alpha$  is not significantly associated with preeclampsia.

**Key words:** Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), Preeclampsia, Normal pregnancy.

## Introduction

The term preeclampsia describes the development of new onset hypertension ( $\geq 140/90$  mmHg) and proteinuria ( $\geq 300$  mg/24h) after the 20th week of gestation which occurs in approximately 5-7% of all pregnancies (1). This disorder is unique to human in which numerous

genetics, immunological and environmental factors interact (2). Therefore, it is a leading cause of maternal and fetal morbidity and mortality throughout the world and still is one of the most complex problems in obstetrics (3). Its etiology is unknown, but endothelial dysfunction can be a causative factor. The new hypothesis regarding the preeclampsia etiology has been focused on immune responses. Cytokines are immune-regulatory substances that may involve in the pathogenesis of preeclampsia. Successful pregnancy is a Th2 phenomenon, which in it Th1/Th2 shifts to Th2 type reaction. Type 1 cytokines including interleukine-2, interferon (IFN),

### Corresponding Author:

Sedigheh Ayati, Department of Obstetrics and Gynecology, Ghaem Hospital, Women's Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

**E-mail:** ayatis@mums.ac.ir

and tumor necrosis factor-alpha (TNF- $\alpha$ ) are more produced in preeclampsia induce inflammation (4). TNF- $\alpha$  cytokine is a 17kd peptide, which is soluble mediator of cellular immunity (5). One postulated mechanism for development of preeclampsia involves abnormal activation of immune system against fetal allograft (6). Cytokines and growth factors have been identified as functional proteins in the placenta, but their roles in normal placental development and therefore in pathological placental disease have not been determined (7, 8). Pathologically secreted TNF- $\alpha$  damages the vascular endothelial cells, causes occlusion of vessels, reduces regional blood flow, and increases permeability of endothelium (9). TNF- $\alpha$  mediated activation of immune system may result in secretion of vasoactive substances due to endothelial injury and lead to vascular permeability and intravascular coagulation (10). TNF- $\alpha$  is a proinflammatory cytokine and its biological activity is inflammation and endothelial cell activation. The sources of TNF- $\alpha$  production in preeclampsia are neutrophils and monocytes and possibly placenta. One possible mechanism in preeclampsia is factors derived from placenta which stimulate monocytes and neutrophils to produce TNF- $\alpha$  that lead to endothelial disturbances (11). Therefore, these findings suggest that increased serum TNF- $\alpha$  may be a part of preeclamptic pathology. In normal pregnancy, TNF- $\alpha$  can modify the growth and invasion of trophoblasts in maternal spiral arteries (12). Moreover, it may contribute to abnormal placentation, oxidative stress and endothelial disturbances (13).

There is increasing interest in possible relationship between endothelial dysfunction and infection, inflammation, and preeclampsia (14, 15). Infection may be a major risk factor for preeclampsia and it may cause increased cytokine levels sufficient to change vascular endothelial function, and 'prime' susceptible individuals for the future development of preeclampsia (16). In one study performed in 2008, increased expression of TNF- $\alpha$  mRNA in the placenta of preeclampsia has been reported (4). Recent studies have demonstrated that some cytokines mediators of inflammatory response may cause endothelial dysfunction through different mechanisms such as oxidative stress and endothelial cell damage (12). Some studies have demonstrated the possible role of these cytokines in the pathophysiology of preeclampsia (11, 17). In a study by Sharma and coworkers performed on 104 cases of preeclampsia, normal pregnancy and non-pregnant

women, the levels of TNF- $\alpha$  were increased significantly in preeclampsia in comparison with the healthy pregnant and non-pregnant groups (11). The aim of this study was comparison of maternal serum TNF- $\alpha$  in severe and mild preeclampsia versus normal pregnancy.

## Materials and methods

This cross-sectional study was performed on 37 women complicated by preeclampsia (17 mild and 20 severe preeclampsia) and 41 normotensive pregnant women who had referred to Maternity ward of Ghaem Hospital Institute of Mashhad University of Medical Sciences in 2006. The study was approved by the Ethical Institutional Committee of Mashhad University. Written informed consent was obtained from all participants. The blood was drawn prior to delivery at the time of admission.

