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SYSTEMATIC REVIEW PAPER

Association of glutathione S-transferase M1 null mutation with male infertility: A pooled data from Iranian studies

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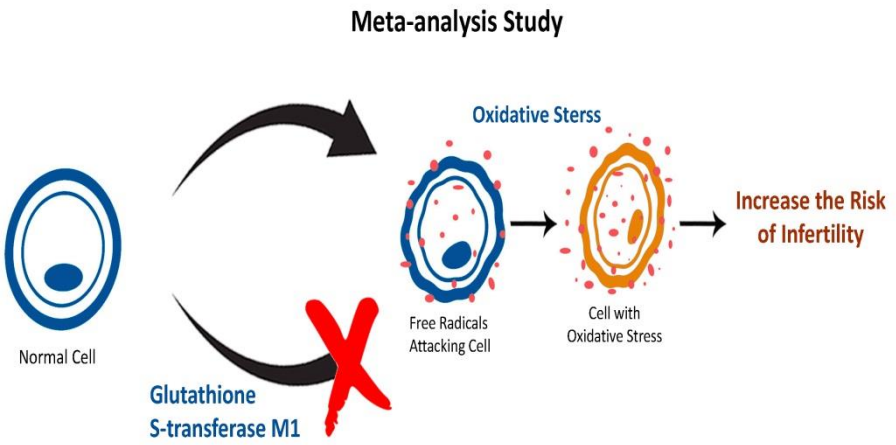
Highlights

- Male infertility is a main public health concern which can affect the couple's life.
- Male infertility could be affected by both genetic and environmental factors.
- Stress oxidative and the related genes involved in this process may influence male infertility.
- Null mutation in GSTM1 as an antioxidant gene could be a considerable risk factor for idiopathic cause of male infertility in Iranian men.

Graphical Abstract



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Abstract

Male infertility is a complex disease in which environmental factors and genetic background are known to be the main causes of pathogenesis. The imbalance in production and elimination of free radicals due to oxidative stress can alter the risk of infertility. There are a number of genes involved in the oxidative stress process, such as the glutathione S-transferase (GST) family of genes, which may play a critical role in combating oxidative stress in the male reproductive system. Any alteration in the structure and function of these genes may increase the risk of infertility. The aim of this study was to investigate the association between GSTM1 null genotype and male infertility in Iranian men using meta-analysis. In this study, valid databases such as Google Scholar, PubMed, SID, and Magiran were used to find appropriate studies. After electronic search and screening of studies, four appropriate studies were included in our meta-analysis. Data analysis revealed that GSTM1 null genotype correlated with increased risk of infertility in Iranian men. No publication bias was detected. Sensitivity analysis showed that exclusion of one study did not significantly affect the pooled odds ratios. These data suggest that the null genotype of GSTM1 may be a significant molecular risk factor for male infertility in the Iranian population. However, further studies with larger samples are needed to confirm the accuracy of these results.

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Introduction

Infertility is a widespread disorder of the reproductive system and affects approximately 12 to 15% of couples (1). The main cause is the low quality of semen in half of the cases. Infertility in men is a complex disease and the cause of this disease is unknown in about 50% of cases that known as idiopathic infertility and current studies show that genetics play role in 30% of idiopathic infertility (2). Recently, the quantity and quality of semen in men have declined on average, which in turn raises global concerns about male fertility (3). Germ cells are highly sensitive to stress in men. Therefore, the most important way to ensure fertility is to protect them from oxidative stress (4).

Glutathione S-transferase (GSTs) belongs to the large family of dimeric cytosolic enzymes that play a key role in the second phase of detoxification in humans against various xenobiotic and physiological substances. This enzyme plays this function by catalyzing the bindings of nucleophilic tripeptide glutathione in a wide range of electrophilic materials (5). Thus, GSTs are a major defense antioxidant system against oxidative stress and reduce the reactive oxygen species that produced by many toxic xenobiotics (6). In humans, GSTs contain many cytosolic, mitochondrial, and microsomal proteins. The cytosolic family is divided into 8 distinct subgroups: α (alpha), κ (kappa), μ (mu), ω (omega), σ (sigma), π (pi), θ (theta), and ζ (zeta) which are encoded by GSTA, GSTK, GSTM, GSTO, GSTS, GSTP, GSTT, and GSTZ genes, respectively (7). Each subgroup includes several genes and enzymes. Some functional polymorphisms or genetic variations have been reported mainly in GSTT1, GSTM1, and GSTP1 genes (8). The Gene locations and the number of exons in GSTM1, GSTT1, and GSTP1 are as follows: GSTT1 (22q11.23 with 6 Exons); GSTM1 (1p13.3 with 8 Exons); GSTP1 (11q13.2 with 7 Exons).

