The relationship between hormones level and body mass index with insertion and deletion (D/I) polymorphism of ACE gene in infertile patients with polycystic ovary syndrome

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ABSTRACT

Background: The prevalence of polycystic ovary syndrome (PCOS) and infertility are rising due to changes in lifestyle. In this study, the polymorphism of insertion and deletion (I/D) in the angiotensin converting enzyme (ACE) gene in PCOS patients and the possibility of its association with PCOS and plasma level of hormones level as well as body mass index was investigated.

Materials and Methods: In this study, three polymorphisms in the ACE gene in Iranian women (Insertion-Insertion (II), Insertion-Deletion (ID), Deletion-Deletion (DD)) at three groups: PCOS, infertile patients and control as well as their relationship with hormones level and Body Mass Index (BMI) were examined. The methods included standard DNA extraction from peripheral blood and polymerase chain reaction (PCR) using specific primers for ACE gene. The data were then statistically analyzed.

Results: Results showed an higher prevalence of ID polymorphism in PCOS and infertility population with statistically significant association (p=0.013). There were also significant associations between the polymorphism with BMI, LH and progesterone level. The number of primordial follicles also found to be three times greater in PCOS group compared with normal ones.

Conclusion: The results showed significant association between polymorphisms of ACE gene, hormones level and BMI in PCOS which might be considered as possible prognostic marker for infertility of PCOS patients.

Keywords: Gene polymorphism, polycystic ovary syndrome (PCOS), Angiotensin converting enzyme gene (ACE gene), Infertility.

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INTRODUCTION

The Polycystic Ovary Syndrome (PCOS) or Stein-Leventhal Syndrome was first diagnosed in women with sclerocystic in 1884 and the attention was not drawn by other variations up to 100 years later, so that the increase in Luteinizing Hormone (LH) was not examined in relation to morbidity of this disease until 1985.1 PCOS syndrome is the most prevalent cause of infertility and endocrine gland disorders in women which ranging from 6 to 10% of fertile women population. The terminological reason of this syndrome is related to the presence of large ovaries with a great number of little cysts located in the outer layer of each ovary. The disease pose significant challenge for health professionals as it increases the risk of breast and endometrial cancer, Type II diabetes mellitus, gestational hypertension, and obesity compare to healthy females. In fact, 40% of the patients are obese and 75% of them are infertile. The diagnosis usually established by history taking, ovarian sonography, and gonadal hormone analysis.2

Angiotensinogen is produced by the liver and released into the blood stream. It cause Juxtaglomerular apparatus to release renin which convert Angiotensin 1 into Angiotensin 2. released renin from kidneys converts angiotensinogen into angiotensin 1. When angiotensin 2 enters into blood circulation it stimulates adrenal cortex to produce aldosterone.3 Changing activity of ACE would affect the homeostasis of RAAS and potentially result in several chronic or degenerative disease. In this study, we evaluate the relationship between D/I type of ACE gene polymorphism toward PCOS risk since PCOS are related with increased risk of type 2 diabetes mellitus, breast cancer, and infertility. However, we only examine the impact of ACE gene polymorphism in relation to infertility related PCOS. Mattei et al determined the position of the ACE gene on chromosome 17q23 through topical hybridization and it was shown that this area was highly involved in the polymorphism but no recombination was observed in this gene.4 Similarly, it was
shown that the diversity in ACE gene is accompanied by deletion and insertion of polymorphism for about 250 base pairs located in intron 16 at the ACE gene. Allele I in ACE gene polymorphism is associated with low activity of the ACE gene and increase the efficiency of muscles in response to physical practices and exercises. The length of Allele-I was determined the same as iterative Alu-allele with 287 base pairs.

