VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2018, vol. 89, no. 10, 573–576

ISSN 0017–0011

DOI: 10.5603/GP.a2018.0098

The powerful association of angiotensin-converting enzyme insertion/deletion polymorphism and idiopathic recurrent pregnancy loss

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ABSTRACT

Objectives: Idiopathic recurrent pregnancy loss (IRPL) is one of the most troublesome complications of pregnancy. Several researches were also conducted to search the possible association with *ACE* I/D polymorphism and IRPL. In the light of these reports, this case-control study was investigated to genotypes and alleles of *ACE* I/D polymorphism in IRPL group and control group.

Material and methods: Overall, 1176 subjects (1007 cases, 169 controls) were investigated. Allele genotype distributions were determined by PCR method in both groups. Differences in genotype and allele frequencies between groups were investigated by Pearson chi-square tests. The odds ratio (OR) and 95% confidence intervals (95% CI) were also determined.

Results: For the *ACE* I/D polymorphism I and D allele frequencies were in the control and case groups respectively; 49.4 and 41.6%, 50.6 and 58.4%. The genotypes of *ACE* for I/D observed in control and case group respectively were as follows; II (27.2 and 17.9), ID (44.4 and 47.4) and DD (28.4 and 34.7). Regarding the distribution of D allele and genotypes containing D allele, we observed significant statistical differences between case and control groups.

Conclusions: Our results showed that the *ACE* I/D polymorphism was associated with IRPL, and that women that carried DD or ID genotypes had a 72% elevated risk of developing IRPL than women with the II genotype (OR (95% CI): 1.72 (1.181–2.5)). This odds ratio was found to be 1.61 in a case-control study and 1.28 in a meta-analysis study compiling 11 separate studies, which is consistent with our study data.

Key Words: ACE, polymorphism, recurrent miscarriages, pregnancy loss

Ginekologia Polska 2018; 89, 10: 573–576

INTRODUCTION

Idiopathic recurrent pregnancy loss (IRPL) is one of the most troublesome complications of pregnancy. IRPL is defined differently by several groups. The diversified description is as follows: loss of two or more consecutive pregnancies before four months and three or more sequent pregnancy loss before the 22nd-24rd week of pregnancy [1–3]. IRPL affects approximately 1–5% of women who are pregnant, according to the 'IRPL definition' of two or three consecutive pregnancy loss. This percentage increases with age [4]. Currently, several well-known risk factors of recurrent miscarriage are described: chromosomal abnormalities, polycystic ovary syndrome, thrombophilia, hypothyroidism, anatomic anomalies, endocrine disturbances, obesity, infections, and environmental and nutritional factors [5]. However, more than 50% of women with IRPL have no precise reason of pregnancy loss [6]. Genetic causes account for only 2–5% of the defined causes [7]. Despite this low level, many different studies have been conducted on genetic changes in the etiology of IRPL. The most marked relationship between these studies is related to numerical and structural chromosome anomalies. However, many gene polymorphisms have also been investigated, especially these related to coagulation cascade, as the successful pregnancy is dependent on a sensitive balance between fibrinolysis and coagulation. Until this date, about the topic the most studied genes and

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polymorphisms were plasminogen activator inhibitor-1 (PAI-1) 4G/5G, methylenetetrahydrofolate reductase (MTHFR) C677T, factor V-Leiden (G1691A), factor-2 20210G > A and angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism [7–9]. The ACE is located on long arm of chromosome of 17 with 25 introns and 26 exons and contains 21 kilobases (kb) of genomic DNA. ACE plays a key role in the rennin-angiotensin-aldosterone system (RAAS) and is related in the transformation of angiotensin I to active angiotensin II, a powerful vasoconstrictor [10]. An I/D polymorphism of the ACE forming of the insertion or deletion of a 287 basepair (bp) Alu sequence component in intron 16 is associated with the serum ACE level (rs4340). The existence of the D/D genotype or D allele is associated with high serum and plasma ACE activity. The D/D genotype also increases procuration of angiotensin II from angiotensin I and is associated with elevated levels of PAI-1, which result in the decreased levels of fibrinolysis. Furthermore, ACE degrades bradykinin, a very potent vasodilator [11]. Several researches were also conducted to search the possible association with ACE I/D polymorphism and IRPL. The potential role of ACE I/D polymorphism in IRPL pathogenesis has been controversial [3, 9, 12–15].

Objectives

This current case-control study was investigated to genotypes and alleles of ACE I/D polymorphism in two groups from the south-eastern part of Turkey. The case group with two unexplained consecutive pregnancy losses and the control group with at least one live birth and no history of pregnancy loss.

