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ORIGINAL ARTICLE

Male Infertility

Metabolic enzyme gene polymorphisms predict the effects of antioxidant treatment on idiopathic male infertility

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To explore the relationship between genetic polymorphisms of metabolic enzymes such as *CYP1A1*, *CYP2D6*, *GSTM1*, *GSTT1*, and *GSTP1* and idiopathic male infertility. By observing the efficacy of antioxidants in the treatment of idiopathic male infertility, the effect of metabolic enzyme gene polymorphisms on antioxidant therapy in patients with idiopathic male infertility was prospectively studied. This case–control study included 310 men with idiopathic infertility and 170 healthy controls. The cytochrome P450 1A1 (*CYP1A1*), cytochrome P450 2D6 (*CYP2D6*), glutathione S-transferase M1 (*GSTM1*), glutathione S-transferase T1 (*GSTT1*), and glutathione S-transferase P1 (*GSTP1*) genotypes in peripheral blood samples were analyzed by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP). The idiopathic male infertility group was treated with vitamin C, vitamin E, and coenzyme Q10 for 3 months and followed up for 6 months. *GSTM1*(–), *GSTT1*(–), and *GSTM1/T1*(–/–) in the idiopathic male infertility groups were more common than those in the control group. The sperm concentration, motility, viability, mitochondrial membrane potential (MMP), and seminal plasma total antioxidant capacity (T-AOC) level in patients with *GSTM1*(–), *GSTT1*(–), and *GSTM1/T1*(–/–) were lower than those in wild-type carriers, and the sperm DNA fragmentation index (DFI), 8-hydroxy-2'-deoxyguanosine (8-OH-dG), and malondialdehyde (MDA) and nitric oxide (NO) levels were higher. Therefore, oxidative damage may play an important role in the occurrence and development of idiopathic male infertility, but antioxidant therapy is not effective in male infertility patients with *GSTM1* and *GSTT1* gene deletions.

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INTRODUCTION

Infertility refers to situations in which couples who have cohabited for more than 1 year and have taken no contraceptive measures are unable to conceive.[1] Fifty percent of cases were partly or completely attributed to male factors.[2,3] To date, no clear cause of idiopathic male infertility, which accounts for 30% of cases of male infertility, has been found.[4] Idiopathic male infertility is not only a clinical medical problem but also a social problem that affects the family, social health services, and stability of the social environment.

Idiopathic male infertility is a multifactorial disease that often involves interactions between genetic factors and environmental factors.[5] The sensitivity of male germ cells to oxidative stress damage is the most important mechanism underlying male infertility.[6] Studies have shown that antioxidant treatments such as the use of vitamin C, vitamin E, and coenzyme Q10 (Co Q10) are effective in some patients and can significantly improve the concentration and morphology of spermatozoa.[6] However, these studies mainly focused on effectiveness but lacked a scientific basis for clinical research, such as which types of patients benefit, a large number of case–control studies, and evidence-based medicine research. Therefore, there is an urgent need for further clinical studies on the clinical efficacy of antioxidants and the selection of suitable male infertility patients.

The metabolic process of the body treats foreign compounds through phase I and phase II reactions. The phase I reaction is mainly related to the biotransfer of foreign compounds, while the phase II reaction is the binding reaction between the electrophilic intermediate produced by the metabolic activation of the phase I reaction and the compounds *in vivo*.

The cytochrome P450 (CYP) enzyme mainly participates in the phase I reaction of drug metabolism *in vivo* and participates in the metabolism of more than 90% of clinical drugs in the process of human drug metabolism.[7] Pharmacokinetic differences in drugs *in vivo* are often caused by individual differences in CYP activities involved in metabolism.

Cytochrome P450 1A1 (CYP1A1 [rs41279188]) and cytochrome P450 2D6 (CYP2D6 [rs16947]) are common oxidative metabolic

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enzymes in CYP that are involved in the pharmacokinetic changes of many commonly used drugs, including antioxidants.[8] At present, 100 alleles of *CYP2D6* have been reported, with approximately 80 mutation sites, and the mutation rate is less than 1% in the Asian population. The main metabolic genotype in the Asian population is *CYP2D6*10*, and the mutation rate of this allele is approximately 50%.[9] Therefore, the efficacy and side effects of drugs metabolized by *CYP2D6* are different among different races.

Glutathione S-transferase (GST) is a related isoenzyme family that catalyzes the binding of reduced glutathione to a variety of electrophilic substrates, including reactive oxygen species (ROS) and carcinogenic compounds and their metabolites. The glutathione S-transferase M1 (*GSTM1* [rs10857795]) and glutathione S-transferase T1 (*GSTT1* [rs140195]) genes are polymorphic, and genetic deletion of the genes leads to the phenotypic loss of enzyme activity. Glutathione S-transferase P1 (*GSTP1* [rs1695]) is an important metabolic enzyme in the human body that is mainly involved in detoxification reactions. Our previous studies have shown that the *GSTT1* zero genotype is a risk factor for sporadic idiopathic azoospermia or oligozoospermia in Northwest China.[10] This polymorphism may reduce the activity and thermal stability of this enzyme.

Previous studies have revealed a potentially stable correlation between metabolic enzyme gene polymorphisms and male infertility[11] that also affects the efficacy of drug treatment for idiopathic oligozoospermia. There may be a stable correlation between metabolic enzyme gene polymorphisms and oxidative stress levels.[12]

The purpose of this study was to prospectively examine the effect of metabolic enzyme gene polymorphisms on antioxidant treatment in patients with idiopathic male infertility to provide a scientific basis for the effectiveness, case selection, and application of clinical antioxidant therapy.

PARTICIPANTS AND METHODS

Selection of research subjects

This study included was performed in Guiyang, China, 310 male patients with idiopathic infertility treated in the Department of Urology of the Affiliated Hospital of Guizhou Medical University (Guiyang, China) from January 2018 to June 2020. One hundred and seventy healthy control members were from the Physical Examination Center of the Affiliated Hospital of Guizhou Medical University. All participants provided written informed consent. This study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University, and all methods were performed in accordance with the relevant guidelines and regulations (reference number: 2013023). The trial has been registered with the Chinese Clinical Trial Registry (registration number: ChiCTR-IPR-14005580).

