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Research Article

Comparison of Polymorphisms of Angiotensin-Converting Enzyme Gene betweenIranian Infertile Women with Polycystic Ovary Syndrome and Healthy Women

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ABSTRACT

The prevalence of infertilityandpolycycticovarian syndrome(PCOS) is dramatically increasing due to the changes in lifestyle that include increasing the interval between pregnancies, increased age at first pregnancy, changes in hormone levels affecting reproductive tissues, contraceptive effect of some medications, poor nutrition, lack of vitamin E, etc.In this research, three polymorphisms in the angiotensin-converting enzyme gene in infertile women were studied in the three groups including patients with PCOS, infertility patients, and normal subjects with hormone information. The location of studied gene on the long arm of chromosome 17 on region 2, band 3, and sub-band 3 was sequenced using in situ hybridization method. The method used involves extracting DNA from peripheral blood, confirmation of DNA extracted on agarosegel, designing primers for the target gene, PCR, and applying electrical current to the products of PCR on agarose gel and comparing it with determination of size guidance, and taking photographsofagarose gel with ultraviolet camera. In this study, it has been shown that increaseinACE gene polymorphisms is related to infertility and PCOS in Iranian women population. This result can be used as prognostic in early detection of POCS and infertility in the Iranian women population.

Keywords: Polymorphism, polycystic ovary syndrome, Infertility, Polymerase chain

INTRODUCTION

Polycyctic ovarian syndrome (PCOS) is the most common cause of infertility and endocrine disorders among women in the childbearing agewith 6 to 10% prevalence.This disease is more common in women with symptoms such as menstrual disorders, acne, hair loss, increased risk of endometrial and breast cancer, increased risk of type 2 diabetes, high blood pressure during pregnancy, and obesity. Ultrasound based diagnosis of the disorder is possible through ovarian, evaluation of serum levels and explanation of patients [1]. The most important

clinical symptom of polycystic ovary syndrome (PCOS) is irregular menstrual periods with the distance between two menstrual periods more than 35 days.Blackacanthosis that is referred to the existence of dark velvety skin and neck, armpits, under the breasts, and external genitals, is also one of the clinical cases that in addition to the association with this syndrome, is the sign of insulin resistance.Etiologyof PCOS is still unknown.It seems thatfulliculogenesisand abnormal steroidogenesis are the main causes of the disease.Hyperactiveinsulinemiaand insulin resistance are the most visible symptoms of patients with PCOS.Genetic causes play important roles in presence of PCOS and are probably inherited as autosomal dominant trait but recent studies show that more than one gene is involved in the etiology of PCOS [2].In this study, a part of the angiotensinogen - renin - aldosterone pathway including the effect of angiotensin converting enzyme effective approach from 1 to 2 through deletionofpolymorphismsand insertion of angiotensin-converting enzyme in the development of PCOSwas investigated [3].Angiotensinogen is produced by the liver and is released in the blood and then rennin is released from the kidney and converts Angiotensinogen to Angiotensin 1.Angiotensin 2 enters to the bloodstream and makesadrenal cortical region to produce the aldosterone hormone because this compound influences in its turn on the reabsorption of sodium and potassium extraction from collecting tubes and this will increase plasma volume and edema. The effect ofAngiotensin1 and 2 on its receptorsisalmost against each other.AT2 is effective to prevent uncontrolled tissue growth and induction of tissues repair and the development. During the fetal period, stimulation of AT1 causes the growth of the fetus and stimulation of AT2 at the end of fetal growth, prevents abnormal fetus growth [4].There is a correlation between angiotensin converting enzyme inhibitors and Angioedema in the African American population [5]. Matthew and his colleagues determined location of ACE gene on chromosome 17 (17q23) through hybridization. It was shown that this is a highly polymorphic region but does not show the ACE gene recombination [6].Important effect of ACE in the homeostasis has been well confirmed [7]. It has been shown that variation in plasma ACE gene is associated withdeletion and addition of polymorphism as about 250 bpinintron 16 of the ACE gene, thereforeit is called I/DACE polymorphism. Allele I from ACE gene polymorphisms is related tolow activity of ACE gene and increasing the efficiency of the muscles in response to the movements and physical exercises [8].It has been reported that allele D from I/D ACE polymorphism leads to higher expression of mRNA, which can be more effective on the renin - angiotensin genes at the same areas [9]. It wasfound that the presence of the allele I in the post- menstrual period cannot be effective in response tothe hormone replacement treatment [10].In this study, it was tried to the frequency of I/D ACE gene polymorphism in infertile women with PCOS in Iranian population to be examined.

