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ORIGINAL ARTICLE

Interaction with angiotensin-converting enzyme-encoding gene in female infertility: Insertion and deletion polymorphism studies

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Abstract Angiotensin-converting enzyme (ACE), a key enzyme in the renin– angiotensin–aldosterone system, converts angiotensin I to angiotensin II. Ethnic origin should be carefully considered in studies pertaining to ACE I/D genotype and disease etiology. This study was evaluated between the ACE gene I/D polymorphism and female infertility in the Saudi population. Out of a A total of 300 women who participated in this study genomic DNA samples from the 150 infertile and 150 fertile women's were isolated who has participated in this study. Genomic DNA was isolated using an Invitrogen kit according to the manufacturer's protocol, and D allele specific primers were used for amplification by polymerase chain reaction. Electrophoresis was carried out on a 2% agarose gel. The mean age and BMI of the cases and controls were similar ($p > 0.05$), and a significant association was noted between the family history and female infertility ($p = 0.0001$). The D allele (OR: 1.67 [95% CI: 1.18–2.35], $p = 0.003$), DD genotype (OR: 2.46 [95% CI: 1.20–5.02], $p = 0.01$) and dominant model (OR: 1.97 [95% CI: 1.00–3.88], $p = 0.04$) were significantly associated with female infertility or fertility. The results of this study show that the ACE polymorphism plays an important role in female infertility in the Saudi population.

1. Introduction

Female infertility is a complex disorder that can be caused by medical conditions such as pelvic inflammatory disease, endometriosis, polycystic ovary syndrome (PCOS), premature ovarian failure, and uterine fibroids (Motovali-Bashi et al., 2015). The incidence of infertility differs between racial and ethnic groups because of the multifactorial nature of the disease. It affects approximately 10% of couples during their reproductive years. Ovulatory disorders (15%), tuboperitoneal factors (35%), male factors (35%), unexplained factors (10%), and both male and female factors (35%) can cause infertility. Ovulatory disorders are more common in young women, whereas unexplained infertility occurs more frequently in older women. Unexplained infertility is diagnosed by the exclusion

of other causes, and its prevalence ranges from 5% to 37% in various populations (Karatas et al., 2014). Age, smoking, obesity, alcohol consumption, and family history are all risk factors for infertility (Seshagiri, 2001). Infertility is defined as either primary or secondary based on the couple's history. Primary infertility is the inability to conceive despite at least 1 year of unprotected intercourse. Secondary infertility is the inability to become pregnant or carry a pregnancy to term when a woman has given birth to a child without the assistance of reproductive medicine in the past (Fatima et al., 2015). Recurrent Pregnancy loss (RPL) is defined as losing of minimum three or among that as consecutive spontaneous abortion before the 20th week of gestation (Li et al., 2016). Specifically, there is no definition of RPL since certain aspects (including number of abortions, consecutive nature of miscarriages, history of live births and gestational age at pregnancy loss) are still controversial (Yang et al., 2015). The pregnancy complications have been found to be related to thrombophilic polymorphisms (examples: MTHFR, FVL, ACE and PII), explains about ∼30% of obstetrical problems including pregnancy complications such as RPL (Goodman et al., 2009). Previous studies have examined the role of the angiotensin-converting enzyme-1 (ACE1) gene in male and female infertility factors including PCOS and endometriosis (Hsieh et al., 2007; Jia et al., 2013; Kowalczyńska et al., 2014; Kucera et al., 2001; Zalata et al., 2012). PCOS is the leading cause of ovulatory infertility, affecting 5–7% of women of reproductive age, and the renin–angiotensin system (RAS) activity in PCOS suggests an important correlation between the RAS and PCOS. ACE1−/− male mice have been found to be sterile, and despite the sperm motility and fusing location of eggs generated in Ace2−/− and Ace3−/− mice, the male mice were slightly abnormal, and both knockouts proved to be fertile. These facts indicate that ACE1, ACE2, and angiotensin-converting enzyme-3 (ACE3) may be among the mechanisms responsible for infertility (Pan et al., 2013). ACE is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. It converts the inactive decapeptide angiotensin I (Ang I or Ang 1–10) to the active octapeptide and potent vasoconstrictor angiotensin II (Ang II or Ang 1–8), which is the main active product of the renin–angiotensin system (RAS). The importance of functional polymorphism of the ACE gene (I/D polymorphism) was discovered through its association with plasma ACE levels (Sayed-Tabatabaei et al., 2006). The insertion and deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE: NM 000789.2) gene is the first genetic variant associated with human physical performance and is in strong linkage disequilibrium with genetic factors that influence serum ACE concentrations (Kowalczyńska et al., 2014; Poornima et al., 2015). The ACE1 gene consists of 26 exons and 25 introns appearing on chromosome 17q23. The ACE gene contains a 287-bp Alu sequence in the 16th intron region (Hsieh et al., 2007; Shanmuganathan et al., 2015). The rs4646994 polymorphism in the ACE gene is characterized by the presence or absence of a 287-bp Alu repetitive sequence, resulting in the II, ID, and DD genotypes (Pan et al., 2013; Zhao et al., 2015). Earlier studies have shown that DD homozygotes have an approximately twofold higher level of tissue and plasma ACE as compared to II homozygotes (Pradhan et al., 2015; Sayed-Tabatabaei et al., 2006). Based on previous reports, this study was evaluated between the

