ORIGINAL ARTICLE

The association of glutathione-S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) with idiopathic male infertility

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The effects of the GST genes (GSTM1, GSTT1 and GSTP1) on male factor infertility have not been well elucidated. A case–control study was performed to investigate the association between functionally important polymorphisms in GST genes and idiopathic male infertility. The study group consisted of 166 men suffering from idiopathic oligoasthenoteratozoospermia (OAT) and an equal number of age-matched healthy fertile male controls. Genomic DNA extracted from the peripheral blood, was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Multivariate regression analysis showed an increase in risk to infertility in the patients with null genotype of GSTM1 (OR: 2.18; 95% CI: 1.64–3.32; $P=0.001$) or GSTT1 (OR: 1.88; 95% CI: 1.12–2.52; $P=0.04$). Our data further showed that combination of deletion genotypes of GST (GSTM1 and GSTT1) pose an even higher risk of infertility. In addition, GSTP1 wild-type genotype in combination with GSTM1 null or GSTT1 null genotype increased probability for infertility (OR: 2.47, 95% CI: 1.46–4.17; and OR: 2.69, 95% CI: 1.39–5.32, respectively). Similarly a much higher risk for infertility was noted in the patients carrying a genotype combination of GSTM1 null, GSTT1 null and GSTP1 (Ile/Ile) (OR: 4.45; 95% CI: 1.59–12.24; $P=0.002$). The variant genotypes of GSTP1 (Ile/Val and Val/Val) resulted in a significant decreased risk to infertility in the cases (OR: 0.44; 95% CI: 0.29–0.78, $P=0.004$). These results support the hypothesis of increased risk of GSTM1 or GSTT1 null genotypes for developing infertility, but the nondeletion GSTM1 and GSTT1 genotypes emerged as protective factors.

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INTRODUCTION

Infertility is a multifactorial disorder affecting one in ten couples, and in 50% of infertile couples, the male factor due to deteriorated semen quality is a major cause.^{1,2} In about 30% of infertile men seeking help for their problem, the etiology and pathogenesis are not yet known and their condition is considered idiopathic.³ Several studies over the past two decades have suggested that the quality and quantity of human semen is declining worldwide in industrialized countries, raising concern about male fertility in the future.^{4,5} Although exact possible causes are not yet understood clearly, interactions between genetic and environmental factors have been suggested to be implicated with deteriorated semen quality.^{6,7} Damage to the genetic component of spermatozoa have a crucial role in a majority of cases where current investigations fail to detect the specific cause of male infertility.⁸ It has been shown that some genetic polymorphisms are associated with impaired spermatogenesis in infertile men with idiopathic oligoasthenoteratozoospermia.⁹ Indeed genetic causes are implicated for 60% of cases of idiopathic infertility.¹⁰ Xenobiotics comprise the majority of the environmental factors.¹¹

Excessive reactive oxygen species (ROS) have been considered to be one of the major factors leading to an infertile status¹² via oxidative DNA damages including base damage and DNA strand breaks. Glutathione-S-transferases (GST, EC 2.5.1.18) are a super-family of phase II antioxidant enzymes involved in the cellular detoxification of various physiological and xenobiotic substances.¹³ GSTs constitute the major defensive antioxidant system against oxidative stress by reducing ROS to less reactive metabolites.¹⁴ Polymorphisms in the GST gene may impair the ability of protection against oxidative stress and lead to the development of a wide range of diseases.¹⁵ Endogenous DNA fragmentation in human sperm (indicated by percentage of DNA fragmentation index) can be modulated by a polymorphism (deletion) in GSTM1.¹⁶

