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# Increased circulating interleukin-17 levels in preeclampsia

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ABSTRACT

Increasing evidence suggests that an exaggerated maternal systemic inflammatory response and an angiogenic imbalance might play a central role in the pathogenesis of preeclampsia. We determined circulating levels of interleukin-17 (IL-17) along with those of angiogenic factors in healthy nonpregnant and pregnant women and preeclamptic patients, and examined whether serum IL-17 levels of preeclamptic patients were related to their clinical features and angiogenic factor concentrations. Fifty-nine preeclamptic patients, 60 healthy pregnant women and 56 healthy nonpregnant women were involved in this case–control study. Serum levels of IL-17A were measured using a high-sensitivity ELISA. Serum total soluble fms-like tyrosine kinase-1 (sFlt-1) and biologically active placental growth factor (PlGF) levels were determined by electrochemiluminescence immunoassay. For statistical analyses, nonparametric methods were applied. Serum IL-17 levels were significantly higher in preeclamptic patients than in healthy nonpregnant and pregnant women. We did not find any relationship between serum IL-17 concentrations of preeclamptic patients and their clinical features and serum sFlt-1 and PlGF levels or sFlt-1/PlGF ratios. However, elevated serum IL-17 level and sFlt-1/PlGF ratio were found to have an additive effect on the risk of preeclampsia, as shown by the substantially higher odds ratios of a combination of the two than of either alone. In conclusion, serum IL-17 levels are increased in preeclampsia, which may contribute to the development of the excessive systemic inflammatory response characteristic of the maternal syndrome of the disease. In addition, elevated serum IL-17 level and sFlt-1/PlGF ratio had an additive (joint) effect on the risk of preeclampsia.



## 1. Introduction

Preeclampsia, characterized by hypertension and proteinuria developing after the 20th week of gestation in a previously normotensive woman, is a severe complication of human pregnancy, with a worldwide incidence of 4.6 (2.7–8.2)% (Abalos et al., 2013). It is one of the leading causes of maternal and perinatal morbidity and mortality, even in developed countries. Despite extensive research, the etiology and pathogenesis of preeclampsia are not completely understood. There is an increasing body of evidence that shows that an exaggerated maternal systemic inflammatory response to pregnancy with activation of both the innate and the adaptive arms of the immune system, and an imbalance between circulating angiogenic and anti-angiogenic factors, plays a central role in the pathogenesis of the disease (Redman et al., 1999; Maynard et al., 2003; Saito et al., 2007; Molvarec et al., 2010a,b; Szarka et al., 2010).

There are six members of the interleukin (IL)-17-family of cytokines (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E [IL-25], and IL-17F), while the IL-17 receptor family comprises five receptor subunits (IL-17RA, IL-17RB/IL-25R, IL-17RC, IL-17RD/SEF, and IL-17RE) (Gaffen, 2009). Of these, IL-17A (referred to as IL-17 in the rest of this paper), also known as cytotoxic T lymphocyte-associated antigen 8 (CTLA-8) in rodents, has been most extensively studied. IL-17 is produced by helper and cytotoxic T cells, macrophages, dendritic cells, natural killer cells, natural killer T cells, lymphoid tissue inducer cells, and γδ T cells (Onishi and Gaffen, 2010). IL-17-producing CD4 cells have been described as a unique subset of helper T cells, which develop via a lineage that is distinct from the T helper type 1 (Th1) and 2 (Th2) lineages (Harrington et al., 2005; Park et al., 2005). Th17 cells are essential for host defense against microbial pathogens, particularly extracellular bacteria and fungi, some protozoa and viruses (O'Quinn et al., 2008).

An important feature of systemic inflammation in preeclampsia is the absence of the Th2 shift characteristic for physiological pregnancy, and thus the predominance of Th1-type immunity

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(Saito et al., 1999a,b). In addition to the imbalance of Th1 and Th2 cells, alterations in the prevalence of Th17 and regulatory T cells may also contribute to the development of systemic inflammation in preeclampsia (Santner-Nanan et al., 2009; Darmochwal-Kolarz et al., 2012). Our research group has formerly demonstrated that not only the prevalence of Th17 cells, but also that of IL-17-producing CD8 (Tc17) and NK cells, are increased in preeclampsia (Toldi et al., 2011).

In the present study, we extended our previous observation and determined serum IL-17 levels in a large number of healthy non-pregnant and pregnant women and preeclamptic patients. We also measured circulating angiogenic factors, and examined whether serum IL-17 levels of preeclamptic patients were related to their clinical features and angiogenic factor concentrations.

