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Idiopathic infertility and GSTM1 gene polymorphism
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·*Original Article*·

Increased oxidative damage of sperm and seminal plasma in men with idiopathic infertility is higher in patients with glutathione S-transferase Mu-1 null genotype

Birsen Aydemir[1], Ilhan Onaran[2], Ali R. Kiziler[1], Bulent Alici[3], Mehmet C. Akyolcu[1]

[1]Department of Biophysics, [2]Department of Medical Biology, [3]Department of Urology, Cerrahpaşa Medical Faculty, İstanbul University, İstanbul 34098, Turkey

Abstract

Aim: To examine whether a relationship exists between glutathione S-transferase Mu-1 (*GSTM1)* gene polymorphism and the susceptibility of sperm and seminal plasma from patients with idiopathic infertility to oxidative stress. **Methods:** Fifty-two men with idiopathic infertility and 60 healthy fertile men were recruited to this study. *GSTM1* gene polymorphism was determined by polymerase chain reaction (PCR) and both the infertile and control individuals were divided into GSTM1 null and GSTM1 positive groups according to their *GSTM1* gene structure. We compared reactive oxygen species (ROS) generation, malondialdehyde (MDA), protein carbonyls and glutathione (GSH) concentrations, and glutathione S-transferase (GST) activity in seminal plasma and spermatozoa from infertile patients and controls with respect to *GSTM1* genotype. **Results:** Significantly higher levels of oxidative stress and damage markers were found in idiopathic infertile men with the *GSTM1* null genotype compared with those with the *GSTM1* positive genotype. There was no significant difference in genotype distribution for the *GSTM1* variant between the idiopathic infertile subjects and fertile subjects. Patients with the *GSTM1* null genotype also had lower sperm concentrations than those with *GSTM1* positive genotype. **Conclusion:** Our results suggest that the susceptibility of sperm and seminal plasma to oxidative stress is significantly greater in idiopathic infertile men with the *GSTM1* null genotype compared with those possessing the gene. Therefore, in patients with idiopathic infertility, *GSTM1* polymorphism might be an important source of variation in susceptibility of spermatozoa to oxidative damage. *(Asian J Androl 2007 Jan; 9: 108–115)*

Keywords: idiopathic infertility; glutathione S-transferase Mu-1; *GSTM1* polymorphism; semen; sperm; oxidative stress

1 Introduction

Oxidative stress is believed to underlie the etiology of numerous human conditions. Organisms are subject to oxidative stress from endogenous and exogenous sources including exposure to solvents, other chemicals and environmental pollutants. All these potential hazards contain components that can induce severe macromolecular, cellular and tissue damage through a) direct cytotoxic effects, b) promotion of primary genotoxic events, or c) generation of reactive oxygen intermediates [1]. Reactive oxygen species (ROS), such as the superoxide anion and

Correspondence to: Dr Birsen Aydemir, Department of Biophysics, Cerrahpaşa Medical Faculty, İstanbul University, İstanbul 34098, Turkey.
Tel: +90-212-551-4935 Fax: +90-212-586-1548
E-mail: birsenay2001@yahoo.com
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hydroxyl radical, can be produced by human spermatozoa [2, 3]. As a result of a high polyunsaturated fatty acid content, human spermatozoa plasma membranes are highly sensitive to ROS-induced damage and hydrogen peroxide appears to be the most toxic ROS for human spermatozoa. There is growing evidence that peroxidative damage to the human spermatozoa membrane is an important pathophysiological mechanism in human male infertility [4]. Human spermatozoa and seminal plasma possess various antioxidant systems to scavenge ROS and prevent ROS-related cellular damage [1, 4]. Failure of antioxidant defences to detoxify excess ROS production can lead to significant oxidative damage including enzyme inactivation, protein degradation, DNA damage and lipid peroxidation. These antioxidant defense systems, which are involved in a variety of detoxification reactions, exhibit baseline levels of activity to ensure the maintenance of the balance between production and removal of endogenous ROS and other pro-oxidants [1].