The inclusion criteria are two or more times semen analysis according to the 2010 World Health Organization (WHO) criteria for classifying sperm.[13] The patients with idiopathic male infertility were divided into the following three categories: (1) idiopathic oligozoospermia: semen volume >1.5 ml, sperm density $<15 \times 10^6$ ml^{-1}, total sperm motility >40% or sperm forward motility >32%, and sperm viability >58%; (2) idiopathic asthenospermia: semen volume >1.5 ml, sperm density $>15 \times 10^6$ ml^{-1}, total sperm motility <40% or sperm forward motility <32%, and sperm viability <58%; (3) idiopathic oligoasthenospermia: semen volume >1.5 ml, sperm density $<15 \times 10^6$ ml^{-1}, total sperm motility <40% or sperm forward motility <32%, and sperm viability <58%.

The testicular volume of participants with idiopathic male infertility was in the normal range of testicular volume (15–25 ml) of Chinese individuals, and the peripheral blood chromosomes were 46,XY. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and testosterone (T) in peripheral serum were in the normal range. His spouse did not conceive without taking any contraceptive measures after more than 1 year of cohabitation. The antisperm antibody test was negative.

The inclusion criteria for healthy men were as follows: reproductive history in the past year and no abnormality in the current sex hormone levels, semen analysis, or other indices.

Exclusion criteria

Diagnostic exclusion criteria for patients with idiopathic male infertility included abnormal sexual life and ejaculatory disorders such as nonejaculation or retrograde ejaculation, chromosomal abnormalities, congenital malformations, cryptorchidism, testicular dysplasia, testicular atrophy, injury, varicocele, hypogonadism, leukospermia, vas deferens obstruction, sex chromosomal aneuploidy/mosaicism, Yq microdeletion, and reproductive system infection (including male accessory gland infection). Those taking anti-epilepsy, antitumor, antirheumatic, and anti-rheumatoid drugs that hinder spermatogenesis and sperm motility, as well as those who had used antioxidants in the past, were excluded. Patients with serious primary diseases, metabolic diseases, and mental disorders (such as cardiovascular, liver, kidney, and hematopoietic system diseases), smoking (more than 10 cigarettes a day for more than half a year), and alcoholism (50 ml pure alcohol daily for more than half a year) were excluded, as well as those who did not agree to participate in the clinical study and did not sign a clinical research agreement.

The exclusion criteria for healthy men were the same as those for patients with idiopathic male infertility, those with abnormal semen analysis and physical examination, and subjects or family members who refused to provide consent to participate in the study.

Selection, dosage, and course of antioxidant treatment

Three different antioxidants with different mechanisms of action, including vitamin C, vitamin E, and Co Q10, were assessed in this study. Studies have shown that the hydrophilicity and lipophilicity of vitamins C and E can be synergistic, and Co Q10 can enhance the effect of vitamins C and E to protect against peroxide attack on spermatozoa.[14,15] The treatment was administered as follows: the idiopathic male infertility group was treated with vitamin C (0.1 g three times a day), vitamin E (0.1 g three times a day), and Co Q10 (10 mg three times a day) for 3 months and followed up for 6 months.

Collection of research specimens

For the collection and treatment of peripheral blood samples, 6 ml of peripheral venous blood was collected by peripheral venipuncture. A total of 3 ml of the blood samples was mixed with ethylenediaminetetraacetic acid (EDTA) anticoagulant and cryopreserved at −80°C, 3 ml was transferred to a common rapid coagulation tube after centrifugation (TGL-16GB, Anting Medical Equipment Co., Ltd., Shanghai, China) at 1000*g* for 10 min to separate plasma, and the blood cells were also cryopreserved at −80°C.

For the collection and treatment of semen samples, all subjects were required to practice abstinence for 3–5 days. The entire semen sample was collected from a single ejaculation by masturbation, semen collector, or *in vitro* ejaculation. The specific collection time was marked, and the sample was placed in an incubator (HH.W21-Cr420, Shantou Medical Equipment Co., Ltd., Shantou, China) at 37°C. After detection, the remaining sperm and seminal plasma were frozen at

−80°C. Semen samples were collected at the time of basal evaluation, 3-month evaluation, and 6-month evaluation.

In the extraction of total genome of peripheral blood, frozen peripheral blood samples were thawed in a water bath at 56°C. The AxyPrep™ human blood genome DNA extraction kit (Axygen Biosciences, Union City, CA, USA) was used to extract total DNA from peripheral blood cells. Based on the method described by the Axygen Biosciences Company, the total genomic DNA of peripheral blood leukocytes was extracted from individuals in the idiopathic male infertility group and the normal control group.

The *CYP1A1*, *CYP2D6*, *GSTM1*, *GSTT1*, and *GSTP1* genes involved in this study were detected by polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (PCR-RFLP). After amplifying the gene by PCR, the PCR product was digested by *Bsm*AI restriction endonuclease, and species-specific RFLP was obtained. The primers were designed by Primer Premier 5.0 software (PREMIER Biosoft International, San Francisco, CA, USA). The primer sequence of the metabolic enzyme gene, the length of the PCR product, annealing temperature, and restriction site are shown in **Table 1**.

Analysis of semen and sperm quality

The semen parameters obtained by the computer-aided semen analysis system (CASA; WLJY-9000; Weili New Century, Beijing, China) included general sperm parameters such as semen volume; sperm density, motility, and viability; average curvilinear velocity; average linear velocity; and average path speed.

The modified Pap staining method recommended by the WHO manual[13] was used for sperm morphology analysis. This method can stain sperm, other cells, sperm head acrosome and postacrosomal areas, cytoplasmic droplets, middle segment, and tail. Bismarck brown in the dye solution is a basic dye, while eosin, bright green, and orange are acid dyes, which can combine with proteins with opposite charges in cells and stain them in different colors. Therefore, various cellular components can be distinguished.

