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Maternal Circulating TNF- α Levels are Highly Correlated with IL-10 Levels, but not IL-6 and IL-8 Levels, in Women with Pre-Eclampsia

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Abstract

Problem—Several lines of evidence have shown that maternal cytokine levels of tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, and IL-10 were altered in women with pre-eclampsia (PE) compared to those from normal pregnancies. In this study, we determined whether these cytokine levels are correlated before and after delivery in patients with PE.

Method of study—Venous blood was obtained from 50 women diagnosed with severe PE at the time of admission and 24 hr after delivery. Plasma concentrations for TNF- α , IL-6, IL-8, and IL-10 were measured by ELISA.

Results—There were no statistical differences for maternal levels of TNF- α , IL-6, IL-8, and IL-10 before and 24 hr postpartum. TNF- α and IL-10, but not IL-6 and IL-8, levels were significantly correlated before and 24 hr after delivery: TNF- α : $y = 19.963 + 0.953 \cdot x$; $r^2 = 0.924$; IL-10: $y = 10.521 + 1.113 \cdot x$; $r^2 = 0.984$, $P < 0.001$, respectively. Furthermore, TNF- α levels were correlated with IL-10 levels, but not with IL-6 and IL-8 levels.

Conclusion—The correlation patterns of TNF- α with IL-10 and TNF- α with IL-6 and IL-8 suggest disparity in functional regulations between these cytokines in maternal circulation in PE.

Keywords

Cytokines; IL-10; pre-eclampsia; TNF- α

Introduction

Pre-eclampsia is a hypertensive and multiple system disorder unique to human pregnancy. Although the etiology of pre-eclampsia remains unknown, there are many proposed theories regarding the pathogenesis of the pre-eclamptic disease processes: oxidative stress; abnormal trophoblast invasion; vascular endothelial dysfunction; genetic predisposition; dietary deficiencies, and defective immunological adaptation to pregnancy.^{1,2}

Several lines of evidence also support the concept that pre-eclampsia is an excessive maternal inflammatory response to normal pregnancy.³ Supporting evidence includes leukocyte and platelet activation, and elevated levels of endothelial adhesion molecules, ICAM, VCAM, and P-selectin in the maternal circulation in women with pre-eclampsia.^{4,5} Furthermore, maternal cytokine and chemokine levels tumor necrosis factor- α (TNF- α), interleukin (IL)-6, and IL-8 are also either increased or decreased during pre-eclampsia.^{6,7} A major role of cytokines in the pathogenesis of pre-eclampsia is considered to contribute to endothelial dysfunction in the maternal vascular system.⁸

Consistent with elevated cytokine levels in the maternal circulation, placental tissue cytokine levels are also altered,^{9,10} which implies inflammatory responses that may occur both in the maternal and the placental compartments. It is believed that some growth factors produced by placental trophoblasts such as VEGF, PlGF, and their soluble receptor sFlt-1 contribute to their maternal levels during pregnancy and in pre-eclampsia. Dramatic fall of increased sFlt-1 levels in the maternal circulation after termination of pregnancy in women who experienced pre-eclampsia¹¹ further supports the idea that the placenta is a source of abnormal elevated bio-molecule levels in the maternal circulation during pre-eclampsia. To study whether placenta-derived cytokines might contribute to their maternal circulating levels during pre-eclampsia, we measured plasma levels of TNF- α , IL-6, IL-8, and IL-10 at admission and 24 hr after delivery in patients with pre-eclampsia. The correlations of these cytokines before and after delivery were determined.

Materials and methods

Patient Information

Patients with pre-eclampsia were recruited when they were admitted to the Labor and Delivery Unit at Louisiana State University Health Sciences Center – Shreveport (LSUHSC-S) with documented severe pre-eclampsia. After consent, venous blood sample was drawn at the time of admission or before administration of magnesium sulfate and 24 hr postpartum. Diagnosis of mild pre-eclampsia is defined as maternal blood pressure $\geq 140/90$ mmHg with proteinuria 1+ or more on dipstick or ≥ 300 mg protein in 24-hr urine collection. Diagnosis of severe pre-eclampsia is defined as follows: sustained systolic blood pressure of ≥ 160 mmHg or a sustained diastolic blood pressure of ≥ 110 mmHg; proteinuria measurement of 3+ or more on dipstick, or 24-hr urine protein collection with ≥ 5 g in specimen; urine output < 30 mL/hr for 2 hr unresponsive to 500 mL intravenous fluid challenge; presence of persistent headache, and visual disturbances, or epigastric pain; patients with one or more abnormal laboratory values reflecting thrombocytopenia, elevated LDH or increased AST; pulmonary edema; or evidence of intrauterine growth restriction. A total of 50 women were recruited in this study. Patients with signs of infection and smokers were excluded. Table I shows the clinical characteristics of the study population. This study was approved by the Institutional Review Board (IRB) for Human Research at LSUHSC-Shreveport.