GSTs are currently categorized into eight separate classes, including alpha, mu, kappa, omega, pi, sigma, theta and zeta, that are encoded by GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT and GSTZ genes, respectively.^{17,18} Three of the GST genes, GSTP1, GSTM1 and GSTT1, coding for GSTs enzymes in the mu, theta and pi classes, respectively, have been found to have functional polymorphisms that are frequently present in the general population.¹⁹ The polymorphism in the GSTT1

and GSTM1 gene loci is caused by a gene deletion and results in the virtual absence of enzyme activity, especially in individuals with deletion in both genes (null genotypes).²⁰ Two genetic variants have been reported in GSTP1 gene, leading to isoleucine (Ile) to valine (Val) polymorphism at codon 105 and alanine to Val transition at codon 114, which leads to significant differences in catalytic activity.²¹

Inconsistent studies are available on the association of polymorphism in GSTM1, GSTT1 and GSTP1 genes and idiopathic male infertility. Aydemir *et al.*²² reported that in patients with idiopathic infertility, GSTM1 polymorphism might be an important source of variation in susceptibility of spermatozoa to oxidative damage. In a study conducted in Russian men, Polonikov *et al.*²³ reported that the nondeletion genotype of the GSTT1 gene is strongly associated with the increased risk of idiopathic male infertility and asthenozoospermia. Wu *et al.*²⁴ reported that there was no statistically significant association between GSTT1 null genotype and Chinese infertile patients with varicocele. In another study from China, GSTT1 null genotype was a predisposing risk factor for sporadic idiopathic azoospermia or oligospermia in northwestern China.²⁵ Another study in Turkish infertile men found that GSTM1, CYP1A1*2C genotypes are associated with infertility.¹¹ The available data are not conclusive and few studies appear to have addressed the issue of association of idiopathic male infertility with GST genes. The aim of this study was to identify potential associations between polymorphism in GST gene (GSTM1, GSTT1, GSTP1), and idiopathic male infertility in an urban Iranian population.

MATERIALS AND METHODS

Studied populations

A case-control study was conducted to examine the association of functionally important polymorphisms in GSTs (GSTM1, GSTT1 and GSTP1), with idiopathic male infertility. Infertile men (mean age 31.4 ± 5.2 years, range 25–40 years) with idiopathic oligoasthenoteratozoospermia ($n=166$) visiting our facility of Urology, included in the study. An equal number of fertile healthy men ($n=166$) (mean age 31.8 ± 5.6 years, range 25–42 years) of the same ethnic group and drawn from the same geographical and linguistic lineage, and similar socioeconomic status were also recruited in the study and served as the controls. All of the participants were recruited during the period from October 2006 to June 2009. The study participants were recruited consecutively after screening for eligibility and meeting the study inclusion and exclusion criteria. Idiopathic oligoasthenoteratozoospermia was diagnosed on the basis of at least two semen analyses with sperm parameters below the cutoff levels accepted by World Health Organization (WHO, 1999)²⁶ alongside with no known reason that could account for their infertility. The criteria for normal semen parameters were sperm density greater than $20 \times 10^6/\text{ml}$, sperm motility greater than 50%, normal morphology greater than 30% and/or semen volume greater than 2 ml. All men in the control group had fathered at least one child, and have had normal semen parameters according to WHO guidelines (WHO, 1999). Infertile subjects did not achieve a pregnancy after at least 2 years of unprotected intercourse with the same partner.

The protocol for research conformed to the guidelines of the Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects and was approved by the local Ethics Committee.

Informed consent was obtained from the study participants before the collection of blood samples and it was also ensured that the subject anonymity was preserved.

Evaluations

All of the participants were subjected to detailed history taking, physical examination, scrotal ultrasonography and laboratory investigations, including standard biochemistry and hematological laboratory tests, and measurement of serum testosterone, luteinizing hormone, follicle-stimulating hormone, prolactin, thyroid-stimulating hormone, and Inhibin B levels. The existence of

varicocele was rolled out by scrotal-colored Doppler ultrasound examination. Testicular volume was measured using scrotal ultrasound with a volume of less than 12 ml considered small. At least two semen analyses after 3 days of sexual abstinence were performed 4 weeks apart. Karyotype analysis and Y chromosome micro-deletion evaluation were performed with use of standard techniques. The mixed agglutination reaction test was also performed for the presence of antisperm antibodies.