Sperm chromatin structure analysis (SCSA) measures the susceptibility of sperm DNA to acid-induced denaturation *in situ* using the metachromatic properties of acridine orange (AO). By quantifying this metachromatic shift of AO from green to red after acid treatment using flow cytometry (CytoFLEX, Beckman Coulter, Indianapolis, IN, USA), the extent of DNA denaturation can be determined. The parameter obtained by SCSA most commonly referred to in the literature is the DNA fragmentation index (DFI), which is a measure of DNA denaturation.[16]

The energy required for sperm movement is mainly provided by mitochondria, and the analysis of mitochondrial function can be used as a direct indicator of the availability and fertilization ability of sperm. The energy supply ability of sperm mitochondria can be easily evaluated by intracellular mitochondrial membrane potential (MMP). The sperm MMP was detected by flow cytometry after staining with 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolyl-carbocyanine iodide (JC-1, molecular probes).[17]

8-hydroxy-2'-deoxyguanosine (8-OH-dG) is a specific product of oxidative DNA damage and a recognized biomarker of oxidative damage of DNA caused by endogenous and exogenous factors.[18] Total sperm DNA was extracted by the Chelex method (Chelex-100, Sigma-Aldrich, Shanghai, China) and then stored at 4°C for follow-up experiments. Sperm total DNA was quantified by an ultramicro microporous plate spectrophotometer (BioTek Epoch Company, Winooski, VT, USA). Then, sperm total DNA samples were used to detect the level of 8-OH-dG.

The contents of malondialdehyde (MDA), total antioxidant capability (T-AOC), and nitric oxide (NO) in plasma were assayed using colorimetric methods with a spectrophotometer (Biomate 5, Thermo Electron Corporation, Rochester, NY, USA). The assays were conducted according to the manufacturer's instructions using assay kits (Jiancheng Institute of Bioengineering, Nanjing, China).

The time points of detection were at the time of the baseline evaluation, the 3-month evaluation, and the 6-month evaluation.

Statistical analyses

All data are expressed as the mean±standard deviation (s.d.), and the Chi-square test was used to assess the distribution of gene polymorphisms in the population. In the analysis of the efficacy of antioxidant therapy in people with different gene polymorphisms, independent-samples *t*-tests and paired-samples *t*-tests were used to compare the differences of different gene subtypes in the same antioxidant therapy. All analyses were performed using SPSS software version 20.0 (SPSS Inc., IBM Company Headquarters, Chicago, IL, USA).

RESULTS

Association between metabolic enzyme gene polymorphisms and idiopathic male infertility

The study found that the frequency of the *GSTM1*(−) genotype in the idiopathic male infertility group was 59.4%, and in the control group, the frequency was 35.9% (odds ratio [OR] = 2.978; 95% confidence interval [CI]: 1.773–5.002; $P < 0.001$). The frequency of the *GSTT1*(−) genotype in the idiopathic male infertility group was 61.9%, and in the control group, the frequency was 45.9% (OR = 1.966; 95% CI: 1.179–3.279; $P = 0.009$). The frequency of the *GSTM1/T1*(−/−) genotype was 37.7% in the case group and 12.4% in the control group (OR = 5.681; 95% CI: 3.105–10.394; $P < 0.001$). The gene frequencies of *GSTM1* and *GSTT1* in the idiopathic male infertility

Table 1: Upstream and downstream primers and product length of each metabolic enzyme gene, annealing temperature, and restriction site

Gene	*Primer 5'–3'*	*Annealing temperature (°C)*	*PCR product (bp)*	*Restriction site*
*CYP1A1*2A* (rs41279188)	F: CAG TGA AGA GGT GTA GCC GCT R: TAG GGA GTC TTG TCT CAT GCC T	60	340	T6235C
*CYP2D6*10* (rs16947)	F: TCA ACA CAG CAG GTT CA R: CTG TGG TTT CAC CCA CC	59	433	C188T
GSTM1 (rs10857795)	F: GAA CTC CCT GAA AAG CTA AAG C R: GTT GGG CTC AAA TAT ACG GTG G	60	219	-
GSTT1 (rs140495)	F: TTC CTT ACT GGT CCT CAC ATC TC R: TCA CCG GAT CAT GGC CAG CA	63	480	-
GSTP1 (rs1695)	F: ACC CCA GGG CTC TAT GGG AA R: TGA GGG CAC AAG AAG CCC CT	58	177	A313G
β-actin	F: ACT CCC CAT CCC AAG ACC R: CCT TAA TGT CAC GCA CGA T	61	400	-

PCR: polymerase chain reaction; *CYP1A1*2A*: cytochrome P450 1A1*2A; *CYP2D6*10*: cytochrome P450 2D6*10; *GSTM1*: glutathione S-transferase M1; *GSTT1*: glutathione S-transferase T1; *GSTP1*: glutathione S-transferase P1

group and the subtype groups were significantly higher than those in the control group. However, there was no significant difference in the distribution of *GSTP1* (OR = 1.006; 95% CI: 0.615–1.645; $P = 0.982$), *CYP1A1* (OR = 0.942; 95% CI: 0.641–1.384; $P = 0.762$), or *CYP2D6* (OR = 0.956; 95% CI: 0.594–1.537; $P = 0.851$) gene polymorphisms among the idiopathic male infertility subtype group and the control group (**Supplementary Table 1**).

Therapeutic effect of antioxidants on idiopathic male infertility

At the time of basal evaluation, it was found that there were significant differences in sperm concentration, sperm motility, sperm viability, sperm MMP depolarizability, seminal plasma T-AOC, sperm DFI, 8-OH-dG, seminal plasma MDA, and NO levels between the case group and the control group. The sperm concentration, sperm motility, sperm viability, sperm MMP depolarizability, and seminal plasma T-AOC were significantly lower than those in the control group ($P < 0.001$), while the DFI, 8-OH-dG, seminal plasma MDA, and NO levels were significantly higher than those in the control group (all $P < 0.001$).

During the treatment stage, sperm concentration, sperm motility, sperm viability, MMP, and seminal plasma T-AOC increased at the 3-month evaluation and 6-month evaluation, while DFI, 8-OH-dG, MDA, and NO levels in seminal plasma decreased significantly (all $P < 0.001$).