ACE gene I/D polymorphism and female infertility in the Saudi population.

2. Materials and methods

2.1. Selection of subjects

This was a hospital-based study carried out by the Department of Obstetrics and Gynecology at the King Khalid University Hospitals (KKUH) in Riyadh and Saudi Arabia. The study took place from January 2015 to November 2015. A total of 300 samples were recruited and 150 infertile women as well as 150 fertile women served as control subjects for this study. The cases were recruited based on their (i) family history of fertility and (ii) inability to conceive for more than 3 years. The infertility cases were examined based on hormonal and biochemical analysis, sexually transmitted diseases, hysterosalpingography, hysteroscopy, laparoscopy, and genetic immunological abnormalities, as well as semen analysis of the partner. The control subjects were confirmed to have conceived without assisted reproductive techniques and were selected based on (i) regular menstrual cycles, (ii) conception of a minimum of one child, and (iii) without family history of infertility. Control subjects with ovarian lesions/endometriosis and family histories of infertility were excluded. Women with endometriosis, Müllerian defects, autoimmune diseases, ovulatory or endocrine disorders, or partners who had male factors such as low sperm count were also excluded as controls. The ethical approval was obtained for this study from KKUH.

2.2. Blood analysis

Four milliliters of peripheral blood were collected from each patient in EDTA vacutainers, and genomic DNA was extracted with an Invitrogen kit (PureLink Genomic DNA Kit; Thermo Fischer Scientific, MA, USA). The genomic DNA was separated from the lymphocytes as per the manufacturer's instructions. The quantification of DNA was performed by NanoDrop spectrophotometer analysis (NanoDrop 2000; Thermo Fischer Scientific, MA, USA). The oligonucleotide sequence (forward and reverse primers) for the insertion and deletion polymorphism of the ACE gene was selected from earlier studies (Khan et al., 2014; Poornima et al., 2015). The genotyping for ACE gene I/D polymorphism was accomplished by direct PCR followed by 2% agarose gel electrophoresis. The 190 bp fragment indicates the deletion allele and the 490 bp fragment indicates the insertion allele. Heterozygous results showed both 490 and 190 bp fragments. The PCR program was performed as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s; and final extension at 95 °C for 5 min (see Table 1).

2.3. Statistical analysis

The genotype and allele frequencies between infertility and fertility women were calculated with odds ratio (OR), followed by 95% confidence intervals (CI's) and p values. The chi-square (χ^2) analysis was calculated between cases and controls. The

Table 1 Characteristics of selected subjects and controls involved in this study.

Variables	Cases (n = 150)	Controls (n = 150)	p Value
Age (Years)	32.1 ± 2.4	31.7 ± 2.3	0.11
BMI (kg/m^2)	33.5 ± 2.7	33.2 ± 2.5	0.18
Women effected with infertility	150 (100%)	0 (0%)	0.0001
Family history of infertility	81 (54%)	0 (0%)	0.0001

statistical analysis was carried out using statistical package for the social sciences, version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). χ^2 test for goodness of fit was used to calculate the agreement of the observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium (HWE). The clinical data were expressed as mean ± standard deviation (SD). The p value which is less than 0.05 was indicated as significant association.