The female partners were diagnosed fertile after thorough clinical and laboratory examinations included, physical examination; baseline body temperature; serum progesterone concentrations at days 21 and 25 of the cycle; karyotyping; serum chemistry, hematology and hormonal profile, including: follicle-stimulating hormone, luteinizing hormone, prolactin, thyroid-stimulating hormone, and thyroxine; immobilizing antibodies in serum; antiphospholipid and anticardiolipin antibodies; lupus anticoagulant; hysterosalpingography; and cervical cultures for Ureaplasma, Mycoplasma, Chlamydia and bacterial vaginosis as needed. Female partners with abnormal findings on hysterosalpingography underwent laparoscopy and/or hysteroscopy.

Inclusion/exclusion criteria

All the infertile subjects had a history of infertility for at least 2 years with no indication of hormonal, medical, or surgical causes for their infertility. Exclusion criteria included azoospermia; a history of epididymo-orchitis, prostatitis, genital trauma and testicular torsion; a testicular volume of less than 12 ml; inguinal or genital surgery; genital disease such as cryptorchidism, urinary tract infection or varicocele; abnormal hormonal profile; presence of any endocrinopathy; use of cytotoxic drugs, immunosuppressants, anticonvulsives, androgens or antiandrogens; Y chromosome microdeletions or karyotype abnormalities; leukocytospermia (more than 10^6 white blood cells per ml), or positive mixed agglutination reaction test; tobacco use; and drug, alcohol or substance abuse. Patients were also excluded from analysis if they had concomitant medical problems known to be associated with decreased fertility; hepatobiliary disease; significant renal insufficiency; a body mass index of 30 kg m^{-2} or greater; and occupational and environmental exposures to potential reproductive toxins.

Genotyping

Genomic DNA was extracted from the peripheral blood of subjects using the QIAmp blood kit (Qiagen, Chatsworth, CA, USA) and according to the method described by Liu *et al.*²⁷ For the GSTM1 gene, DNA samples were amplified with the primers: 5'-CTGCCCTACTTGATTGATGGG-3' and 5'-CTGGATTGTAGCAGATCATGC-3', using polymerase chain reaction (PCR). The paired primers for GSTT1 were: 5'-TTCCTTACTGGTCCTC ACATCTC-3' and 5'-TCACCGGATCATGGCCAGCA-3'. Amplification of human β -globin (110bp) with the primers 5'-ACACAAGTGTGTTTAC TAGC-3' and 5'-CAACTTCATCCACGTTTACC-3' was used as an internal control. PCR was performed in a final volume of 50 μl , consisting of DNA (0.5- μl), and a PCR buffer containing 200 ng of the following primers: dNTP (0.2 mM each), MgCl_2 (1.5 mM), KCL (50 mM), Tris-HCl (10 mM, pH 8.3), and 0.1% of bovine serum albumin. Reaction mixtures were heated at 94 °C for 5 min followed by 35 cycles of amplification as follows: a denaturing step at 94 °C for 90 s, an annealing step at 59 °C for 1 min, and final extension step at 72 °C for 1 min. Reaction products were analyzed by 2% agarose gel. The fragment lengths of the PCR products were 273-bp for individuals with one or more GSTM1 alleles, and 480-bp for individuals with one or more GSTT1 alleles.

The GSTP1 genotype was also determined by the method of Liu *et al.*²⁷ using a PCR-restriction fragment length polymorphism technique. An 177 bp fragment of the GSTP1 gene containing Ile to Val substitution in exon 5 (codon 105) was amplified using the primer pair 5'-ACCCAGGGCTCTATGGGAA-3' and 5'-TGAGGGCACAAGAAGCCCT-3'. The amplification cycles included an initial denaturing at 94 °C for 30 s, followed by an annealing step at 61 °C for 30 s, and a final extension step at 72 °C for 30 s. The PCR products were then digested with *Alw26I*. Homozygous Ile/Ile individuals had a single fragment of 177-bp, and homozygous Val/Val individuals had both 92- and 85-bp fragments. The presence of all three fragments corresponded to heterozygous Ile/Val individuals.

