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ORIGINAL ARTICLE

Pregnancy-associated plasma protein A gene polymorphism in pregnant women with preeclampsia and intrauterine growth restriction

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Abstract Preeclampsia (PE) and intrauterine growth restriction (IUGR) are still among the most commonly researched titles in perinatology. To shed light on their etiology, new prevention and treatment strategies are the major targets of studies. In this study, we aimed to investigate the relation between gene polymorphism of one of the products of trophoblasts, pregnancy-associated plasma protein A (PAPP-A) and PE/IUGR.A total of 147 women (IUGR, $n = 61$; PE, $n = 47$; IUGR + PE, $n = 37$; eclampsia, $n = 2$) were compared with 103 controls with respect to the sequencing of exon 14 of the PAPP-A gene to detect (rs7020782) polymorphism. Genotypes "AA" and "CC" were given in the event of A or C allele homozygosity and "AC" in A and C allele heterozygosity. Our findings revealed that the rate of AA, CC homozygotes, and AC heterozygotes did not differ between groups. Moreover, there was no difference in the distribution of PAPP-A genotypes among the patients with IUGR, PE, IUGR + PE, or eclampsia. Finally, birth weight, rate of the presence of proteinuria, and total protein excretion on 24-hour urine were similar in the subgroups of AA, AC, and CC genotypes in the study group. Our study demonstrated no association between PAPP-A gene rs7020782 polymorphism and PE/IUGR.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

Although several associated theories have been proposed about the underlying mechanisms of preeclampsia (PE), a disease generally defined as pregnancy-induced hypertension with albuminuria arising after the 20th week of gestation, its etiology has yet to be definitively defined [1]. Today, PE is thought to result from a composition of immunologic, inflammatory, nutritional, and genetic factors that lead to the failure of the action of migratory interstitial and endovascular trophoblast into the walls of the spiral arterioles [2–4].

Intrauterine growth restriction (IUGR), affecting ~5–10% of pregnancies, is usually defined as the situation when the fetus is below the 10th percentile on the curve of weight for gestational age [5]. Maternal, fetal, placental, and external factors combined with the genetically predetermined growth potential organize the normal fetal growth, and failures of these factors result in IUGR and associated disorders [6].

The insulin-like growth factors (IGFs) play a considerable role in the growth of fetoplacental tissues throughout gestation [7]. Six insulin-like growth factor binding proteins (IGFBPs) organize IGF activities by escalating the half-life, localization, and migration of IGFs in particular tissues [7,8]. Pregnancy-associated plasma protein-A (PAPP-A), a zinc-binding metalloproteinase, releases IGF1 by cleaving the IGFBP-4/IGF 1 complex in order to modulate local IGF1 bioavailability [9]. Moreover, the potential of the differential regulation of decidual PAPP-A in arranging IGF 2 bioavaibility at the throphoblast–decidual interface reveals that maternal PAPP-A in the endometrial stroma and the trophoblastic tissues may have an influence on placental and fetal growth and development during implantation and pregnancy [10,11].

Proper interference between maternal endometrium, and trophoblastic and embryonic tissues is mandatory to ensure successful pregnancy outcomes. The failures in the processing of proteins such as PAPP-A, which may result in defective placentation, may pose unfavorable situations such as PE/IUGR. We performed this study to evaluate the potential association between the gene polymorphism (rs7020782) of PAPP-A and PE/IUGR.

Methods

This prospective study was conducted on 250 women who presented for prenatal care between January 2010 and December 2013 to the Gynecology and Obstetrics Department of Ege University, Izmir, Turkey. The protocol of the study was approved by the Ethics Committee for Human Studies at Ege University, and informed consent was obtained from all participants. The inclusion criterion was a diagnosis of PE, IUGR, PE with IUGR, or eclampsia. Women with multiple pregnancies and pregnancies with congenital and chromosomal abnormalities were excluded from the study. The control group consisted of patients with normal blood pressure and an appropriate weight for gestational age fetus.

PE was defined as a persistent systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg or higher, taken at least 6 hours apart, after 20 weeks of gestation in a previously normotensive patient. Proteinuria was defined as urine protein concentration of $\geq$ 300 mg/dL or 1+ on a urine dipstick in at least two random specimens collected at least 4 hours apart [12]. Total protein excretion on 24-hour urine was also calculated. IUGR was defined as estimated fetal weight below the 10th percentile on ultrasound together with birth weight below the 10th percentile of the standard growth curve [5]. Finally, eclampsia was defined as the development of convulsions and/or unexplained coma during pregnancy or postpartum in patients with signs and symptoms of PE [13].

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood cells using standard techniques. Forward 5′-GCACTTCGCATGTGAGAAAA-3′ and reverse 5′-GAGCTCAGCACCTGCACATA-3′ were used in order to amplify the region surrounding rs7020782 polymorphism in the PAPP-A gene [10,14]. Polymerase chain reaction (PCR) was performed in a 25-μL reaction mix containing 5 μL of extracted DNA, 2.5 μL buffer (10× Taq Buffer with KCl), 2 μL $MgCl_2$ (25mM), 1 μL dNTP (10mM), and 1.25 μL (10 pmol/μL) forward and reverse primer 0.5 μL (5 U/μL) Taq-Polimerase, and 11.5 μL distilled water. PCR conditions were as follows: 95°C for 11 minutes, then 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds, 72°C for 30 seconds, followed by extension at 72°C for 5 minutes. PCR products were visualized on a 2% agarose gel by electrophoresis and purified before cycle sequencing. All PCR products were sequenced with the dye termination method using a DNA sequencing kit (Perkin-Elmer, Foster City, CA, USA) and analyzed using the ABI Prism 3100 sequence analyzer (Applied Biosystems, Foster City, CA, USA). Genotypes "AA" and "CC" labels were given in the event of A or C allele homozygosity and "AC" in the event of A and C allele heterozygosity.

Statistical analysis was performed with the SPSS version 11.0 statistical software package (SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used to detect normal distribution. Continuous parameters were expressed as mean ± standard deviation when normally distributed. Categorical results were expressed as percentages. For comparisons, Chi-square, analysis of variance, Kruskal–Wallis, and Mann–Whitney *U* tests were used, where appropriate.

Results

A total of 147 women (61 IUGR, 47 PE, 37 IUGR + PE, 2 eclampsia) were compared with 103 controls. Patients in the study group delivered at earlier gestational ages and had infants with significantly lower birth weights (Table 1). The rate of AA, CC homozygotes, and AC heterozygotes did not differ between study and control groups ($p = 0.966$, Chi-square test; Table 2).

Furthermore, there was no difference in the distribution of PAPP-A genotypes among the patients with IUGR, PE, IUGR + PE, or eclampsia ($p = 0.942$, Chi-square test; Table 3). Additionally, birth weight, rate of the presence of proteinuria, and total protein excretion on 24-hour urine were similar in the subgroups AA, AC, and CC genotypes in the study group (Table 4).