MATERIALS AND METHODS:

One hundred twenty one subjects consisting of 52 cases of PCOS and infertile, 30 cases of infertile, and 39 healthy subjects were investigated in this project.The study was approved by the local Institutional Ethics committee and written consent was obtained from all healthy individuals and patients. In this study, DNA extraction kit was prepared fromArashTebPishroo Co (Terhan, Iran) medicine. Ethanol, isopropanol, and chloroform were prepared from MERK Co. (Germany). Moreover, thermal cycler devices (EPPENDORF), PCR (CONVERGENCY) and microcentrifugation (SIGMA) were used. Primers in order to perform PCR analysis were prepared from the MASTER MIX Company,Primarily, for this study human samples required were prepared from infertile patients with POCS, infertility, and healthy referred totheShariatiHopital, Tehran, Iran and infertility clinics and were collected in the period 2014 to 2015.Blood samples were collected using

ethylene-di-amine tetra-acetic acid (EDTA) as anti- coagulation. The desired gene at 17q23 position was selected and DNA was extracted using DNA extracted kit, and then the agarose gel was used to confirm and DNA extractionand finally DNA extraction from peripheral blood was confirmed by applying an electrical voltage.At the next stage, oligonucleotides used for PCR were designed using Primer express,GENERUNNER, andBlast softwares.The sequences of genes that their primers have been designed for studying polymorphism on DNA were obtained from the NCBI site,andthen the polymorphic region of the interest gene in the studied exon was found and primers were designed on both sides of the polymorphic region.Primers were prepared fromtheTakapuzist Company and primers, extracted DNA, and other ingredients for mix were provided and it was reached to 30 ml volume and PCR was performed.Finally, PCR productswere applied on agarose gel and after applying electrical pulses, sequences with the length of 490 and 190 bpwere compared on the gel to Ladder and statistical analysis was performed after the shooting. Forward primers sequence was GAGCCACTCCCATCCTTTCT and reverse primers sequences was GTGGCCATCACATTCGTCAG.

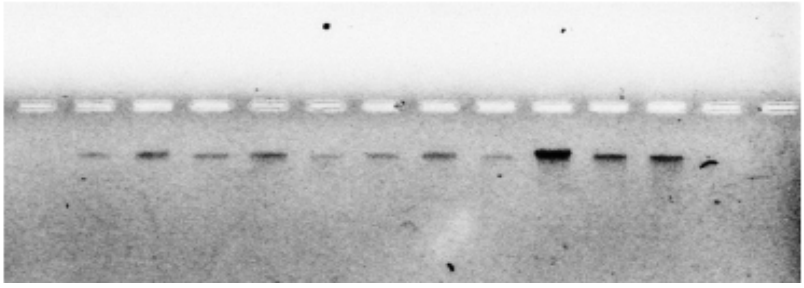

Figure 1.Confirmation of DNA extraction on the agarose gel

STATISTICAL ANALYSIS

Results were analyzed using SPSS software and the relatedfigures were represented.

Data obtained from normal distribution were analyzed with the statistical package SPSSusing student t-test and analysis of variance for data with normal distribution tests for nonparametric Mann-Whitney and Kruskalwallis, Chi-square, fisher exact test, and independent sample-test and if the difference between groups have the higher the confidence level than 95% or p<% 95 was considered statistically significant.