Statistical analysis

Results are expressed as mean $\pm$ s.d. Desired power of the present study was set at 80% as analyzed by power genetic association analysis software (<http://dceg.cancer.gov/bb/tools/pga>) at the level of significance $\alpha=0.05$. Infertile men and healthy fertile controls were compared for differences in demographic characteristics using the Student's *t*-test for continuous variables and the χ^2 -test

Table 1 Baseline demographics, serum hormones and semen parameters of study groups

Characteristics	Patients (n=166)	Controls (n=166)	P-value
Age (year)	31.4 $\pm$ 5.2	31.8 $\pm$ 5.6	NS
Duration of marriage	7.8 $\pm$ 2.7	7.5 $\pm$ 2.4	NS
Infertility duration (year)	4.8 $\pm$ 2.2	4.6 $\pm$ 2.5	NS
BMI (kg m ⁻²)	26.7 $\pm$ 2.6	26.7 $\pm$ 2.5	NS
Occupational status No. (%)			
Employed	145 (87.3)	146 (88.0)	NS
Unemployed	21 (12.7)	20 (12.0)	NS
Educational level			
None	0	0	—
Primary school	8 (4.8)	9 (5.4)	NS
High school	105 (63.3)	103 (62.0)	NS
Graduate	53 (31.9)	54 (32.5)	NS
Serum hormones			
Testosterone (nmol l ⁻¹)	15.4 $\pm$ 4.8	15.6 $\pm$ 4.6	NS
LH (IU l ⁻¹)	12.2 $\pm$ 2.4	12.4 $\pm$ 2.2	NS
FSH (IU l ⁻¹)	16.8 $\pm$ 4.2	16.4 $\pm$ 4.6	NS
PRL (pmol l ⁻¹)	363 $\pm$ 117	367 $\pm$ 122	NS
TSH (mIU ml ⁻¹)	1.7 $\pm$ 0.7	1.7 $\pm$ 0.7	NS
Inhibin B (pg ml ⁻¹)	189.6 $\pm$ 21.4	186.8 $\pm$ 22.6	NS
Semen parameters			
Ejaculate volume (ml)	2.7 $\pm$ 1.3	2.8 $\pm$ 1.4	NS
Total sperm/ejaculate ($\times 10^6$)	46.7 $\pm$ 11.8	197.2 $\pm$ 32.2	0.001
Sperm density ($\times 10^6$ /ml)	21.4 $\pm$ 4.2	70.8 $\pm$ 16.6	0.001
Motility (% motile)	24.8 $\pm$ 2.4	58.8 $\pm$ 4.6	0.001
Morphology (% normal)	17.6 $\pm$ 4.2	62.4 $\pm$ 4.2	0.001

Abbreviations: BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; NS, not significant; PRL, prolactin; TSH, thyroid stimulating hormone.

for discrete variables. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated to measure the risk associated with variant genotypes by unconditional logistic regression method. Tests for Hardy–Weinberg equilibrium were conducted by χ^2 -test. The χ^2 and Fisher's exact tests were performed to compare genotype frequencies between patients and controls. Correlation between two parameters was determined by Pearson's correlation coefficient (*r*). A *P*-value less than 0.05 was considered statistically significant. The data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 16.0; SPSS Inc., Chicago, IL, USA).

RESULTS

The distribution of selected demographic and clinical characteristics in infertile patients and controls are shown in Table 1. Mean age ($\pm$ s.d.) was 31.4 $\pm$ 5.2 years among cases, age range 25–40 years, and 31.8 $\pm$ 5.6 years among controls, age range 25–42 years (*P*=0.1). No significant difference in the demographic characteristics was observed between cases and controls. The genotype distributions in the controls and patients were in agreement with the Hardy–Weinberg equilibrium (*P*=0.512, *P*=0.668, respectively).

