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Serum Levels of Neopterin and Interleukin-2 Receptor in Women With Severe Preeclampsia

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Preeclampsia continues to be a major cause of maternal and perinatal mortality and morbidity worldwide. The etiopathogenesis of preeclampsia is not fully understood. Neopterin and interleukin-2 (IL-2) production reflects cellular immunity. Our purpose was to determine the levels of neopterin and interleukin-2 receptor (IL-2R) in pregnant women with severe preeclampsia, and assess the implications of these findings in the pathophysiology of preeclampsia. Fourteen women with preeclampsia were compared with 14 healthy pregnant women. Serum levels of neopterin were measured by an enzyme-linked immunoassay (ELISA), and IL-2R levels were determined by an immunoassay method with an Immulite analyzer. The levels of neopterin and IL-2R were significantly higher in the preeclamptic subjects than in normotensive women ($P<0.05$). There was a significant correlation between neopterin and IL-2R. We found that serum neopterin and IL-2R levels are increased in women with severe preeclampsia. The results of this study suggest that a T-helper 1 (Th1) type immune mechanism is involved in the pathogenesis of preeclampsia. J. Clin. Lab. Anal. 19:36–39, 2005.

Key words: preeclampsia; pregnancy; neopterin; IL-2R; immunity

INTRODUCTION

Preeclampsia is a common complication of pregnancy, affecting 5–7% of pregnant women. Although it continues to be major cause of maternal and perinatal mortality and morbidity worldwide (1), the etiopathogenesis of preeclampsia is not fully understood.

Preeclampsia is characterized by enhanced cell-mediated immunity. Immunological factors may affect the pathogenesis of preeclampsia, and imbalances in the T-helper 1/T-helper 2 (Th1/Th2) ratios may play an important role in the production of leukocyte endothelial adhesion molecules, which mediate the adherence of inflammatory cells. Such an imbalance may induce endothelial injury and eventually cause and/or worsen preeclampsia (2).

Neopterin and interleukin-2 (IL-2) production reflects cellular immune activity, and IL-2 receptors (IL-2R) mediate the action of IL-2. Our purpose was to determine the levels of neopterin and IL-2R in pregnant women with severe preeclampsia, and assess the implications of these findings in the pathophysiology of preeclampsia.

MATERIALS AND METHODS

This study was carried out at the Department of Obstetrics and Gynecology, University Hospital of Pamukkale. Twenty-eight pregnant women were enrolled and divided into two groups: severely preeclamptic (n = 14) and normotensive (n = 14). None of the pregnant women had any chronic or acute physical illness, and none were taking any medication other than iron and folic acid supplements.

Severe preeclampsia was determined as defined by the American College of Obstetricians and Gynecologists guidelines, and included blood pressure measurements of $\geq$160/110 mm Hg, proteinuria levels of 5 g in $\geq$24 hr, thrombocytopenia ($\geq$100,000/mL), oliguria (500 mL in $\geq$24 hr), elevated liver function tests, and seizures

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(eclampsia) (3). The control group was comprised of 14 normotensive pregnant women matched for age, gestational age, weight, and parity.

Blood samples were obtained from all of the pregnant women by venipuncture. Sera were obtained by centrifugation at 4,000 rpm for 10 min. The samples were stored at -20°C until they were analyzed. Routine assays for blood biochemistry were performed with the Synchron LX20 analyzer (Beckman Coulter). Total platelet concentrations were established with a Coulter LH 750 analyzer (Beckman Coulter, Brea, CA).

IL-2R was determined with the use of Immulite IL2R kits based on immunometric assays with the Immulite analyzer (DPC). The calibration range of the kit was up to 7200 U/mL, and the analytical sensitivity was 5 U/mL.

Serum neopterin levels were measured following the basic principle of competitive enzyme-linked immunosorbent assay (Neopterin ELISA; Immuno Biological Laboratories, Hamburg, Germany), i.e., the competition between a peroxidase-conjugated and nonconjugated antigen for a fixed number of antibody binding sites (rabbit anti-neopterin). The peroxidase-conjugated antigen-antibody complexes were bound to the wells of the microtiter strips, which were then coated with a goat anti-rabbit antibody. Unbound antigen was then removed by washing. After the substrate reaction was obtained, the optical density was measured at 450 nm. We quantified the samples by comparing the enzymatic activity of the samples with a response curve prepared using standards ranging from 0 to 111 nmol/L. Serum specimens were stored at –20°C in the dark until they were assayed. Statistical analysis was performed with the SPSS 11.0 computer statistics programmer licensed by Pamukkale University. We tested the data for normal distribution, and used Student's *t*-test or Mann-Whitney's U-test to compare the study and control groups when appropriate. We conducted a correlation analysis between the neopterin and IL-2R levels in preeclampsia sera with Spearman's rank test.

