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RESEARCH ARTICLE

SERUM TUMOR NECROSIS FACTOR ALPHA LEVELS IN PREECLAMPTIC AND NORMOTENSIVE PREGNANT WOMEN. A COMPARATIVE STUDY IN A SEMI URBAN NORTH INDIAN POPULATION

[1]Dr. Fozia Amin Patigaroo, [2]Dr. Waseem Iqbal, [3]Dr. Rajiv Gulati, [4]Dr. Zehra Mohsin and [5]Dr. Suhail Amin Patigaroo

[1]Demonstrator, Dept of Physiology, Government Medical College, Baramulla, JK
[2]Assistant Professor, Department of Pediatrics, Government Medical College, Baramulla, JK
[3]Professor, Department of Physiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, UP
[4]Associate Professor, Department of Obstetrics & Gynaecology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, UP
[5]Assistant Professor, Department of Otirhinolaryngology, Government Medical College, Srinagar, JK

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ABSTRACT

Background: Preeclampsia is a multisystem disorder of pregnancy hypothesized to be due to endothelial cell dysfunction that increases the maternal and fetal morbidity and mortality. Although intensive research has been done, the etiopathogenesis of preeclampsia (PE) remains uncertain. Inflammatory cytokines are thought to be involved in the pathogenesis of preeclampsia.Preeclampsia occurs in two stages. In the first stage there is reduced placental perfusion because of abnormal implantation and development of the placental vasculature. In the second stage there is widespread inflammation resulting in maternal endothelial cell dysfunction. TNF alpha is an inflammatory cytokine expressed by neutrophils, monocytes and macrophages. **Material and methods:** The present study isa non-randomized prospective case control study comparing the serum levels of TNF alpha in thirty preeclamptic and thirty normotensive pregnant women during third trimester of pregnancy. Preeclamptic women were divided according to severity (BP and level of proteinuria) into mild (n=15) and severe (n=15). TNF alpha estimation was done by ELISA. **Results:**TNF alpha levels were significantly higher in preeclamptic women compared to normotensive pregnant women (p=0.001). Serum TNF alpha levels were higher in severe preeclampsia than mild preeclampsia (p=0.043). These results suggest that normal pregnancy is a state of mild inflammation which becomes exaggerated in preeclampsia. **Conclusion:** The levels of TNF alpha are related with severity of preeclampsia. TNF alpha can be used as a predictive marker for preeclampsia.

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INTRODUCTION

Preeclampsia is defined as hypertension (systolic blood pressure of ≥140mm Hg and /or diastolic blood pressure of ≥90 mm Hg) and substantial proteinuria (≥300mg in 24 h) at or after 20 weeks of gestation.[1] Preeclampsia is a frequent and potentially severe disease that affects about 3-8% of all pregnancies and increases the mothers risk for morbidity.[2-4]The worldwide incidence of preeclampsia is 3–4% of all pregnancies[5] and in India it is 2.8%.[6]The risk factors associated with preeclampsia include nulliparity, preexisting medical conditions (e.g. hypertension, diabetes mellitus and anti-phospholipid syndrome), older maternal age, multifetal gestation and obesity.[2,3] The etiology of preeclampsia is not fully understood but various mechanisms have been proposed to be involved in the disease pathophysiology. The only definitive cure is delivery[7].During normal pregnancy when endovascular trophoblasts invade the uterine spiral arterioles, those are extensively remodeled replacing the vascular endothelial and muscular linings enlarging the vascular diameter. In preeclampsia this invasion is incomplete and superficial (abnormal implantation) so that the decidual vessels are invaded but deeper myometrial arterioles are not affected and their diameter is only half that of vessels in normal placenta.[8] Placental oxidative stress directly and indirectly leads to oxidative stress in the maternal circulation because of spillage of placental debris and syncytiotrophoblast microvesicles in the maternal circulation which leads to activation of maternal neutrophils.[9] Activated neutrophils release tumor necrosis factor alpha and interleukin-6 which contribute to the pathogenesis of preeclampsia. [10] TNF alpha is a polypeptide cytokine produced by activated neutrophils, monocytes and macrophages and has a potentially cytotoxic effect to trophoblasts.[11] TNF alpha cytokine is a 17 KD peptide, which is a soluble mediator of cellular immunity.[12] TNF alpha circulates throughout the body responding to infective stimuli

Corresponding author: **Waseem Iqbal,**
Government Medical College, Kanth Bagh, Baramulla, India.

or tissue injury by activation of neutrophils and altering the vascular endothelial cells.[13,14]In normal pregnancy TNF alpha can modify the growth and invasion of trophoblasts in maternal spiral arteries.[15] T helper cells play an important role in immunoregulation.