It was reported that Allele D of ID ACE gene polymorphism might be led to increase the expression of the corresponding gene and this might be effective in the regions of the same gene in the renin-angiotensin system. It has been studied that those patients who had received alternative hormonal therapy resulted in favorable symptoms in terms of muscle response and contraction. Furthermore, the I-allele of the ACE gene was observed to influence bone mineral density. It affected mostly backbone with no impact observed in hip and pelvic which lead to the conclusion that this allele improves bone mineral density and muscle contraction, especially during postmenopausal period.

There are 13 polymorphisms that have been found in ACE gene. The importance of ACE gene was described in experimental mice. ACE gene deletion or inactivation in experimental mice resulted in impairment of sperm movement. Nonetheless, ACE gene was also observed to have important role in PCOS development. Therefore, we examined then effect of ACE polymorphism toward PCOS risk as well as several other variables especially BMI and reproductive related hormone.

MATERIALS AND METHODS

121 subjects were enrolled which consist of 52 cases of infertile polycystic ovary syndrome patients, 30 cases infertile and 39 cases of control subjects obtained from Shariati hospital (Tehran,Iran), Cytogenome laboratory (Tehran,Iran) and infertility clinics within period of 2014 to 2016 years. The study was approved by local institutional ethics committee and written consent was obtained from all subjects who were investigated in this project. Diagnosis of the disease was established under the supervision of gynecologist and according to the clinical signs, symptoms, and laboratory findings.

Blood samples were obtained from subjects and the level of hormones that related to fertility including LH, FSH, TSH, Estradiol, Progesterone and Prolactin were examined. Then, the values of these hormones were compared to normal and infertileindividual. BMI was also measured and its relationship to PCOS was analyzed.

DNA was extracted from venous blood according to manufacturer’s procedure (ATP Bioscience, Iran). Electrophoresis by 2% agarose gel was performed to confirm the presence of extracted DNA. Oligonucleotide sequences of forward primer of ACE gene was 5’-GAGCCACTCCCATCCTTTCT-3’ and reverse primer was 5’-GTGGCCATCACATTCGTCAG-3’ (Takapouzist Co., Iran). DNA was amplified by PCR for 35 cycles with denaturation at 94°C for 40s, annealing at 56°C for 40s and extension at 72°C for 40s (Convergency , Germany) using 30 µl PCR mixture which consist

Figure 1 Etiology and clinical traits including metabolic and reproductive indices in PCOS

Figure 2 Confirmation for extraction of DNA in infertile group with PCOS syndrome (T) before proceeding to Polymerase Chain Reaction (PCR)

Figure 3 Selection of optimum temperature by using gradient PCR

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of both primer, 10x PCR buffer, DNA, dNTPs, Taq polymerase enzyme, MgCl2 and ddH2O (Figure 3). The PCR products were then separated by electrophoresis on agarose gel.

In the presence of D allele, the PCR procedure would produce 190 bp products. In contrast, if I allele was present, a 490 bp DNA will be produced. In case of heterozygous individual, both fragment will present and resulted in the formation of two band in electrophoresis.

Data were analyzed using the statistical package for social sciences (SPSS ver.20). For those data which were normally distributed student t-test and Analysis of Variance (ANOVA) were performed and non-parametric tests were used for data with abnormal distribution including Mann-Whitney, Chi-square, Fisher’s exact test, and independent sample test. P value less than 0.05 was considered as significant.

RESULTS

The result of this study showed that all three possible genotypes were present in all three groups (Infertile with PCOS, Infertile, and control) with different proportion in each group (Table 1). More than half of subject with infertility and PCOS was observed to have ID genotype. The same cases also reported from Infertility groups. However, in control group, DD genotype was the dominant ones. The presence of the allele type was determined by PCR followed by electrophoresis as represented in Figure 4, 5, and 6. Comparison between Infertile group with or without PCOS to control resulted in statistically significant difference (p-value= 0.013). Meanwhile, when all three groups were compared each other, the result also statistically significant but almost approach 0.05 (p=0.048)