The including criteria was the patients who were primigravid, had third trimester pregnancy, BP  $\geq$  140/90 mmHg, and proteinuria  $\geq$  300 mg in a 24-h urine sample. Patients with renal diseases, chronic hypertension, renal and urinary infection, fetal disorders, multiple pregnancy and immunologic diseases were excluded from the study.

Pregnant women without above criteria were considered as control group. The preeclamptic women were divided into two groups of mild (17 cases) and severe (20 cases) preeclampsia. Mild preeclampsia was defined as blood pressure  $\geq$  140/90mmHg and  $<$ 160/110 mmHg with proteinuria  $\geq$  300-2000 mg/24h after the 20th week of gestation and severe preeclampsia was defined as blood pressure  $\geq$ 160/110mmHg with massive proteinuria  $>$  2 g/24h or other signs and symptoms of severe preeclampsia such as persistent headache, visual disturbances, epigastric pain and thrombocytopenia (2).

Two groups were separately compared with control group. A questionnaire was completed for each patient including patient's age, gestational age, parity, the history of hypertension, diabetes mellitus, hypertension in family, diabetes mellitus in family, tobacco consumption, weight and body mass index (BMI). A total of 5CC peripheral venous blood was taken from each woman and sent to the central laboratory of Ghaem Hospital. The blood was centrifuged for 10 minutes and serum was separated and stored at  $-20^{\circ}$  C. The level of marker (TNF- $\alpha$ ) was measured by Enzyme-linked immune Sorbent assay (ELISA) (TNF- $\alpha$  Austria-Bederved Kit).

**Statistica analysis:**

Data was analyzed by SPSS software version 11.5, using analysis of One-way ANOVA and Tukey HSD tests.  $p \leq 0.05$  was considered statistically significant.

**Results**

Level of TNF- $\alpha$  was measured in serum samples from 78 pregnant women (17 mild and 20 severe preeclampsia) (case group) and 41 healthy pregnant women (control group). There was no significant difference in the mean age between severe, mild preeclampsia and control group ( $p=0.53$ ). Two groups were not different in the view of parity ( $p=0.06$ ). Gestational age was  $36 \pm 3.3$  in mild preeclampsia,  $34 \pm 3.8$  in severe

preeclampsia and  $39 \pm 1.0$  in the control group. Three groups were significantly different when they were compared in the view of gestational age ( $p < 0.001$ ). Birth weight was  $2716 \pm 825$  g in mild preeclampsia,  $2195 \pm 942$  g in severe preeclampsia and  $3177 \pm 431$  in the control group. They were significantly different in the view of newborn weight ( $p < 0.001$ ) (Table I).

The mean and variance of TNF- $\alpha$  concentration in mild, severe and control group was  $2.89 \pm 3.70$  pg/ml,  $3.70 \pm 3.11$  pg/ml and  $3.58 \pm 3.99$  pg/ml, respectively. When compared with mild preeclampsia and normal pregnancy, the mean of TNF- $\alpha$  concentration was higher in severe preeclampsia. As it has been shown in Table II, this increase in severe cases was not statistically significant ( $p = 0.31$ ).

**Table I.** Demographic characteristics of the women referred to Ghaem Hospital.

Parameters	Mild preeclampsia		Severe preeclampsia		Control		p-value
	Mean	Variance	Mean	Variance	Mean	Variance	
Age (years)	27.7	5.7	28.2	6.6	26.3	5.2	0.53
Gestational age (weeks)	36	3.3	34	3.8	39	1	0.000
Parity	1.6	1.1	2.1	1.5	1.4	1.1	0.06
Newborn weight (gr)	2716	825	2195	942	3177	431	0.000

**Discussion**

The present study demonstrated an elevated mean level of TNF- $\alpha$  in the maternal plasma of severe preeclamptic patients compared with mild preeclampsia and normal pregnancy. But this increase in concentration of TNF- $\alpha$  was not statistically significant.