Based on the analysis of the data after 6 months of antioxidant treatment, paired-samples *t*-tests showed that there were significant differences in the therapeutic effects of different genotypes ($P < 0.01$). The indices of patients with *GSTM1*(−), *GSTT1*(−), and *GSTM1/T1*(−/−) genotypes exhibited a similar overall distribution, but the values of the variables were lower than the average levels, showing that the therapeutic effect was not satisfactory. Sperm concentration, sperm motility, sperm viability, MMP, and T-AOC levels in the *GSTM1*(−), *GSTT1*(−), and *GSTM1/T1*(−/−) genotypic subgroups were lower than those in the *GSTM1*(+), *GSTT1*(+), and *GSTM1/T1*(+/+) subgroups and control groups (all $P < 0.01$). The levels of DFI, 8-OH-dG, seminal plasma MDA, and NO were higher in patients with the *GSTM1*(+), *GSTT1*(+), and *GSTM1/T1* genotypes (all $P < 0.001$), as shown in **Figure 1** and **Supplementary Table 2**.

DISCUSSION

Male infertility is a multifactorial disease, and the interaction between genetic factors and environmental factors plays an important role in its pathogenesis.[19] Oxidative stress (OS) refers to the imbalance between oxidation and antioxidation in the body, which leads to oxidation and the production of numerous oxidation intermediates, which are called ROS.[20] ROS lead to sperm DNA damage and even sperm DNA breakage, which is considered to be one of the most important factors leading to infertility.[20–22] Moreover, the high sensitivity of male germ cells to OS damage is the most important mechanism leading to male infertility.[6,20] Therefore, among numerous factors of male infertility, OS is considered to be a critical factor.[23]

The metabolic process of foreign compounds includes phase I and phase II reactions. The phase I reaction is mainly the biotransformation of foreign compounds, while the phase II reaction is the binding reaction of electronic intermediates produced by metabolic activation of the phase I reaction with compounds *in vivo*. Phase I metabolic enzymes are mainly CYP enzymes encoded by the *CYP* gene family, which is the main enzyme system that catalyzes the biotransformation of foreign compounds in the body.[24] To date, seven genetic polymorphisms of *CYP* genes have been identified: *CYP1A1*, cytochrome P450 2A6 (*CYP2A6*), cytochrome P450 2C9 (*CYP2C9*), cytochrome P450 2C18 (*CYP2C18*), cytochrome P450 2C19 (*CYP2C19)*, cytochrome P450 2D6 (*CYP2D6*), and cytochrome P450 2El (*CYP2El*).[25] Among the phase II metabolic enzymes, GST is a dimer protein that belongs to the phase II detoxification enzyme system.[26] GST participates in all physiological stages of cell detoxification of exogenous chemicals and synthetic organic substances against damage caused by OS and free electrons.[27] Detoxification is achieved by catalyzing the binding of various exogenous chemicals, electrophilic substances, carcinogens, poisons (or their metabolic intermediates), and oxidizing substances with reduced glutathione (GSH).[28] At present, the *GSTM1*, *GSTT1*, and *GSTP1* genes are known to have functional gene polymorphisms in the general population.[29]

Salehi *et al*.[30] analyzed the genetic polymorphisms of the *GSTT1*, *GSTM1*, and *CYP1A1*2A* genes in 150 patients with male infertility and 200 healthy controls in Iran. The results showed that the frequencies of *GSTM1* and *GSTT1* gene deletions in patients with idiopathic male infertility were 61.0% and 33.0%, respectively, while those in healthy controls were 33.0% and 17.0%, respectively. The rate of deletion of the *GSTT1* and *GSTM1* genes in the male infertility group was significantly higher than that in the normal group ($P < 0.0001$). The genotypes of *CYP1A1* genes *TT*, *TC*, and *CC* were 42.5%, 45.5%, and 12.0%, respectively, in the healthy control group, and 38.7%, 48.0%, and 13.3%, respectively, in the idiopathic male infertility group. There was no difference between the male infertility group and the normal control group ($P > 0.05$).[30] Aydos *et al*.[11] found that individuals with cytochrome P450 1A1 Val/Val (*CYP1A1 Val/Val*), cytochrome P450 1A1 Ile/Val (*CYP1A1 Ile/Val*), and *GSTM1* gene deletions were 6.9 times more likely than *CYP1A1 Ile/Ile* and *GSTM1* gene wild-type carriers to have male infertility (OR = 6.90, 95% CI: 2.29–19.3). Moreover, our previous study suggested that the *GSTM1* and *GSTT1* null genotypes may predispose sperm to increased oxidative damage in infertile men with varicoceles.[10] Barati *et al*.[31] analyzed the *GSTM1* and *GSTT1* null genotypes that could be considered genetic risk factors for male infertility, interfering with some oxidative stress markers in infertile men. All these studies proved that *GST* was a direct or indirect risk factor for infertility. In this study, we also found that the proportion of *GSTM1*(−), *GSTT1*(−), and *GSTM1/T1*(−/−) in the case group was significantly higher than that in the control group. In addition, we detected and analyzed the polymorphisms of the *GSTM1* and *GSTT1* genes and the OS level in all patients with idiopathic male infertility. The levels of MDA and NO in the seminal plasma of patients with *GSTM1*, *GSTT1*, and *GSTM1/T1* gene deletions were higher than those of wild-type patients, while the level of T-AOC was lower than that of wild-type patients. Therefore, *GSTM1* and *GSTT1* gene deletions may increase the body's susceptibility to OS body and the risk of infertility.