6 hormones including FSH, LH, TSH, progesterone, prolactin, estradiol were analyzed in all three groups. The results are described in Table 2. The result shown that there were significant relationship between BMI, increase in LH and progesterone level with PCOS. The difference in BMI only became statistically significant if both PCOS and
Infertile group were compared to control. Same situation also found in progesterone variable (p= 0.007). In LH variable, a slightly different situation was observed which was statistically significant differences were detected when both PCOS and Infertile group compared to control (P=0.019) but also detected when all three groups were compared with each other (P=0.007). Meanwhile, no statistically significant differences were detected when all variables were compared between genotypes. In general, the statistically significant results were obtained when corresponding variables were compared among PCOS, Infertile, and control group. However, no statistically significant differences were observed among different genotype groups.

Table 2  The results of statistical analysis of the studied hormones and Body Mass Index (BMI) in the studied groups

| Studied parameters        | Normal values | Infertile affected to polycystic ovary syndrome | Control |
|---------------------------|---------------|--------------------------------------------------|---------|
| Number                    | -             | 52                                               | 39      |
| Age                       | 20-50 years   | 4.9 (sd ± 29.4)                                  | 3.9 (sd ± 30.8) | 4.2 (sd ± 27.2) |
| BMI                       | 18.5 – 24.9   | 4.2 (sd ± 27.3)                                  | 4.1 (sd ± 24.6) | - |
| FSH Level                 |               |                                                  |         |
|                           |               | p-value = 0.007 (pcos and infertile compared with control) |
|                           |               | p-value = 0.155 (pcos and infertile compared to control) |
|                           |               | p-value = 0.371 (comparison of infertile group with different polymorphism of ACE gene) |
|                           |               | p-value = 0.509 (pcos and infertile and control compared together) |
|                           | 10- 60        | 3.7 (sd ± 7.1)                                   | 3.5 (sd ± 7.2) | 1.95 (sd ± 6.4) |
|                           | In some cases, it is slightly greater than 30. (At the middle of menstrual cycle, during pregnancy after menopause) |
|                           | 5-20          | 7.1 (sd ± 8.3)                                   | 2.9 (sd ± 5.1) | 1.31 (sd ± 5.4) |
|                           | In matured females |
|                           | 5-25 (after menopause) | 35.1 (sd ± 43.8)        | 18.65 (sd ± 49.7) | - |
|                           | 20–400 (before menopause) |
| Estradiol Level           |               | p-value = 0.393 (pcos and infertile comparison together) |
|                           |               | p-value = 0.738(comparison of pcos group with different polymorphism of ACE gene) |
|                           |               | p-value = 0.433 (comparison of infertile group with different polymorphism of ACE gene) |
|                           | Less than 500 | 257.9 (sd ± 325.1)                               | 232.3 (sd ± 296.7) | - |
| Progesterone Level        | 5-20          | 5.45 (sd ± 5.59)                                 | 8.65 (sd ± 10.5) | - |
|                           | Before ovulation and at the middle of menstrual cycle |
| TSH Level                 | 0.4–4.2       | p-value = 0.693 (pcos and infertile comparison together) |
|                           |               | p-value = 0.188 (comparison of pcos group with different polymorphism of ACE gene) |
|                           |               | p-value = 0.153 (comparison of infertile group with different polymorphism of ACE gene) |
| Cycle Length              | 21-35days     | 2.5 (sd ± 26.7)                                 | 3.3 (sd ± 27.1) | 2.6 (sd ± 28.5) |
DISCUSSION

Table 1 shows the significant relationship among ACE gene ID polymorphism and PCOS in Iranian female population. These findings are consistent with Kioka et al. in Greek population and meta-analysis conducted by Jia et al. in Caucasian population which stated that there was a statistically significant association between ACE polymorphism and PCOS in Caucasian population but not in Asian population. Furthermore, it also reveal that those with D allele poses greater risk of PCOS compared to I allele. However, the small number of sample involved in these studies, hindered the generalization of these studies. On the other hand, case-control study by Sun et al. in Chinese population found that DD genotype increase the risk of PCOS as high as 5.77 times than normal when combined with PAI-1 polymorphism 4G/5G. This finding is further confirmed by Deepika et.al and our current study which strengthen the evidence of the influence of D/I ACE polymorphism to PCOS risk.

