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Original Research Article

Predictive value of inflammatory Cytokines in preeclampsia

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Abstract

Preeclampsia (PE) is a multisystem disorder associated with maternal hypertension, placental abnormalities and adverse fetal outcomes. Endothelial dysfunction and immunological imbalance are the proposed etiological factors for the development of PE. The aim of the study was to assess the concentration of cytokines (IL-1β,IL-6,IL-8 and TNF-α) with progression of normal pregnancy and development of PE.40 primigravidas with uncomplicated normal pregnancies in first trimester which were followed till the last trimester (control) and 35 primigravidas who developed PE (study group) in the third trimester were selected by random sampling. A demographic characteristic along with assessment of cytokines was determined in normotensive and preeclamptic pregnant females. The levels of TNF-α, IL-6 and IL-8 are significantly increased ($p<0.001$) in PE when compared with the levels in normal healthy controls and normal pregnant females in the third trimester. Also, there is a significant progression ($p<0.001$) in the levels of TNF-α from the first trimester in females who subsequently developed PE. The present data indicates that measurement of TNF-α early in pregnancy can be used to predict the progression of PE.

Keywords: Preeclampsia, Interleukins

1. Introduction

Pre-eclampsia (PE) is a multi system disorder specific to pregnancy characterized by blood pressure exceeding 140/90mmHg after the twentieth week of gestation and proteinuria exceeding 0.3g/24 hours. In the Antenatal Care Trial by the World Health Organization, 9.1% of pregnant women had either PE or gestational hypertension (HTN).[1] If untreated, PE can lead to eclampsia, a life-threatening maternal neurovascular complication with severe HTN and convulsions.[2] It affects around 5% of all the pregnancies in the western world and remains a major cause of fetal and maternal morbidity and mortality. Numerous theories like increased serum levels in the fibronectin[3], disturbance in the thromoxane A2/prostacyclin balance, increased levels of factor VIII-related antigen to factor VIII coagulation activity[4], disturbance of nitric oxide production[5] hypothesize that endothelial cell damage of the maternal vascular endothelium is involved in the pathogenesis of PE.

The maternal immune system is said to have an influence on the placentation and subsequent systemic reaction. Dadelszen[6] suggested that PE may be a result of an infectious trigger. The cytokine network is known to elicit a wide spectrum of biological activities. They are known vasoconstrictors capable of producing endothelial cell activation and dysfunction. The placenta expresses a variety of pro and anti-inflammatory cytokines, adipokines and cytokine-like angiogenic growth factors, production of which is altered in PE, driven (at least in part) by hypoxia. Studies[7] in hypertensive pregnant women and experimental animal models suggest that reduction in utero-placental perfusion pressure and the ensuing placental ischemia/hypoxia during late pregnancy may trigger the release of placental factors that initiate a cascade of cellular and molecular events leading to endothelial and vascular smooth muscle cell dysfunction and thereby increased vascular resistance and arterial pressure.

Inadequate cytotrophoblast invasion of the uterine spiral arteries cause a reduction in uterine perfusion pressure and the ensuing placental ischemia. Oxidants and inflammatory cytokines overproduced by the placenta in response to hypoxia may then lead to increased plasma levels and endothelial activation and dysfunction in PE. Though studies[8][9] have indicated variations in the levels of cytokines in PE, most of them have involved women with established PE. Little is known regarding the levels of these immunomodulators with progression of pregnancy leading to PE and it remains uncertain whether enhanced levels of these immunomodulators are present before the clinical signs of PE develop.

1.1 Objectives

1) To assess the levels of cytokines (IL-1β, IL-6, IL-8 and TNF-α) with progression of normal pregnancy and those who developed PE.
2) To evaluate a possible association between the levels of interleukins in the prediction of PE early enough.

2. Materials and Methods

2.1 Study population

A prospective study approved by the institutions ethics committee (NKPSIMS/7/2010 dated 20/8/2010) was carried out at NKP Salve Institute of Medical Sciences, Nagpur, India. 40 primigravidas with uncomplicated normal pregnancies in first trimester which were followed till the last trimester (control) and 35 primigravidas who developed PE (study group) in the third trimester were selected by random sampling.

2.2 Inclusion criteria

All subjects within the age group of 18-35 years were selected for the study. PE was defined as persisting elevated diastolic blood pressure (90mmHg), a proteinuria (>300 mg in a 24 urine sample) and the presence of edema. Subjects willing to participate were included in the study. The females with normal pregnancy were included as the control group.

2.3 Exclusion criteria

Subjects with non-confirmed PE, essential hypertension, malaria, haemolytic anemia, any other infection such as urinary tract infection or upper respiratory tract infection.