Tavakol Afshari and co workers in 2005 reported no increased serum concentration of TNF- $\alpha$  in 24 preeclamptic patients compared to 18 control healthy pregnant women (18). Their finding is in consistent with our finding. Several investigators have reported that serum concentration of TNF- $\alpha$  were significantly higher in the first and second trimester among pregnant women who subsequently developed preeclampsia compared to those in control group. In the study performed by Kocyigit, the concentration of TNF- $\alpha$  was significantly higher in preeclampsia group (19). In other prospective studies, first trimester TNF- $\alpha$  was significantly higher in women who subsequently developed preeclampsia compared with those who did not (20, 21). Some other researchers also reported the same results (22-26). However, our data indicated that increased TNF- $\alpha$  concentration in severe preeclampsia was not statistically significant when compared with mild preeclampsia and control group. Statistical

difference in various studies may be due to the effect of genetics and environmental factors in preeclampsia.

Some studies suggested that infection and inflammatory processes are related to preeclampsia (27, 28). The role of inflammation and infection in the pathogenesis of preeclampsia is significant in developing countries, where the high incidence of chronic subclinical infection may contribute to the high incidence of preeclampsia (18). Because of the heterogeneous nature of patients with severe preeclampsia and small number of patients in this study, it would be necessary to undertake further studies with more samples in different regional areas. So far, management of preeclampsia was concentrated on signs like hypertension, whereas treatment of immune responses may be a possibility in the future.

**Conclusion**

In conclusion, the use of cytokines to predict preeclampsia is still controversial. These findings and previous studies demonstrated that TNF- $\alpha$  may be involved in the pathogenesis of PE and may identify the patients who are at high risk of PE. However, for close information, further studies in a large volume from different population are required.