3. Results

For this study, 300 subjects were selected, including 150 infertile women (cases) and 150 fertile women (controls). The mean age of the cases was 32.1 ± 2.4 years, and the mean age of the controls was 31.7 ± 2.3 years. The ages and BMIs of both groups were found to be similar ($p > 0.05$). All the cases were affected by infertility and a statistical significant was found between the cases and controls ($p < 0.05$). The family history was found in the 54% of the cases and none of them in the controls, associated positively in both the cases and controls ($p < 0.05$). The allelic frequency and genotype distribution of the ACE polymorphism is associated with infertility in women. The genotype distributions of the I/D polymorphism in the infertile cases and fertile controls are shown in Table 2. The genotype frequencies of II, ID, and DD in infertile women were 10%, 35.3% and 54.7%, respectively. The percentage of

the D allele was 0.72%, and the percentage of the I allele was 0.28% among the infertile cases. The genotype distributions of II, ID, and DD in normal subjects were 18%, 42%, and 40% respectively. The allelic frequencies of the D and I alleles in the control subjects were 0.61% and 0.39%, respectively. The genotype frequencies of the I/D polymorphism were consistent with HWE in the study group. The statistical analysis of genotype results indicated that DD vs. II (OR: 2.46 [95% CI: 1.20–5.02], $p = 0.01$), and DD + ID vs. II (OR: 1.97 [95% CI: 1.00–3.88], $p = 0.04$) of the cases and controls was significantly associated. The allele frequencies of the cases and controls were statistically associated as well (D vs. I, OR: 1.67 [95% CI 1.18–2.35], $p = 0.003$) (Table 3).

4. Discussion

There are no prior or current studies carried out with female infertility and ACE gene I/D polymorphism in any ethnic groups. The aim of the present study was to identify the association between the ACE gene I/D polymorphism and female infertility in the Saudi population. To the knowledge, this is the first such study to be carried out on this topic in infertile female subjects in Saudi Arabia. The results showed a positive association within the alleles and genotypes ($p < 0.05$). The dominant and recessive models also showed a statistical association. Age vs. genotypes and BMI vs. genotypes showed an insignificant association among the cases ($p > 0.05$).

Infertility has become a global problem affecting 15% of couples having unprotected intercourse; it affects males and females equally. Idiopathic infertility is diagnosed when couples are unable to conceive for more than 2 years, with no abnormalities found during repeated investigations of tubes or in ovulation, the luteal phase, cervical mucus, semen, sperm–oocyte interaction, or intercourse. The major genetic causes of infertility are chromosomal abnormalities, microdeletions, chromosomal abnormalities, cystic fibrosis transmembrane conductance regulator mutations, follicle stimulation hormone receptor mutations, and other genetic factors. The major causes of infertility in women are ovulation disorders and tubal damage, which account for 50% of the cases. Endometriosis, hyperprolactinemia, and reproductive tract

Table 2 Genotype and allele frequencies between cases and control subjects.

Genotypes	Cases (n = 150)	Controls (n = 150)	p Value	OR (95% CI)	X^2
II	15 (10%)	27 (18%)	Reference		
ID	53 (35.3%)	63 (42%)	0.26	1.51 (0.73–3.14)	1.24
DD	82 (54.7%)	60 (40%)	0.01	2.46 (1.20–5.02)	6.27
I	83 (0.28)	117 (0.39)	Reference		
D	217 (0.72)	183 (0.61)	0.003	1.67 (1.18–2.35)	8.65

Table 3 Different model of statistical analysis with the cases and controls.

Model	Cases (%)	Controls (%)	X^2	OR (95% CI)	p Value
Dominant	15 (10%)	27 (18%)	3.97	1.97 (1.00–3.88)	0.04
Co-dominant	53 (35.3%)	63 (42%)	1.40	1.32 (0.83–2.11)	0.23
Recessive	82 (54.7%)	60 (40%)	6.45	0.55 (0.34–0.87)	0.001

diseases are other causes of infertility in women. The other major risk factors affecting female fertility are pelvic inflammatory disease, sexually transmitted diseases, damaged Fallopian tubes, pelvic surgery, ectopic pregnancy, and unsafe abortions. Additionally, it has been reported that cigarette smoking can lead to tubal diseases and abnormal cervical mucus, resulting in an increased risk of infertility and childhood cancers (Ferlin et al., 2006; Zenzes, 2000).