One of the defense systems against the damaging effects of oxidative stress in human semen are the glutathione S-transferases (GST; EC 2.5.1.18), which catalyze the conjugation of glutathione (GSH) with various electrophilic substances, and play a role in preventing oxidative damage by conjugating breakdown products of lipid peroxides to GSH [5]. It is known that GST activity is widely distributed in hepatic and extrahepatic tissues including the ovaries, testes and serum, and it has been shown that GST might have a relevant protective role during spermatogenesis [6]. Among the substrates of the enzyme are toxic products generated from tissue damage; for example, alkenes, epoxides, hydroperoxides and aldehydes which are produced as a result of lipid peroxidation of biological membranes [7]. GST have been grouped into at least six classes called Alpha, Mu, Pi, Theta, Sigma and Zeta. Genes encoding the glutathione S-transferase Mu-1 and Theta-1 (GSTM1 and GSTT1, respectively) isoforms are polymorphic. Homozygotes for the mutated inactive alleles of each gene are devoid of any specific enzymatic activity (null genotypes). Up to 50% of the Caucasian population are null genotypes for the *GSTM1* gene. The *GSTM1* gene deletion might, therefore, modify the risk of individuals to expose to toxins. Several epidemiological studies have reported that the *GSTM1* null genotype is correlated with an increased susceptibility to diseases associated with oxidative stress and proposed that *GSTM1* might be a critical isozyme in the detoxification of oxidative stress products [7–12]. Chen *et al.* [8] have shown that sperm of varicocele patients with the *GSTM1* null genotype are more vulnerable to oxidative damage. It has been also reported that seminal plasma and spermatozoa from men with idiopathic infertility have higher ROS levels than those from the fertile men [13]. If enzymatic deficiency in the *GSTM1* isoform is correlated with increased risk of certain diseases associated with oxidative damage, then it is possible that there is an association between *GSTM1* genotypes and idiopathic infertility. Although the potential role of different detoxification mechanisms such as superoxide dismutase, catalase, GSH peroxidase and GSH have been investigated in idiopathic infertility [13, 14], there are no reports regarding *GSTM1* polymorphism and idiopathic infertility. Therefore, the general aim of the present study was to determine whether the *GSTM1* null genotype is associated with altered susceptibility to oxidative stress, and the damage of sperm and seminal plasma in patients with idiopathic infertility.

2 Materials and methods

2.1 Collection and preparation of samples

The present study was approved by the institutional review board of the Infertility Central Urology Department of Cerrahpaşa Medical Faculty, İstanbul, Turkey. Blood and semen specimens were obtained from 52 men aged 25–49 years with idiopathic infertility, between 2004 and 2006. Specimens were also obtained from 60 male volunteers aged 25–49 years with normal semen analysis according to World Health Organization (WHO) guidelines [15] to serve as the fertile control. The Institutional Ethical Committee approval was taken in accordance with the principles of Declaration of Helsinki. Informed consent was obtained from each study subject. Individuals with a significant medical history or signs suggestive of defective androgenisation or abnormal testicular examinations were excluded from this study. Further exclusion criteria for both groups included chromosomal disorders related to a fertility disorder, cryptorchidism, vasectomy, anormal liver function and hormone tests, cigarette smoking, alcohol consumption and the use of folic acid, glutathione, vitamin C, vitamin E supplements or medication within three months before recruitment. Criteria for study inclusion were infertility for at least 12 months with at least one semen parameter abnormality, semen leukocyte count less than 1×10^6/mL and negative semen antisperm antibody on a mixed agglutination

reaction test. Semen specimens were collected by masturbation into a sterile wide-mouth metal-free plastic container after at least 3 days (3–5 days) of abstinence and liquified at 25ºC for 30 min.