MATERIALS AND METHODS

Overall, 1176 subjects (1007 cases, 169 controls) were investigated between October 2016 and August 2018. Of those, 1007 women had a history of ≥ 2 consecutive pregnancy losses and 169 fertile women had at least one live birth and no history of pregnancy loss. For this purpose, the participants with following evidence were excluded from each group; structural uterine abnormality, chromosomal abnormalities, hormonal imbalance (TSH, FSH, LH, prolactin), anti-nuclear antibodies, antiphospholipid antibodies and lupus anticoagulant. All women were from Sanliurfa province in the south-east of Turkey. The study was approved by the Institutional Review Board of Faculty of Medicine in Harran University (Ethics Committee Document Number: 04.01.2018/01-12) and the study was conducted in accordance with 2013 Declaration of Helsinki. All women included in the study signed the informed consent form. Complete blood samples were taken from the antecubital vein via vacutainer tubes comprising ethylenediaminetetraacetic acid (EDTA) (BD, Franklin Lakes, NJ). DNA was isolated from blood samples using Magpurix Blood DNA Extraction Kit 200 (Zinexts LSC, New Taipei City, Taiwan [R.O.C.]). Quantitative-purity determinations and fluorometry analysis were performed (NanoDrop 8000, Thermo-Fisher Scientific, DE, USA). To analyze ACE I/D polymorphism, genomic DNA was amplified by polymerase chain reaction (PCR) using specific primers: forward: 5′-CTGGAGACCACTCCCATCCTTTCT-3′; reverse:5′ GATGTGGCCATCACATTCGTCAGAT-3′ (Sentegen Biotech, Ankara, TR). The PCR method used for this polymorphism was performed as previously described [16]. DNA fragments were separated by 3% agarose electrophoresis and described by ethidium bromide staining. Three types of ACE PCR products were described a 190 bp band corresponding to the D/D genotype, a 490 bp band corresponding to I/I genotype, or a compound of 190 and 490 bp bands corresponding to the I/D genotype. Allele and genotype frequencies were checked for deviation from Hardy-Weinberg equilibrium by Pearson chi-square analysis. Differences in genotype and allele frequencies between groups were investigated by Pearson chi-square tests. The odd ratio (OR) and 95% confidence intervals (95% CI) were also determined. All statistical data were analyzed using statistical package for social sciences (SPSS) software version 23.0 (IBM SPSS, Chicago, IL, USA) program. For all tests $p < 0.05$ was considered as significant.

Results

The mean age of the control group and case group was 26.41 ± 6.29 years (ranged between 19–45, median 26), and 25.88 ± 5.76 years (ranged between 19-43, median 25), respectively. There was no significant difference between the case and control groups ($p > 0.05$). For the ACE I/D polymorphism I and D allele frequencies were in the control and case groups respectively; 49.4 and 41.6%, 50.6 and 58.4%. The genotypes of ACE for I/D observed in control and case group respectively were as follows; II (27.2 and 17.9), ID (44.4 and 47.4) and DD (28.4 and 34.7). The genotype and allele frequencies in the control and case groups were checked for deviation from Hardy-Weinberg equilibrium. No deviation was observed (Tab. 1). The odds ratio, confidence interval, chi-square test and p value are displayed in Table 1. The most frequent genotype was ID in both groups. Regarding the distribution of D allele and genotypes containing D allele, we observed significant statistical differences between case and control groups.

DISCUSSION

In this study, we have tried to examine ACE I/D polymorphism allelic frequencies and genotypes and its association with IRPL among south-eastern Turkish women. ACE I/D polymorphism has so far been associated with many conditions such as stroke, coronary heart disease, diabetic nephropathy,

Table 1. The allelic frequencies and genotype of ACE I/D polymorphisms in both groups

Tests for deviation from 'Hardy-Weinberg' equilibrium		Tests for association (C.I.: 95% confidence interval)			
Controls	**Cases**	**Allele frequency difference**	**Heterozygous**	**Homozygous**	**Allele positivity**
		[I] - [D]	[II] - [ID]	[II + ID] - [DD]	[II] - [ID + DD]
p = 0.1443	p = 0.4305	Odds_ratio = 1.373 C.I. = [1.090–1.730] chi2 = 7.29 p = 0.00694	Odds_ratio = 1.625 C.I. = [1.084–2.437] chi2 = 5.59 p = 0.01806	Odds_ratio = 1.863 C.I. = [1.197-2.901] chi2 = 7.75 p = 0.00537	Odds_ratio = 1.718 C.I. = [1.181–2.500] chi2 = 8.14 p = 0.00433

athletic performance, hypertension, preeclampsia and IRPL [15, 17, 18]. The probable association between IRPL and ACE I/D polymorphism is based on the theory that degenerated hemostasis, excess fibrin accumulations in spiral arteries and impedes perfusion secondary to platelet aggregation could lead to pregnancy loss. The D allele was shown to be associated with a high level of ACE in serum, which enhances the formation of angiotensin II from angiotensin I, thus increasing the risk of thrombotic episodes. Some studies have reported a link between the D allele and risk of thrombosis [11, 13, 15]. Data by several regions demonstrated that the D allele and DD genotype leads to a high PAI-1 level which reduced fibrinolysis and thus correlated with an increased risk of IRPL [9, 12, 15]. Many studies have been managed to explore the link between ACE I/D polymorphism alleles and genotypes and IRPL, but the results of these studies differ from each other [13–15, 19–24]. Our results showed that the ACE I/D polymorphism was associated with IRPL, and that women that carried DD or ID genotypes had a 72% elevated risk of developing IRPL than women with the II genotype [OR (95% CI): 1.72 (1.181–2.5)]. This odds ratio was found to be 1.61 in a case-control study and 1.28 in a meta-analysis study compiling 11 separate studies, which is consistent with our study data [13, 25]. In a study conducted in our country in 2012, the relationship between ACE I/D polymorphism and IRPL was found to be significant at a high level [26].

CONCLUSIONS

The genotype and allele distribution of the ACE I/D polymorphism influenced the risk of IRPL in the south-east of Turkey, confirming the findings in the north-west of Turkey. The most valuable aspect of our work is that it has a large sample and is a prospective case-control study. Examination of the PAI-1 level could add value to this study, but unfortunately, this was not possible. Polymorphism studies in different societies, with a high number of participants, will undoubtedly become more valuable with the work to be done in the coming years.

Conflict of Interest.

The authors declare no conflicting of interest.

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