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Role of C677T polymorphism in the MTHFR gene in Saudi females affected with infertility

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ABSTRACT

The methylenetetrahydrofolate reductase (MTHFR) gene plays a major role in folate metabolism in infertile women. To date, limited studies have implicated the C677T polymorphism in MTHFR with female infertility, which is defined as the failure to accomplish clinical pregnancy with consistent unprotected sexual intercourse for 1 year; this disorder is influenced by various factors, including obesity and it affects in ~15% of couples during the reproductive period. However, limited molecular studies have been conducted pertaining to female infertility. The current study aimed to investigate the correlation between the C677T polymorphism in MTHFR and infertility in a Saudi population. In this study, 300 genomic DNA samples were extracted from 150 infertile and 150 fertile women, and genotyping was performed for the C677T polymorphism in MTHFR by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The results revealed that the mutant T allele was significantly associated with infertility (odds ratio [OR] = 1.86; 95% CI = 1.15–2.99; p = 0.009). Additionally, the dominant and recessive modes of inheritance were positively associated with infertility, i.e., TT vs. CT+CC (OR = 2.98; 95% CI = 1.33–6.66; p = 0.005) and TT+CT vs. CC (OR = 1.89; 95% CI = 1.09–3.26; p = 0.02). The current results indicate that the T allele of the C677T polymorphism in MTHFR is a significant risk factor for infertility in women from a Saudi population.

1. Introduction

Female infertility is defined as a complex disorder affected by medical conditions, such as pelvic inflammatory disease, polycystic ovarian syndrome (PCOS), endometriosis, uterine fibroids, and premature ovarian failure (Al-Mutawa, 2018). Female infertility is caused by anatomical, hormonal, thrombotic, autoimmune, genetic, and unknown infectious factors (Artini et al., 2013). Infertility affects 10.7–15.5% of couples, explaining the reproductive system disorder (Maddirevula et al., 2017). Infertility is categorized as either primary or secondary infertility. Primary infertility is defined as the failure to conceive after 1 year of unprotected intercourse in a couple. In secondary infertility, couples take a long time to conceive and continue with the pregnancy or may face a miscarriage (Eniola et al., 2017). The global prevalence of infertility is ~ 15% worldwide, and it is not affected by ethnicity or racial background. Interestingly, the prevalence of infertility has increased nearly 50% in the last decade (Ashraf et al., 2015). The most common causes of fertility impairment are menstrual, ovulatory, and uterine factors (Masoumi et al., 2015). In reproductive aged women, obesity is well established as one of the major risk factors for infertility (Gaskins, 2018). Approximately 5–10% of infertile women carry originating genetic abnormalities, such as chromosome abnormalities, deletions, insertions, aberrations, mutations, and polymorphism analysis. Gene identification is important for understanding the disease pathophysiology and refining prevention, diagnosis, and treatment. Genetic polymorphisms are confirmed through multiple alleles at distinct DNA loci (Hanson et al., 2017; Khan et al., 2016), and human phenotypic variants occur due to genetic and environmental factors (Fucharoen, 2007). A combination of human genome sequencing and single nucleotide polymorphism (SNP) has provided

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the required amount of genetic data for human diseases (Romero-Sánchez et al., 2015). Numerous studies have verified homocysteine as an important biomarker in human diseases, along with the biological function in the folate metabolism pathway (Wang et al., 2016), and it plays a pivotal role in the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which converts the remethylation of homocysteine to methionine (Khan et al., 2015). Primarily, there are C677T and A1298C SNPs, which are commonly studied in global populations along with meta-analysis studies of human diseases. The complementary DNA sequence of methylenetetrahydrofolate reductase (MTHFR) is ~2.2 kb, which consists of 11 exons (103-432 bp) at the end of the locus of chromosome 1p36.3 and translates to a 77kDa protein in human (Qin et al., 2014). The relation between MTHFR and female infertility is connected with folate deficiency, which often occurs with associated hyperhomocysteinemia (Kim et al., 2015). Presently, no studies have implicated the role of MTHFR in Saudi Arabian women affected with infertility. Therefore, the current study aimed to investigate the role of the C677T polymorphism in MTHFR in Saudi women with infertility.