ACE plays a critical role in the RAS, involved in the conversion of angiotensin I to active angiotensin II, a potent vasopressor. ACE also has a physiological function in the fibrinolysis pathway as it regulates the concentrations of plasminogen activator Inhibitor I (PAI-1), an important determinant in the control of the fibrinolytic process. The appearance of D or DD allele/genotype was correlated with elevated plasma and tissue-specific ACE activity and the deletion genotype (DD) also enriches production of angiotensin II from angiotensin I, is associated with high levels of circulating PAI-1, which result in reduced levels of fibrinolysis. The mutant of D allele in the ACE gene may compromise placental formation and trophoblastic invasion because of increased PAI-1 expression and concomitant reduced fibrinolytic activity (Su et al., 2013). The gravidas with thrombophilic defects, risks for pregnancy-associated thromboembolism, and other vascular complications, such as pre-eclampsia and abortion, will be improved (Yang et al., 2012). Earlier studies carried out with ACE gene I/D polymorphism studies with RPL failed to show the association in different ethnicities (Bagheri et al., 2010; Goodman et al., 2009; Vettriselvi et al., 2008) and it was supported by one of the meta-analysis studies by Yang et al. (2012) carried out in Asian and Caucasian populations. However, Su et al. (2013) meta-analysis study revealed the positive association in RPL. The present study is in accordance with the Su et al. (2013) studies.

The association between genetic polymorphisms and clinical disease has long been recognized. Many aspects of female reproductive function are strongly influenced by genetic factors, and numerous studies have attempted to identify susceptibility genes for disorders affecting female fertility (Tempfer et al., 2009). The associations between several polymorphisms in the human genome and the infertility of women with endometriosis have been demonstrated.(Kowalczyńska et al., 2011) The ACE gene has a significant association with male infertility and the ID polymorphism (Kucera et al., 2001; Zalata et al., 2012). The strength of our current study was its recruitment of women between the ages of 20 and 40 years, since strong evidence suggests that couples who are trying to conceive become less fertile as they age. The failure to measure ACE levels was the one of the limitations of this study.

Results of current study indicate that the ACE polymorphism plays an important role in female infertility in the Saudi population. Additional studies are needed to explore this relationship in more detail in subjects with primary or secondary infertility.

Acknowledgement

References

Bagheri, M., Abdi Rad, I., Omrani, M.D., et al, 2010. Polymorphisms of the angiotensin converting enzyme gene in Iranian Azeri Turkish women with unexplained recurrent pregnancy loss. Hum. Fertil. (Camb) 13, 79–82.

Fatima, P., Rahman, D., Hossain, H.B., et al, 2015. Psychosocial consequences of infertility on infertile women. Mymensingh Med. J. 24, 704–709.

Ferlin, A., Arredi, B., Foresta, C., 2006. Genetic causes of male infertility. Reprod. Toxicol. 22, 133–141.

Goodman, C., Hur, J., Goodman, C.S., et al, 2009. Are polymorphisms in the ACE and PAI-1 genes associated with recurrent spontaneous miscarriages? Am. J. Reprod. Immunol. 62, 365–370.

Hsieh, Y.Y., Lee, C.C., Chang, C.C., et al, 2007. Angiotensin I-converting enzyme insertion-related genotypes and allele are associated with higher susceptibility of endometriosis and leiomyoma. Mol. Reprod. Dev. 74, 808–814.

Jia, H., Wang, B., Yu, L., et al, 2013. Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with polycystic ovary syndrome: a meta-analysis. J. Renin Angiotensin Aldosterone Syst. 14, 255–262.

Karatas, A., Eroz, R., Bahadır, A., et al, 2014. Endothelial nitric oxide synthase gene polymorphisms (promoter −786 T/C, exon 894 G/T and intron G10T) in unexplained female infertility. Gynecol. Obstet. Invest. 77, 89–93.

Khan, I.A., Jahan, P., Hasan, Q., et al, 2014. Angiotensin-converting enzyme gene insertion/deletion polymorphism studies in Asian Indian pregnant women biochemically identifies gestational diabetes mellitus. J. Renin Angiotensin Aldosterone Syst. 15, 566–571.