The distributions of GSTM1, GSTP1 and GSTT1 genotypes in cases and controls are shown in Table 2.

The frequency of null genotype of GSTM1 was significantly higher in patients (44%) when compared with the cases (27.7%) resulting in significant association between idiopathic male infertility and GSTM1 null genotype (adjusted OR: 2.18; 95% CI: 1.64–3.32; *P*=0.001). Similarly the GSTT1 null genotype was also found to be prevalent in the infertile men, which significantly increased the risk to infertility (OR: 1.88; 95% CI: 1.12–2.52; *P*=0.04). This increase in risk persisted even when the data was adjusted for age, duration of infertility, body mass index and occupational status, in multivariate logistic regression analysis (GSTM1, OR: 2.04; 95% CI: 1.51–3.12; GSTT1, OR: 1.42; 95% CI: 1.22–2.18, after adjusting). The frequency of the Ile/Ile genotype of GSTP1 was found to be higher in cases (61.5%) when compared with the controls (51.8%). As the frequency of the homozygous mutant (Val/Val) genotype of GSTP1 was very rare both in the cases (3%) or the controls (2.4%), the heterozygous and homozygous mutant genotypes were combined together and are referred to as variant genotypes (Ile/Val+Val/Val) of GSTP1. The frequency of the variant genotypes of GSTP1 (Ile/Val and Val/Val) was found to be higher in controls (48.2%) when compared with the cases (38.6%), and resulted in a significant decreased risk to infertility in the cases

Table 2 Distribution of GSTM1, GSTT1 and GSTP1 genotypes among infertile patients and controls

Genotype and allele frequency	Control (N=166)	Cases (N=166)	Crude OR (95% CI)	P-value	Adjusted OR (95% CI) ^a	P-value
GSTM1						
Present	120 (72.3%)	93 (56.0%)	1.0 (referent)			
Null	46 (27.7%)	73 (44.0%)	2.18 (1.64–3.32)	0.001	2.04 (1.51–3.12)	0.001
GSTT1						
Present	134 (80.7%)	119 (71.7%)	1.0 (referent)			
Null	32 (19.3%)	47 (28.3%)	1.88 (1.12–2.52)	0.04	1.42 (1.22–2.18)	0.04
GSTP1						
Ile/Ile	86 (51.8%)	102 (61.5%)	1.0 (referent)			
Ile/Val	76 (45.8%)	59 (35.5%)	0.71 (0.42–1.06)	0.08	0.42 (0.22–0.78)	0.001
Val/Val	4 (2.4%)	5 (3.0%)	1.22 (0.34–4.56)	1.0	1.74 (0.39–8.7)	0.47
Ile/Val or Val/Val	80 (48.2%)	64 (38.6%)	0.72 (0.44–1.05)	0.12	0.44 (0.29–0.78)	0.004

Abbreviations: CI, confidence interval; GST, glutathione-S-transferase; OR, odds ratio.

^aAdjusted OR: adjusted in multivariate logistic regression models including age, duration of infertility, body mass index, occupational status and GST genotypes.

(adjusted OR: 0.44; 95% CI: 0.29–0.78, $P=0.004$). When the genotypes of GSTM1 and GSTT1 were combined, three genetic combinations were practical; (1) carriers of functional gene (GSTM1 and GSTT1), (2) carriers of either GSTM1 or GSTT1 as functional genotype and, (3) carriers for both the null genotypes.

About 33% of the infertile patients and 30% of controls carried the combination of null genotype of both GSTM1 and GSTT1, which increased the risk to 1.44-fold (adjusted OR: 1.43; 95% CI: 0.88–2.24) though not significant in the cases carrying both the null genotypes ($P=0.15$). The risk associated with these variant genotypes was found to be further increased (approximately 3-fold) in cases carried both the null genotypes of GSTM1 and GSTT1 (adjusted OR: 2.92; 95% CI: 1.57–5.54; $P=0.0007$).