There are also protective substances called antioxidants that eliminate OS in the body, and an imbalance between antioxidants and ROS leads to molecular and cellular damage, which affects the whole body.[14] Studies have shown that antioxidants have extensive effects on the male system,[32,33] such as reducing sperm damage by ROS, scavenging ROS produced by leukocytes,[34] preventing the formation of sperm DNA fragments,[35] improving the semen quality of smokers,[36,37] combating the effects of obesity and aging on sperm quality,[38] reducing sperm freezing damage,[39,40] preventing premature sperm maturation, stimulating sperm, and improving the success rate of assisted reproductive technology (ART).[41] The commonly used clinical antioxidants are as follows. (1) Vitamin C is an important chain antioxidant that accounts for approximately 65% of the intracellular and total antioxidant capacity in seminal plasma. It can neutralize hydroxyl groups, superoxides, and hydrogen peroxide free radicals, prevent sperm adhesion, prevent lipid

Figure 1: Sex hormone levels, sperm parameters, oxidation levels, MMP, and DFI distribution before and after treatment in the idiopathic male infertility group. *CYP1A1*: cytochrome P450 1A1; *CYP2D6*10*: cytochrome P450 2D6*10; *GSTM1*: glutathione S-transferase M1; *GSTT1*: glutathione S-transferase T1; *GSTM1/T1*: glutathione S-transferase M1/T1; *GSTP1*: glutathione S-transferase P1; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; MDA: malondialdehyde; NO: nitric oxide; T-AOC: total antioxidant capability; 8-OH-dG: 8-hydroxy-2'-deoxyguanosine; MMP: mitochondrial membrane potential; DFI: DNA fragmentation index.

peroxidation, promote the recycling of vitamin E, and protect sperm DNA from damage by free radicals such as H_2O_2.[42] (2) Vitamin E is the most important chain antioxidant of the sperm membrane and has a dose-dependent effect. It can scavenge three types of free radicals: peroxide, H_2O_2, and hydroxide free radicals.[39,43] (3) Co Q10 is a type of nonenzymatic antioxidant that reduces the concentration of lipoprotein and protects the body from peroxidation.[44,45] Our previous study found that tamoxifen combined with Co Q10 can significantly improve sperm concentration, motility, and morphology in patients with idiopathic oligozoospermia.[46] As common antioxidants in the clinical treatment of male infertility, vitamin C, vitamin E, and Co Q10 have been proven to be effective, cheap, and easy to obtain, which makes this study more useful for clinical treatment. Therefore, we used the above drugs for antioxidant treatment.

Analysis of the patient indices of each subtype group at baseline, the 3-month evaluation, and the 6-month evaluation showed that the semen quality (sperm concentration, sperm motility, MMP, DFI, and 8-OH-dG), the recovery of OS (seminal plasma MDA, NO, and T-AOC), and the pregnancy rate of individuals with *GSTM1*, *GSTT1*, and *GSTM1/T1* gene deletion types were lower than those of *GSTM1*, *GSTT1*, and *GSTM1/T1* gene wild-type carriers. The antioxidant therapy was not efficacious. Therefore, we believe that the deletion of the *GSTM1* and *GSTT1* genes may be a risk factor for the aggravation of OS damage in patients with idiopathic male infertility and may inhibit the efficacy of antioxidant therapy.

Genetic polymorphisms of the *GSTM1* and *GSTT1* genes are a risk factor for idiopathic male infertility, while oxidative stress also plays an important role in the occurrence and development of idiopathic male infertility. *GSTM1* and *GSTT1* gene deletions increase damage due to oxidative stress, resulting in poor efficacy of antioxidant therapy. However, the single-center design and small sample size of this study were limitations, and large-sample and multicenter studies are required for further clarification.

AUTHOR CONTRIBUTIONS

KFT conceived of and performed the experiments and secured funding. HYZ and YM wrote the manuscript. HYZ, YM, PC, DDL, QY, KHC, and JH performed experiments. JPX and FS provided expertise and feedback. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Cytochrome P450 1A1, cytochrome P450 2D6, glutathione S-transferase M1, glutathione S-transferase T1, and glutathione S-transferase M1/T1 gene frequency distribution

Group	*Control (%)*	*Case (%)*	*Oligozoospermia (%)*	*Asthenospermia (%)*	*Oligoasthenospermia (%)*	χ^2	*P*	*OR (95% CI)*
N	170	310	90	96	124	-	-	-
CYP1A1m1m1	66 (38.8)	116 (37.4)	31 (34.4)	33 (34.4)	52 (41.9)	0.092	0.762	0.942 (0.641–1.384)
m1m2	78 (45.9)	144 (46.4)	42 (46.7)	46 (47.9)	56 (45.2)	0.482	0.487	0.828 (0.484–1.411)
m2m2	26 (15.3)	50 (16.1)	17 (18.9)	17 (17.7)	16 (12.9)	0.520	0.471	0.825 (0.490–1.391)
						0.289	0.591	1.138 (0.710–1.824)
*CYP2D6*10 C/C*	33 (19.4)	58 (18.7)	16 (17.8)	21 (21.9)	21 (16.9)	0.035	0.851	0.956 (0.594–1.537)
C/T	96 (56.5)	177 (57.1)	52 (57.8)	50 (52.1)	75 (60.5)	0.103	0.749	0.898 (0.464–1.738)
T/T	41 (24.1)	75 (24.2)	22 (24.4)	25 (26.0)	28 (22.6)	0.230	0.631	1.162 (0.628–2.151)
						0.293	0.588	0.846 (0.463–1.548)
GSTM1(+)	109 (64.1)	126 (40.6)	41 (45.6)	36 (37.5)	49 (39.5)	24.207	<0.001	2.609 (1.772–3.842)
(–)	61 (35.9)	184 (59.4)	49 (54.4)	60 (62.5)	75 (60.5)	8.307	0.004	2.136 (1.270–3.592)
						17.530	<0.001	2.978 (1.773–5.002)
						17.456	<0.001	2.735 (1.697–4.408)
GSTT1(+)	92 (54.1)	118 (38.1)	33 (36.7)	36 (37.5)	49 (39.5)	11.497	0.001	1.919 (1.314–2.803)
(–)	78 (45.9)	192 (61.9)	57 (63.3)	60 (62.5)	75 (60.5)	7.179	0.007	1.768 (1.048–2.985)
						6.787	0.009	1.966 (1.179–3.279)
						6.125	0.013	1.805 (1.129–2.888)
GSTM1/T1(+/+)	52 (30.6)	51 (18.4)	16 (17.8)	17 (17.7)	18 (14.5)			
(+/–)	57 (33.5)	75 (24.2)	25 (27.8)	19 (19.8)	31 (25.0)	1.241	0.265	1.342 (0.800–2.251)
						0.906	0.341	1.425 (0.686–2.962)
						0.003	0.960	1.020 (0.479–2.168)
						1.649	0.199	1.571 (0.787–3.138)
(–/+)	40 (23.5)	67 (21.6)	17 (18.9)	19 (19.8)	31 (25.0)	3.660	0.056	1.708 (0.985–2.960)
						0.632	0.426	1.381 (0.622–3.066)
						0.901	0.343	1.453 (0.670–3.149)
						5.007	0.025	2.239 (1.098–4.564)
(–/–)	21 (12.4)	117 (37.7)	32 (35.6)	41 (42.7)	44 (35.5)	34.743	<0.001	5.681 (3.105–10.394)
						16.898	<0.001	4.952 (2.258–10.863)
						22.786	<0.001	5.972 (2.795–12.760)
						23.915	<0.001	6.053 (2.869–12.771)
GSTP1 (*A/A*)	114 (67.1)	210 (67.7)	63 (70.0)	64 (66.7)	83 (66.9)	0.023	0.879	0.969 (0.651–1.444)
A/G+	40 (23.5)	69 (22.3)	24 (26.7)	19 (19.8)	26 (21.0)	0.234	0.628	0.872 (0.502–1.516)
G/G	16 (9.4)	31 (10.0)	3 (3.3)	13 (13.5)	15 (12.1)	0.004	0.948	1.018 (0.598–1.732)
						<0.001	0.982	1.006 (0.615–1.645)