Cytokine Assays

Cytokine TNF- α , IL-6, IL-8, and IL-10 levels were measured in the plasma samples using commercially available enzyme-linked immunosorbent assay (ELISA) DuoSet development system kits (R & D, Minneapolis, MN, USA). 96-well plates were purchased from the Nunc International Company (Rochester, NY, USA). For assays, plates were coated overnight with the capture antibody and the assay procedures were followed according to the manufacturer's instructions. The standard was serially diluted and used to generate a standard curve for each ELISA assay. The ranges of the standard curve are: TNF- α : 1.0–1000 pg/mL; IL-6: 0.6–600 pg/mL; IL-8: 0.5–500 pg/mL; and IL-10: 2.0–2000 pg/mL, respectively. An aliquot of 100 μ L plasma sample were assayed. All samples were measured

in duplicate at the same time. Within assay variations were <7% for these assays. The plates were read at 492 nm with an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Statistical Analysis

A statistical computer software program StatView (SAS Institute Inc., Cary, NC, USA) was used for data analysis. Paired *t*-test was used for analysis of TNF- α , IL-6, IL-8, and IL-10 concentration between pre- and post-delivery levels. Simple regression was used to analyze regression coefficients. A probability level of $P < 0.05$ was considered significant. Data are presented as the mean $\pm$ S.D. for the demographic characteristics and mean $\pm$ S.E. for cytokine productions.

Results

The demographics and pregnancy characteristics of the study are presented in Table I. The mean blood in the study patients was $164 \pm 3/104 \pm 2$ mm/Hg. There is no difference in the concentrations of TNF- α , IL-6, IL-8, and IL-10 between admission values and 24 hr postpartum. Fig. 1 demonstrates these results. The values comparing admission to 24 hr postpartum are: TNF- α : 135.08 ± 30.09 versus 120.74 ± 30.33 pg/mL; IL-6: 17.99 ± 6.35 versus 21.01 ± 3.12 pg/mL; IL-8: 6.63 ± 1.78 versus 6.24 ± 1.60 pg/mL; and IL-10 310.58 ± 127.37 versus 259.54 ± 109.65 pg/mL. All these results had P values greater than 0.05.

An analysis was then undertaken to evaluate the relationship between TNF- α and IL-10, IL-6, and IL-8 levels both before and after delivery. TNF- α and IL-10, but not IL-6 and IL-8, levels were significantly correlated before and 24 hr after delivery: TNF- α : $y = 19.963 + 0.953*x$; $r^2 = 0.924$; IL-10: $y = 6.932 + 1.083*x$; $r^2 = 0.96$, $P < 0.001$, respectively (Fig. 1). A relationship exists between TNF α and IL-10 at admission and 24 hr postpartum, at admission: $y = 24.606 + 0.339*x$; $r^2 = 0.842$; 24 hr postpartum: $y = 18.281 + 0.366*x$; $r^2 = 0.888$, respectively. Fig. 2 represents these data. No correlation existed between TNF- α and IL-6 or IL-8 before and after delivery. Analysis was then undertaken to see if cytokine levels correlated with maternal blood pressure. No such relationship existed in our analysis.

Discussion

Endothelial cell dysfunction has long been theorized as an etiology of pre-eclampsia.¹ Cytokines have long been implicated in the pathogenesis of the dysfunction.² Understanding how different cytokines affect the disease may ultimately help us to understand better the disease process and possibly to develop an intervention strategy for pre-eclampsia. Little information exists about the role of placenta-derived cytokines and how this may affect the maternal levels. If the placenta plays a major role, once the placenta is delivered, the maternal levels should drop rapidly. Our study failed to reveal this. All cytokine levels TNF- α , IL-6, IL-8, and IL-10 did not differ when comparing concentrations before delivery with those 24 hr after delivery. These data suggest that the placenta may not be the major source of these cytokines in the maternal circulation during pregnancy, even though increased placental tissue levels and altered cytokine productions for TNF- α , IL-6, IL-8, and IL-10 have been found in placentas from women with pre-eclampsia.¹²⁻¹⁴ In this study, we did not directly test the source of these inflammatory cytokines. However, published works have shown that immunoactive cells and circulating leukocytes including macrophages, monocytes, and lymphocytes produce TNF- α , IL-6 and IL-8.^{15,16} In addition, vessel endothelium is also a source of inflammatory cytokines.¹⁷