When combinations of GSTM1 and GSTP1 were studied, the frequency of GSTM1+GSTP1 (Ile/Val or Val/Val) in infertile patients was 40 of 166 (24.1%), which was lower than in control subjects (55 of 166, 33.1%). This difference did not reach statistical significance ($P=0.06$). Regarding the combination of GSTM1 null genotype with GSTP1 (Ile/Ile), distribution of the patients with null GSTM1 genotype+GSTP1 (Ile/Ile) polymorphisms was found to be higher (27.7%) as compared with the controls (13.3%), which resulted in significant increase in infertility risk (adjusted OR: 2.47; 95% CI: 1.46–4.17; $P=0.0006$) (Table 3).

Similarly, when genotype combinations of null GSTT1 genotype and GSTP1 (Ile/Ile) were studied, significantly increased risk (adjusted OR: 2.69; 95% CI: 1.39–5.32) was observed in infertile patients when compared with normal controls ($P=0.003$). However, the frequency of GSTM1 null+GSTP1 (Ile/Val or Val/Val) was nearly equal in cases and control groups (11.4, and 10.2%, respectively; $P=1.0$). The risk associated with all the three high-risk GST genotypes compared with no risk genotypes was also investigated (Table 4). When the genotypes of GSTM1, GSTT1 and GSTP1 were combined, eight possible combinations were observed. Individuals with combination of the null genotypes of GSTT1, GSTM1 and the wild type (GSTP1 gene) were at significantly higher risk to be infertile when compared with individuals having no high-risk GSTs genotypes (adjusted OR: 4.45; 95% CI: 1.59–12.24, $P=0.002$). The combination of other GSTs genotype combinations has not been found to be associated with significant infertility risk (Table 4).

Correlation with semen parameters

The null GSTM1 genotype was strongly negatively correlated with sperm density ($r=-0.68$, $P=0.004$), sperm motility ($r=-0.72$, $P=0.003$) and sperm morphology ($r=-0.78$, $P=0.001$). There was also weak but statistically significant inverse correlation between null GSTT1 genotype and sperm density ($r=-0.38$, $P=0.04$), sperm

Table 3 Distribution of double GST genotypes among infertile patients and controls

Double GST genotypes	Control (N=166) (%)	Infertile patients (N=166) (%)	Odds ratio (OR)	95% CI	P-value
<i>GSTM1 and GSTT1</i>					
Both present	102 (61.4%)	79 (47.6%)	1.0 (referent)		
Either null	50 (30.1%)	55 (33.1%)	1.44	0.88–2.24	0.15
Both null	14 (8.4%)	32 (19.3%)	2.92	1.57–5.54	0.0007
<i>GSTM1 and GSTP1</i>					
M1(+/+) and P1(Ile/Ile)	65 (39.1%)	54 (32.5%)	0.72	0.50–1.17	0.18
M1(+/+) and P1(Ile/Val or Val/Val)	55 (33.1%)	40 (24.1%)	0.66	0.20–0.98	0.06
M1(–/–) and P1(Ile/Ile)	22 (13.3%)	46 (27.7%)	2.47	1.46–4.17	0.0006
M1(–/–) and P1(Ile/Val or Val/Val)	24 (14.5%)	26 (15.7%)	1.14	0.66–1.93	0.78
<i>GSTT1 and GSTP1</i>					
T1(+/+) and P1(Ile/Ile)	77 (46.4%)	72 (43.4%)	0.92	0.65–1.43	0.81
T1(+/+) and P1(Ile/Val or Val/Val)	60 (36.2%)	47 (28.3%)	0.72	0.47–1.12	0.17
T1(–/–) and P1(Ile/Ile)	12 (7.2%)	28 (16.9%)	2.69	1.39–5.32	0.003
T1(–/–) and P1(Ile/Val or Val/Val)	17 (10.2%)	19 (11.4%)	0.97	0.52–1.86	1.00

Abbreviations: CI, confidence interval; GST, glutathione-S-transferase; OR, odds ratio.

