Transforming Growth Factor-β₁ (TGF-β₁) in Plasma Is Associated With Preeclampsia Risk in Peruvian Women With Systemic Inflammation

Martin Muy-Rivera, Sixto E. Sanchez, Surab Vadachkoria, Chunfang Qiu, Victor Bazul, and Michelle A. Williams

**Background:** In a case-control study of 100 preeclamptics and 100 controls, we assessed plasma transforming growth factor-β₁ (TGF-β₁) concentrations in relation to preeclampsia risk among Peruvian women with and without systemic inflammation.

**Methods:** Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

**Results:** The OR of preeclampsia increased across quartiles of TGF-β₁ concentrations. Women with elevated TGF-β₁ and a proinflammatory profile experienced the highest risk of preeclampsia (OR = 15.4, 95% CI 4.7–50.4).

**Conclusions:** Our results confirm an association between TGF-β₁ and risk of preeclampsia and extend the literature by indicating a strong association in women with systemic inflammation. Am J Hypertens 2004;17:334–338 © 2004 American Journal of Hypertension, Ltd.

**Key Words:** Cytokines, Peru, preeclampsia, pregnancy, soluble receptors, transforming growth factor-β₁, tumor necrosis factor-α.

The transforming growth factor-β (TGF-β) “superfamily” of growth factors constitutes a large family of protein growth factors that play important roles in physiologic processes ranging from differentiation and development, to the regulation of cell growth and extracellular matrix biology. Transforming growth factor-β is also known to have powerful immune regulatory properties, including their capacity to modulate inflammatory events in leukocytes and vascular endothelial cells. As recently noted by Topper, the role of TGF-β growth factors in regulating immune functions was dramatically illustrated by Shull et al, who observed that mice lacking the TGF-β₁ gene die in utero or in the perinatal period due to widespread, uncontrolled inflammation. Notably, these adverse events can be prevented with the systemic administration of active soluble TGF-β₁.

Transforming growth factor-β₁, a 25-kD homodimeric protein, secreted by several cell types including peripheral blood monocytes, endothelial cells, vascular smooth muscle, platelets, renal and decidual cells, is regarded as the prototypical member of the TGF-β superfamily. Transforming growth factor-β₁ has been implicated in the pathogenesis of renal and cardiovascular disorders including renal fibrosis, end-stage renal disease, atherosclerosis, myocardial hypertrophy, and essential hypertension in humans and in several animal models. Li et al recently reported a statistically significant positive correlation between plasma TGF-β₁ concentrations and blood pressure in patients with end-stage renal disease.

A rapidly accumulating literature suggests that TGF-β₁ is likely to play several important roles in human parturition. Transforming growth factor-β₁ has been noted to have antiproliferative effects of trophoblast cells, which may in turn influence placental implantation. Although not all, investigators have reported that increased TGF-β₁ concentrations are predictive of preeclampsia, a hypertensive disorder of pregnancy that is characterized by shallow endovascular cytotrophoblast invasion in spiral arteries, chronic systemic inflammation, diffuse endothelial dysfunction and activation, renal dysfunction, and hypertension. Previous investigators, however, have rarely controlled for confounding factors, and even fewer have reported the magnitude of association between preeclampsia risk and varying concentrations of TGF-β₁.
TGF-\(\beta_1\). We, therefore, used available data from a case-control study of Peruvian women\(^{13}\) to evaluate the extent to which plasma TGF-\(\beta_1\) concentrations are associated with an increased risk of preeclampsia. We also evaluated the extent to which the relation between TGF-\(\beta_1\) and preeclampsia risk is modified by elevated concentrations of soluble tumor necrosis factor p55 (sTNF-p55), a stable marker of elevated tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) synthesis and release.

**Methods**

Subjects for this analysis were recruited between June 1997 and January 1998 as part of a case control study designed to study the epidemiology of preeclampsia among Peruvian women. Details regarding data collection methods have been previously described.\(^{13}\) During the study period, women with preeclampsia and normotensive women were recruited from Labor and Delivery wards at the Materno-Perinatal Institute and the Dos de Mayo Hospital in Lima, Peru. The Ethical Committee of the Dos De Mayo Hospital, the Materno-Perinatal Institute of Lima, and the Human Subjects Committee of the University of Washington Medical Center approved this investigation.

From the original study population of 169 women with preeclampsia (according to the then-current diagnostic criteria) and 201 normotensive control subjects, we randomly selected 100 women with preeclampsia according to recently revised diagnostic criteria.\(^{14}\) Controls (\(n = 100\)) were randomly selected from women with pregnancies uncomplicated by pregnancy-induced hypertension and proteinuria. Women with pregestational diabetes and women with a physician diagnosis of chronic hypertension were not eligible for this study.