There are also several evidence regarding the prevalence of ACE polymorphism in far east Asian population. Bayram et al reported that the frequency of allele D was as high as 68% among PCOS population and 51.5% in control group. Similar result was also obtained by Karabulut et.al that reported that the frequency of DD polymorphism was as high as 63% in PCOS patients in Turk. These findings were further strengthen by Celik et.al that stated DD polymorphism has been more frequently observed in PCOS group than control group in Turkish population. These findings clearly opposed reports by Jia et.al and Sun et.al as stated above.

The proposed mechanism for increased risk among ACE polymorphism was first highlighted by Tiret et.al that found lower gene expression rate among those with allele I. However, opposite finding was proposed by Arafi et.al that reported increase ACE gene activity among PCOS patients in Iranian population and Qin et.al who found similar result in Chinese population. Observation in Turkish population also yielded similar result which lead to conclusion that ACE gene ID polymorphism might be a risk factor for PCOS patients in Iranian, Caucasian, and Greek populations, meanwhile, ACE gene DD polymorphism could be considered a predictor for PCOS patients in Chinese, Indian, and Turkish populations.

Regarding the level of gonadotropin hormones in those with PCOS, Ann et.al reported elevated LH and LH to LSH ration in PCOS subjects. Seventy five percent of the PCOS patients had an elevated plasma LH level and 94% had elevated LH to FSH ratio. In addition, Joanne et al. reported that in the women with PCOS both the amplitude and frequency of LH secretion were increased compared to those with normal menstrual cycle throughout the follicular phase as well as the relative suppression of FSH secretion. Pastore et al. observed no difference between the true and placebo acupuncture protocols for the women with PCOS so that both groups had a similar improvement in their LH/FSH ratio but the level of plasma LH was elevated. Lastly, Cook et al. demonstrated that women with PCOS had higher serum LH level than normal women. All of the aforementioned studies have indicated an elevated LH level among women with PCOS.

In this study we found that I-allele were inversely associated with BMI. We found no other study that evaluate the association between ACE polymorphism with BMI, but our finding regarding of the relationship between BMI and PCOS was in conjunction with several other studies. Gambineri et al. reported that approximately 50% of PCOS women were overweight or obese and most of them had the abdominal obesity type. This finding highlighted the possible role of obesity in pathophysiology of PCOS or vice versa. This finding was in conjunction with the earlier one which suggested that elevated BMI at age 18 years old, even at lower level than those which considered to be obese, might be a risk factor for subsequent ovulatory infertility. Therefore, Pasquali et al. recommended weight loss as the first-line therapeutic option in all PCOS women with obesity. Furthermore, study Kiddy et al. reported that 35% of the women in the study were obese and obese women with PCOS had greater prevalence of hirsutism. In response, Hamilton-Fairley et al. concluded that
it is important to encourage weight reduction in obese women with PCOS before considering therapy to induce ovulation. In addition, Kiddy et al. also reported that the improvement in menstrual function and fertility may be the consequent upon an increase in insulin sensitivity which, directly or indirectly, affects ovarian function.

CONCLUSION

The results of this study clearly confirm that the ID polymorphism was predominant within infertile groups either with or without PCOS with statistically significant difference with control group and highlighting its potential as PCOS and infertility biomarker. Furthermore, it also revealed the hormonal change resulted from PCOS including higher level of LH and Progesterone. In addition, there was also an association between BMI and PCOS with higher BMI was associated with higher risk of PCOS based on several literatures. However, further researches are required to confirm the association of ACE gene polymorphism with PCOS and infertility in other population in order to reveal its global impact and also to determine its interaction with other gene related to ovulation.

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