RESULTS

The characteristics of both the preeclamptic and normotensive groups are shown in Table 1. There were no significant differences in maternal age, parity, or gestational age at the time of blood sampling between preeclamptic women and normotensive women ($P > 0.05$). As expected, the preeclamptic subjects had higher diastolic blood pressure and lower mean birth weight than the normotensive controls. Women with preeclampsia also had a higher mean body weight than healthy controls. Eight patients (57.1%) in the study group were primigravid, and six (42.9%) were parous. In the control group, seven women (50%) were primigravid, and seven (50%) were parous ($P > 0.05$).

Four women in the study group were eclamptic, and one of them was postpartum eclamptic. In the study group, four patients showed evidence of having hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome.

The levels of neopterin and IL-2R are shown in Table 2. In this study we found that the median serum neopterin level increased significantly from 7.6 to 14.5 nmol/L in preeclamptic women compared to controls ($P < 0.05$). We found an IL-2R level of 279 U/mL in normal pregnant women. The IL-2R level increased significantly to 682 U/mL in preeclamptic subjects compared to normal controls ($P < 0.05$). The serum neopterin and IL-2R levels are shown in Figs. 1 and 2. In all groups, significant correlations were found between IL-2R and neopterin ($r = 0.552$, $P = 0.002$).

TABLE 1. Clinical characteristics of patients

	Normotensive (n = 14)	Preeclampsia (n = 14)
Maternal age (years)	25.7 ± 5.8	26.2 ± 6.7*
Maternal weight (kg)	70 ± 9	80 ± 9**
Birth-weight (g)	30.96 ± 498	2592 ± 553**
Gestational age at sampling (weeks)	34.1 ± 3.2	34.2 ± 3.6*
Diastolic blood pressure (mmHg)	7.2 ± 0.9	11 ± 1.3***

Data are presented as measn ± SD.
*$P > 0.05$ compared with the normotensive group.
**$P < 0.05$ compared with the normotensive group.
***$P < 0.0001$ compared with the normotensive group.

TABLE 2. Serum neopterin and IL-2R levels of normotensive and preeclamptic pregnancies

	Normotensive (n = 14)		Preclampsia (n = 14)	
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)
Neopterin (nmol/1)	7.7 ± 2.4	7.6 (3.9–12)	20.1 ± 18.9*	14.5 (2.3–62)
IL-2R (U/ml)	319.5 ± 95.0	279 (185–441)	739.1 ± 485.0*	682 (250–2042)

*$P < 0.05$ compared with the normotensive group.

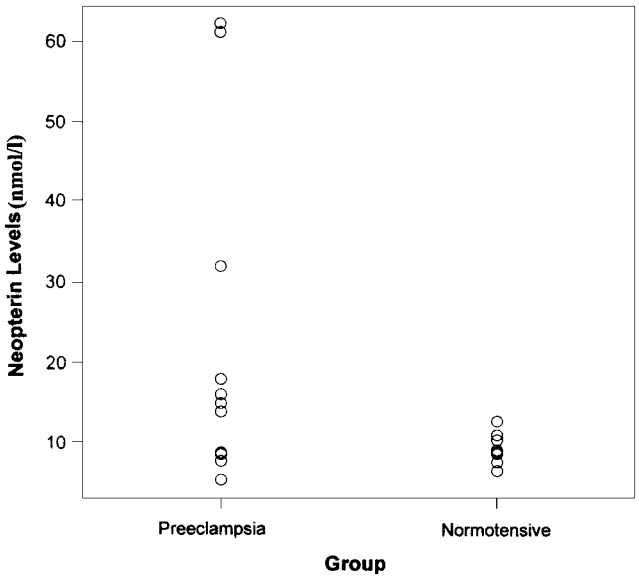

Fig. 1. Serum neopterin levels in preeclamptic (n = 14) and normotensive (n = 14) women.

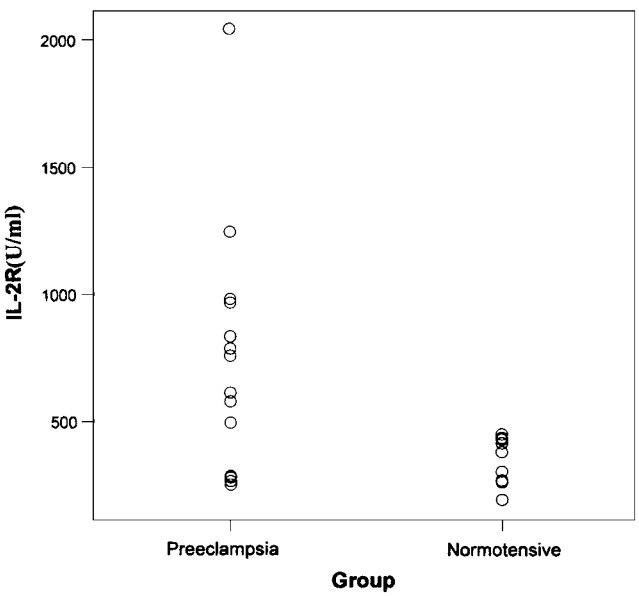

Fig. 2. Serum IL-2R levels in preeclamptic (n = 14) and normotensive (n = 14) women.

