

1984A/G adrenomedullin (rs3814700) gene polymorphism: can it be responsible for unexplained recurrent early pregnancy loss?

Burcu Artunc Ulkumen¹, Safiye Ulucay², Burak Batir², Fatma Eskicioglu¹, H. Gursoy Pala¹, Sirri Cam²

Abstract

Objective: The etiology of recurrent miscarriage (RM) is heterogeneous and the current data cannot be able to cover all the aspects of RM. Adrenomedullin (ADM) has been very popular with the discovery of vital functions in maintaining an uneventful pregnancy. Idiopathic RM may result due to defective placentation and implantation process associated with altered ADM function and levels. So, we hypothesized that increased ADM gene polymorphism could play a role in idiopathic RM cases

Methods: This prospective case-control study consisted of 60 women; 30 of whom had three consecutive pregnancy losses, who admitted to the outpatient clinic of department of obstetrics and gynecology or department of genetic at our tertiary center. Genomic DNA was extracted from peripheral blood and the frequency of genotypes and alleles of -1984A>G ADM (rs3814700) gene polymorphism was examined by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method

Results: The mean ages were 29.36±6.22 and 32.15±5.43 in RM and control group, respectively (p=0.314). For -1984A>G polymorphism, the frequency of A allele was 91.7% and 93.3% in RM and control group, respectively (p=0.72). The frequency of G allele was 8.3% and 6.7% in RM and control group, respectively (p=0.72). Regarding the incidence of genotype; AA genotype was 83.3% and 86.7% in RM and control group, respectively (p=0.71). AG genotype was 16.7% and 13.3% in RM and control group, respectively (p=0.71).

Conclusions: -1984A>G ADM gene polymorphism does not seem to be associated with idiopathic RM

Keywords: Recurrent pregnancy loss, Adrenomedullin, gene polymorphism, -1984A>G polymorphism

Introduction

Recurrent miscarriage (RM) is three or more consecutive pregnancy losses before 22 gestational weeks [1]. The prevalence is approximately 0.8-1.4%, if only clinical RM (evidence of pregnancy with ultrasonographic and histologic findings) is taken into account; the prevalence is 2-3%, if also biochemical losses (urinary HCG positivity with no evidence of sonographic or histologic endometrial findings) are taken into consideration (2). In fact, there is a "rule of 30%'s" regarding the outcome of conceptions: 30% of them are lost before they implant into the endometrium. Further 30% of them are lost after the implantation; however, before the next menstrual period begins. So, only 30% of them end up in live birth (3). The etiology of RM is heterogeneous and the current data cannot be able to cover all the aspects of RM. Still approximately 50% of RM is unexplained. The well-known factors are classified as genetic, immunologic factors, thrombophilia, endocrinological causes, uterine malformations and obesity (2-5). Due to

exaggerated oxidative stress, uterine natural killer cells via increased angiogenesis during the implantation period have been thought as a cause for idiopathic RM (6). Recent studies have showed that adrenomedullin (ADM) was important in angiogenesis, extracellular cytotrophoblast migration and placentation (7, 8). The normal physiological maternal adaptation to the pregnancy occurs with the increased serum ADM levels (8). In compromised pregnancies such as preeclampsia –a result of defective placentation-, the increase in ADM levels does not occur (8).

It is showed that GG genotype for ADM gene causes a decrease in ADM production, whereas AA genotype is associated with increased ADM levels. -1984A>G variant in the promoter region of the ADM gene, which probably decreases adrenomedullin transcription and, in consequence, decreases ADM concentration (9). From that point of view, idiopathic RM may result due to defective placentation and implantation process associated with altered ADM function and levels. So, we

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¹Celal Bayar University, School of Medicine, Department of Obstetrics and Gynecology, Manisa, Turkey

²Celal Bayar University, School of Medicine, Department of Genetics, Manisa, Turkey

*Corresponding Author: Burcu Artunc Ulkumen E-mail: artunc.burcu@gmail.com

hypothesized that ADM gene polymorphism could play a role in idiopathic RM cases and we aimed to investigate the frequency of ADM -1984A>G polymorphism in idiopathic RM cases.

Material and Methods

This prospective case-control study consisted of 60 women; 30 of whom had three consecutive pregnancy losses, who admitted to the outpatient clinic of department of obstetrics and gynecology or department of genetic at our tertiary center. The other 30 women were age-matched healthy multiparous women admitted to the outpatient clinic at our department during the study period. Women with congenital uterine anomalies, large uterine myomas, cervical insufficiency, hereditary thrombophilia or genetic abnormalities were excluded from the study. Institutional Ethic Committee approved the study. All participants signed informed consent.

Genomic DNA was extracted from peripheral blood by commercial Invitrogen Genomic DNA extraction kit following the manufacturer's instructions and stored at -20°C . The frequency of genotypes and alleles of -1984A>G ADM (rs3814700) gene polymorphism was examined by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method.

PCR was performed in a 25 μl reaction containing 150 ng DNA, 10x PCR buffer, 2.5 mM MgCl_2 , 20 μM dNTPs, Primer Forward (10 pmol/ μl), Primer Reverse (10 pmol/ μl), 5U/ μL Hot Start Taq polymerase. Amplification conditions were set like as; an initial activation step of 94°C for 15 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, extension at 72°C for 1 min and 45 s and a final extension step at 72°C for 10 min. PCR products (250 bp) were digested with restriction enzyme HpyCH4III at 37°C for two hours and digested fragments were analyzed by agarose gel electrophoresis.