Table 4 Distribution of triple GST genotypes among infertile patients and controls

Triple GST genotypes	Control (N=166) (%)	Infertile patients (N=166) (%)	Odds ratio (OR)	95% CI	P-value
M1 and T1(+/+) and P1(Ile/Ile)	57 (34.3%)	44 (26.5%)	0.72	0.47–1.20	0.16
M1 and T1(+/+) and P1(Ile/Val or Val/Val)	46 (27.7%)	32 (19.3%)	0.66	0.43–1.11	0.12
M1(–/–), T1(+/+) and P1(Ile/Ile)	17 (10.3%)	26 (15.7%)	1.64	0.82–2.95	0.11
M1(–/–), T1(+/+) and P1(Ile/Val or Val/Val)	14 (8.4%)	14 (8.4%)	1.01	0.46–2.10	1.05
M1(+/+), T1(–/–) and P1(Ile/Ile)	10 (6.0%)	13 (7.8%)	1.45	0.65–3.32	0.42
M1(+/+), T1(–/–) and P1(Ile/Val or Val/Val)	9 (5.4%)	7 (4.2%)	0.74	0.28–1.84	0.64
M1(–/–), T1(–/–) and P1(Ile/Ile)	4 (2.4%)	17 (10.2%)	4.45	1.59–12.24	0.002
M1(–/–), T1(–/–) and P1(Ile/Val or Val/Val)	9 (5.4%)	13 (7.8%)	1.12	0.50–2.42	0.81

Abbreviations: CI, confidence interval; GST, glutathione-S-transferase; OR, odds ratio.

motility ($r=-0.34$, $P=0.04$) and sperm morphology ($r=-0.42$, $P=0.03$). As well a strong negative correlation existed between GSTP1 (Ile/Ile) polymorphism and sperm density ($r=-0.56$, $P=0.02$), sperm motility ($r=-0.54$, $P=0.03$) and sperm morphology ($r=-0.62$, $P=0.01$).

DISCUSSION

This study is the first report on the association between GSTM1, GSTT1 and GSTP1 polymorphisms and susceptibility to infertility in the Iranian population. This study investigated GSTM1, GSTT1 and GSTP1 gene polymorphisms in Iranian subjects and showed that the frequency of GSTM1, GSTT1 and GSTP1 (Ile/Val) genotypes was significantly higher in a normal population than in infertile subjects, whereas the frequency of GSTM1 null, GSTT1 null and GSTP1 (Ile/Ile) in infertile patients was significantly higher as compared with normal controls. The frequency of GSTM1 null genotype ranges from 42 to 60% in Europeans, 42–54% in Asians and 16–36% in Africans; and for GSTT1 null genotype, the reported frequencies are 13–26% and 35–52% in Caucasians and Asians, respectively.²⁸ This study revealed that in normal controls the frequency of GSTM1 null genotype was 27.7%, the frequency of GSTT1 null genotype was 19.3% and the frequencies of GSTP1-Ile/Ile, -Ile/Val and -Val/Val genotypes were 51.8, 45.8 and 2.4%, respectively. The distribution of GSTP1 genotypes followed Hardy–Weinberg equilibrium. Such marked ethnic differences may have important clinical and health implications. Different ethnic groups may have different exposure and susceptibility to environmental factor-induced diseases such as infertility due to distinct GST gene polymorphism. Gene–environment interaction between GSTM1 and air pollution has been reported by Rubes *et al.*¹⁶ In the available literature, we could not find similar studies from Iranian population for comparison.