2.2 Semen analysis

A semen analysis was carried out according to the WHO guidelines to obtain volume, pH, sperm concentration, motility and morphology. Sperm concentration was determined using a Makler Counting Chamber (Seti-Medical Instruments, Haifa, Israel). Motility was expressed as the percentage of motile spermatozoa and their mean velocity. Morphology was determined according to the WHO criteria after incubation of the sample with trypsin for 10 min at 25ºC, using the methylene blue eosin staining procedure, feathering and fixation by flame. At least 100 cells were examined at a final magnification of × 1 000 [15].

2.3 Spermatozoa preparation

After liquefaction, spermatozoa were fractionated on Percoll gradients (40–95%) according to WHO guidelines [15]. Semen was layered on top of the gradient and centrifuged at 400 × *g* for 20 min at 25ºC. Spermatozoa in the 95% Percoll layer were collected, and washed twice at 400 × *g* for 6 min at 25ºC with added Tris, sodium and EDTA (TNE) buffer (0.15 mol/L NaCl, 0.01 mol/L Tris-HCl, 1 mmol/L Na_2EDTA, pH 7.4) [13]. ROS levels within the spermatozoa were determined immediately after washing. The remaining spermatozoa were frozen without preservatives and stored for up to 1 month at –70ºC before being assayed for GST, GSH, malondialdehyde (MDA) and protein carbonyls.

2.4 Measurement of ROS

ROS were measured in spermatozoa, immediately after collection and washing, using a luminol (5-amino-2, 3,-dihydro-1, 4-phthalazinedione)-enhanced chemiluminescence method [13, 16]. Luminol was prepared as 5 mmol/L stock in dimethyl sulfoxide (DMSO). 10 μL of the stock was added to 500 μL of the sperm suspension (1×10^6 spermatozoa/mL). Negative control was prepared by adding an equal amount (10 μL) of luminol to 500 μL of TNE buffer. The levels of ROS were assessed by measuring the luminol-dependent chemiluminescence with the Luminoskan TL luminometer (Labsystems Inc., Helsinki, Finland) in the integrated mode for 10 min. Results were expressed as relative light units (RLU) per 1×10^6 spermatozoa/mL.

2.5 Measurement of lipid peroxidation

The lipid peroxide levels in the seminal plasma and spermatozoa were measured using a thiobarbituric acid reactive substances (TBARS) assay, which monitors MDA production based on the method of Beuge *et al.* [17]. Briefly, to 100 μL sample of seminal plasma (1×10^6 spermatozoa/mL), 200 μL of cold 1.15% (w/v) KCl was sonicated for 30 s on ice, and added to 1.8 mL of 3% phosphoric acid and 0.6 mL of 0.6% TBA. These mixtures were heated in boiling water for 45 min. After cooling, the MDA was extracted by centrifugation at 1 500 × *g* for 10 min at 25ºC and the intensity was measured at 535 nm using ultraviolet-visible spectrophotometry (Shimadzu UV-1601, Tokyo, Japan). The MDA level was determined using the molar absorption coefficient of the MDA at 535 nm 1.56×10^5 mol/L/cm.

2.6 Measurement of GSH

GSH concentration was determined in a modified coupled optical test system [18]. In this system, GSH was oxidized by 5,5' dithiobis-2 nitrobenzoic acid (DTNB) and then reduced by GSH reductase with NADPH as hydrogen donor. The oxidation of GSH by DTNB was detected photometrically by a change of absorption at 412 nm. Briefly, to 100 μL of sample seminal plasma or spermatozoa (1×10^6 /mL), 150 μL of 5% sulphosalisilic asid (w/v) was added to induce lysis. Then, 20 μL of lysate was taken and added to 980 μL of reaction buffer (100 nmol/L potassium phosphate buffer, 1 mmol/L NADPH, 0.5 mmol/L DTNB, 0.5 U GSH reductase, pH 7.4). The change of absorption was recorded at 412 nm on ultraviolet-visible spectrophotometry. GSH level was determined using the molar absorption coefficient of the GSH at 412 nm 13.6×10^{-4} mol/L/cm.