The gene frequencies of *GSTM1* and *GSTT1* in the idiopathic male infertility group or its subtype groups were higher than those in the control group. However, there was no significant difference in the distribution of *GSTP1* and *CYP2D6* gene polymorphisms between the idiopathic male infertility subtype group and the control group. *CYP1A1*: cytochrome P450 1A1; *CYP2D6*10*: cytochrome P450 2D6*10; *GSTM1*: glutathione S-transferase M1; *GSTT1*: glutathione S-transferase T1; *GSTM1/T1*: glutathione S-transferase M1/T1; *GSTP1*: glutathione S-transferase P1; OR: odds ratio; CI: Confidence interval

Supplementary Table 2: Sex hormone levels, sperm parameters, oxidation levels, mitochondrial membrane potential, and DNA fragmentation index distribution before and after treatment in the idiopathic male infertility group

Group	*Number of participants*	*FSH (Iu l^{-1})*	*LH (Iu l^{-1})*	*T (nmol l^{-1})*	*Sperm concentration (×10^6)*	*Sperm viability (%)*	*Sperm motility (%)*	*MDA (nmol ml^{-1})*	*NO (nmol ml^{-1})*	*T-AOC (Iu ml^{-1})*	*8-OH-dG (pg ml^{-1})*	*MMP (%)*	*DFI (%)*
Before													
Control	170	8.04±1.13	7.41±1.08	14.63±3.06	64.15±5.81	76.2±11.6	62.2±9.8	18.71±5.06	21.69±7.01	23.17±6.17	57.48±15.81	83.9±21.1	8.0±3.7
Case	310	7.98±1.75	7.51±1.84	14.47±4.01	10.06±3.47	61.8±6.0	52.5±6.1	25.07±7.90	33.02±9.17	16.38±5.09	129.24±28.75	51.2±11.6	26.5±5.1
CYP1A1m1m1	116	8.02±2.04	7.86±1.03	15.14±3.08	11.02±1.46	63.1±5.7	54.6±5.1	23.17±7.58	32.02±8.17	18.03±5.16	118.69±29.02	59.1±8.6	25.7±4.1
m1m2	144	7.78±2.13	7.48±2.18	13.95±3.86	9.68±2.32	62.2±3.1	53.9±4.1	25.17±8.01	32.70±6.28	16.18±4.29	131.14±27.68	50.8±11.3	26.9±3.7
m2m2	50	7.69±3.31	7.39±3.01	13.89±4.47	9.62±4.24	61.0±4.0	53.9±7.0	27.18±5.90	34.15±3.17	16.21±6.14	130.94±31.81	51.0±12.1	27.0±4.0
CYP2D6˚10 C/C	58	8.11±1.04	7.79±1.14	15.43±2.16	11.14±1.71	63.5±5.1	54.7±5.2	22.89±7.61	32.51±8.07	18.56±5.26	119.41±30.14	58.6±9.5	24.7±5.1
C/T	177	7.69±2.06	7.53±2.07	14.08±3.45	9.77±2.13	62.0±3.1	53.8±5.1	25.69±8.21	33.02±6.47	17.09±6.34	129.37±29.90	54.7±12.1	25.9±3.7
T/T	75	7.73±3.29	7.41±3.31	13.96±4.85	9.60±4.58	61.0±5.2	53.0±7.3	26.92±6.81	34.25±2.49	16.98±8.04	131.79±30.59	53.7±11.3	25.1±4.3
GSTM1(+)	126	8.46±2.11	8.01±1.12	15.48±3.46	11.79±2.54	63.6±5.4	54.9±5.1	23.70±7.69	32.11±6.11	19.03±6.01	118.49±29.57	59.4±8.7	25.5±3.1
(–)	184	7.90±2.09	7.79±2.22	13.08±4.06	9.36±2.58	62.0±4.1	53.0±4.3	26.09±8.11	34.88±6.21	16.21±4.90	132.09±28.59	53.6±11.5	26.8±3.7
GSTT1(+)	118	8.21±1.11	7.99±3.13	15.89±2.46	11.21±1.70	63.7±5.1	54.9±5.5	22.99±7.77	32.47±8.39	19.09±5.70	119.98±31.13	59.0±9.6	24.9±5.7
(–)	192	7.61±2.31	7.31±6.05	14.11±3.06	9.81±2.07	61.4±8.1	51.8±5.9	25.57±8.35	34.01±5.47	16.82±6.11	129.80±29.70	54.2±12.1	25.8±6.1
GSTM1/T1(+/+)	51	8.57±2.59	8.47±1.59	15.58±3.49	11.89±2.49	64.0±5.6	56.9±5.2	22.75±7.09	32.09±6.14	21.01±6.11	118.21±26.51	59.9±9.0	24.5±3.1
(+/–)	75	8.11±1.23	7.98±4.37	15.45±2.38	11.12±1.71	62.1±5.0	54.9±5.0	23.16±7.17	33.71±8.09	18.31±5.69	121.32±31.08	58.8±9.5	25.2±5.6