The increase in frequency of GSTM1 null, and GSTT1 null genotypes among infertile patients when compared with the controls has indicated that GSTM1 and GSTT1 polymorphisms are associated with an increase in infertility risk. Wu *et al.*²⁴ believe that GSTT1 null genotype predisposes to over oxidative damage to spermatocytes of infertile patients with varicocele. Similar increase in risk for infertility was also reported in Turkish¹¹ and Brazilian populations.¹⁰ Finotti *et al.*¹⁰ examined GSTM1 and GSTT1 polymorphism in 233 Brazilian men with idiopathic infertility. They found that individuals polymorphic for GSTM1 and GSTT1 genotypes are susceptible to decrease in sperm quality and infertility. However, one study in Russian men with idiopathic infertility reported an increase in risk for infertility with nondeletion genotype of the GSTT1 gene.²³ Also, there are two case–control studies in which the role of GSTT1, GSTM1 genotypes in male infertility has been addressed in patients with varicocele.^{29,30} Okubo *et al.*²⁹ concluded that the response rate to varicocelectomy is significantly better in patients with the GSTT1 genotype than the GSTT1-null genotype. In the second study, Chen *et al.*³⁰ showed that GST M1-genotype predisposes to increased oxidative damage to sperm of patients with varicocele. Such discrepancy could be explained by a different study population, different analytical methods, study sample sizes, effect of other genes and/or genotypes or a different study design. Glutathione transferases involve in the metabolic pathways of both methylation and transsulfuration.³¹ This could be another explanation for these discrepancies. Methylenetetrahydrofolate reductase is a gene involved in the process of DNA synthesis and methylation.³² Subjects carrying the homozygous variant methylenetetrahydrofolate reductase genotype and of a functional copy of GSTM1 has a significantly higher risk of infertility. Such a risk becomes non-significant when the GSTM1 deletion is also present.³¹ In our study, all of the

infertile patients had abnormal sperm parameters in all three domains (density, motility and morphology).

The importance of oxidative stress in spermatogenesis has recently received increasing attention. Human studies have generally supported the hypothesis that impaired spermatogenesis is associated with increased seminal plasma oxidative stress.^{32,33} Under pathological circumstances, ROS production exceeds the seminal plasma antioxidant capacity and causes increased oxidative stress.³⁴ GST a multi-gene family of phase II detoxification enzymes, acts as an antioxidant through inactivation of endogenous unsaturated epoxides, aldehydes, hydroperoxides and quinines, formed as secondary metabolites during the oxidative stress, thus having a key role in protecting cells against oxidative damage.³⁵

Taken together, our findings suggested that a genetic background (GST gene polymorphisms) may contribute to the development of infertility. The most likely explanation is based on the strong antioxidant activity of GST enzyme against the damaging effects of oxidative stress. GSTM1 and GSTT1 polymorphisms cause disparities on enzyme activities that can also result in oxidative stress potentiation³⁶ through changes on GSH metabolism. Individuals with GSTM1 null, and GSTT1 null have complete absence of activity of these genes.³⁷ The patients carrying the risk genotypes of GSTM1 null, and GSTT1 null simultaneously could be subjected to increased oxidative stress, which may account for the increase in risk to be infertile. Similarly several fold increased risk in the infertile patients with the combination of null genotypes of GSTT1, GSTM1 and the wild type (GSTP1 gene) could be attributed to the increased accumulation of ROS, because excessive ROS may not be detoxified due to deletion or variant genotype of GSTs.

Our study has several limitations. We did not include in our analysis the completed information about the familial history of infertility. Another limitation of the study is the low power (80%) of the study for gene–gene interactions and stratified analyses. Therefore the probability of chance findings in these analyses should be considered. As a result, it is worthwhile to design multi-center study to collect data on large numbers of cases for such analysis.

CONCLUSIONS

In conclusion, our findings suggest that GST gene polymorphism is associated with an increased probability of infertility. Risk to infertility was further found to increase in subjects carrying the combination of null or variant genotypes of GSTs. Also, several fold increased risk in the infertile cases carrying combinations of risk genotype of GSTs implies that interaction between the genes involved in detoxifying ROS may be important in detecting the susceptibility to infertility. Further studies should be done on a larger scale and different ethnic background for confirmation of our results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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