Maternal Second Trimester Serum Tumor Necrosis Factor-α-soluble Receptor p55 (sTNFp55) and Subsequent Risk of Preeclampsia

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Preeclampsia is characterized by diffuse vascular endothelial dysfunction. Tumor necrosis factor-α (TNF-α), which plays a key role in the cytokine network responsible for immunoregulation, is also known to contribute to endothelial dysfunction and other metabolic disturbances noted in preeclampsia. Results from cross-sectional studies and one longitudinal study indicate that TNF-α (or its soluble receptor, sTNFp55) is increased in the peripheral circulation and amniotic fluid of women with preeclampsia as compared with normotensive women. Between December 1993 and August 1994, prediagnostic sTNFp55 concentrations (a marker of excessive TNF-α release) were measured in 35 women with preeclampsia and 222 normotensive women to determine whether elevations precede the clinical manifestation of the disorder. Logistic regression procedures were used to calculate maximum likelihood estimates of odds ratios and 95% confidence intervals. Mean second trimester (15–22 weeks' gestation) serum sTNFp55 concentrations, measured by enzyme-linked immunosorbent assay, were 14.4% higher in preeclamptic women than in normotensive controls (716.6 pg/ml (standard deviation 193.6) vs. 626.4 pg/ml (standard deviation 158.0); p = 0.003). The relative risk of preeclampsia increased across successively higher quintiles of sTNFp55 (odds ratios were 1.0, 1.3, 2.1, and 3.7, with the lowest quintile used as the referent; p for trend = 0.007). After adjustment for maternal age, adiposity, and parity, the relative risk between extreme quintiles was 3.3 (95% confidence interval 0.8–13.4). These findings indicate that the level of TNF-α in maternal circulation is increased prior to the clinical manifestation of the disorder, and they are consistent with the hypothesized role of cytokines in mediating endothelial dysfunction and the pathogenesis of preeclampsia. Further work is needed to identify modifiable risk factors for the excessive synthesis and release of TNF-α in pregnancy, and to assess whether lowering of TNF-α concentrations in pregnancy alters the incidence and severity of preeclampsia. Am J Epidemiol 1999;149:323–9.

cytokines; pre-eclampsia; pregnancy; receptors, tumor necrosis factor; tumor necrosis factor
Although the causes of endothelial cell activation and dysfunction in preeclampsia are presently unknown, investigators have proposed that placental hypoxemia, secondary to abnormal trophoblast implantation, gives rise to bloodborne products which directly or indirectly activate and damage endothelial cells (5). Cytokines, soluble peptide protein mediators of the immune system, are involved in immune response, inflammatory reactions, the control of maternal immune response, and fetoplacent development. They are known to be mediators of endothelial cell properties, and they have been suggested to be the bloodborne product capable of damaging endothelial cells and contributing to many of the pathophysiological changes associated with preeclampsia (16, 18).

Tumor necrosis factor-α (TNF-α), a powerful 17-kilobase proinflammatory polypeptide, is active at picomolar concentrations. It plays a central role as an endogenous mediator of endotoxic shock, elicits a wide spectrum of biologic activities, and is the most well-studied cytokine. Originally identified as a product of activated macrophages which inhibit tumor cell proliferation, TNF-α is now known to be produced by other cells. It has pleiotropic effects which modulate cellular growth and differentiation and the synthesis of other substances. Importantly, TNF-α has been shown to be synthesized by the placenta (19). The effects of TNF-α are mediated by the TNF-α-soluble receptors sTNFp55 and sTNFp75, each of which can be shed as a soluble protein binding specifically to TNF-α. If released in large amounts, TNF-α can modify the anticoagulant properties of endothelial cells, stimulate platelet-derived growth factor production, activate neutrophils, promote hypertriglyceridemia, increase lipid peroxide formation, modulate cell adhesion molecules, and induce the release of other proinflammatory cytokines such as interleukin-6 (20, 21). The shedding of TNF-α-soluble receptors is thought to limit the responsiveness of cells to the effects of TNF-α, and they are therefore thought to play an important role in modulating the activity of TNF-α (20).

The collective biologic properties and activities of TNF-α, coupled with the consistently noted metabolic disturbances documented in preeclamptic pregnancies, have led investigators to postulate that TNF-α may be important in preeclampsia (22–24). Some cross-sectional studies (16, 18, 25), though not all (26), have demonstrated that serum or plasma TNF-α concentrations are increased among preeclamptic women as compared with normotensive pregnant women. However, since concentrations of TNF-α were determined after the diagnosis of preeclampsia, it is not possible to infer from these studies the longitudinal relation between excessive TNF-α synthesis and the risk of preeclampsia. Results obtained from two very small longitudinal studies are conflicting (27, 28). Additionally, previous investigators have not controlled for potential confounding, nor have they reported the magnitude of associations between varying levels of TNF-α and preeclampsia.