2.7 Measurement of protein carbonyls

Because carbonyl groups (aldehydes and ketones) might be introduced into proteins by ROS and free radicals, quantitation of protein carbonyls was carried out by incubating equal volumes of the sample (seminal plasma, 1×10^6 spermatozoa/mL) and 2,4-dinitrophenylhydrazine (3.4 mg per 10 mL 1 mol/L HCl) at 50ºC for 1 h. After the reaction, proteins were precipitated with 20% trichloroacetic acid and the unreacted dye was removed by centrifugation. The pellet was dissolved in 1 mol/L NaOH and the absorbance at 450 nm was

recorded. The molar absorbance coefficient (ε = 25 500 mol/L/cm) was used to calculate the carbonyl content [19]. Protein concentrations were determined by the Lowry method, with bovine serum albumin (BSA) as the standard [20].

2.8 Measurement of GST

GST activities of the samples (seminal plasma and 1×10^6 spermatozoa/mL) were measured by the method described by Habig *et al*. [21], using 1-choloro-2-4 dinitrobenzene (CDNB) as substrate. Briefly, 80 μL of sample (seminal plasma or 1×10^6 spermatozoa/mL), was added to 720 μL of reaction buffer. The reaction buffer consisted of 0.1 mol/L KH_2PO_4, 1 mmol/L EDTA, 20 mmol/L GSH and 20 mmol/L CDNB (pH 7.4). Formation of the S-conjugate was identified by its absorbance at 340 nm and the extinction coefficient of CDNB (9600 mol/L/cm) was used to calculate GST activity.

2.9 GSTM1 polymorphism

For the determination of the genetic status, DNA was prepared from peripheral lymphocytes of anticoagulated blood (EDTA) by proteinase K digestion and a salting out procedure with a saturated NaCl solution described by Miller *et al.* [22]. The polymerase chain reaction (PCR) method was used to detect the presence or absence of the *GSTM1* gene as described previously [23]. The GSTM1 primers used were: forward, 5'-GAACTCCC TGAAAAGCTAAAGC-3'; reverse, 5'-GTTGGGCTCAA ATATACGGTGG-3'. The β-globulin primers used were: forward, 5'-CAACTTCATCCACGTTCACC-3'; reverse, 5'-GAAGAGCCAAGGACAGGTAC-3'. Polymerase chain reaction was carried out for 35 cycles in a DNA thermal cycler using a thermal profile of denaturation at 94ºC for 1 min, annealing at 55ºC for 1 min and primer extension at 72ºC for 1 min. The PCR products were then separated on a 2% agarose at 150 V for 1.5 h, and stained with 1 μg/mL ethidium bromide at 25ºC for 10 min. DNA from individuals with positive GSTM1 and β-globulin alleles yielded 215- and 268-bp products, respectively. The absence of amplifiable GSTM1 (in the presence of β-globulin PCR product) indicates a *GSTM1* null genotype. The presence of amplifiable *GSTM1* indicates positive genotype (homozygous or heterozygous for the *GSTM1* gene).

Values reported are mean ± SD. All data were normally distributed and underwent equal variance testing. Statistical significance of differences was determined by SPSS version 11.5 for windows (SPSS, Chicago, IL, USA). Statistical analysis was performed by Mann-Whitney *U*-test and ANOVA with Tukey's post test. $P < 0.05$ was considered statistically significant.

3 Results

There was no statistically significant difference in frequency of the *GSTM1* null genotype between the idiopathic infertile group (51.9%) and the control group (46.7%). Table 1 shows the population characteristics and results of classic semen analysis in idiopathic infertile patients and control donors according to *GSTM1* genotype.