RESULTS

A total of 121 peripheral blood samples from three groups of infertile women with PCOS, infertile, and healthy respectively were examined with the numbers of 52, 30, 39 for each of the three ACE gene polymorphism in each group. Blood samples of studied subjects in this study were collected from infertility centers of theShariati Hospital, Tehran, Iran.

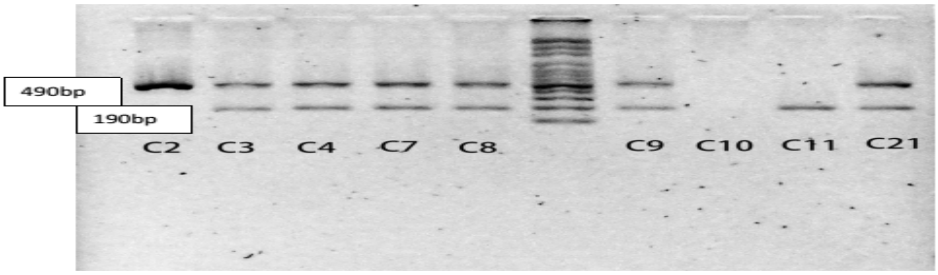

Figure 2. Infertile group - people with both bands of 490 bpand 190 bp bands, ID and people who have 490 bpband were considered as II and DD are people who have 190 bpband.

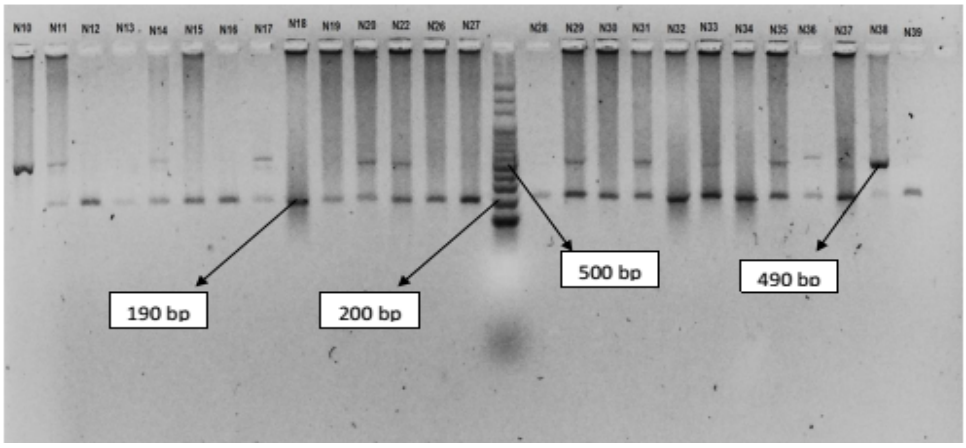

Figure 3.The control group- the band lengths of190 bpand 490bpbeside Ladder 200bpand 500 bp, respectively.

Finally, 52 samples having infertility and PCOS, 30 samples of infertile subjects, and 39 healthy controls were genotyped for polymorphisms of interest gene. The results are presented as genotypic frequencies of allele in each group and the results of agarose gel image, tables and statistics charts are listed.