A structured interview questionnaire was used to collect information regarding maternal sociodemographic, medical, reproductive, and lifestyle characteristics. Maternal and infant records were reviewed to collect detailed information concerning antepartum, labor, and delivery characteristics, and conditions of the newborn. Nonfasting, prelabor blood samples were collected in EDTA 10-mL Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Plasma TGF-\(\beta_1\) concentrations were determined using enzyme immunoassay (R&D Systems, Minneapolis, MN). Intra-assay and interassay coefficients of variation were both below 10%.

Comparisons of categoric variables were made between case and control subjects using \(\chi^2\) or Fisher’s exact tests. Unadjusted mean differences in maternal plasma TGF-\(\beta_1\) concentrations and other continuous variables such as maternal age and pregnancy body mass index (BMI) were assessed using the Student \(t\) test. Spearman correlation coefficients were used to determine the relationship between maternal TGF-\(\beta_1\) and selected maternal characteristics.

To estimate the relative association between preeclampsia and concentrations of plasma TGF-\(\beta_1\), we categorized each subject according to quartiles determined by its distribution in control subjects. Using the lowest quartile category of TGF-\(\beta_1\) for the reference group, odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated. Logistic regression procedures were used to calculate maximum likelihood estimates for the coefficients and their standard errors were used to calculate ORs and 95% CIs, adjusted for confounders. We explored the possibility of a nonlinear relation between plasma TGF-\(\beta_1\) concentrations and preeclampsia risk using generalized additive modeling procedures. All analyses were performed using Stata 7.0 statistical software (Stata, College Station, TX).

**Results**

Table 1 lists the characteristics of study participants. Mean maternal plasma TGF-\(\beta_1\) concentrations were 42% higher in cases than in controls (mean ± SE: 18.4 ± 1.0 ng/mL vs 13.0 ± 0.8 ng/mL, \(P < .001\)). We examined the association between maternal plasma TGF-\(\beta_1\) concentrations with covariates, including maternal prepregnancy BMI, plasma sTNF-p55 concentrations, infant birth weight, and gestational age at blood collection among preeclampsia cases. Analyses were repeated for controls. Plasma sTNF-
p55 concentrations were positively correlated with plasma TGF-β1 concentrations among case and control subjects, respectively (r = 0.24, P = .016 for cases; r = 0.23, P = .024 for controls). Maternal plasma TGF-β1 concentrations did not correlate with maternal prepregnancy BMI, infant birth weight, or gestational age at blood collection (all P values > .05; data not shown).

As listed in Table 2, the relative risk of preeclampsia (as estimated by the OR) increased across successively higher quartiles of TGF-β1 (ORs: 1.0, 2.1, 3.3, 6.1 with the lowest quartile as referent; P for trend < .001). After adjusting for potential confounding by maternal age, parity, prepregnancy BMI, and plasma sTNF-p55 concentration, the OR for each of the upper three tertiles increased slightly (adjusted ORs: 1.0, 3.4, 4.0, 7.2, with the lowest quartile as referent) and the test for trend remained statistically significant (P for trend < .001). Compared to women with TGF-β1 concentrations <8.1 ng/mL (the lowest quartile), those with concentrations of 15.8 ng/mL (the upper quartile) experienced a 7.2-fold increased risk of preeclampsia (adjusted OR = 7.2; 95% CI 2.2 to 23.8).

Then, we modeled the risk of preeclampsia in relation to maternal plasma TGF-β1 concentrations expressed as a continuous variable using a generalized additive model. For these analyses we excluded 8 subjects with TGF-β1 concentrations >40 ng/mL that were thought to be outliers. From these analyses, we noted a linear relationship between preeclampsia risk and plasma TGF-β1 (Fig. 1). On the basis of this observation, we modeled plasma TGF-β1 concentrations expressed as a continuous variable. From this analysis, we noted that a 5 ng/mL increase in plasma TGF-β1 concentration was associated with a 50% increase in preeclampsia risk (adjusted OR = 1.5; 95% CI 1.1 to 1.9), after adjusting for maternal age, race/ethnicity, nulliparity, prepregnancy BMI, and sTNF-p55 concentrations.

Because available data suggest that TGF-β1 has immuno-regulatory properties, and because results from this and other study populations indicate that preeclampsia risk is increased among women with elevated proinflammatory cytokines including TNF-α, sTNF-p55, and interleukin-2, we examined the independent and joint associations of elevated TGF-β1 concentrations (>10.4 ng/mL) and elevated sTNF-p55 (>598 pg/mL) with risk of preeclampsia. For these analyses women with TGF-β1 concentrations ≥10.4 ng/mL and with sTNF-p55 concentrations ≥598 pg/mL served as the referent group. Women with elevated TGF-β1 concentrations, but who did not have elevated sTNF-p55 concentrations, as compared with the referent group, experienced a 3.1-fold increased risk of preeclampsia (95% CI 0.8 to 12.5). The OR for women with elevated sTNF-p55, but without elevated TGF-β1, was 5.1 (95% CI 1.4 to 17.8). Women with elevated TGF-β1 and sTNF-p55 concentrations experienced a 15.4-fold increased risk of preeclampsia (95% CI 4.7 to 50.4). The excess risk of