In India, Tirumala Vani et al. identified a link between GSTM1 null genotype and idiopathic male infertility (9). In the US, Olshan et al. revealed that the non-null genotype GSTT1 was correlated with decreased sperm count in semen (10). In China, Wu et al., found that the null genotype GSTT1 is a risk factor for idiopathic oligospermia or azoospermia (11). Tang et al., showed that the null genotype GSTM1 and GSTT1 in fertile men with varicocele predispose sperm to oxidative damage, while allelic changes in GSTP1 make no difference between the control and patient groups (12). High frequency of null genotypes GSTM1 and GSTT1 in different populations observed, also the lack of enzymatic activity in this genotype. The study of GSTM1 and GSTT1 gene polymorphisms in relation to infertility has received more attention. Some of previous studies have shown that polymorphisms of these genes as an important source, predispose spermatozoa to oxidative damage, and lead to the fragmentation of sperm DNA (12). Due to the relationship between infertility and ethnicity and race, as well as the lack of genetic studies in the population of infertile people in Iran, molecular studies can help to diagnose and high-risk people who are susceptible to infertility. This study aimed to investigate the relationship between null genotype of GSTM1 gene and idiopathic infertility in Iranian men with a meta-analysis approach.

Materials and Methods

Literature search

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) procedures were used forsystematic review and meta-analysis. The included papers were obtained from the electronic standard databases including PubMed, Scopus, Web of science, Google Scholar, Magiran, and Scientific Information Database (SID) databases. The following terms were used to electronic search: ("GSTM1" or "Glutathione s-transferase"), ("male infertility" or "idiopathic male infertility), ("Iran or Iranian"), and ("polymorphism" or "gene" or "variant" or "mutation"). No language limitations were executed on the electronic search. The literature search was updated on July 2021.

Study selection

The papers were selected based on the following inclusion criteria: a) case-control design, b) provided data on the distribution of GSTM1 null genotypes, Iranian studies, and c) contained an assessment of the GSTM1 null mutation and male infertility risk in Iran. The papers that did not provide suitable data on selection criteria

which were excluded from the meta-analysis. Also, case reports, letters, review articles, and the same cases were excluded from our study.

Data extraction

Data were independently extracted from all eligible papers by two of the authors based on the above-described criteria. Some features were collected from each paper: name of the first author's, year of publication, sample size, and genotype frequency for both case and control groups.

Statistical analysis

The Comprehensive Meta-Analysis software (version 2) was employed for statistical analyses. The pooled odds ratio (OR) with 95% confidence interval (CI), was applied to compute and evaluate the strength of the correlation between GSTM1 null mutation and male infertility risk in the Iranian population. Heterogeneity status was tested using the Q-test. The Random-effects model (dersimonian-Laird method) and fixed-effects model (Mantel-Haenszel method) were employed to estimate the pooled ORs. Sensitivity examination was also done by omitting each individual data to indicate the effect of the individual study on the pooled OR. Publication bias was measured via Egger's tests and Begg's funnel plots. A p-value <0.05 was considered significant for the association of GSTM1 null mutation and male infertility risk.

Results

Study characteristics

A total of 16 papers were obtained from the electronic search of the mentioned electronic databases. Seven papers were excluded because they were irrelevant or duplicated studies. Four others were also excluded because they were meta-analysis or review articles. One of the studies did not provide adequate information and then was excluded. Finally, four studies (13-16) were included in our meta-analysis. The flowchart of search strategy was illustrated in Figure 1. Three of these studies had a sample size of more than 300 individuals while one of them had a sample size of less than 300. In addition, one of the studies was in the Persian language while three of them were in English. These outcomes are summarized in Table 1.

