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The role of -1984A> G adrenomedullin gene polymorphism in tubal ectopic pregnancy

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Abstract

Objective: Tubal ectopic pregnancy is a health issue that can cause maternal death in first trimester and our knowledge about its pathogenesis is limited. Adrenomedullin (ADM) is responsible from regulation of ciliary motility in fallopian tubes. Its expression is known to be reduced in tubal tissue in ectopic pregnancy. In this study, it was aimed to investigate the relationship between -1984A>G functional polymorphism affecting the expression level of the ADM gene and tubal ectopic pregnancies.

Material and Methods: This prospective case-control study consisted of 64 women. Peripheral blood samples were obtained from 31 women diagnosed with tubal pregnancy (the study group) and 33 fertile women without a history of ectopic pregnancy (the control group). Genomic DNA was extracted from peripheral blood. 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: For -1984A>G polymorphism the study group had an A allele frequency of 90% and a G allele frequency of 10%; the control group had an A allele frequency of 91.7% and a G allele frequency of 7.3% (p=0.75). AA genotype frequency was 80% and AG allele frequency was 20% in the study group while they were 83.3% and 16.7%, respectively, in the control group (p=0.73). There were no significant differences between the study and control groups with respect to allele and genotype frequencies.

Conclusion: This study found no significant relationship between tubal ectopic pregnancy and -1984A>G ADM gene polymorphism. Further studies are needed to explore other factors that affect ADM expression.

Keywords: Adrenomedullin, Tubal ectopic pregnancy, Genetic polymorphism

Introduction

Ectopic pregnancy (EP) is the implantation of fertilized ovum outside the uterine cavity. This occurs in fallopian tubes in 98% of cases (1). The incidence of the disease is 1-2% in America and Europe. It is the most common cause of maternal mortality in first trimester in western countries (2). Its incidence is even higher in developing countries and 1 of every 10 patients admitted to clinics are lost because of this disease (3).

Recent studies on pathogenesis of EP have suggested that retention of embryo within fallopian tube as a result of defective tubal embryonal transport may be responsible from EP development (2). Tubal embryonal transport is controlled by smooth muscle contraction and ciliary beating (CB) throughout fallopian tubes. Factors affecting muscle cell contraction and ciliary activity have been considered to be responsible from tubal EP development (4).

Adrenomedullin (ADM) is a peptide hormone secreted from several tissues. It induces vasodilation via smooth muscle relaxation. It has been thought to play a role in a number of pathophysiological processes in circulatory, respiratory, neuroendocrinologic, and immunological systems (5). Recent studies have shown that ADM induces ciliary motility in human fallopian tubes (6). Tubal embryonal retention and implantation in tubal ectopic pregnancy have been thought to result from a reduction in CB and muscle contraction caused by low ADM levels in fallopian tubes (7).

The gene coding for ADM is localized on chromosome 11. It has been shown that -1984A>G polymorphism located in the promotor region of the ADM gene affects ADM expression (8). There are recent studies investigating the role of -1984G

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allele in gestational hypertension and preeclampsia, some pathological processes of pregnancy (9,10).

As far as we know, there no studies in the literature that have specifically investigated the role of -1984A>G ADM gene polymorphism in development of tubal EP. The aim of the present study was to investigate the relationship between tubal EPs and -1984A>G ADM gene polymorphism known to affect the expression of adrenomedullin that plays a role in tubal EP pathogenesis.

Materials and Methods

This prospective case-control study was approved by Celal Bayar University Institutional Review Board and all the participants gave informed consent before study onset. Consent approval was obtained from patients participating in the study. The study group was composed of 31 women with a mean age of 28 (range 21-42) years who were either admitted to the department of gynecology and obstetrics with the diagnosis of ectopic pregnancy or had a previous history of ectopic pregnancy. The control group consisted of 33 women with a mean age of 30.4 (range 23-38) years who had at least two previous pregnancies with none being ectopic. Patients having infertility, pelvic inflammatory disease, previous tubal surgery, hypertension, renal failure, type 2 diabetes, congestive heart failure, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, or those who had a smoking history or were active smokers were excluded from the study.

All blood samples obtained from the participants were collected in tubes containing EDTA (ethylenediaminetetraacetate) which served as an anticoagulant agent.

Genetic Analysis: Genomic DNA was extracted from peripheral blood by commercial Invitrogen Genomic DNA extraction kit following the manufacturer's instructions and stored at -20^{0}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.

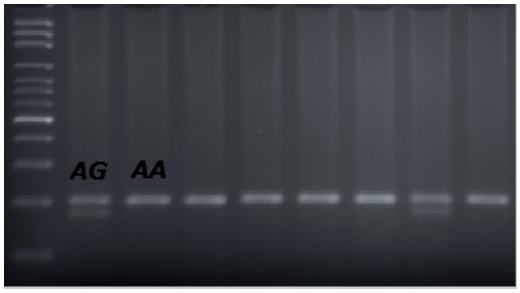
PCR was performed in a 25 µl reaction containing 150 ng DNA, 10x PCR buffer, 2.5 mM MgCl2, 20 µM dNTPs, Primer Forward (10 pmol/ µl), Primer Reverse (10 pmol/ µl), 5U/µL Hot Start Taq polymerase. Amplification conditions were set like as; an initial activation step of 94°C for 15 min followed by 35 cycles 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 (193 bp) were digested with restriction enzyme HpyCH4III at 37^{0}C for two hours and digested fragments were analyzed by agarose gel electrophoresis. The AA genotype was identified at the presence of 193 bp, heterozygous AG genotype in presence of 193 bp, 164 bp and homozygous GG genotype in presence of 164 bp (Figure 1).