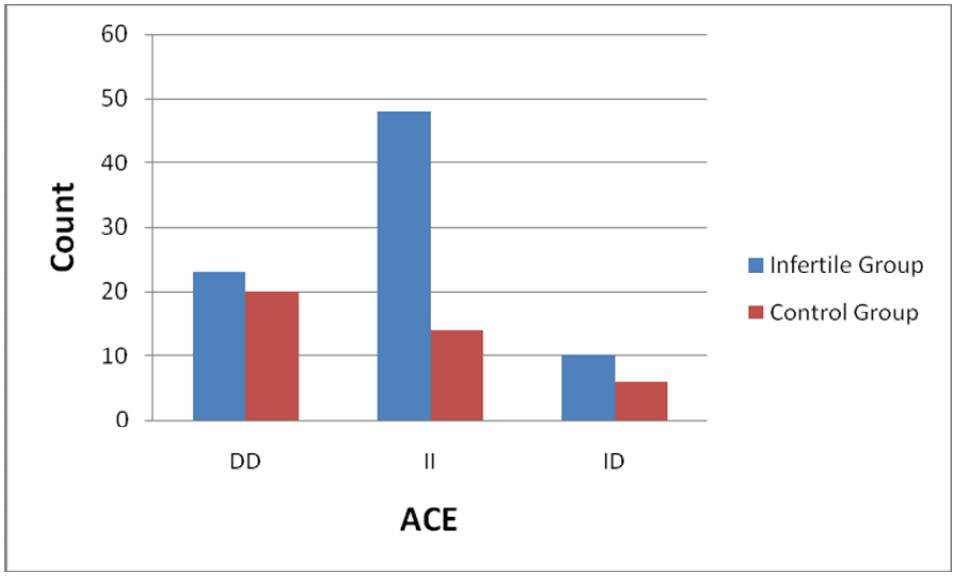

Figure 4.ACE gene polymorphism frequency ID in infertile group compared with the control group

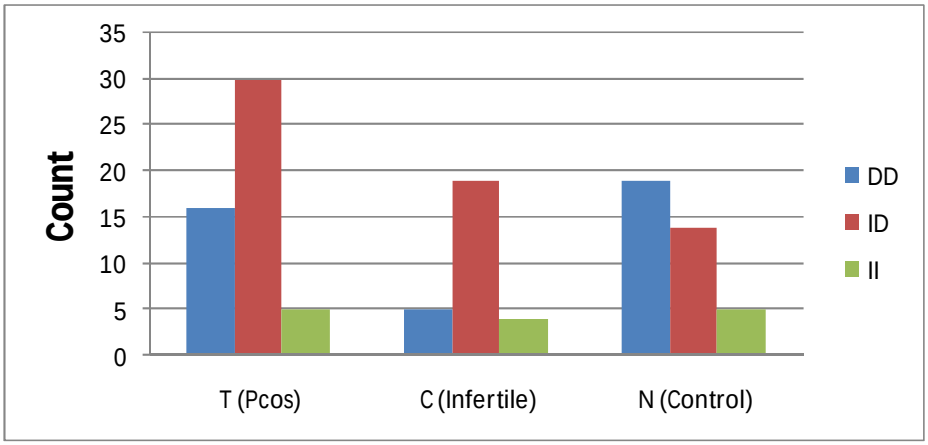

Figure 5.ACE gene polymorphism frequency in groups T (PCOS) - C (Infertile) - N (Control)

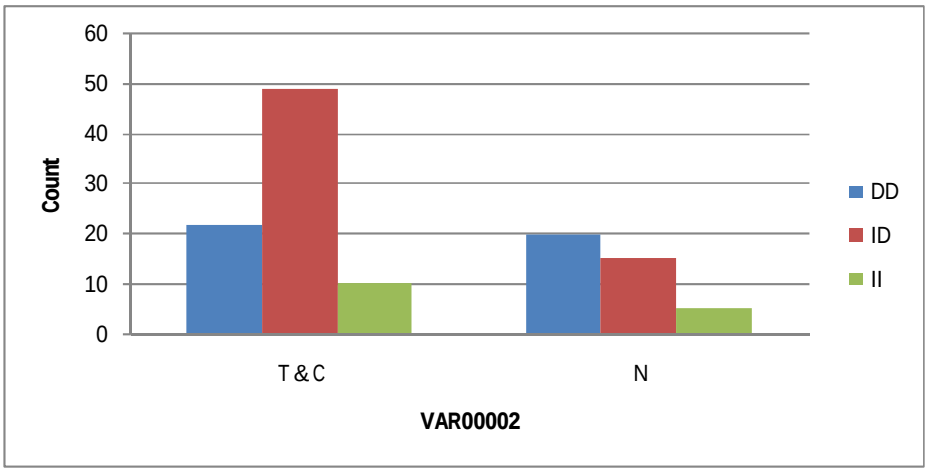

Figure 6.Comparison of the ACE gene polymorphism ID in infertile group (C-T) and control group (N)