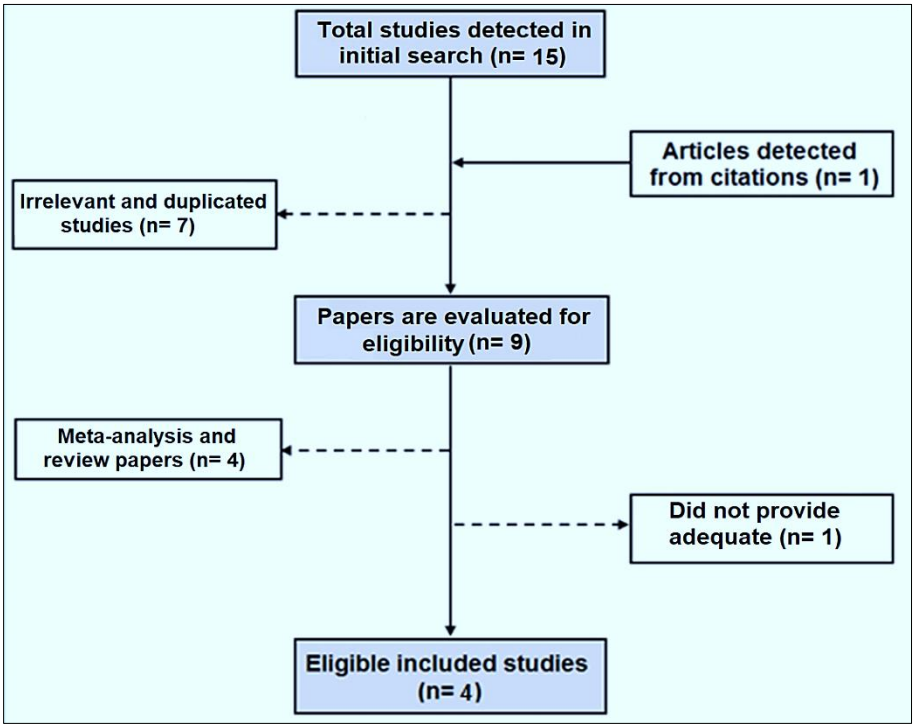

Figure 1. Flowchart of search strategy.

Table 1. Features of included studies in the meta-analysis.

First author	Year	Province	Genotyping method	Sample size	Case		Control	
					Present	Null	Present	Null
Safarinejad (13)	2010	Tehran	Multiplex-PCR	332	93	73	120	46
Salehi (14)	2012	Guilan	Multiplex-PCR	350	58	92	134	66
Fatahi (15)	2015	Hamadan	Multiplex-PCR	103	37	14	47	5
Barati (16)	2020	Isfahan	Multiplex-PCR	350	184	9	157	0

Correlation of the GSTM1 null mutation with susceptibility to male infertility

Four studies consisting of a total of 1135 subjects assessed the effect of the GSTM1 null mutation on the male infertility risk. The results of the genetic association analysis are summarized in Table 2. Data revealed that there is a significant correlation between GSTM1 null mutation and male infertility in Iranian population in fixed-effects (OR=2.812, 95%CI=2.079-3.803, P <0.001) model (Figure 2). Also, true heterogeneities were not found among studies (I^2=20.445, $H_{heterogeneity}$ =0.287 for random effect and 0.154 for fixed effect model). Whereas the I^2 was estimated 20.445, the association output was just evaluated for fixed effects model.

Table 2. Meta-analysis results including, odds ratio, heterogeneity, and publication bias.