Using a nested case-control study design, we sought to determine the extent to which elevations of maternal serum TNF-α-soluble receptor p55 (sTNFp55) during the second trimester were predictive of subsequent preeclampsia risk. Because the half-life of TNF-α is less than 30 minutes (29, 30)—resulting in poor detection even in instances of toxic shock (31)—we elected to measure the soluble receptor, sTNFp55. The half-life of sTNFp55 is reported to be 45 minutes to 2 hours (31), thus presenting a more favorable biologic marker for excessive TNF-α release in epidemiologic studies with only one measurement of exposure. Specifically, the longer half-life diminishes, to some extent, the likelihood of subject misclassification according to excessive TNF-α release.

**MATERIALS AND METHODS**

A cohort of 1,383 pregnant women who provided a second trimester serum sample to a central laboratory between December 1993 and August 1994 and who later gave birth at the Swedish Medical Center in Seattle, Washington, constituted the base cohort study population, wherein the present case-control study was nested. Each blood sample, collected at 15–22 weeks’ gestation, was centrifuged, and serum was separated. Serum remaining after routine biochemical analyses were completed was stored at 20°C. After 1–3 months, serum samples were transferred to the Swedish Medical Center and stored at −70°C until analysis.

From this base cohort population of 1,383 pregnant women, we identified 39 patients who subsequently developed preeclampsia. The diagnosis of preeclampsia was made in accordance with the guidelines of the American College of Obstetricians and Gynecologists (32). Using serial maternal blood pressure readings and maternal urinary protein results abstracted from medical records, preeclampsia was defined as a sustained (≥26 hours) 15-mmHg rise in diastolic blood pressure or a 30-mmHg rise in systolic blood pressure. If first trimester blood pressures were unknown, preeclampsia was defined as a sustained (≥26 hours) blood pressure greater than or equal to 140/90 mmHg. Proteinuria was defined as a urinary protein concentration of 0.1 g/liter or more in at least two random specimens collected at least 4 hours apart. Nulliparity was not a diagnostic criterion for this investigation. In this base study population of 1,383 nulliparous and multiparous gravidae, the incidence of preeclampsia was
**Maternal Serum sTNFp55 and Risk of Preeclampsia**

Absorbance was determined at 450 nm using a microplate reader (Vmax; Molecular Devices, Sunnyvale, California). Unknown sTNFp55 values were calculated from a standardized curve using recombinant human sTNFp55 (R & D Systems) in high-salt assay buffer by four-parameter analysis (SoftMax Pro; Molecular Devices). Inter- and intra-assay coefficients of variation were determined to be <10 percent with an assay sensitivity of <2 pg/ml. Analyses of all samples, standards, and controls were run in duplicate. All laboratory analyses were performed without knowledge of pregnancy outcomes.

Information on maternal sociodemographic characteristics, reproductive and medical histories, and labor and delivery characteristics, as well as anthropometric measurements (maternal height, prepregnancy weight, and weight at 18–22 weeks’ gestation), was abstracted from maternal medical records. Gestational age was based on the date of the last menstrual period and was confirmed by ultrasound examination, conducted prior to 20 weeks’ gestation. Body mass index, used as a measure of overall maternal adiposity, was calculated as weight in kilograms divided by height in meters squared. Maternal pregnancy weight gain (up to 18–22 weeks’ gestation) was calculated by subtracting maternal prepregnancy weight from maternal weight measured at 18–22 weeks’ gestation.

To estimate the relative association between preeclampsia and second trimester sTNFp55 concentrations, we categorized each subject according to quintiles determined by the distribution of sTNFp55 concentrations in control subjects. Using the lowest quintile as the referent group, we estimated odds ratios and 95 percent confidence intervals. The Mantel extension test for linear trend in proportion was used in univariate analyses to test for a linear component of trend in preeclampsia risk in relation to sTNFp55 receptor concentrations in maternal serum (33). Logistic regression procedures were used to calculate maximum likelihood estimates for the coefficients, and their standard errors were used to calculate odds ratios and 95 percent confidence intervals adjusted for confounding factors. In multiple logistic regression models, significance for monotonic trends was assessed by treating the five quintiles as a continuous variable after assigning a score for each quintile.