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## References

- Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. *Am J Obstet Gynecol* 2005; 193: 860-863.
- Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC, Wenstrom KD. Williams Obstetrics. 22th ed. Philadelphia: McGraw Hill; 2005: 765-768.
- Vahid Roudsari F, Ayati S, Torabizadeh A, Ayatollahi H, Esmaeli H, Shahabian M. Serum calcium and magnesium in preeclamptic and normal pregnancies; A comparative study. *Journal of Reproduction & Infertility* 2008; 9: 256-262.
- Mirahmadian M, Kalantar F, Heidari G, Safdarian L, Mansouri R, Amirzargar AA. Association of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in Iranian patients with preeclampsia. *Am J Reprod Immunol* 2008; 60: 179- 185.
- Gulti R. Raised serum TNF-alpha, blood sugar and uric acid in preeclampsia in third trimester of pregnancy. *J Nep Med Assoc* 2005; 44: 36-38.
- Sibai BM. Immunological aspects of preeclampsia. *Clin Obstet Gynaecol* 1991; 34: 27-34.
- Tranchot-Diallo J, Gras G, Parnet-Mathieu f, Benveniste O, Marce D, Roques P, et al. Modulations of cytokine expression in pregnant women. *Am J Reprod Immunol* 1997; 37: 215-226.
- Kaumba S, Matt D, Sytrom S, Eierman D, Turner T. Interleukin-1beta, human leukocyte antigen HLA-DR alpha, and transforming growth factor-beta expression in endometrium, placenta and placental membranes. *Am J Obstet Gynecol* 1990; 163: 1430-1437.
- Hunt JS. Cytokines network in uteroplacental unit, macrophages as pivotal regulatory cell. *J Reprod Immunol* 1989; 16: 1-17.
- Ari KI, Lee F, Miyajima A, Miyatake S, Ari N, Yokata T. Cytokines: coordinators of immune and inflammatory responses. *Annu Rev Biochem* 1990; 59: 783-836.
- Sharma A, Satyam A, Sharma JB. Leptin, IL-10 and inflammatory markers (TNF-alpha, IL-6 and IL-8) in preeclamptic, normotensive pregnant and healthy non-pregnant women. *Am J Reprod Immunol* 2007; 58: 21-30.
- Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med* 1986; 163:740-745.
- Kupfermink MJ, Peaceman AM, Wington TR, Tamura RK, Rehnberg KA, Socol ML. Immunoreactive tumor necrosis factor-alpha is elevated in maternal plasma but undetected in amniotic fluid in the second trimester. *Am J Obstet Gynecol* 1994; 171: 976-979.
- Vallance P, Collier J, Bhagat K. Infection, inflammation and infraction: dose acute endothelial dysfunction provide a link? *Lancet* 1997; 349: 1391-1392.
- Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Mclaughlin MK, Hubel CA. Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol* 1989; 161: 1200-1204.
- Herrera JA, Chaudhuri G, Lopez-Jaramillo P. Is infection a major risk factor for preeclampsia? *Med Hypotheses* 2001; 57: 393-397.
- Teran E, Escudero C, Moya W, Flores M, Vallance P, Lopez-Jaramillo P. Elevated C-reactive protein and pro-inflammatory cytokines in Andean women with preeclampsia. *Int J Gynecol Obstet* 2001; 75: 243-249.
- Tavakol Afshari J, Ghomian N, Shameli A, Shakeri MT, Fahmidehkar MA, Mahajer E, et al. Determination of interleukin-6 and tumor necrosis factor-alpha concentrations in Iranian-Khorasanian patients with preeclampsia. *BMC Pregnancy and Childbirth* 2005; 5: 14.
- Kocyigity Y, Atamer Y, Atamer A, Tuzcu A, Akkus Z. Changes in serum levels of leptin, cytokines and lipoprotein in preeclamptic and normotensive pregnant women. *Gynecol Endocrinol* 2004; 19: 267-273.
- Hamai Y, Fujii T, Yamashita T, Nishina H, Kozuma S, Mikami Y et al. Evidence for an elevation in serum interleukin-2 and tumor necrosis factor-alpha levels before the clinical manifestations of preeclampsia. *Am J Reprod Immunol* 1997; 38: 89-93.
- Omu AE, Al-Qattan F, Diejomach ME, Al-Yatama M. Differential levels of T helper cytokines in preeclampsia: pregnancy, labor and puerperium. *Acta Obstet Gynecol Scand* 1999; 78: 675-680.
- Kupfermink MJ, Peaceman AM, Aderka D, Wallach D, Payser Mr, Lessing JB, Socol ML. Soluble tumor necrosis factor receptors in maternal plasma and second-trimester amniotic fluid. *A J Obstet Gynecol* 1995; 173: 900-905.
- Teran E, Escudero C, Moya W, Flores M, Vallance P, Lopez-Jaramillo P. Elevated C-reactive protein and pro-inflammatory cytokines in Andean women with preeclampsia. *Int J Gynaecol Obstet* 2001; 75: 243-249.
- Greer IA, Lyall F, Perera T, Boswell F, Macara LM. Increased concentrations of cytokines interleukin-6 and interleukin-1 receptor antagonist in plasma of women with preeclampsia: a mechanism for endothelial dysfunction? *Obstet Gynecol* 1994; 84: 937-940.
- Heyl W, Handt S, Reister F, Gehlen J. Elevated soluble adhesion molecules in women with preeclampsia. Do cytokines like tumor necrosis factor-alpha and interleukin-1 beta cause endothelial activation. *Eur J Obstet Gynecol Reprod Biol* 1999; 86: 35-41.
- Ellis J, Wennerholm UB, Bengtsson A, Lilja H, Pettersson A, Sultan B, et al . Levels of dimethyl-arginines and cytokines in mild and severe preeclampsia. *Acta Obstet Gynecol Scand* 2001; 80: 602-608.
- Hill JA, Devoe LD, Bryans CI Jr. Frequency of asymptomatic bacteriuria in preeclampsia. *Obstet Gynecol* 1986; 67: 529-532.
- Hsu CD, Witter FR. Urogenital infection in preeclampsia. *Int J Gynecol Obstet* 1995; 49: 271-275.