Model	OR	95% CI	P-value	I^2	P $_{Heterogeneity}$	P $_{Egger}$
Fixed effect	2.812	2.079-3.803	< 0.001	-	0.154	0.337

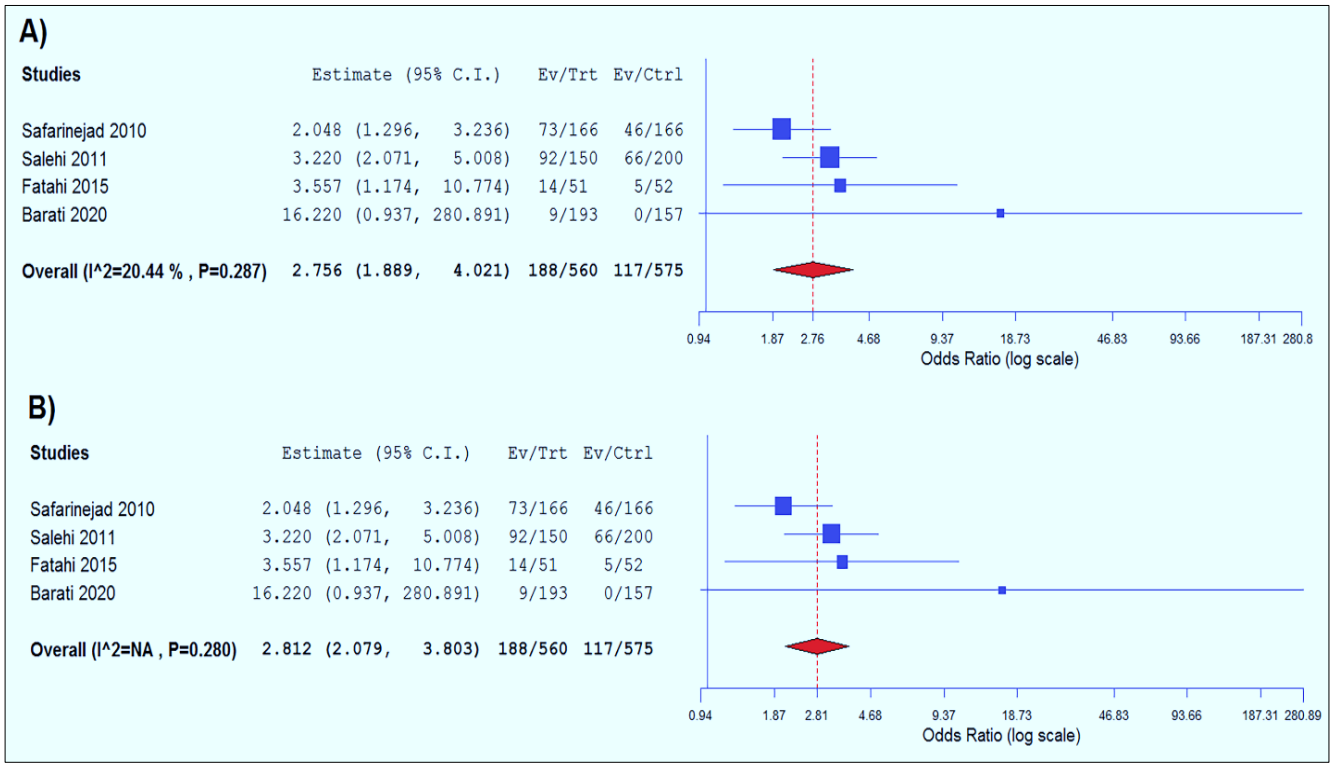

Figure 2. Forest plot. The data showed that there is significant association between GSTM1 null mutation and male infertility in Iranian population in both random (A) and fixed (B) effects models.

Sensitivity assessment and publication bias

Publication bias was evaluated for the GSTM1 null mutation by funnel plots and Egger's test. The form of the funnel plot did not show any indication of apparent asymmetry (Figure 3). Moreover, Egger's test proposed no evidence of publication bias (P=0.337) (Table 2). The sensitivity evaluation was done to compute the pooled ORs by neglecting one dataset each time. The outcomes showed that no individual paper affected the overall pooled ORs (data are not shown), representing that the results of this meta-analysis are robust and reliable.