Many studies have been reported on maternal cytokine levels in pre-eclampsia.^{8,9} The results of these studies have not always been consistent.^{8,12} Most studies have shown that

pre-eclamptic patients have an increase in maternal serum levels of TNF- α , IL-6, and IL-8, but a decrease in IL-10 when compared to normal controls.¹²

Our study evaluated plasma cytokines to see if any relationship existed. TNF- α concentration was found to correlate with IL-10, but not IL-6 or IL-8, concentration at admission and 24 hr after delivery. This correlation makes sense physiologically because of the known biological effects of these substances. TNF- α is responsible for endothelial cell dysfunction,¹⁰ while IL-10 is an anti-inflammatory cytokine. Other than anti-inflammatory effect, IL-10 could also down-regulate adhesion molecules, inhibit superoxide production, and reduce metalloproteinase release. Although it is not known if the correlation of IL-10 to TNF- α is because of a compensative anti-inflammatory response of IL-10 to TNF- α in the maternal circulation, the correlation patterns of TNF- α with IL-10, and TNF- α with IL-6 and IL-8 suggest that disparity in functional regulation exists in these cytokines in the maternal circulation in women with pre-eclampsia. Hennessey et al.¹⁸ found a correlation between an increase in TNF- α and decrease in IL-10 concentrations in pre-eclamptic patients. Although TNF- α , IL-6 and IL-8 are all considered as inflammatory cytokines. The reason for no correlation of TNF- α and IL-6 or IL-8 in patients with pre-eclampsia is not clear. A possible explanation could be due to the differential target cell types and function of TNF- α , IL-6 and IL-8 in the immune and vascular systems. Table II shows major sources and function of TNF- α , IL-6, IL-8, and IL-10.

In conclusion, our study demonstrated no significant change in the concentrations of the plasma cytokine TNF- α , IL-6, IL-8, and IL-10 concentrations when comparing levels before delivery to 24 hr after delivery. This suggests that the placenta is not a major source of TNF- α , IL-6, IL-8, and IL-10 in the maternal circulations of patients with pre-eclampsia. Our study also demonstrated a correlation that exists between TNF- α and IL-10, but not between TNF- α and IL-6 or IL-8. We also failed to find a correlation of cytokine levels and blood pressure in pre-eclamptic patients, which suggests that altered cytokine levels may not have a direct effect on vaso-activity in the maternal systemic vasculature. These data help to further our knowledge of cytokine function and interaction associated with increased inflammatory response in women with pre-eclampsia.

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Fig. 1.

Plasma concentrations of tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, and IL-10 in women with pre-eclampsia at admission and 24 hr postpartum. a: TNF- α ; b: IL-6; c: IL-8; and d: IL-10. Upper panel: the mean concentration; and lower panel: the correlation of the plasma cytokine levels at admission and 24 hr postpartum.

Fig. 2.

Correlations of plasma concentrations of tumor necrosis factor- α (TNF- α) and IL-10 in women with pre-eclampsia at admission and 24 hr postpartum. Upper panel: at admission and lower panel: 24 hr postpartum.

Table I**Demographic Data for the Study Patients ($n = 50$)***

Maternal age (years)	23 ± 6
Racial status	Black $n = 33$; White $n = 16$; Other $n = 1$
Blood pressure (mmHg)	
Systolic	164 ± 16
Diastolic	104 ± 12
Gestational age (weeks)	
<34	$n = 19$
34–37	$n = 15$
>37	$n = 16$
Nulliparous	$n = 27$ (54%)
Mode of delivery	V/D $n = 25$; C/S $n = 25$
History of PE	$n = 6$

V/D, vaginal delivery; C/S, cesarean section.

* Data are expressed as mean $\pm$ S.D.

Table IIMajor Sources and Function of TNF- α , IL-6, IL-8, and IL-10

Cytokine	Producing cells	Gene location	Target cells	Function
TNF- α	Macrophages	Chromosome 6	Macrophages	Inflammatory response
	Leukocytes (mast cells, NK cells)		Tumor cells	Induce cell death
	Placental cells		Placental cells	Stimulate acute phase reaction
IL-6	Th2 cells	Chromosome 7	Plasma cells	Acute phase response
	Monocytes		B cells	Antibody secretion
	Endothelial cells		Endothelial cells	Plasma cell differentiation
	Macrophages		Stem cells	Various cell differentiation
	Stromal cells		Placental cells	
	Placental cells			
IL-8	Endothelial cells	Chromosome 4	Endothelial cells	Chemotaxis
	Macrophages		Neutrophils	
	Placental cells		Macrophages	
			Placental cells	
IL-10	Monocytes	Chromosome 1	Macrophages	Offset Th1 cytokine production
	Th2 cells		B cells	B Cell activation
	Placental cells		Placental cells	