(–/+)	67	7.98±3.01	7.90±2.01	14.90±4.11	10.90±2.41	62.0±4.6	54.0±4.9	24.09±8.02	34.01±4.22	18.29±4.98	121.69±28.31	58.6±11.6	25.4±4.0
(–/–)	117	7.44±2.12	7.39±5.04	13.47±3.01	9.77±2.11	60.2±7.1	51.0±4.9	26.79±8.31	35.61±5.90	16.66±6.02	129.95±29.67	55.0±10.1	27.3±6.2
GSTP1 (*A/A*)	210	8.12±2.12	7.88±1.14	15.35±2.91	10.92±2.01	63.0±5.4	54.8±5.1	23.59±7.60	32.14±8.31	18.13±4.56	118.57±28.41	58.8±8.0	25.8±3.3
A/G+	69	7.91±2.04	7.47±2.22	14.02±2.98	9.84±1.97	62.2±4.1	53.9±5.1	26.11±7.26	33.21±5.71	16.77±4.21	130.21±28.09	54.1±11.1	26.8±3.7
G/G	31	7.67±3.42	7.41±2.98	13.91±4.58	9.71±3.80	61.1±5.0	53.9±6.9	27.08±6.09	34.21±2.91	16.59±6.01	130.98±30.24	52.0±16.0	27.0±2.9
3 months													
Case	310	8.21±2.80	7.92±2.54	15.89±5.02	12.01±2.40	64.2±5.3	54.7±6.0	23.79±4.92	31.11±8.12	18.28±5.28	127.29±27.74	56.2±12.6	24.8±4.1
CYP1A1m1m1	116	8.35±2.24	8.05±2.02	16.12±3.21	13.80±2.41	65.1±6.0	56.4±5.0	21.09±6.54	29.98±6.12	19.11±4.14	116.61±27.04	61.1±8.1	23.4±2.0
m1m2	144	7.91±2.43	7.56±2.17	14.08±3.76	10.03±1.38	63.2±3.2	54.3±2.1	22.08±6.01	31.79±6.31	18.78±3.28	129.21±25.67	52.7±12.3	25.5±3.2
m2m2	50	7.72±2.52	7.42±2.08	13.97±4.29	9.70±4.11	61.4±4.2	54.0±4.0	24.08±4.92	32.11±3.39	17.35±5.14	129.07±28.85	51.6±11.0	26.1±4.1
CYP2D6˚10 C/C	58	8.42±2.02	8.12±2.11	16.03±2.09	12.80±2.72	66.2±4.1	56.0±5.2	22.90±5.29	28.74±6.37	19.69±5.90	117.49±29.19	59.0±9.8	23.2±2.1
C/T	177	7.87±2.12	7.69±2.14	14.36±3.23	10.11±2.12	63.7±3.6	54.7±5.2	24.36±3.25	32.11±5.47	17.94±6.39	128.60±29.91	55.4±11.0	25.1±2.7
T/T	75	7.75±2.21	7.57±3.29	14.06±4.85	9.82±4.67	61.8±6.0	53.3±7.2	25.59±4.82	33.11±3.59	17.04±7.31	129.59±29.01	54.7±11.1	26.0±5.3
GSTM1(+)	126	8.50±1.97	8.35±3.13	16.03±2.41	13.10±1.51	65.6±4.5	56.0±4.1	21.49±2.65	29.97±5.11	21.13±4.81	116.19±23.54	61.0±7.8	23.0±3.0
(–)	184	8.01±1.81	8.19±1.23	14.05±3.06	10.14±1.54	62.0±3.1	53.3±3.4	27.08±5.41	35.07±4.43	15.02±3.92	136.03±22.59	52.2±6.5	27.9±4.0
GSTT1(+)	118	8.49±2.01	8.23±2.90	16.13±3.01	12.91±2.11	66.2±4.2	56.0±6.3	20.97±5.87	30.10±5.29	20.92±4.50	115.72±29.13	60.8±7.5	22.2±4.4
(–)	192	7.79±2.04	7.80±4.80	14.62±2.16	10.04±1.87	62.0±4.1	52.4±4.7	26.92±4.95	36.02±4.39	17.04±5.31	130.09±31.31	53.0±6.9	28.0±4.4
GSTM1/T1(+/+)	51	8.98±2.19	8.63±2.09	16.98±4.19	13.23±3.03	69.7±4.8	57.6±5.0	20.97±5.93	29.93±5.90	22.82±5.70	116.21±25.42	60.0±7.9	23.6±2.3
(+/–)	75	8.22±1.04	8.02±2.87	15.70±3.18	11.69±2.11	63.0±4.1	55.0±4.3	22.98±5.57	32.03±6.71	18.49±4.90	120.12±26.18	58.8±6.9	24.9±4.4
(–/+)	67	8.02±1.18	7.98±1.81	15.15±3.91	11.05±3.11	62.5±3.9	54.7±3.9	23.86±4.92	32.95±3.71	18.38±5.03	120.61±26.11	58.7±12.0	24.8±2.8
(–/–)	117	7.47±1.96	7.42±3.14	14.02±2.91	9.98±2.91	61.3±6.1	51.4±3.0	25.49±6.81	34.81±4.89	17.01±5.72	129.31±21.07	55.4±11.1	26.9±5.4
GSTP1 (*A/A*)	210	8.41±2.32	8.12±2.01	16.10±3.97	13.04±1.96	65.9±5.0	55.9±4.8	21.48±5.71	30.01±6.72	20.31±5.02	116.28±27.68	59.9±6.3	23.1±2.9
A/G+	69	8.11±2.01	7.59±1.97	14.49±2.31	10.21±1.01	62.8±5.0	54.0±4.6	25.72±5.93	32.97±4.52	17.81±3.17	129.31±27.10	55.2±10.7	25.3±2.5
G/G	31	7.78±2.90	7.51±1.73	14.02±3.81	9.99±2.71	61.9±3.5	54.0±5.9	26.52±4.79	33.02±2.47	17.04±5.48	129.82±21.07	53.5±9.3	25.7±2.9

Contd...