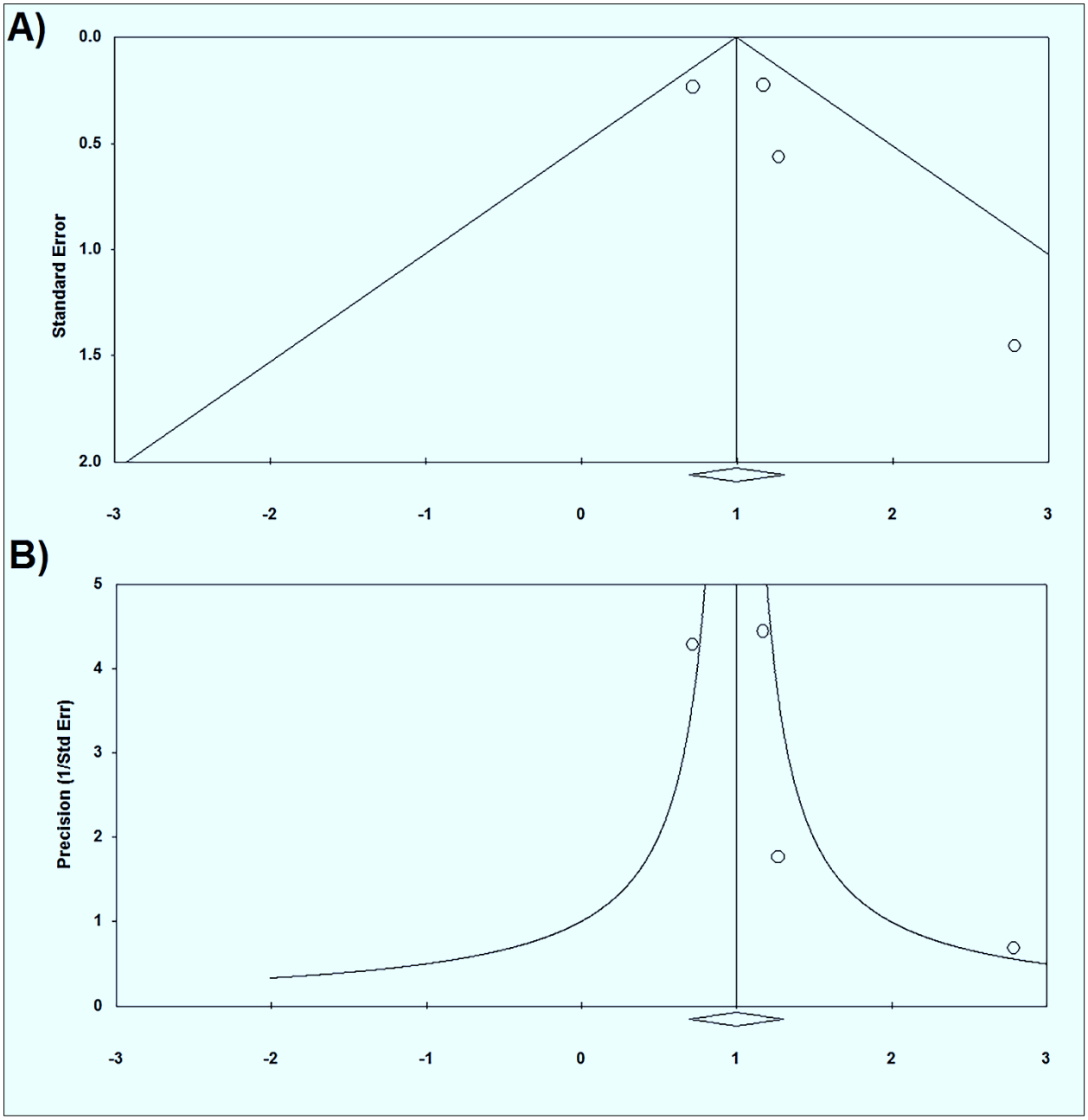

Figure 3. Funnel plot. Funnel plot of standard error by log odds ratio (A). Funnel plot of precision by log odds ratio (B).

Discussion

Defects in genes involved in sperm production can cause infertility in men (17). Environmental factors and genetic background affect the male fertility. In fact, both of these factors are related to sex hormone levels, genome stability, and quality of sperm. About 20% of the existing changes in the count of sperm, levels of the hormone, the morphology of sperm, and parameters of sperm chromatin are affected by genetic factors (18). The ROS is produced by environmental and endogenous factors and could cause significant damage to sperm DNA quality. Antioxidant enzymes play a critical role in counteracting oxidative stress during the spermatogenesis process and fertilization, so study of enzymes polymorphism that is involved in coping with oxidative stress is important (19). In this study, the relationship between null mutation of the GSTM1 antioxidant gene and male infertility in the Iranian population was investigated with a meta-analysis approach. Our study revealed a true relationship between GSTM1 null genotype and male infertility. Also, no publication bias was found. The sensitive analysis also showed that omitting a study had no significant effect on overall OR. So, these results can be reliable and robust.