DISCUSSION

The present study demonstrates that serum neopterin and IL-2R levels increase in women with severe preeclampsia. These results suggest that increased serum neopterin and IL-2R levels may be related in part to the stimulated cell-mediated immune status during preeclampsia.

Cytokine-secreting T-cells play a central role in the immune response, and have been classified into subsets based on their type of cytokine production. Th1 cells synthesize mainly IL-2 and interferon-γ, which induce cellular immunity (4). Th2 cells produce predominantly IL-4, −5, −6, and −10, which promote humoral immunity (5). The shift from a Th1/Th2 balance to Th2 predominance occurs in normal pregnancy. It appears to protect the fetus and placenta from being rejected, and to aid in the maintenance of normal pregnancy (6). Normal pregnancy is characterized by depressed cell-mediated immunity in conjunction with enhanced humoral immunity. The production of Th2 cytokines by decidual T-cells contributes to the maintenance of pregnancy, while an exaggerated Th1 response appears to be harmful to the survival of the fetus (7,8). Enhanced progesterone production during pregnancy promotes Th2 secretion (7,9). Th1 dominance may be associated with pathologic conditions such as recurrent abortions and preeclampsia (10–12).

Neopterin is synthesized from guasine triphosphate in macrophages and monocytes by the activity of guanosine triphosphate cyclohydrolase I (13). The activity of this enzyme is greatly enhanced by interferon-γ and other cytokines. Serum concentrations of neopterin are increased in a variety of infections (e.g., malaria, measles, and septic shock), chronic inflammatory states (e.g., Crohn's disease and ulcerative colitis), and autoimmune disorders (e.g., rheumatoid arthritis and thyroiditis) (13,14). Neopterin is a sensitive indicator of the cell-mediated immune activator (15).

Fuith et al. (16) showed that neopterin levels exceed the normal range during pregnancy. Bichler et al. (17) demonstrated increased urinary neopterin concentrations in normal pregnancy. Neopterin values increase with pregnancy up to the third trimester and are higher than in normal nonpregnant women (18,19). Because neopterin production reflects cellular immunity, one may hypothesize that during pregnancy immunogenic stimuli are increasingly induced by the fetus and placenta (20,21). Only a few studies have mentioned neopterin levels and preeclampsia. In two different studies, Schröcksnadel et al. (22,23) showed that neopterin levels are increased in hypertensive pregnant women.

IL-2Rs mediate the action of IL-2, an immune system growth hormone. Normal resting T- and B-cells do not normally display significant numbers of these receptors on their cell surface, but when they are stimulated by a change to the immune system the number of IL-2 receptors on the cells' plasma membrane increases and a form of the IL-2R protein is released into the surrounding fluid by the activated cells (19). Activation of the T-cells is associated with increased cell-surface expression of IL-2R, which binds IL-2. Like neopterin, IL-2R typically is elevated during allograft rejection and the development of certain malignancies, infectious diseases, and autoimmune disorders (24). IL-2, which is an important cytokine in the T-cell pathway, is

increased in preeclampsia (2,25,26). The source of the Th1 cytokine may be decidual leukocytes and placenta (27). Decidual production of IL-2 is increased in preeclampsia, which may lead to increased proliferative and cytotoxic activities of uterine large granular lymphocytes, and conversion to lymphokine-activated killer cells (28). Placental cells express erythropoietin, the prototype molecule for transcriptional regulation of hypoxia in mammals. TNF-α and IL-1 have DNA sequences homologous to the hypoxia-responsive enhancer element of the erythropoietin gene, and thus provide a potential molecular link between placental hypoxia and stimulation of cytokine production (29). Dysfunctional endothelial cells undergo activation and produce leukocyte-endothelial adhesion molecules that are mediated by cytokines produced by inflammatory cells and activated endothelial cells (2). Preeclampsia is associated with increased levels of these adhesion molecules, and this increase may be an early event (30).

In our study we found that serum neopterin and IL-2R levels are increased in women with severe preeclampsia. The results of this study suggest that a Th1-type immune mechanism is involved in the pathogenesis of preeclampsia.