Supplementary Table 2: Contd...

Group	*Number of participants*	*FSH (Iu l^{-1})*	*LH (Iu l^{-1})*	*T (nmol l^{-1})*	*Sperm concentration (×10^6)*	*Sperm viability (%)*	*Sperm motility (%)*	*MDA (nmol ml^{-1})*	*NO (nmol ml^{-1})*	*T-AOC (Iu ml^{-1})*	*8-OH-dG (pg ml^{-1})*	*MMP (%)*	*DFI (%)*
6 months													
Case	310	9.31±2.72	8.42±2.38	19.62±4.59	18.04±3.81	72.7±5.4	58.4±5.4	20.32±3.38	29.03±5.72	21.04±4.49	120.29±27.91	68.5±9.4	21.1±4.0
CYP1A1m1m1	116	14.21±3.72	8.58±2.31	21.38±4.14	20.69±2.96	78.4±8.4	60.2±4.8	18.93±5.49	25.31±4.97	24.31±5.03	114.20±24.82	71.3±7.4	20.0±2.2
m1m2	114	8.92±2.57	7.97±2.09	17.03±2.86	17.08±2.17	69.9±7.3	56.1±3.2	21.49±5.92	29.71±5.49	19.89±3.48	127.62±26.01	61.0±12.0	23.3±2.3
m2m2	50	8.08±2.61	7.81±2.18	16.04±3.98	12.97±3.59	64.3±6.9	55.9±5.0	22.49±4.48	30.36±4.439	18.87±4.68	128.32±26.60	57.3±12.2	24.2±3.6
*CYP2D6*10 C/C*	58	15.03±3.17	8.68±1.99	22.49±3.29	21.08±3.31	75.4±5.2	60.1±5.0	18.07±3.79	26.31±4.97	23.49±5.31	114.70±26.08	72.1±10.2	20.1±3.1
C/T	177	8.97±1.97	7.82±2.71	17.09±2.97	14.79±2.66	68.1±4.0	56.0±4.4	23.02±3.91	30.02±4.39	20.02±4.97	123.62±26.11	59.8±12.2	23.2±2.0
T/T	75	8.36±2.04	7.72±2.95	16.86±3.95	12.79±3.79	67.0±5.0	54.5±5.7	24.14±3.21	32.86±4.01	18.89±3.97	126.21±23.29	57.8±10.0	24.8±4.3
GSTM1(+)	126	13.02±3.07	8.72±4.02	22.38±4.03	21.07±2.29	76.0±5.9	61.0±3.5	18.89±3.04	23.09±4.98	23.71±5.10	113.91±21.39	72.0±8.8	19.9±4.0
(–)	184	8.45±2.69	8.21±2.21	17.12±2.97	14.10±2.69	67.4±4.0	55.3±4.1	24.39±3.49	33.17±5.08	17.18±2.96	129.39±19.96	60.2±7.8	24.8±4.0
GSTT1(+)	118	12.39±3.12	8.51±2.17	20.86±3.29	20.39±3.01	75.0±3.7	60.3±5.9	19.02±4.79	24.18±4.29	24.02±3.97	112.96±19.94	71.7±7.0	20.2±3.8
(–)	192	8.91±3.28	8.12±3.20	16.35±3.01	15.05±2.69	68.1±4.0	55.0±5.2	25.03±5.09	32.97±6.64	18.62±4.49	128.18±27.74	61.0±5.7	24.5±4.1
GSTM1/T1(+/+)	51	14.69±3.09	8.63±2.09	22.49±3.86	23.02±3.72	73.8±6.0	63.3±4.1	18.37±4.28	23.41±4.91	25.14±4.43	110.91±28.01	75.6±5.4	18.5±3.0
(+/–)	75	8.42±2.03	8.24±1.98	17.31±2.96	14.59±2.39	66.7±4.4	56.8±4.0	21.08±3.96	30.41±5.30	19.96±3.86	118.21±23.95	62.0±6.0	22.1±3.2
(–/+)	67	8.18±1.23	8.03±2.01	17.40±2.95	13.96±3.19	66.3±4.0	56.9±4.0	21.86±4.02	30.79±4.03	19.82±4.02	118.04±24.10	62.7±10.3	22.7±2.7
(–/–)	117	7.69±2.02	7.81±2.42	16.05±2.32	11.21±2.01	63.1±4.4	54.6±3.3	24.12±5.04	33.28±4.40	18.31±3.99	126.39±19.79	57.2±13.5	25.9±4.4
GSTP1 (A/A)	210	12.05±2.70	8.50±1.87	20.19±3.49	20.03±2.21	75.2±5.0	60.4±5.0	19.04±3.98	28.03±4.90	23.82±4.50	114.39±20.29	70.2±6.2	20.2±2.9
A/G	69	8.98±1.83	8.10±2.05	17.80±2.50	15.21±2.95	65.7±4.8	56.7±5.2	22.08±4.82	31.29±3.96	19.02±2.29	125.47±23.79	58.1±11.7	23.2±3.1
G/G	31	7.90±2.48	7.73±1.83	15.07±2.91	11.03±2.07	63.2±5.0	55.2±4.8	24.82±4.20	32.29±3.08	18.06±4.39	128.03±27.19	55.3±5.0	24.5±2.1

The indices of patients with *GSTM1*(–), *GSTT1*(–), and *GSTM1/T1*(–/–) genotypes followed the overall distribution, but values of the variables were lower than the average levels. *CYP1A1*: cytochrome P450 1A1; *CYP2D6*10*: cytochrome P450 2D6*10; *GSTM1*: glutathione S-transferase M1; *GSTT1*: glutathione S-transferase T1; *GSTM1/T1*: glutathione S-transferase M1/T1; *GSTP1*: glutathione S-transferase P1; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; MDA: malondialdehyde; NO: nitric oxide; T-AOC: total antioxidant capability; 8-OH-dG: 8-hydroxy-2'-deoxyguanosine; MMP: mitochondrial membrane potential; DFI: DNA fragmentation index