To assess confounding, we entered variables into a logistic regression model one at a time, then compared the adjusted and unadjusted odds ratios (34, 35). Factors assessed as potential confounders included maternal age (continuous and categorical), race, receipt of public assistance (welfare), marital status, cigarette smoking status, parity, gravidity, prepregnancy body mass index, and gestational age at specimen.
collection. Final logistic regression models included covariates that altered unadjusted odds ratios by at least 10 percent (34). The final modeling of confounding variables was based on the categorizations that accounted for the greatest degree of confounding. Effect modification by parity and adiposity (as measured by maternal prepregnancy body mass index) was evaluated in stratified analyses and by including appropriate interaction terms in logistic regression models (35). All reported \( p \) values are two-tailed.

The research was approved by the Institutional Review Board of the Swedish Medical Center.

RESULTS

Sociodemographic, medical, and reproductive characteristics of the study subjects are presented in table 1. Women who developed preeclampsia, as compared with control subjects, tended to be heavier and to be nulliparous. Preeclampsia cases and controls had gained similar amounts of weight by the second trimester. The average gestational age at blood collection was similar for the two study groups. The mean maternal second trimester sTNFp55 concentration was statistically significantly higher among women who later developed preeclampsia than among those women who remained normotensive throughout pregnancy (716.6 pg/ml (SD 193.6) vs. 626.4 pg/ml (SD 158.0); \( p = 0.003 \)). In absolute terms, sTNFp55 concentrations were, on average, 14.4 percent higher among women who later developed preeclampsia than in women who remained normotensive throughout pregnancy.

The relative risk of preeclampsia increased significantly across increasing quintiles of concentrations of maternal serum sTNFp55 (table 2) (for linear trend in risk across quintiles, unadjusted \( p \) = 0.007). Women in the highest quintile experienced a 3.7-fold increased risk of preeclampsia compared with women in the lowest quartile (unadjusted odds ratio = 3.7, 95 percent confidence interval 1.0–14.4). After adjustment for potential confounding by maternal age, adiposity, and parity, the relative risk between extreme quintiles decreased slightly and was no longer statistically significant (adjusted odds ratio = 3.3, 95 percent confidence interval 0.8–13.4; for linear trend in risk, adjust-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preeclampsia cases (n = 35)</th>
<th>Control subjects (n = 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (years)</td>
<td>31.1 (6.5)*</td>
<td>29.6 (4.7)</td>
</tr>
<tr>
<td>&lt;19</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>19–34</td>
<td>22</td>
<td>62.9</td>
</tr>
<tr>
<td>≥35</td>
<td>12</td>
<td>34.3</td>
</tr>
<tr>
<td>Black race</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Receipt of public assistance (welfare)</td>
<td>7</td>
<td>20.0</td>
</tr>
<tr>
<td>Single marital status</td>
<td>9</td>
<td>25.7</td>
</tr>
<tr>
<td>Cigarette smoker</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Primigravida</td>
<td>15</td>
<td>42.9</td>
</tr>
<tr>
<td>Nullipara</td>
<td>25</td>
<td>71.4</td>
</tr>
<tr>
<td>Mean prepregnancy body mass index†</td>
<td>24.8 (3.2)</td>
<td>22.6 (3.8)</td>
</tr>
<tr>
<td>Quartile 1 (&lt;19.9)</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Quartile 2 (19.9–21.6)</td>
<td>4</td>
<td>11.4</td>
</tr>
<tr>
<td>Quartile 3 (21.7–23.4)</td>
<td>9</td>
<td>25.7</td>
</tr>
<tr>
<td>Quartile 4 (≥23.4)</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Mean body mass index at 18–22 weeks</td>
<td>27.4 (3.4)</td>
<td>24.9 (4.0)</td>
</tr>
<tr>
<td>Mean weight gain (kg) by 18–22 weeks</td>
<td>2.7 (1.5)</td>
<td>2.4 (1.5)</td>
</tr>
<tr>
<td>Mean gestational age (weeks) at specimen collection</td>
<td>16.9 (1.9)</td>
<td>16.9 (1.1)</td>
</tr>
<tr>
<td>Mean gestational age (weeks) at delivery</td>
<td>36.7 (3.4)</td>
<td>38.6 (2.0)</td>
</tr>
<tr>
<td>Mean infant birth weight (g)</td>
<td>2,809 (968)</td>
<td>3,400 (550)</td>
</tr>
<tr>
<td>Mean sTNFp55‡ level (pg/ml)</td>
<td>716.6 (193.6)</td>
<td>626.4 (158.0)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, standard deviation.
† Weight (kg)/height (m)².
‡ sTNFp55, tumor necrosis factor-α-soluble receptor p55.
ed \( p = 0.055 \)). There was no evidence of effect modification by parity or maternal prepregnancy adiposity.