Sperm concentrations were significantly lower in the patients with idiopathic infertility compared with individuals in the control group. Furthermore, in the infertile patient group, sperm concentrations were higher in those with the *GSTM1* positive genotype. No such differences in sperm concentration were noticed between *GSTM1* positive and null control donors. No significant differences in sperm motility and morphology were observed between infertile patients and control donors. Furthermore, these sperm parameters did not differ by *GSTM1* genotype.

Considering the oxidative stress biomarkers, we compared the levels of MDA and protein carbonyls in spermatozoa and seminal plasma between infertile patients and controls, with respect to *GSTM1* genotype. As shown in Table 2, protein carbonyls and MDA levels in the seminal plasma and spermatozoa were significantly higher in the infertile group. Furthermore, protein carbonyls and MDA levels were found to be significantly higher in *GSTM1* null infertile patients compared with *GSTM1* positive infertile and control donors (both *GSTM1* null and positive). Protein carbonyls and MDA levels in the control group were also higher in *GSTM1* null individuals compared with *GSTM1* positive donors. Levels of ROS were also measured in washed sperm suspensions, using chemiluminescence assay. ROS levels in the spermatozoa samples were also significantly higher in specimens from infertile patients than that from controls (24.54 ± 10.90 RLU 1×10^6 spermatozoa/mL *vs.* 9.30 ± 4.71 RLU/ 1×10^6 spermatozoa/mL) ($P < 0.001$). In individuals of the idiopathic infertility group with *GSTM1* null genotype, ROS levels were 34.22 ± 4.47 RLU/1×10^6 spermatozoa/mL *vs.* 14.08 ± 3.39 RLU/1×10^6 spermatozoa/mL in *GSTM1* positive genotype ($P < 0.001$). In those of

Table 1. The population characteristics and semen variables in idiopathic infertile men in terms of glutathione S-transferase Mu-1 (*GSTM1*) genotype. [a]*P,* for direct comparison of *GSTM1* null and positive genotypes (ANOVA, Tukey's Range [HSD] test); [b]*P,* compared with the control donors; [c]*P,* compared with the *GSTM1* positive control donors, [d]*P,* for direct comparison with *GSTM1* null and positive genotypes (among idiopathic infertile individuals). Population characteristics and semen parameters are given as mean ± SD.

Genotype	Age (years)	Abstinence period (days)	Sperm concentration ($\times 10^6$/mL)	*P* value	Sperm motility (%)	*P* value	Sperm morphology (%)	*P* value
Control fertile group								
Total (*n* = 60)	40.00 ± 7.00	3.72 ± 0.80	61.80 ± 17.70		52.73 ± 6.59		48.57 ± 4.16	
GSTM1 positive (*n* = 32)	41.03 ± 7.31	3.69 ± 0.82	62.75 ± 16.68		53.84 ± 5.53		49.25 ± 3.09	
GSTM1 null (*n* = 28)	38.61 ± 6.51	3.75 ± 0.80	60.71 ± 19.05	0.664[a]	51.46 ± 7.53	0.174[a]	47.79 ± 5.07	0.191[a]
Idiopathic infertile group								
Total (*n* = 52)	39.12 ± 5.94	3.65 ± 0.76	30.27 ± 7.98	< 0.001[b]	51.44 ± 4.81	0.235[b]	47.44 ± 7.37	0.333[b]
GSTM1 positive (*n* = 25)	40.04 ± 5.14	3.72 ± 0.79	34.40 ± 7.60	< 0.001[c]	53.08 ± 4.46	0.566[c]	49.32 ± 5.10	0.952[c]
GSTM1 null (*n* = 27)	38.26 ± 6.57	3.59 ± 0.75	26.44 ± 6.32	< 0.001[d]	49.93 ± 4.69	0.056[d]	45.70 ± 8.72	0.072[d]

Table 2. The levels of malondialdehyde (MDA) and protein carbonyls in seminal plasma and spermatozoa of idiopathic infertile men with respect to glutathione S-transferase Mu-1 (*GSTM1*) genotype. [a]*P,* for direct comparison of *GSTM1* null and positive genotypes (ANOVA, Tukey's Range [HSD] test); [b]*P,* compared with the control donors; [c]*P,* compared with the *GSTM1* positive control donors; [d]*P,* for direct comparison with *GSTM1* null and positive genotypes (among idiopathic infertile individuals). The values are given as mean ± SD. MDA, malondialdehyde.