The results of the ACE gene polymorphisms

1. The frequency of DD, ID, II ACE gene polymorphisms in patients with PCOS, infertility, and controls, respectively (11%,58%,31%), (20%,67%,13%) and (51%, 33%, 16%).
2. The frequency of DD, ID, II ACE gene polymorphisms in infertile groups and control subjects (27%, 61%, 12%) and (51%, 33%, 16%).
3. Studying ACE gene polymorphism in female population, polymorphism II in almostthe infertile and control groups, but ID and DD values for the studied groups with P-value=0.013 have a significant difference.
4. ACE gene polymorphism in the population of women in the infertile group with PCOS, infertile, and control showed a significant difference with P-value=0.048.
5. Studying the infertile and control groups for any of the ACE gene polymorphisms in statistical analysis showed a significant difference with P-value=0.013.

DISCUSSION

The results obtained in this study that represent significant correlation between ID polymorphism of ACE gene and PCOS in Iranian women is consistent with the results of the study of Kioka et al. on Greece population (11) and meta-analysis conducted by Jia et al. in the Caucasian population corresponded and on the other hand, Sun L et al.has announced the Chinese population (12) the polymorphism as a risk factor of DD while in the study conducted byDeepika et al. (13) ACE gene ID polymorphism is observed in case group and the control group as 51% of of India's population in the PCOS as 37% and people who have the D allele more likely show symptoms of PCOS in younger age groups.Celik et al. (14) stated that DD polymorphism in PCOS group than control group has more been observed in the Turkish population.Jia et al. meta-analysis reported that no correlation was found in the population of the Far East between patients with PCOS and ID polymorphism.Tiret et al. revealed that the frequency of I allele of ACE gene with low activity and increasing efficiency of the muscles is associated with response to the movements and physical exercises; as well as the Arefi et al.have reported increase in ACE gene activity in patients with PCOSand Qin et al. (15) have reported increase in the activity of this gene in patients with PCOS in the Chinese population.AdditionallyAlphan et al. (16) has declared the activity of genes in the group of patients with PCOS and control group in the population of Turkey.Totally, according to the results, it can be concluded that the ID polymorphism of ACE gene has been stated as a

risk factor for patients with PCOS deletions in Iran, the Caucasus and Greece population. On the other hand, DD polymorphism of ACE gene has been be considered as a risk factor for PCOS patients in populations of China, India and Turkey as a prognosis.

CONCLUSION

ID polymorphism in infertile groups and DD polymorphism in the control group were observed.ACE gene polymorphism in the studied groups in statistical analysis showed a different distribution. According to the obtained results we can conclude that polymorphisms of infertilitygenes are significantly associated with POCS and therefore the achieved results from the population of women in the childbearing age can be used as a prognosis. It is suggested that in the future studies, studyingthe candidate genes polymorphisms in women with PCOS undergoing IVF such as genes including LHB-LHR-CYP19A1-PGR-AMH-GDF9-BMP15 and genes involved in folate cycle such as MTHFR-TCN2FOLR1 and CTH, evaluation of the expression of other candidates, such as Anti-Mullerian Hormone and Androgen Receptor in granulosa cells due to increased expression of these two genes in granulosa cells, ACE gene polymorphism in a gene resources around the world has not been done, as well as the gene therapy methods have been studied on infertile patients with PCOS.

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