**DISCUSSION**

We have shown that excessive TNF-\( \alpha \) release (as measured by detection of the soluble receptor sTNFp55 in maternal serum collected at an average gestational age of 16 weeks) into the maternal circulation precedes the clinical manifestation of preeclampsia. After we controlled for potential confounders, women in the highest quintile of serum sTNFp55 concentration as compared with women in the lowest quintile experienced a 3.3-fold increased risk of preeclampsia. These findings are consistent with the hypothesis that excessive cytokine release, particularly release of TNF-\( \alpha \), mediates risk of preeclampsia. Our reported associations are in general agreement with results from some previous cross-sectional investigations (16, 18, 25), but not all (26). In a case-control study of 31 women with preeclampsia and an equal number of normotensive controls matched on maternal age, parity, and gestational age (average gestational age was approximately 33 weeks), Vince et al. (18) noted that TNF-\( \alpha \) concentrations were not detectable for a majority of cases and controls. Median concentrations of sTNFp55 were reported to be statistically significantly higher in cases than in controls (1.22 ng/ml vs. 0.72 ng/ml; \( p < 0.001 \)). The investigators further noted that concentrations of sTNFp55 were correlated with the severity of preeclampsia.

Our results are at variance with those of Meekins et al. (27), who reported that TNF-\( \alpha \) concentrations were not elevated prior to the clinical manifestation of preeclampsia. In their study of 10 preeclamptic and 10 normotensive pregnant women, they noted that maternal serum TNF-\( \alpha \) concentrations (at 32 weeks’ gestation, on average) were below the level of detection (<80 pg/ml) in all instances. In one report that has been published since we conducted our study, investigators provided evidence which corroborates our findings. In a study of Japanese women, Hamai et al. (28), using a very sensitive assay, measured TNF-\( \alpha \) in serum samples collected at 11–13 weeks’ gestation for 16 preeclamptic women and 16 normotensive pregnant controls. The investigators reported that median TNF-\( \alpha \) concentrations were statistically significantly higher in cases than in controls (9.5 pg/ml vs. 4.5 pg/ml; \( p < 0.05 \)).

Results from several other studies also support our observations. Placental TNF-\( \alpha \) protein levels are increased for preeclamptic women as compared with controls (36). Wang and Walsh (36) recently reported that mean levels of TNF-\( \alpha \) were 49 percent higher in preeclamptic placentas than in the placentas of normotensive pregnant women (17.32 pg/mg protein (SD 1.97) vs. 11.62 pg/mg protein (SD 0.93)). Using reverse transcription polymerase chain reaction, the investigators also showed that placental TNF-\( \alpha \) mRNA was expressed in preeclamptic placentas but not in normotensive placentas. Interestingly, Chen et al. (37), in their study of 14 women with preeclampsia, 12 normotensive pregnant women, and 15 nonpregnant women, reported that TNF-\( \alpha \) mRNA expression (RNA from peripheral blood leukocytes) was significantly elevated in preeclamptic women as compared with normotensive pregnant women and nonpregnant women, respectively. Moreover, in their evaluation of the distribution of subjects according to the TNF-\( \alpha \) polymorphism, Chen et al. noted that the frequency of the TNF-\( \alpha \) allele, which is associated with significantly higher TNF-\( \alpha \) mRNA expression, was significantly increased in preeclamptic cases (37).

Many of the consistently documented pathophysiology features of preeclampsia may result from or be augmented by the biologic activities of TNF-\( \alpha \). For instance, disseminated intravascular coagulation which is seen in preeclampsia (5) may result from the actions of TNF-\( \alpha \) on platelets and its promotion of procoagulative conditions over the vascular endothelium (20, 38). Furthermore, TNF-\( \alpha \) is a potent activator of

**TABLE 2. Second trimester serum concentrations of tumor necrosis factor-\( \alpha \)-soluble receptor p55 (sTNFp55) in women who developed preeclampsia and those who remained normotensive throughout pregnancy, Seattle, Washington, 1993–1994**