![FIG. 1. Relationship between maternal plasma transforming growth factor-β1 (TGF-β1) concentrations and risk of preeclampsia (solid line), with 95% confidence intervals (dotted lines) after adjusting by maternal age, parity, prepregnancy body mass index, and sTNF-p55 concentrations. The vertical bars along the plasma TGF-β1 concentration axis indicate distribution of study subjects.](https://academic.oup.com/ajh/article-abstract/17/4/334/206803/321x106)
preeclampsia associated with having both elevated TGF-β1 and sTNF-p55 concentrations was greater than the sum of the excess risk for each cytokine considered independently. Hence, in this population, there was some evidence of a greater-than-additive effect between elevated TGF-β1 and elevated sTNF-p55 on the risk of preeclampsia, although the interaction term did not reach statistical significance (P = .89) due to the small sample size.

Discussion

Results from our study are consistent with some, 7–9 although not all, previous reports. 10–12 In a case-control study of Norwegian women, Djurovic et al11 reported that TGF-β1 concentrations were elevated in women with severe and mild preeclampsia late in gestation (mean gestational age, 40 weeks) compared with normotensive pregnant women. This finding of elevated TGF-β1 among preeclamptics compared with normotensive pregnant women was corroborated in subsequent studies of women in Istanbul, Turkey,8 and Durban, South Africa.8 Results of our study of Peruvian women are consistent with these reports. Our results however, are not consistent with those reported by Huber et al,12 who reported no statistical difference in maternal serum concentrations of TGF-β1.

There are several possible explanations why the existing studies are not in complete agreement, the most likely being differences in study design and analytical techniques. As recently noted by Naicker et al13 differences in procedures used when processing blood samples and in assay techniques can result in considerable variability in TGF-β1 concentrations. For instance, investigators have noted that determination of TGF-β1 in serum may not reflect plasma concentrations as the cytokine is released from platelets during clotting.7 Differences in population characteristics such as maternal systemic inflammatory status, as well as dissimilar distributions of the severity of preeclampsia could also account for some of the variation. In addition, distortion from uncontrolled confounding secondary to whether blood samples were collected before, after, or during labor, and other maternal factors may have been present in many of the previous studies. Last, several studies may have been limited by the small sample size, having too little statistical power to detect true differences between case and control subjects.

Our present study has several important strengths. First, the relatively large size of our study allowed us to assess the risk of preeclampsia in relation to varying concentrations of maternal plasma TGF-β1 while controlling for confounding factors. Second, the high participation rates achieved for cases and controls (>95% for cases and controls, respectively) minimized possible selection bias. Third, specimens were collected before labor onset, and were analyzed without knowledge of pregnancy outcome, thus reducing the likelihood of confounding and systemic error in determining maternal TGF-β1 concentrations, respectively. However, an important limitation merits discussion and consideration. Because of the retrospective design of our study, we cannot determine whether the observed elevations in TGF-β1 concentrations preceded preeclampsia, or whether the differences may be attributed to disease-related alterations in synthesis and release of the growth factor. Prospective studies that allow for the longitudinal assessment of maternal TGF-β1 concentrations throughout pregnancy are needed to more thoroughly evaluate the extent to which dysregulation of TGF-β1 synthesis and release contributes to the pathogenesis of preeclampsia.

The increased risk of preeclampsia with increasing maternal plasma concentrations of TGF-β1 is biologically plausible. Investigators have shown that the cytokine is involved in the paracrine regulation of trophoblast–endometrial interaction and trophoblast differentiation.15 Hence, dysregulation of TGF-β1 synthesis and release may play a role in the pathogenesis of poor placental implantation, known to be pathogenic in preeclampsia.

We have shown that Peruvian women with preeclampsia are more likely to have high plasma TGF-β1 concentrations compared with normotensive controls. The risk of preeclampsia is particularly elevated in women with a concomitant elevation in plasma sTNF-p55—a marker of systemic inflammation. Although the biological mechanisms for this association are presently unknown, our results, when taken together with those of others, 7–9 suggest that excessive TGF-β1 may play an important role in the pathogenesis of preeclampsia. More information is needed, however, to determine whether TGF-β1 concentrations, measured in early pregnancy, may be used to identify women at high risk of developing preeclampsia, and to evaluate genetic and nongenetic determinants of pregnancy-associated elevations in TGF-β1 concentrations.

References


Downloaded from https://academic.oup.com/ajh/article-abstract/17/4/334/206803 by guest on 08 February 2018