2.3 Collection of sample

2ml blood sample was collected from the anticubital vein under strict aseptic precautions. The blood was allowed to clot for 30 minutes at room temperature and centrifuged at 3000rpm for 15 minutes. The serum was then pipette and placed in sterilized vials free of endotoxins at -20^0C until analysis.

2.4 Biochemical analysis

IL-1β, TNF-α, IL-6 and IL-8 were assayed by Ray Bio ®(Ray Biotech, Inc., USA) human ELISA (Enzyme Linked Immunosorbent Assay) kits which employs an antibody specific for human IL coated on a 96 well plate. Standards and samples are pipette into the wells and IL present the sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated antihuman IL`s antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipette to the wells. The wells are again washed, a TMB substrate solution is added to the wells and colour develops in proportion to the amount of IL`s bound. The stop solution changes the colour from blue to yellow and the intensity of colour was measured at 450nm.

2.5 Statistical Analysis

Statistical analysis was performed using EPI info software. p value was calculated. A value of $p<0.001$ was considered to be significant.

3. Results

Clinical data from normal and PE pregnant women, and from their neonates, are documented in Table 1. Proteinuria and high blood pressure were characteristic of PE patients. Fetal (birth) weight, placental weight, and gestational age were significantly lower in PE cases, and the number of cases with Apgar ≤7 at 1 minute was significantly higher in PE cases, as compared to normal cases; SGA newborns were observed only in the PE group. The two groups presented similar values for maternal age and body mass index (BMI).

Table I: Demographic characteristics of normotensive and preeclamptic pregnant females

Characteristics	Normotensive (n=40)	Preeclamptic (n=35)	P value
Age (years)	23.5 ± 3.19	25.5 ± 4.17	
Blood pressure (mm Hg) Systolic Diastolic	 120.1± 11.5 69.0 ± 7.2	 157.1 ± 14.6 97.1 ± 6.3	 <0.001 <0.001
Cases presenting proteinuria [n (%)] Cases with 1+ Cases with 2+ Cases with 3+ Cases with 4+	 - - - -	 16 (45%) 6(17.1%) 8(22.85%) 5 (14.28%)	
Gestational age (weeks)	37.5 (37.0; 38.3)	37.0 (34.0; 38.0)	<0.001
Parity			
Body Mass Index (kg/m2)	29.2 (27.2; 30.8)	29.8 (26.8; 32.8)	
Fetal (Birth) weight (kg)	3.3 (3.0; 3.7)	2.5 (1.7; 3.0)	<0.001
Placental weight (g)	625.0 (541.5; 779.8)	509.0 (390.5; 565.0)	<0.001
Apgar Score ≤ 7 [n (%)]: 1min 5min	 1 (2.4%) 0 (0%)	 7 (15.2%) 0 (0%)	
SGA/AGA/LGA (n)	0/40/0	6/29/0	
Cesarean section [n (%)]	31 (73.8%)	36 (78.3%)	

SGA/AGA/LGA – Small for gestation/ average for gestation / large for gestation

As shown in table II, the levels of TNF-α, IL-6 and IL-8 are significantly increased (p<0.001) in preeclamptic patients when compared with the levels in normal healthy controls and normal pregnant females in the third trimester. However there is no difference in the levels of IL-1β.

Also, there is a significant progression (p<0.001) in the levels of TNFα from the first trimester in females who subsequently developed PE.

Table II: Levels of cytokines in healthy normal controls, normotensive and preeclamptic females

	Healthy normal controls (n=40)	Normotensive females (n=40)			Preeclamptic females (n=35)		
		Ist trimester	II trimester	III trimester	Ist trimester	II trimester	III trimester
IL-1β (pg/ml)	27.4 ± 3.52	28.17 ± 3.9	28.37 ±3.3	27 ± 3.08	30.28 ± 4.6	27.88 ± 3.0	26.91 ± 2.50
TNF –α (pg/mL)	9.25 ± 1.34	9.13 ± 0.59	9.47 ± 0.6	9.4 ± 0.69	9.64 ± 0.73	41.8 ± 6.8[b]	78.11 ± 7.36[ac]
IL – 6 (pg/mL)	6.86 ± 0.89	7.63 ± 1.23	7.53 ± 0.88	7.57 ±1.11	7.62 ± 1.25	7.81 ± 1.15	15.56 ± 4.06 [ac]
IL – 8 (pg/mL)	26.49 ± 5.02	25.74 ± 2.71	27.23 ± 1.80	27.99 ± 3.56	26.71 ± 2.83	27.01 ± 3.00	75.51 ± 14.06 [ac]

a- P<0.001 – III trimester PE compared with III trimester normotensive females and healthy normal controls.
b- P<0.001 – II trimester PE compared with II trimester normotensive females and healthy normal controls.
c- P<0.001 – III trimester PE compared with II trimester preeclamptic females.

