

## INTERLEUKINS IN PREECLAMPSIA

Samuel O. Olusi, MBBS, FRCPath, PhD; Michael Diejomaoh, MBBS, FRCOG;  
Alexander Omu, MBBS, FRCOG; Amal Abdulaziz, BM, ChB, CABOG;  
Kinatamith Prabha, MBBS, MD; Sunila George, MSc

**Background:** Preeclampsia is a multisystemic disorder of unknown etiology. Recently, endothelial damage has been implicated in its cause. The objective of this study was to determine the role of interleukins in the etiology of preeclampsia.

**Patients and Methods:** Thirty-two primigravidas with preeclampsia but without any clinical evidence of infection and 32 age-matched primigravidas with uncomplicated normal pregnancies were investigated. Phlebotomy was performed at 32 weeks of gestation and blood collected for immunoassays of interleukin-2 (IL-2), interleukin-2 receptor (IL-2R), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10), using commercially available immunoassay kits.

**Results:** Although the maternal plasma concentrations of IL-2 and IL-2R were slightly higher in normal pregnant women ( $76.3 \pm 13.7$  pg/mL and  $526.3 \pm 47.1$  pg/mL, respectively) than in women with preeclampsia ( $57.8 \pm 10.8$  pg/mL and  $476.9 \pm 33.9$  pg/mL, respectively), the differences were not statistically significant ( $P > 0.05$ ). However, maternal plasma IL-6 and IL-8 concentrations were significantly higher ( $P < 0.05$ ) in normal pregnancy ( $158.0 \pm 35.4$  pg/mL and  $5163.6 \pm 800$  pg/mL, respectively) than in pregnancy complicated with preeclampsia ( $60.0 \pm 13.7$  pg/mL and  $2495.8 \pm 729.4$  pg/mL, respectively). On the other hand, maternal plasma concentration of IL-10 was significantly higher ( $P < 0.05$ ) in preeclampsia ( $93.2 \pm 24.1$  pg/mL) than in normal pregnancy ( $31.0 \pm 7.0$  pg/mL).

**Conclusion:** It is concluded that the elevated maternal plasma IL-10 concentration in preeclampsia may be a protective response to maternal immunorejection.

*Ann Saudi Med 2000;20(1):4-7.*

**Key Words:** Maternal plasma, interleukins, preeclampsia.

Preeclampsia is a multisystemic disorder of pregnancy, with the clinical diagnostic features of hypertension and proteinuria. Although the cause of this disorder is still unknown, current concepts of preeclampsia suggest that generalized dysfunction of the maternal vascular endothelium is a central pathogenic feature. Increased serum levels of factor VIII-related antigen to factor VIII coagulation activity,<sup>1</sup> increased serum levels of fibronectin,<sup>2,3</sup> a disturbance of thromboxane A2 (TXA2)/prostacyclin balance,<sup>4</sup> and disturbance of nitric oxide production<sup>5</sup> all support the hypothesis that endothelial cell damage is ultimately involved in the etiology of preeclampsia. Recently, some authors<sup>6</sup> have reported that carriers of the factor V Leiden mutation, the most frequent genetic cause of resistance to activated protein C, are at

increased risk of preeclampsia because of their increased predisposition to deep vein thrombosis.

The trigger for the endothelial dysfunction in eclampsia is not known, but neutrophil activation can result in similar vascular damage in the non-pregnant state.<sup>7</sup> Greer et al.<sup>8</sup> have reported that concentrations of neutrophil elastase, a specific marker for neutrophil activation *in vivo*, are elevated in the peripheral circulation of women with preeclampsia. Greer et al.<sup>9</sup> also reported a significant correlation between plasma neutrophil elastase and von Willebrand factor, a marker of endothelial damage, in patients with preeclampsia.

Although Greer et al.<sup>8</sup> have demonstrated that neutrophil activation occurs in preeclampsia, the mechanism underlying this activation remains unknown. Cytokines could trigger neutrophil activation, expression of von Willebrand factor, and cell adhesion on the endothelium with resultant vascular damage. But there are conflicting reports in the literature on serum concentrations of interleukins in preeclampsia. For example, while Silver et al.<sup>10</sup> and Kupferminc et al.<sup>11</sup> reported a significant decrease in the amniotic fluid concentration of IL-6 in

---

From the Departments of Pathology and Obstetrics and Gynecology, Faculty of Medicine, Kuwait University, Safat, Kuwait.

Address reprint requests and correspondence to Prof. Olusi: Department of Pathology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait.