Th1 cells stimulate inflammatory cytokine secretion and Th2 cells promote humoral immunity. In non-pregnant woman there is balance between Th1 and Th2 response. In normal pregnancy there is shift of maternal immune response from Th1 towards Th2 [16-18] which is termed as Type 2 bias.[19] There is predominant Th1 type immunity present in preeclampsia. [20] In this study, we compared the serum levels of TNF alpha in preeclamptic women with the normotensive pregnant women.

MATERIALS AND METHODS

This study was a non-randomized prospectivecase control study conducted on women attending the Gynaecology & Obstetrics Inpatient and Outpatient Department of J.N. Medical College Hospital, A.M.U., Aligarh. The study design was observational, nonetheless proper consent and approval from the patients and caretakers was taken as per preformed protocol. Necessary ethical clearance was obtained from the Institute Ethics Committee.

Thirty preeclamptic cases and an equal number of age, parity and gestational age matched normotensive pregnant women in third trimester of pregnancy were included in the study. The cases were considered to have preeclampsia if previously they were normotensive but after 20 weeks of gestation their BP was raised to or above 140 mmHg systolic and/or 90 mmHg diastolic and when proteinuria of ≥300 mg/24 h (or ≥1+ on dipstick) of urine sample was present.

Exclusion criteria

- Renal disease or renal and urinary tract infection.
- Chronic hypertension or cardiac disease or patients on antihypertensive treatment.
- Clinical or biochemical evidence of metabolic disorders like diabetes mellitus.
- Lipid disorders.
- Maternal age <20 years or >40 years.
- Pregnancy of <28 weeks or >41 weeks.
- Multiple pregnancy
- Patients already in labour.

5 ml random venous blood sample from antecubital vein was collected. Serum was obtained by centrifugation at 1500 RPM for 10 minutesand it was cryopreserved at-20ºC. Human TNF alpha ELISA kit (Gen-Probe Diaclone) was used for the quantitative measurement of TNF alpha in the serum of selected subjects.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences Version 22 (SPSS 22) for Windows software and Microsoft Office Excel 2007. The results were expressed as Mean ± Standard Deviation (S.D.). Unpaired t tests for independent samples were used in comparing continuous data between two groups. p value of less than 0.05 was taken as statistically significant.

RESULTS

The anthropometric parameters of the subjects is are depicted in table 1. There was no significant variation between preeclamptic women(cases) and women with normal pregnancy (controls) in terms of maternal age, parity and gestational age. Maximum number of subjects in both the groups were in 20-24 year age group with gestational age of 35-40 weeks. Systolic and diastolic blood pressure (Table 2), maternal weight, Body Mass Index and Mid Arm Circumference was significantly higher in cases (Table 1). The serum Tumor Necrosis Factor alpha (TNF alpha) levels in caseswas91.58 ± 17.79 pg/ml and in controls was 19.93 ± 6.58 pg/ml (Table 3).

In the preeclamptic group 60% (n=18) were at < 37 weeksand 40% (n=12) were ≥ 37 weeks of gestation. While in the control group 56.66% (n=17) were < 37 weeks and 43.33% (n=13) were ≥ 37 weeks of gestation. There was no significant difference (p>0.05) in the tumor necrosis factor alpha levels within the groups (Gestational age < or ≥ 37 weeks) (Table 4). In the preeclamptic group, there were 50% (n=15) subjects each with mild and severe preeclampsia. The TNF alpha levels in mild group was 85.13 ± 19.64 pg/ml and in severe preeclampsia 98.02 ± 13.46 pg/ml (p=0.043) (Table 5).