4. Discussion

Redman [4] has hypothesized that normal pregnancy and PE are a part of the same continuum i.e PE is a more severe level of a mild proinflammatory state pregnancy. This down regulation of the immune system in pregnancy is to protect the mother from infection. Alterations in the maternal immune system in the form of altered Th1-cytokine and CD4 cell expression are known to occur in PE.[10]

Placental ischaemia (hypoxia) induces marked increases in TNF-α, IL-6 and IL-8 synthesis in vascular smooth muscle (VSM) cells[11] leading to endothelial cell dysfunction, generalized vascular changes and hypertension[12] In this study, we confirmed an enhanced inflammatory process i.e significantly higher levels for TNF-α, IL-6 and IL-8 in maternal circulation in PE when compared with normal pregnant women.

TNF-α has a major role in the cytokine network with a widest spectrum of biological activities. Normally it is produced by the placental trophoblast cells and fetoplacental macrophages thus upregulating the endothelial expression of platelet derived growth factor, endothelial-1 and the plasminogen activator inhibitor. TNF-α induces structural and functional alterations in endothelial cells, enhances the formation of endothelin-1, reduces acetylcholine-induced vasodilatation and destabilizes eNOS mRNA.[13] Our study demonstrates that the levels of TNF-α are significantly higher in preeclamptic patients in the second and third trimester. Sharma [8] and Muzammil [12] have demonstrated elevated values of TNF- α in PE, however Roudsari FV [9] inferred that the increase in the levels of TNF-α in preeclamptic patients are not statistically significant.

IL-6, a proinflammatory cytokine produced by mononuclear phagocytes, endothelial cells, fibroblasts and T cells is involved in immune activation, vascular wall function and modulation of TNF-α production.[13] The significant increase in the levels of IL-6 in our study is consistent with that of Greer *et al* [14], Munno *et al*[15] and Teran *et al*[16] It is hypothesized that IL-6 may increase the permeability of endothelial cells by changing the cell shape and rearrangement of intracellular actin fibers[17]. IL-6 also increase the thromboxane A2 to prostacyclin ratio, reduce prostacyclin (PG I2) synthesis by inhibiting the cyclooxygenase enzyme[18] and stimulate platelet-derived growth factor.

This cytokine could trigger neutrophil activation, expression of von Willebrand factor, and cell adhesion on the endothelium with resultant vascular damage. It is also stated that proinflammatory cytokines associated with the genesis of PE (i.e., tumor necrosis factor-α and interleukin-1) enhanced IL-6 mRNA levels and increased secreted IL-6 levels in first trimester leukocyte free decidual cell incubations which may be the cause for the increased levels of IL-6 in PE. However Olusi [19] demonstrated a decrease in the levels of serum IL-6 and IL-8 where as Greer *et al*[14] reported a normal concentration of IL-8 in PE.

IL-1β produced by activated macrophages is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. IL-1β is a multifunctional mediator, able to modulate bone resorption by the activation of osteoclasts and by stimulating prostaglandin synthesis [PGE2].

Our study is in concordance with that of Casart YC[20] indicating that the levels of IL-β is similar in PE patients with that of normal pregnancy.

Also, Hefler LA[21] did not support a role for polymorphisms of the interleukin-1 beta and interleukin-1 receptor antagonist genes in the pathogenesis of PE among Hispanic women. However, Siljee *et al*[22] in their study have demonstrated that L-1*β* has potential to improve first trimester prediction of PE.

Conflicting results also exist concerning cytokine placental production[23] suggesting that placenta becomes a considerable source of cytokines along with pregnancy that is disturbed in PE and may contribute to the higher levels of those cytokines, in maternal circulation. Interleukins are multifunctional cytokines regulate hematopoiesis as well as the acute phase reaction[24] and modulate both pro- and anti-inflammatory events[25] which may be responsible for the development of PE.

TNF-α, IL-8 and IL 1-β are inflammatory mediators, likely to synergize with elevated plasma IL-6 levels to promote systemic vascular damage, particularly in the kidney, that results in the characteristic proteinuria and hypertension of the maternal syndrome of PE.

The great variability in the levels of cytokines in different studies may be attributed to the difference in the study design, number of subjects, assay sensitivity, type of sample analyzed, time of collection of sample etc. The probable limitation of our study is the small patient size considered which warrants undertaking further studies to consider the levels of interleukins individually in prediction of these parameters for the early diagnosis of PE in pregnancy.

4. Conclusion

Based on the findings of the present study, the levels of TNF-α, IL-6 and IL-8 are significantly increased in PE. Search for a biochemical parameter early in the first trimester which could help in the management of PE is the need of the hour. Our study failed to find any alteration in the levels of cytokines in the first trimester, however, the increase in TNF-α individually in the second trimester may represent an impending PE.

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