## 2. Materials and methods

### 2.1. Study patients

Our study was designed as a case–control study. Fifty-nine preeclamptic patients, 60 healthy pregnant women with uncomplicated pregnancies, and 56 healthy nonpregnant women were involved in the study. The study participants were enrolled in the First Department of Obstetrics and Gynecology and in the Department of Obstetrics and Gynecology of Kútvölgyi Clinical Center, at the Semmelweis University, Budapest, Hungary. All women were Caucasian and resided in the same geographic area in Hungary. Exclusion criteria were multifetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease, angiopathy, renal disorder, maternal or fetal infection, and fetal congenital anomaly. None of the pregnant women was in active labor, and none had rupture of the amniotic membranes. The healthy nonpregnant women were in the early follicular phase of their menstrual cycle (between cycle days 3 and 5), and none of them received hormonal contraception.

Preeclampsia was defined by increased blood pressure (≥140 mmHg systolic or ≥90 mmHg diastolic on ≥2 occasions at least 6 h apart) that occurred after the 20th week of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥0.3 g/24 h or ≥1 + on the dipstick in the absence of urinary tract infection). Blood pressure returned to normal by the 12th postpartum week in each preeclamptic study patient. Preeclampsia was regarded as severe if any of the following criteria was present: blood pressure ≥160 mmHg systolic or ≥110 mmHg diastolic, or proteinuria ≥5 g/24 h (or ≥3 + on the dipstick). Pregnant women with eclampsia or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) were not enrolled in this study. Intrauterine growth restriction (IUGR) was diagnosed if the fetal birth weight was below the 10th percentile for gestational age and gender, based on Hungarian birth weight percentiles.

The study protocol was approved by the Regional and Institutional Committee of Science and Research Ethics of the Semmelweis University, and written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

### 2.2. Laboratory methods

Fasting blood samples were taken from an antecubital vein into plain tubes, and then centrifuged at room temperature with a relative centrifugal force of 3000 × *g* for 10 min. The aliquots of serum were stored at −80 °C until the analyses. Serum levels of interleukin-17 (IL-17A) were measured using a high-sensitivity ELISA (eBioscience, San Diego, CA, USA, Cat. No. BMS2017HS) according to the manufacturer's instructions. Serum total soluble fms-like tyrosine kinase-1 (sFlt-1) and biologically active placental growth factor (PlGF) levels were determined by electrochemiluminescence immunoassay (Elecsys, Roche, Mannheim, Germany, Cat. No. 05109523 and 05144671 respectively) on a Cobas e 411 analyzer (Roche, Mannheim, Germany).

### 2.3. Statistical analysis

The normality of continuous variables was assessed using the Shapiro–Wilk *W*-test. As the continuous variables were not normally distributed, nonparametric statistical methods were used. To compare the continuous variables of the two groups, the Mann–Whitney *U*-test was applied, whereas to compare continuous variables of multiple groups, the Kruskal–Wallis analysis of variance by ranks test was performed. Multiple comparisons of mean ranks for all groups were carried out as post-hoc tests. Fisher's exact and Pearson's Chi-squared tests were used to the compare categorical variables of the groups. Analysis of covariance (ANCOVA) and multivariate logistic regression were undertaken with adjustment for BMI at blood draw.

Statistical analyses were carried out using the following software: STATISTICA (version 12; StatSoft, Tulsa, OK, USA) and Statistical Package for the Social Sciences (version 22 for Windows; SPSS, Chicago, IL, USA). For all statistical analyses, $p < 0.05$ was considered statistically significant.

In the article, data are reported as median (interquartile range) for continuous variables and as number (percentage) for categorical variables.

## 3. Results

### 3.1. Patient characteristics

The clinical characteristics of the study participants are described in Table 1. There was no statistically significant difference in terms of age among the three study groups. Furthermore, no significant differences were observed in gestational age at blood collection and the percentage of primiparas between preeclamptic patients and healthy pregnant women. However, all of the other clinical features presented in Table 1 differed significantly among our study groups. Fetal growth restriction was absent in healthy pregnant women, whereas the frequency of this condition was 18.6% in the preeclamptic group. Twenty-one women (35.6%) had severe preeclampsia and 30 patients (50.8%) experienced preterm onset (<37 weeks) of the disease.

### 3.2. Laboratory parameters

The laboratory parameters of the study subjects are displayed in Table 2. Serum IL-17 levels were significantly higher in preeclamptic patients than in healthy nonpregnant and pregnant women. In addition, the proportion of women with a measurable IL-17 concentration was significantly higher in the preeclamptic group than in the groups of healthy nonpregnant and pregnant women. The differences in serum IL-17 levels between preeclamptic patients and healthy nonpregnant and pregnant women remained significant even after adjustment for BMI at blood draw in ANCOVA (data not shown). Serum levels of sFlt-1 and PlGF, and sFlt-1/PlGF ratio, were significantly higher in healthy pregnant than in nonpregnant women. Preeclamptic patients had significantly higher sFlt-1 levels and sFlt-1/PlGF ratio and significantly lower PlGF concentrations compared with healthy pregnant women. Moreover, their sFlt-1 and PlGF levels and sFlt-1/PlGF ratio were significantly higher than those of healthy nonpregnant women.

In the group of preeclamptic patients, no statistically significant differences were found in serum IL-17 concentrations between patients with mild and those with severe preeclampsia, between