Genotype	Seminal plasma				Spermatozoa			
	Protein carbonyls (nmol/mg protein)	*P* value	MDA (nmol/mL)	*P* value	Protein carbonyls (nmol/10^6 sperm)	*P* value	MDA (nmol/10^6 sperm)	*P* value
Control fertile group								
Total (*n* = 60))	1.36 ± 0.34		0.40 ± 0.06		1.83 ± 0.38		0.23 ± 0.05	
GSTM1 positive (*n* = 32)	1.14 ± 0.20		0.38 ± 0.07		1.66 ± 0.32		0.21 ± 0.04	
GSTM1 null (*n* = 28)	1.62 ± 0.27	< 0.001[a]	0.42 ± 0.05	0.009[a]	2.02 ± 0.35	< 0.001[a]	0.25 ± 0.04	< 0.001[a]
Idiopathic infertile group								
Total (*n* = 52)	2.82 ± 0.79	< 0.001[b]	0.62 ± 0.16	< 0.001[b]	2.99 ± 0.63	< 0.001[b]	0.38 ± 0.11	< 0.001[b]
GSTM1 positive (*n* = 25)	2.12 ± 0.26	< 0.001[c]	0.48 ± 0.07	< 0.001[c]	2.47 ± 0.29	< 0.001[c]	0.28 ± 0.04	< 0.001[c]
GSTM1 null (*n* = 27)	3.48 ± 0.47	< 0.001[d]	0.76 ± 0.09	< 0.001[d]	3.47 ± 0.47	< 0.001[d]	0.48 ± 0.07	< 0.001[d]

control group, ROS levels were 13.50 ± 3.24 RLU/1 × 10^6 spermatozoa/mL *vs*. 5.63 ± 1.81 RLU/1 × 10^6 spermatozoa/mL in *GSTM1* positive genotype ($P < 0.001$).

GSH content and GST activity were determined in spermatozoa and seminal plasma from both fertile and idiopathic infertile males. As shown in Table 3, GST activities and the levels of GSH in seminal plasma and spermatozoa did not differ significantly between infertile patients and control donors. In addition, these parameters are not affected by *GSTM1* genotype.

4 Discussion

Male subfertility affects 1/10 males and in 30% of cases the origin of reduced male fertility is unknown. It is a heterogeneous disorder, with several genetic and environmental factors contributing to impaired spermatogenesis [24]. Increasing evidence suggests that polymorphisms in several genes are associated with male infertility, although genetic factors that could mediate the pathogenesis of male infertility are mostly unclear. There is also growing evidence to suggest that seminal stress is involved in many aspects of male infertility [25]. Genetic tests have been developed for polymorphisms in several important enzymes that are involved in the protection against oxidative stress. These include polymorphic large deletions causing inactivation of two genes, *GSTM1* and *GSTT1*, that have previously been associated with several conditions where oxidative stress has been implicated [7, 12, 26, 27].