Accepted for publication 25 September 1999. Received 27 April 1999.

preeclampsia, Greer et al.<sup>12</sup> reported increased plasma concentrations of IL-6, but normal concentration of IL-8 in preeclampsia. As a result of this discrepancy, we decided to measure the serum concentrations of IL-2, IL-2 receptor, IL-6, IL-8 and IL-10 in women with preeclampsia, and to determine if they differ from those of normal age-matched pregnant women. To the best of our knowledge, this is the first report of all these serum interleukins in the same group of women with preeclampsia.

## Patients and Methods

### Patients and Controls

Thirty-two primigravidas with preeclampsia and 32 primigravidas with uncomplicated normal pregnancies were investigated. Preeclampsia was defined as persistent diastolic blood pressure above 90 mm Hg, with proteinuria (above 300 mg/24 hrs) and hyperuricemia (above 0.33 mmol/L) in pregnant women who were normotensive before 20 weeks' gestation. The 32 controls who had normal, uncomplicated pregnancies were age-matched with the patients. None of the women (patient or control) had evidence of any active infective process such as urinary tract infection or upper respiratory tract infection. Informed consent was obtained from each subject at the beginning of the study. The clinical and biochemical characteristics of the groups are shown in Table 1.

Venous blood obtained at 32 weeks' gestational age

TABLE 1. Clinical and biochemical characteristics of preeclamptic patients and controls.

Characteristics	Preeclamptic pregnancy	Normal pregnancy	Significance
Parity	Primigravida	Primigravida	
Maternal age (years±SD)	25.6±4.9	26.2±5.2	NS
Diastolic blood pressure (mm Hg)	80 (60-85)*	71 (65-80)	NS
	104 (95-130)**	75 (70-80)	P<0.001
Gestational age at phlebotomy (weeks±SD)	32.5±2.5	33.6±3.8	NS
Plasma uric acid (mmol/L±SD)	0.38±0.06	0.28±0.04	P<0.001
Proteinuria (>2+ on urine testing)	30/30	0/25	P<0.001
Urine culture for microorganisms	No significant growth	No significant growth	

NS=not significant; \*at first antenatal visit, mean (range); \*\*at study entry, mean (range).

TABLE 2. Plasma concentrations of interleukins (mean ± SEM) in preeclampsia and in normal pregnant controls.

Interleukin	Preeclampsia	Normal pregnant control	P-value
IL-2 (pg/mL) (mean ± SEM)	57.8 ± 10.8	76.3 ± 13.7	0.299
IL-2R (pg/mL)	476.90±33.90	526.3±47.1	0.402
IL-6 (pg/mL)	60.0±13.7	158.0±35.4	0.015
IL-8 (pg/mL)	2495.8±729.4	5163.6±800.0	0.019
IL-10 (pg/mL)	93.2±24.1	31.0±7.0	0.03

from patients and controls were drawn into specimen tubes containing trisodium citrate as anticoagulant. Plasma was prepared by centrifugation and stored at -70°C until it could be analyzed for interleukins.

### Assay of Plasma Interleukin-2 Concentration

Commercial enzyme immunoassay kits (obtained from ICN Pharmaceuticals Inc., California, USA) were used for the assay of plasma concentrations of IL-2. The manufacturer's instructions were followed. The sensitivity for each interleukin was 5 pg/mL.

### Assay of Plasma Interleukin-6 and Interleukin-10 Concentrations

Plasma IL-6 concentrations in the samples were measured with the CYT Elisa™ 6 kit (CYT Immune Sciences Inc., College Park, MD 20740, USA), a commercial sandwich enzyme immunoassay which measures the free forms of IL-6. The detection limit of the assay was 0.92 pg/mL, while the intraassay and interassay variations in our study were 8.3% and 10%, respectively. Plasma IL-10 concentrations were measured with the CYT Elisa™ 10 kit. In each case, the manufacturer's instructions for the assay were followed.

### Assay of Plasma IL-2R and IL-8 Concentrations

Plasma IL-2R concentration was measured using the Immulite IL-2R, a solid-phase, two-site chemiluminescent enzyme immunometric assay for use with the Immulite Automated Analyser (Diagnostic Products Corporation, California, USA), while plasma IL-8 was measured using the Immulite IL-8 assay. The intraassay and interassay CV for IL-2R and IL-8 were less than 3.6% and 6.5%, respectively.

### Statistical Analysis of Data

Data were analyzed using Statgraphics Plus (version 6) software. The difference between the mean value for each variable among patients and controls was tested for significance by Student's *t*-test, and *P*-values <0.05 were considered significant. Pearson's correlation coefficient was used to test the significance of relationships between variables.