REFERENCES

1. Robillard PY. Interest in preeclampsia for researchers in reproduction. J Reprod Immunol 2002;53:279–287.
2. Dekker GA, Sibai BM. The immunology of preeclampsia. Seminars Perinat 1999;23:24–33.
3. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Technical bulletin no. 219. Washington, DC; American College of Obstetricians and Gynecologists. 1996.
4. Yoneyama Y, Sawa R, Suzuki S, et al. Relation between adenosine deaminase activities and cytokine-producing T cells in women with preeclampsia. Clin Biochem 2002;35:303–306.
5. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th and more. Immunol Today 1996;17:138–146.
6. Wegman TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a T_h2 phenomena? Immunol Today 1993;14:353–356.
7. Piccini MP, Maggi E, Romagnani S. Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy. Biochem Soc Trans 2000;28:212–215.
8. Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. Immunol Today 1997;18:478–482.
9. Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 1996;31:81–95.
10. Hill JA, Polger K, Anderson DJ. T helper I type immunity to trophoblasts in women with recurrent spontaneous abortion. JAMA 1995;2/3:1933–1936.
11. Saito S, Sakai M, Sasaki M, Tanabe K, Tsuda H, Michihama T. Quantitative analysis of peripheral blood Th0, Th1, Th2 and the Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clin Exp Immunol 1999;117:550–555.
12. Darmochwal-Kolarz D, Leszczynska-Gorzelak B, Rolinsli J, Oleszczuk J. T helper1- and T helper2-type cytokine imbalance in pregnant women with preeclampsia. Eur J Obstet Gynecol Reprod Biol 1999;86:165–170.
13. Muller MM, Curtius HC, Herold M, Huber CH. Neopterin in clinical practice. Clin Chim Acta 1991;201:1–16.
14. Fuchs D, Weiss G, Wachter H. Neopterin, biochemistry and clinical use as a marker for cellular immune reactions. Int Arch Allerg Immunol 1993;101:1–6.
15. Huber C, Batchelor JR, Fuchs D, Hausen A, Lang A, Niederwieser D. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon gamma. J Exp Med 1984;160:310–316.
16. Fuith LC, Fuchs D, Hausen A, et al. Neopterin, a marker of cell-mediated immune activation in human pregnancy. Int J Fertil 1991;36:372–375.
17. Bichler A, Fuchs D, Hausen A, Hetzel H, Reibnegger G, Wachter H. Measurement of urinary neopterin in normal pregnant and nonpregnant women and in women with benign and malignant genital neoplasms. Arch Gynecol 1983;233:121–130.
18. Schröcksnadel H, Baier-Bitterlich G, Dapunt O, Wachter H, Fuchs D. Decreased plasma tryptophan in pregnancy. Obstet Gynecol 1996;88:47–50.
19. MacLean MA, Wilson R, Thomson JA, Krishnamurthy S, Walker JJ. Immunological changes in normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1992;43:167–172.
20. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter H. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol Today 1988;9:150–155.
21. Munn DH, Zhou M, Attwood JT, et al. Prevention of allogenic fetal rejection by tryptophan catabolism. Science 1998;281: 1191–1193.
22. Schröcksnadel H, Herold M, Steckel-Berger G, Fuchs D, Wachter H, Dapunt O. Zellvermittelte immunitet bei schwangerschaftsinduzierten hochdruckerkrankungen. Geburtsh U Frauenheilk 1992;52:592–595.
23. Schröcksnadel H, Fuchs D, Herold M, Wachter H, Dapunt O. Aktivierte makrophagen im pathomechanismus schwangerschaftsinduzierter hochdruckerkrankungen. Zentralbl Gynacol 1994;116:274–275.
24. Wachter H, Fuchs D, Hausen A, Reibnegger G, Werner ER. Neopterin as marker for activation of cellular immunity: immunologic basis and clinical application. Adv Clin Chem 1989;27:81–141.
25. Darmochwal-Kolarz D, Rolinski J, Leszczynska-Gorzelak B, Oleszczuk J. The expressions of intracellular cytokines in the lymphocytes of preeclamptic patients. Am J Reprod Immunol 2002;48:381–386.
26. Madazlý R, Aydýn S, Uludag S, Vildan O, Tolun N. Maternal plasma levels of cytokines in normal and preeclamptic pregnancies and their relationship with diastolic blood pressure and fibronectin levels. Acta Obstet Gynecol Scand 2003;82:797–802.
27. Vince GS, Johnson PM. Immunobiology of human uteroplacental macrophages–friend and foe. Placenta 1996;17:191–199.
28. Hamai Y, Fujii T, Yamashita T, Kozuma S, Okai T, Taketani Y. Pathogenic implication of interleukin 2 expressed in preeclamptic decidual tissues: a possible mechanism of deranged vasculature of the placenta associated with preeclampsia. Am J Reprod Biol 1997;38:83–88.
29. Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. Am J Reprod Biol 1997;37:240–249.
30. Krauss T, Kuhn W, Lakoma C, Augustin HG. Circulating endothelial adhesion molecules as diagnostic markers for the early identification of pregnant women at risk for development of preeclampsia. Am J Obstet Gynecol 1997;177:443–449.