Kowalczyńska, L.J., Tomasz, F., Wojciechowski, M., et al, 2011. ACE I/D polymorphism in Polish patients with endometriosis. Ginekol. Pol. 82, 102–107.

Kowalczyńska, L.J., Ferenc, T., Wojciechowski, M., et al, 2014. Endometriosis and RAS system gene polymorphisms: the association of ACE A2350G polymorphism with endometriosis in Polish individuals. DNA Cell Biol. 33, 328–335.

Kucera, M., Crha, I., Vasků, A., et al, 2001. Polymorphism of angiotensin converting enzyme (ACE) and TNF-beta genes in men with disorders of spermatogenesis–pilot study. Ceska Gynekol. 66, 313–317.

Li, H.H., Xu, X.H., Tong, J., et al, 2016. Association of TNF-α genetic polymorphisms with recurrent pregnancy loss risk: a systematic review and meta-analysis. Reprod. Biol. Endocrinol. 14, 6.

Motovali-Bashi, M., Sedaghat, S., Dehghanian, F., 2015. Association between Serum Paraoxonase 1 Activities (PONase/AREase) and L55M Polymorphism in Risk of Female Infertility. Avicenna J. Med. Biotechnol. 7, 173–178.

Pan, P.P., Zhan, Q.T., Le, F., et al, 2013. Angiotensin-converting enzymes play a dominant role in fertility. Int. J. Mol. Sci. 14, 21071–21086.

Poornima, S., Subramanyam, K., Khan, I.A., et al, 2015. The insertion and deletion (I28005D) polymorphism of the angiotensin I converting enzyme gene is a risk factor for osteoarthritis in an Asian Indian population. J. Renin Angiotensin Aldosterone Syst. 16, 1281–1287.

Pradhan, V., Kemp, E.H., Nadkar, M., et al, 2015. Association between the angiotensin-converting enzyme gene insertion/deletion polymorphism and susceptibility to systemic lupus erythematosus in an Indian population. Scand. J. Rheumatol. 44, 425–427.

Sayed-Tabatabaei, F.A., Oostra, B.A., Isaacs, A., et al, 2006. ACE polymorphisms. Circ. Res. 98, 1123–1133.

Seshagiri, P.B., 2001. Molecular insights into the causes of male infertility. J. Biosci. 26, 429–435.

Shanmuganathan, R., Kumaresan, R., Giri, P., 2015. Prevalence of angiotensin converting enzyme (ACE) gene insertion/deletion

polymorphism in South Indian population with hypertension and chronic kidney disease. J. Postgrad. Med. 61, 230–234.

Su, M.T., Lin, S.H., Chen, Y.C., et al, 2013. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. Thromb. Haemost. 109, 8–15.

Tempfer, C.B., Simoni, M., Destenaves, B., et al, 2009. Functional genetic polymorphisms and female reproductive disorders: part II–endometriosis. Hum. Reprod. Update 15, 97–118.

Vettriselvi, V., Vijayalakshmi, K., Paul, S.F., et al, 2008. ACE and MTHFR gene polymorphisms in unexplained recurrent pregnancy loss. J. Obstet. Gynaecol. Res. 34, 301–306.

Yang, C., Fangfang, W., Jie, L., et al, 2012. Angiotensin-converting enzyme insertion/deletion (I/D) polymorphisms and recurrent pregnancy loss: a meta-analysis. J. Assist. Reprod. Genet. 29, 1167–1173.

Yang, Y., Luo, Y., Yuan, J., et al, 2015. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. Arch. Gynecol. Obstet., Epub ahead of print

Zalata, A.A., Morsy, H.K., Badawy, Ael-N., et al, 2012. ACE gene insertion/deletion polymorphism seminal associations in infertile men. J. Urol. 187, 1776–1780.

Zenzes, M.T., 2000. Smoking and reproduction: gene damage to human gametes and embryos. Hum. Reprod. Update 6, 122–131.

Zhao, W., Ma, S.T., Cui, L.Q., 2015. Meta-analysis of angiotensin-converting enzyme insertion/deletion polymorphism and myocardial infarction in Han Chinese. Genet. Mol. Res. 14, 8068–8076.