2. Methods

Written informed consent was obtained from study participants following the receipt of approval of ethical consent from King Saud University (KSU). In brief, 150 infertile women were recruited from the outpatient Department of Obstetrics and Gynecology clinic in King Khalid University Hospitals (KKUH). These women underwent hormonal and biochemical analysis; investigations of sexually transmitted diseases and genetic immunological abnormalities; and hysterosalpingography, hysteroscopy, and laparoscopy. In addition, their partners underwent semen analyses. All tests were performed in the hospital premises, and all women were of Saudi origin. In parallel, 150 Saudi fertile (control) women were selected from KKUH premises. The inclusion and exclusion criteria of the infertile and fertile women were documented in a prior publication (Al-Mutawa, 2018).

2.1. Molecular analysis

From 300 women, 2 mL of the leukocytes were collected in an ethylenediaminetetraacetic acid (EDTA) tube, and genomic DNA was extracted using the PureLink Genomic DNA kit (Thermo Fisher Scientific, Walthman, USA), based on the manufacturer's protocol. DNA quality was calculated using a NanoDrop spectrophotometer (ThermoFisher Scientific, USA), and the samples were stored at -20°C. Genotyping for C677T polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The forward and reverse primers along with restriction enzyme usage were adopted from a prior publication (Khan et al., 2015). Based on the PCR amplification, a 198 bp product was obtained. Subsequently, using HinfI enzyme, the PCR product was digested and analyzed by 2% ethidium bromide stained agarose gel electrophoresis. The wild type allele represented 198 bp, whereas the mutant allele represented 175/23 bp, respectively.

2.2. Statistical analysis

Comparison of allele and genotype analysis was performed with odds ratios, 95% confidence intervals, and p-values using the statistical package for social sciences program (SPSS) version 19 (SPSS, Inc. Chicago, IL, USA). The continuous variable results were reported as mean ± standard deviation (m ± sd). Hardy-Weinberg equilibrium (HWE) was tested with genotype frequencies. A p-value less than 0.05 was considered statistically significant.

3. Results

The baseline characteristics of Saudi women are shown in Table 1. Infertile women were older (32.1 ± 2.4) with higher Body Mass Index (BMI) (33.5 ± 0.7) than controls (31.7 ± 2.3), and age with BMI (33.2 ± 2.5) was negatively associated ($p > 0.05$). Family history and affected women of infertility were significantly associated with the study group ($p < 0.0001$). HWE violation was not documented for genotype frequencies ($p > 0.05$). C677T polymorphism genotype frequency of CC, CT, and TT in the infertile women was 70.7%, 23.3%, and 6%, respectively. In the control subjects, the genotype frequencies of CC, CT, and TT were 82%, 15.3%, and 2.7%, respectively. The allelic frequencies of T and C in the controls were 0.11% and 0.89%, and in cases were 0.18% and 0.82%, respectively. The allelic association was found to be significant (OR-1.86; 95% CI: 1.15–2.99; p =0 .009) (Table 2).

As shown in Table 3, a different mode of inheritance was calculated with the statistical analysis. A significant observation was appeared in dominant (OR-1.89; 95% CI: 1.09–3.26; p = 0.02) and recessive (OR-7.63; 95% CI: 1.33–6.66; p = 0.005) modes of inheritance.

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

BMI= Body Mass Index

Table 2: Genotype and allele frequencies of C677T polymorphism between cases and control subjects

Genotypes	Cases (n=150)	Controls (n=150)	p value	OR (95% CI)	X^2
CC	106 (70.7%)	123 (82%)	Reference		
CT	35 (23.3%)	23 (15.3%)	0.05	1.76 (0.98-3.17)	3.64
TT	09 (6%)	04 (2.7%)	0.10	2.61 (0.78-8.72)	2.58
C	247 (0.82)	269 (0.89)	Reference		
T	53 (0.18)	31 (0.11)	0.009	1.86 (1.15-2.99)	6.68

Table 3: Different pattern of mode of inheritance

Model	Cases (%)	Controls (%)	X^2	OR (95% CI)	p value
Dominant	44 (29.3%)	27 (18%)	5.31	1.89 (1.09-3.26)	0.02
Co-dominant	115 (76.7%)	127 (84.7%)	3.06	1.68 (0.93-3.01)	0.07
Recessive	141 (91%)	126 (97.3%)	7.63	2.98 (1.33-6.66)	0.005

4. Discussion

The current study is the first to investigate the association between the C677T polymorphism in the MTHFR gene in Saudi infertile women. Comparison between 150 infertile women and 150 normal fertile women revealed significant association between dominant (TT+CT vs CC: OR-1.89; 95% CI: 1.09–3.26; p = 0.02) and recessive modes of inheritance (TT vs CC+CT; OR-2.98; 95% CI; 1.33–6.66; p = 0.005), and the T allele (OR-1.86; 95% CI: 1.15-–2.99; p = 0.009).