## Results

The concentrations (mean±SEM) of all the interleukins are shown in Table 2. Although the plasma concentration of IL-2 was slightly higher in normal pregnant women (76.3±13.7 pg/mL) than in preeclampsia (57.8±10.8 pg/mL), the difference was not statistically significant (*P*=0.3). Similarly, there was no significant difference (*P*=0.4) between plasma IL-2R concentration in normal pregnancy (526.3±47.1 pg/mL) and preeclamptic pregnancy (476.9±33.9 pg/mL). These results would appear



FIGURE 1. Plasma concentrations of IL-6 in normal pregnancy and pregnancy complicated with preeclampsia.

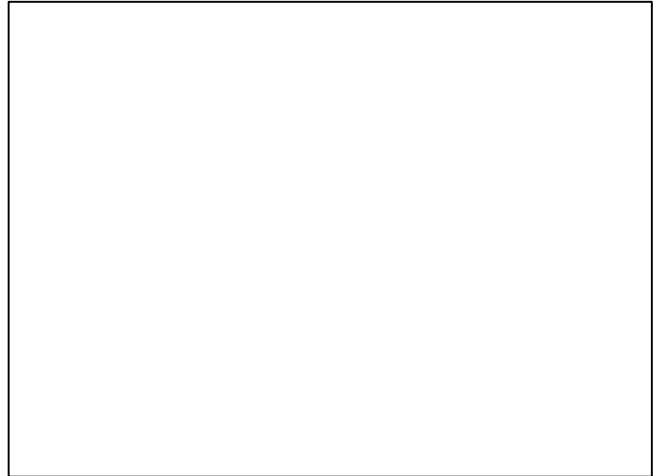


FIGURE 2. Plasma concentrations of IL-8 in normal pregnancy and pregnancy complicated with preeclampsia.

to suggest that IL-2 and IL-2R do not play a role in the etiology of preeclampsia. Plasma concentrations of IL-6 in normal pregnancy and in pregnancy complicated with preeclampsia are shown in Figure 1. Of the 32 control samples, 10 had plasma IL-6 concentrations that were substantially higher than those for the majority of the group. When these “outliers” with plasma IL-6 levels greater than 600 pg/mL were excluded, the mean±SEM in controls (158.0±35.4 pg/mL) was still significantly greater ( $P=0.015$ ) than the level in preeclampsia (60.0±13.7 pg/mL). Similarly, plasma IL-8 concentration was significantly higher ( $P=0.019$ ) in normal pregnancy (5163.6±800 pg/mL) than in preeclamptic pregnancy (2495.8±729.4 pg/mL). This is illustrated in Table 2 and Figure 2. These results would appear to suggest that plasma IL-6 and IL-8 concentrations are reduced in preeclampsia. Figure 3 shows the plasma concentrations of IL-10 in patients with preeclampsia and in normal pregnant controls. Unlike IL-6 and IL-8, plasma IL-10 concentration was significantly higher ( $P=0.03$ ) in women with preeclampsia (93.2±24.1 pg/mL) than in normal pregnant women (31.0±7.0 pg/mL).

### Discussion

Our results demonstrate that preeclampsia is associated with significant decrease in plasma IL-6 and IL-8, but significant increase in plasma IL-10 concentrations. There are no significant alterations in plasma IL-2 and IL-2R concentrations in pre-eclampsia. Our results on interleukin-6 are at variance with those of Greer et al.<sup>12</sup> and Kupferminc et al.,<sup>11</sup> who found increased serum interleukin-6 in women with preeclampsia, but agree with those of Silver et al.,<sup>10</sup> who found decreased amniotic fluid levels in women with preeclampsia. Different reasons may explain the variances between our results and those of Greer and Kupferminc. For example, it was not clear in

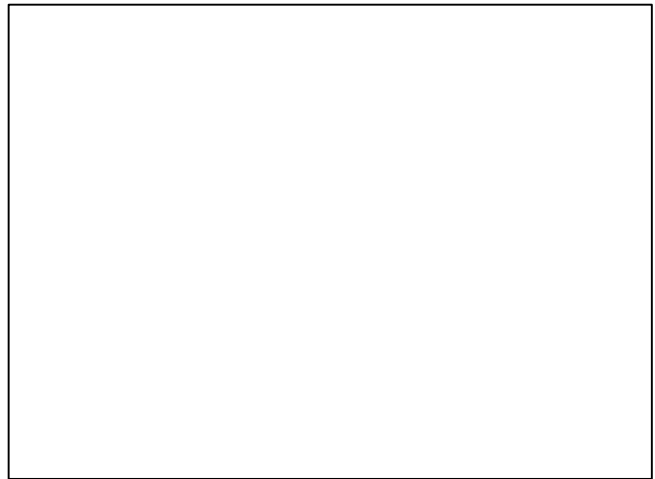


FIGURE 3. Plasma concentrations of IL-10 in normal pregnancy and pregnancy complicated with preeclampsia.