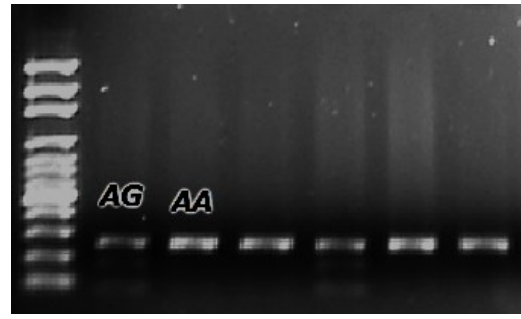
The AA genotype was identified at the presence of 250 bp, heterozygous AG genotype in presence of 250,166 ve 84 bp and homozygous GG genotype in presence of 166,84 bp (Figure-1).

Results

The mean ages were 29.36 ± 6.22 and 32.15 ± 5.43 in RM and control group, respectively ($p=0.314$). Regarding the -1984A>G polymorphism, the frequency of A allele was 91.7% and 93.3% in RM and control group, respectively ($p=0.72$). The frequency of G allele was 8.3% and 6.7% in RM and control group, respectively ($p=0.72$). Regarding the incidence of genotype; AA genotype was 83.3% and 86.7% in RM and control group, respectively

($p=0.71$). AG genotype was 16.7% and 13.3% in RM and control group, respectively ($p=0.71$).

Figure 1: The band appearance of ADM gene -1984A>G polymorphism in agarose gel. There was no GG genotype in study group.



Discussion

In the present study, we examined -1984A>G ADM (rs3814700) gene polymorphism in the RM group and compared it with the healthy multiparous Turkish women. Adrenomedullin (ADM) is a 52 amino acid peptide, which was firstly discovered in pheochromocytoma in 1993 (6). It was shown that ADM had role in vasodilatation and angiogenesis (7,8). It is also well-known that an uneventful pregnancy depends on adequate blood supply between fetomaternal interfaces. The alterations in uterine vasculature – especially around the implantation site – is one of the main physiologic adaptations in pregnancy (8). Recent studies pointed that ADM has important role in placentation and also in maintaining a successful pregnancy.

To the best of our knowledge, our study is first by evaluating ADM gene polymorphism in idiopathic recurrent pregnancy losses. Recent studies regarding ADM and pregnancy losses focused mainly on ADM levels in plasma. Nakatsuka et al evaluated plasma ADM levels and pulsed Doppler ultrasonography measurements in RM with positive antiphospholipid and anti-nuclear antibodies and in control groups. They found increased plasma ADM levels in RM group (10). The study of El-Mashad et al consisted of 40 idiopathic RM and 43 control cases. They showed that plasma ADM levels were also increased in idiopathic RM group (11).

Both studies agreed with that plasma ADM levels were correlated with the uterine artery pulsatility index (10,11). These findings may be regarded as the evidence of that oxidative stress caused ADM levels to increase.

Kato and co-workers showed that ADM was produced mainly by the vascular tissue, especially endothelium and vascular smooth muscle (12). ADM has a complex interactive relation with various molecules such as prostaglandins (PG's), nitric oxide (NO), atrial natriuretic peptide (ANP),

renin aldosterone system (RAS), norepinephrine, arginine vasopressin, endothelin-1 and adrenocorticotrophic hormone (ACTH) (13). There are a few studies in the literature regarding the possible gene polymorphisms and RM. The data about the association of the polymorphisms of the eNOS gene and methylenetetrahydrofolate reductase (MTHFR) and RM is conflicting: Tempfer et al conducted a prospective study with 105 RM cases and evaluated the endothelial nitric oxide synthase (eNOS) gene polymorphisms. They found that eNOS gene polymorphisms could be a genetic determinant for the developing idiopathic RM (14). Similarly, Suryanarayana et al. showed that eNOS gene polymorphisms were associated with an increased risk of RM (15). Makino et al. found that only NO concentration but not the polymorphism of MTHFR and eNOS gene are associated with RM (16). In accordance with that, Zammiti et al. pointed that there was no association between the eNOS gene polymorphisms and the risk of RM (17).

Kato and co-workers suggested that ADM increased during the pregnancy as a result of the physiologic adaptations which in turn increases the utero-placental blood supply (12). Recent studies provided further data that ADM had a key role for placentation (18-20). The placentas of murine fetuses which lack the ADM gene showed typical pathological features of preeclampsia which is a result of improper placentation (21).

We hypothesized that patients with idiopathic RM may have had problems regarding the early period of pregnancy including the implantation and placentation window. With this aim, we evaluated these RM patients with the frequency of genotypes and alleles of -1984A>G ADM (rs3814700) gene polymorphism. Compared with parous control group, we could not show any significant difference. The main limitation of our study was the sample size. The second limitation was that we could not investigate the tissue expression of ADM. And also we had no data about the smoking status of the study population.

Our preliminary results showed no difference between the RM and parous women in terms of -1984A>G ADM (rs3814700) gene polymorphism. Further prospective studies with greater sample size may be conducted to investigate any other relations

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