DISCUSSION

Pro-inflammatory cytokines have been found to be elevated in normal pregnancy,[21] but such elevation is exaggerated in preeclampsia.[22] Normal pregnancy is accompanied by a mild pro-inflammatory status [23], whereas preeclampsia is marked with an intense maternal inflammatory response. [24] TNF alpha is a proinflammatory cytokine which has a role in the pathogenesis of preeclampsia.[25,26] In preeclampsia abnormal implantation causes placental oxidative stress which leads to activation of maternal neutrophils. Activated neutrophils release TNF alpha. It may cause tissue damage either directly through activation of protease, collagenase or phospholipase A2 or through oxygen radicals .[27]

Direct damage to vascular endothelial cells, reduced regional blood flow, occlusion of vessels and increased endothelial permeability are characteristic effects of pathologically secreted TNF alpha.[28] The present study found significant increase (p=0.001) in TNF alpha levels in preeclamptic pregnancies and these findings are in accordance with those of Serin et al.[29] , Sharma et al.[30] and Muzzamil et al.[31]The findings of Catarino et al.[32]and Kalantar et al.[33] are also similar to our study suggesting the role of TNF alpha in the pathogenesis of preeclampsia. However, Afshari et al.[34] and Roudsari et al.[35] found no significant difference in TNF alpha concentrations in preeclamptic women compared to normal pregnant women. In the present study there was no significant difference (p>0.05) in TNF alpha levels with advancing gestational age both in normal pregnancy and preeclampsia. Our findings are similar to Kupfermic et al.[36] who found no correlation of TNF alpha with gestational age. Although labor appeared to increase the concentration of TNF alpha in normal pregnancy, further elevations in TNF alpha concentrations were seen in preeclamptic parturients.

Table 1. Anthropometric parameters

Parameters	Cases (n=30)	Controls (n=30)	p value
Age (years)	26.13 ± 4.35	24.63 ± 2.98	0.124
GA (weeks)	35.63 ± 2.17	36.17 ± 1.5	0.266
Weight (Kg)	63.87 ± 5.61	57.35 ± 1.35	0.001*
Height (cm)	150.37 ± 2.79	151.15 ± 3.76	0.364
BMI (Kg/m²)	28.26 ± 2.60	25.14 ± 1.17	0.001*
MAC (cm)	28.20 ± 2.52	25.65 ± 1.89	0.001*

All the parameters in the table are expressed as Mean ± S.D. GA=gestational age, BMI=Body mass index , MAC=Mid arm circumference *p value significant

Table 2 Blood Pressure

	Cases (n=30)	Controls (n=30)	p value
SBP (mm Hg)	157.4 ± 11.24	117.67 ± 6.56	0.001*
DBP (mm Hg)	105.33 ± 7.62	76.4 ± 5.49	0.001*

SBP=systolic blood pressure, DBP=diastolic blood pressure

Table 3. TNF alpha in cases and controls

Parameter	Cases (n=30)	Controls (n=30)	p value
TNF alpha (pg/ml)	91.58 ± 17.79	19.93 ± 6.58	0.001*

Table 4. TNF alpha levels according to gestational age

Parameter	Cases Controls	GA <37 weeks (n=18) (n=17)	GA ≥37 weeks (n=12) (n=13)	p value
TNF alpha (pg/ml)	Cases	90.01 ± 14.92	93.93 ± 21.93	0.564
	Controls	20.44 ± 6.88	19.26 ± 6.38	0.635

GA=gestational age

Table 5. TNF alpha levels in mild and severe preeclampsia

Parameter	MPE (n=15)	SPE (n=15)	Controls (n=30)	p value
TNF alpha (pg/ml)	85.13 ± 19.64	98.02 ± 13.46	19.93 ± 6.58	0.043*

MPE=mild preeclampsia, SPE=severe preeclampsia

The present study found that severe preeclamptics had higher concentrations of TNF alpha compared to mild preeclamptics. Similar findings were reported by Tosun et al.[37] TNF alpha has an inhibitory effect on nitric oxide [38] and stimulatory action on endothelin-1.[39] This may explain the elevated blood pressure in preeclampsia.

The sensitivity to pressor agents is also increased.[40]TNF alpha increases endothelial permeability and causes intravascular protein leakage leading to proteinuria. Endothelial dysfunction activates the coagulation cascade resulting in formation of microthrombi and loss of fluid from intravascular space.[41] However Meekins et al.[42] and Roudsari et al.[35]found no correlation of TNF alpha with the severity of preeclampsia. Ellis et al.[43] also reported no significant difference between the mild and severe preeclamptic group. This statistical difference may be due to genetic and environmental factors in preeclampsia. Preeclampsia causes maternal mortality and morbidity. TNF alpha has a role in the pathogenesis of preeclampsia. Use of cytokines in early detection of patients at risk of preeclampsia is still controversial. Further studies are needed to investigate its role in preeclampsia.

Conclusion

The levels of Serum TNF alpha are positively related with severity of preeclampsia and therefore Serum TNF alpha can be utilized as a useful marker in late pregnancy to predict the severity of preeclampsia and plan management.

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