<table>
<thead>
<tr>
<th>Quintile of sTNFp55 (pg/ml)</th>
<th>Preeclampsia cases (n = 35)</th>
<th>Control subjects (n = 222)</th>
<th>Unadjusted odds ratio</th>
<th>95% confidence interval</th>
<th>Adjusted* odds ratio</th>
<th>95% confidence interval</th>
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<tbody>
<tr>
<td>&lt;502.6</td>
<td>4</td>
<td>11.4</td>
<td>45</td>
<td>20.3</td>
<td>1.0†</td>
<td>1.0†</td>
</tr>
<tr>
<td>502.6–573.4</td>
<td>4</td>
<td>11.4</td>
<td>45</td>
<td>20.3</td>
<td>1.0</td>
<td>0.2–5.7</td>
</tr>
<tr>
<td>573.5–650.3</td>
<td>5</td>
<td>14.3</td>
<td>45</td>
<td>20.3</td>
<td>1.3</td>
<td>0.3–6.0</td>
</tr>
<tr>
<td>650.5–744.0</td>
<td>8</td>
<td>22.9</td>
<td>44</td>
<td>19.8</td>
<td>2.1</td>
<td>0.5–8.8</td>
</tr>
<tr>
<td>&gt;744.0</td>
<td>14</td>
<td>40.0</td>
<td>43</td>
<td>19.4</td>
<td>3.7</td>
<td>1.0–14.4</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>p trend</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
<td>0.055†</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for maternal age, parity, and body mass index.
† Referent.
neutrophils (20). Greer et al. (39) have shown previously that activated neutrophils precede the clinical manifestation of preeclampsia. It is notable that TNF-α-induced hyperlipidemia is part of the acute-phase response induced by TNF-α and other proinflammatory cytokines in response to infection and inflammation (40). Feingold et al. (41) have shown TNF-α substantially augments hepatic synthesis of fatty acids and triglycerides. Results from studies in humans (42) and animals (41) indicate that administration of TNF-α promotes increases in serum triglyceride concentrations. Feingold et al. (41) have shown that TNF-α acutely stimulates de novo fatty acid synthesis and hepatic triglyceride synthesis. The latter has been shown to result in increased secretion of lipids into the circulation. Moreover, Semb et al. (43) have shown that TNF-α is capable of decreasing adipose lipoprotein lipase activity, which in turn may lead to decreased clearance of triglyceride-rich lipoproteins, resulting in hypertriglyceridemia. Investigators have shown that hyperlipidemia, particularly hypertriglyceridemia, precedes the clinical manifestation of preeclampsia (8).

Cells treated with TNF-α release arachidonic acid, an omega-6 fatty acid that can be metabolized to prostaglandin (44) and the potent lipolytic enzyme phospholipase A₂. We have shown previously that arachidonic acid is increased in preeclampsia, and other investigators have reported that phospholipase A₂ is increased in preeclampsia (4). Supplementation with omega-3 fatty acids has been shown to significantly attenuate the production of proinflammatory cytokines, including TNF-α (45, 46). Antioxidants have been suggested to selectively inhibit the release of TNF-α because they control glutathione, which is regarded as an endogenous modulator of TNF-α production (47). Finally, TNF-α, which is synthesized by both decidual and trophoblast cells, has been shown to significantly inhibit trophoblast cell motility in vitro (48); this suggests that the excessive release of the cytokine may, in part, contribute to restricted endovascular cytotrophoblast invasion along the uterine spiral arteries in preeclampsia (6).

In summary, TNF-α interacts in complex ways with the immune compartment and with vascular endothelial cells to release other cytokines, to augment the expression of adhesion molecules, to promote the release of neutrophils, and to promote the synthesis of lipid peroxides and eicosanoids, which are likely to be important mediators of pathophysiologic lesions seen in preeclampsia. The results of our study indicate that women destined to develop preeclampsia have elevated serum sTNFp55 concentrations in the second trimester (a marker for excessive TNF-α release) as compared with women who remain normotensive throughout pregnancy. Anti-TNF-α therapy, including administration of medications which prevent the synthesis of endogenous TNF-α, has been shown to attenuate organ rejection, graft-versus-host disease (20), and other conditions, including rheumatoid arthritis (49) and adult respiratory distress syndrome (30), which are known to be mediated by TNF-α. Additional work is needed to confirm our findings, to identify modifiable risk factors for the excessive synthesis and release of TNF-α in pregnancy, and to assess whether lowering of TNF-α concentrations in pregnancy alters the incidence and severity of preeclampsia.

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REFERENCES

14. FitzGerald DJ, Entman SS, Mulloy K, et al. Decreased prosta-