Greer’s study if there was any maternal infection in their patients with preeclampsia, since maternal infection is a potent stimulus for IL-6 production. In our study, care was taken to exclude preeclamptic patients with any clinical evidence of infection. Another reason may be differences in gestational age at which phlebotomy was performed. In this study, phlebotomy was performed in all cases at 32 weeks.

Our finding of a significant elevation of maternal plasma IL-10 in preeclampsia compared with controls has not been previously reported, although there are reports of maternal serum IL-10 concentrations during pregnancy and labor<sup>13</sup> and in small-for-gestational-age neonates.<sup>14</sup> Interleukin-10, a potent immunosuppressive cytokine, has several functions, including the inhibition of macrophage activity and function, *in vivo* suppression of cell-mediated immunity and the inhibition of nitric oxide production.<sup>15,16</sup> Interleukin-10 has been found in pregnant women and localized by immunohistologic methods to the interface area between maternal and fetal tissues and expressed at

high concentrations in placental tissues.<sup>15</sup> Although the role of interleukin-10 in the physiology of human gestation has not been properly elucidated, it may play a role in protecting the fetus from maternal immunorejection.<sup>14</sup> The high concentration of this interleukin in women with preeclampsia, as found in this study, may be due to the fact that preeclampsia is a manifestation of immune rejection which IL-10 tries to suppress.

### References

1. Redman CWG, Beilin LJ, Denson KWE, Bolton FG, Stirrat GN. Factor VIII consumption in preeclampsia. *Lancet* 1997;2:1249.
2. Stubbs TM, Lazarchick J, Horger EO III. Plasma fibronectin levels in preeclampsia: a possible biochemical marker for vascular endothelial damage. *Am J Obstet Gynecol* 1984;150:885-7.
3. Lockwood CJ, Peters JH. Increased plasma levels of EDI cellular fibronectin precede the clinical signs of preeclampsia. *Am J Obstet Gynecol* 1990;162:358-62.
4. Dekker GA. Prediction and prevention of pregnancy-induced hypertensive disorders: a clinical and pathophysiologic study (thesis). Rotterdam: Erasmus University, 1989.
5. Davidge ST, Stranko CP, Roberts JM. Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. *Am J Obstet Gynecol* 1996;174:1008-13.
6. Dizon-Townson DS, Nelson LM, Easton K, Ward K. The factor V Leiden mutation may predispose women to severe preeclampsia. *Am J Obstet Gynaecol* 1996;175:902-5.
7. Harlan JD. Neutrophil-mediated vascular injury. *Acta Med Scand Suppl* 1987;715:123-9.
8. Greer IA, Haddad NG, Dawes J, Johnstone FD, Calder AA. Neutrophil activation in pregnancy-induced hypertension. *Obstet Gynecol* 1991;78:28-32.
9. Greer IA, Leask R, Hodson BA, Dawes J, Kilpatrick DC, Liston WA. Endothelin, elastase, and endothelial dysfunction in preeclampsia (letter). *Lancet* 1991;337:558.
10. Silver RM, Schwitzer B, McGregor JA. Interleukin-6 levels in amniotic fluid in normal and abnormal pregnancies: preeclampsia, small-for-gestational-age fetus, and premature labor. *Am J Obstet Gynecol* 1993;169:1101-5.
11. Kupferminc MJ, Peaceman AM, Aderka D, Wallach D, Socol ML. Soluble tumor necrosis factor receptors and interleukin-6 levels in patients with severe preeclampsia. *Obstet Gynecol* 1996;88:420-7.
12. 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 Gynaecol* 1994;84:937-40.
13. Dudley DJ, Hunter C, Mitchell MD, Varner MW. Amniotic fluid interleukin-10 (IL-10) concentrations during pregnancy and with labor. *J Reprod Immunol* 1997;33:147-56.
14. Spong CY, Sherer DM, Ghidini A, Jenkins CB, Seydel FD, Eglinton GS. Second-trimester amniotic fluid or maternal serum interleukin-10 levels and small-for-gestational-age neonates. *Obstet Gynaecol* 1996; 88:24-8.
15. Mosman TR. Interleukin-10. In: Thomson AW, editor. *The cytokine handbook*. California: Academic Press, 1994;223-37.
16. Greig PC, Herbert WNP, Robinette BL, Teot LA. Amniotic fluid interleukin-10 concentrations increase through pregnancy and are elevated in patients with preterm labor associated with intrauterine infection. *Am J Obstet Gynecol* 1995;173:1223-7.