Some antioxidant genes are involved in the spermatogenesis process include NFR2, GPX, SOD, CAT, GRX, PRX, NOS, and TRX. Enzymatic proteins encoded by the mentioned genes are extensively involved in antioxidant responses, GSH synthesis and reduction, and the thiol reduction-oxidation cycle during spermatogenesis. Also, the ARE motifs exit in the promoter of most of these genes that facilitate regulation of the NRF2 transcription pathway in oxidative stress (20). NRF2, a nuclear transcription factor, is an essential gene in antioxidant defenses that stimulate antioxidant enzyme via ARE element (21). In response to oxidative stress, NRF2 binds to AREs and regulates defense mechanisms against oxidative stress. Among the regulated genes by the NRF2-ARE signal pathway is CATs and SODs, which sustain sperm cells from damages of oxidative stress by hydrogen peroxide and superoxide. The SODs cause the degradation of superoxide radicals in hydrogen peroxide and molecular oxygen. In humans, three following families of SOD isoenzymes have been known: soluble SOD (SOD1), mitochondrial SOD (SOD2), and extracellular SOD (SOD3) (22). Among them, SOD2 isoenzyme is more expressed in the human sperms. The SOD gene has some key variants, and studies show that rs4880 single nucleotide polymorphism is strongly correlated with an increased male infertility risk (23). The seminal CAT converts H2O2 to oxygen and water, ultimately maintaining normal ROS levels and protecting sperm from ROS. This gene also has a single-nucleotide C-262T mutation in the promoter region, which is effectively associated with increased male infertility (24).

Glutathione s-transferases are another group of proteins that catalyze the binding of GSH to xenobiotic electrophilic substrates as ROS-causing agents. The GST family includes three major cytosolic, mitochondrial, and microsomal subtypes (25). In humans, GSTs include microsomal MGST1-MGST3, mitochondrial GSTK1, and cytosolic GSTA1-GSTA5, GSTO1-GSTO2, GSTP1, GSTZ1, GSTM1-GSTM5, and GSTT1-GSTT4 (13). Studies on different genotypes of GST show that deletion of GSTM1 and GSTT1 genes, and also point variations in the GSTP1 gene, are correlated with an increased risk of male infertility (26).

The key role of GSTs in cells is to detoxify the cell, for example, to protect macromolecules against the attack of reactive electrophiles, including environmental carcinogens, ROS, and chemotherapeutic agents (27). This role of detoxification is manifested by the fact that the expression profile of GST alters by xenobiotics (28). Sperm appears to use extracellular GSH and membrane GSTs to maintain mitochondrial status, sperm motility and viability, the capacity of egg binding, and fertility when exposed to H2O2 and/or lipid peroxidation products (29). GSTM interfered with acrosin releasing and binding to zona pellucida (ZP) (30, 31), and it was proposed that GSTM sperm attaching to zona pellucida is an important step in achieving successful fertilization. Since no covalent interaction was found between the plasma membrane and GSTM, it has been proposed that these proteins anchor via an integral membrane protein, which can amplify a signaling pathway that causes acrosome reaction and membrane fusion (32, 33). These show that any changes in the function and structure of GSTM may influence male infertility.

Conclusion

Male infertility is a complex disorder that is influenced by both genetic and environmental factors. Oxidative stress could increase the male infertility risk. Many genes are involved in relieving oxidative stress, and one of the most important is the glutathione S-transferase gene family. The null genotype of the GSTM1 gene could increase the risk of male infertility. Some scattered studies have been conducted in this field in the Iranian population, which have presented different results. Our meta-analysis of these Iranian studies showed that this polymorphism could be a considerable genetic risk factor for Iranian male infertility. But there were some limitations in our study such as low sample size. Of course, subsequent futures studies with larger sample sizes can help us to achieve more validate results.

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