Female infertility is defined as decreased fertility failing to achieve pregnancy within 12 exposed cycles. Female infertility occurs mainly due to pelvic adhesion, endometriosis, and unexplained infertility anovulation fallopian tube disease. Further, unexplained infertility is defined as the lack of an identical cause of infertility (Brazdova et al., 2016). In this study, obesity was found in the recruited samples of both fertile and infertile women. Obesity is reportedly associated with female infertility and is one of the major factors, especially in Saudi Arabia, because females are highly obese when compared with males, and the overall prevalence of obesity in the Saudi Arabia is 35.5% (Rafique and Nuzhat, 2016). However, recent lifestyle of female reproductive health is protected towards BMI, nutrients, physical activity, and stress-related jobs (Silvestris et al., 2018). Dağ and Dilbaz (2015) concluded that dropping weight would be beneficial for the reproductive effect in infertile women.

Frosst et al. (1995) reported a common C-T mutation at position 677 of the MTHFR cDNA, which results in thermolability of a protein with reduced enzyme activity. However, homozygous 677T genotype (T/T) has been associated with congenital anomalies and compromised pregnancies. Mothers as carriers of the TT genotype pass the risk to their babies, including neural tube defects, Down's syndrome, and others connected with a pre-conception increase in folic acid intake (Thaler et al., 2006). Mfady et al. (2014) have recognized that the susceptibility to male infertility is due to MTHFR polymorphism. The enzyme MTHFR is found to be important for folic acid metabolism, which is crucial for reproductive function (Enciso et al., 2016). Limited genetic studies have been carried out on C677T polymorphism in infertile females of different ethnicities (Enciso et al., 2016; Murto et al., 2015; Settin et al., 2011). Some of the global studies have shown a negative association between C677T polymorphism and infertility (Altmäe et al., 2010; Murto et al., 2015; Settin et al., 2011; Szczepańska et al., 2011). The current study revealed a positive association, and this could be due to variability in the recruited subjects, i.e., the current study subjects were infertile Saudi women. For example, Murto et al. (2015) studied women undergoing infertility treatment, those that were non-pregnant; fertile healthy women with unexplained fertility were selected as subjects in the studies by Altmäe et al. (2010). Moreover, Szczepańska et al. (2011) opted to investigate women with endometriosis-associated infertility, and Settin et al. (2011) studied those with unexplained pregnancy loss. Several studies carried out in different ethnicities regarding the C677T polymorphism in male infertility found a significant association (Aarabi et al., 2015; Kim et al., 2015; Li et al., 2014; Ni et al., 2015). Our study also found a positive association, but in regards to female infertility. There are no meta-analysis studies on the MTHFR gene with female infertility. However, Gong et al. (2015), Nikzad et al., (2015), and Zhu et al. (2016) performed meta-analysis studies on male infertility and reported a negative association. Amalgamation of C677T polymorphism and meta-analysis studies were carried out in limited human diseases, such as male infertility, recurrent pregnancy loss, pre-eclampsia, ovarian cancer, colorectal cancer, prostate cancer, endometrial cancer, type 2 diabetes, diabetic nephropathy, and coronary artery disease.

Evaluations of homocysteine levels would have added strength to the current study results. However, they were not performed, and this is a limitation of the current study. Opting to investigate a single SNP and the lack of biochemical and hormonal tests performed in the KKUH are other limitations of this study. In conclusion, the T allele in the C677T polymorphism and genotype associates were found to be associated with infertility in Saudi women. Further research should be carried out with other SNPs in the MTHFR gene in conjugation with evaluations of homocysteine levels in different ethnicities to confirm these results.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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