Statistical analysis: The statistical package SPSS for Windows 15.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) was used to analyze the data. Statistical comparisons between groups were performed using the X^2 test. P values less than 0.05 were accepted as significant.

Results

For -1984A>G polymorphism the study group had an A allele frequency of 90% and a G allele frequency of 10%; the control group had an A allele frequency of 91.7% and a G allele frequency of 7.3% (p=0.75). AA genotype frequency was 80% and AG allele frequency was 20% in the study group and 83.3% and 16.7%, respectively, in the control group (p=0.73). The study and control groups were not significantly different with respect to allele and genotype frequencies.

Figure 1: Agarose gel band images of -1984A>G ADM gene polymorphism



Discussion

ADM is a peptide that belongs to calcitonin/calcitonin-gene-related peptide / amylin family. It regulates smooth muscle relaxation and vasodilation, as well as electrolyte balance, inflammatory response, cellular development, differentiation, apoptosis, and neoangiogenesis. Additionally, it inhibits steroidogenesis and inhibin secretion and increases fluid secretion in epididymis. In high concentration, it increases sperm progressive motility. ADM expression is greater in uterus and endometrial glands compared to endometrial stroma and myometrium. This may mean that ADM regulates uterine contraction and participates in development of pregnancy by promoting endometrial angiogenesis. ADM acts via calcitonin receptor-like receptor, via modulation by receptor-modifying proteins (6).

Spermatozoas that accumulate in vagina after sexual intercourse first reach uterus and then

fallopian tubes where capacitation, fertilization, and early preimplantation embryonic development take place. Fallopian tubes provide the necessary microenvironment for early embryonic development. It has been shown that existence of spermatozoa in mice upregulates ADM expression in oviduct (11). Different regions of fallopian tubes have different roles in reproductive processes. The fimbrial portion is responsible from oocyte capture and transit, the ampullary portion from fertilization, and the isthmic portion from transport of spermatozoa and embryo. ADM is expressed more in isthmic ampullary region compared to ampullary and fimbrial regions (6). CB is important for gamet/embryo transport in fallopian tubes. CB function increases in isthmus and ampulla in fallopian tubes after ovulation (12). A higher ADM expression in the isthmic region indicated the stimulating role of ADM on CB and its active participation in gamet/embryo transport in fallopian tubes (6). Steroids assume a key role in ciliary functions. Estrogen induces ciliary formation in fallopian tubes, thereby increasing CB function (13, 14). It has been shown that higher estrogen levels in peri-ovulatory phase compared to follicular phase induce ADM expression in fallopian tubes (6). Higher progesterone levels, on the other side, cause ciliary dysfunction, leading to EP (14). However, confusing results have been reported about progesterone and ADM expression. Kobayashi et al., (15) reported a positive correlation between ADM concentration and progesterone levels in pregnancy.

It has been though that ADM may take part in EP pathogenesis via its available actions on CB in fallopian tubes (16). Liao et al., (7) showed that CB and muscle contraction frequency in fallopian tubes are lower in ectopic pregnancy compared to normal pregnancy. The authors also reported that ADM expressions in fallopian tubes and plasma were also lower. The same study also found an improvement in fallopian tube CB and muscle contraction frequency after in vitro ADM administration (7). WS et al., (17) also reported that plasma ADM concentrations were lower in tubal EP cases compared to normal pregnancies.

Varying plasma levels of ADM with different pathological processes have led to performance of studies on the factors affecting adrenomedullin's plasma levels (18,19). Cheung et al., (19) showed that IL-6, which is a proinflammatory cytokine, suppresses ADM expression through the promotor region of the ADM gene. It is also believed that ADM gene promotor region polymorphisms may affect the molecule's plasma level (19). ADM gene promotor region -1984A>G polymorphism and ADM plasma level have been investigated by various studies and higher plasma levels have been detected in persons with AA genotype, albeit statistically non-significant (10, 19).

To our knowledge, no study to date has investigated the relationship between EP and ADM gene polymorphism. This study failed to show a relationship between EP and -1984G allele frequency that was expected to have lower plasma levels. This may have stemmed from the fact that EP has a multifactorial origin. We believe that genetic variability is only one of the available risk factors for EP. To increase the reliability of our results, we excluded other risk factors that increase ADM levels, such as renal failure, hypertension, and diabetes mellitus, as well as predisposing factors for EP, such as smoking, tubal surgery, and pelvic inflammatory disease. A limited sample size of our study may be another reason of lack of a statistically significant association. Our study was conducted in a Turkish patient population. Genotypes and allele frequencies may vary by population and races. Further studies with larger sample size that will include more diverse populations are clearly needed in this field. It is of vital importance for clarifying the role of ADM in EP to determine other ADM gene sequence alterations that are capable of affecting ADM plasma level, and to reveal the relationship between such alterations and ADM expression, plasma ADM level, and other pro-inflammatory factors.

Conclusion

ADM is a peptide that plays a role in EP physiopathogenesis. A defect in its expression causes reduced ciliary activity that is responsible from gamet transport in tubal epithelium. However, no relationship could be shown between EP and -1984A>G ADM gene polymorphism. This topic should be investigated by further studies.

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