Polymorphism in the genes *GSTM1, GSTM3* and *GSTM5* have been shown to be associated with male infertility [8, 28–31]. With respect to *GSTM1*, it has been suggested that polymorphism of the gene might be an important factor in determining the susceptibility of patients to the development of alcohol-induced disorders of human spermatogenesis [28]. Chen *et al.* [8] also showed that polymorphism of GSTM1 was related to a susceptibility to infertility in men with varicocele testes. In addition, Okuno *et al.* [29] showed that the *GSTM1* null genotype was associated with a favorable response to varicocelectomy, using an increase in sperm concentration as the outcome. However, there are no reports regarding *GSTM1* polymorphism and idiopathic infertility, which has been shown to be associated with an overproduction of reactive oxygen species and an impairment of antioxidant defensive capacity [13]. Thus, in the present study, we measured protein carbonyl and malondialdehyde levels in spermatozoa and seminal plasma from patients with idiopathic infertility to evaluate the effect of *GSTM1* polymorphism on oxidative damage. Lipid peroxidation and protein oxidation are well-defined mechanisms of cell injury for the monitoring of free radical damage in cells and biological fluids. Therefore, MDA and protein carbonyls were selected as markers for measuring lipid peroxidation and protein oxidation in seminal plasma and spermatozoa. The role of GSTM1 in defense against oxidative damage was also further evaluated by assessing its ability to reduce to intracellular ROS [32].

When we compared the genotype distribution for the *GSTM1* variant between idiopathic infertile subjects and control subjects, we found that there was not any significant difference between our populations. However,

Table 3. Glutathione S-transferase (GST) activities and glutathione (GSH) concentrations in seminal plasma and spermatozoa of idiopathic infertile men with respect to *GSTM1* genotype. Results were expressed as mean ± SD (ANOVA, Tukey's Range [HSD] test).

Genotype	Seminal plasma		Spermatozoa	
	GST (nmol/min/mL)	GSH (pmol/mL)	GST (nmol/min/10^6 sperm)	GSH (pmol/10^6 sperm)
Control fertile group				
Total (n = 60)	38.77 ± 5.11	36.52 ± 3.59	33.92 ± 6.40	36.13 ± 6.50
GSTM1 positive (n = 32)	39.22 ± 3.67	37.09 ± 3.71	33.63 ± 6.72	34.50 ± 6.23
GSTM1 null (n = 28)	38.25 ± 6.42	35.86 ± 3.40	34.25 ± 6.13	38.00 ± 6.41
Idiopathic infertile group				
Total (n = 52)	37.69 ± 5.60	35.69 ± 5.46	33.79 ± 5.64	38.50 ± 7.05
GSTM1 positive (n = 25)	38.12 ± 5.09	36.16 ± 6.00	34.68 ± 5.18	40.12 ± 8.50
GSTM1 null (n = 27)	37.30 ± 6.10	35.26 ± 5.00	32.96 ± 6.02	37.00 ± 5.11

the present study shows an association between *GSTM1* gene polymorphism, and markers of oxidative stress and damage in spermatozoa and seminal plasma from subjects with idiopathic male infertility. Spermatozoa and seminal plasma from infertile individuals with the *GSTM1* null genotype exhibited greater susceptibility to oxidative stress and damage than that from GSTM1 positive infertile patients. Furthermore, the *GSTM1* null genotype was associated with higher ROS, protein carbonyl and MDA levels in the control group. The mean semen sperm concentration was lower in patients with the *GSTM1* null genotype than in those possessing the gene. There were no further differences in the semen analysis parameters between the GSTM1 null and positive groups.

These findings suggested that the seminal plasma and spermatozoa of idiopathic infertile men with the *GSTM1* null genotype appear to be affected by oxidative stress. Therefore, the *GSTM1* null genotype might predispose spermatozoa of patients with idiopathic infertility to increased oxidative damage. Our findings might also suggest that the effect of the *GSTM1* genotype might be potentiated in the presence of an additional oxidative burden that might be apparent in idiopathic infertility.

As a result of studies on epithelial ovarian cancer, Sarhanis *et al.* [11] proposed that *GSTM1* might be a critical factor in the detoxification of the products of oxidative stress produced during the repair of the ovarian epithelium. It has also been shown that the GSTM1 enzyme has the highest catalytic efficiency in the detoxication of HAE, which are produced as a result of free radical-initiated lipid peroxidation [33]. Therefore, in individuals with the *GSTM1* null genotype, the lack of GSTM1 activity might affect the antioxidant potential within spermatozoa and seminal plasma. However, our findings might also be influenced by uncontrolled factors, such as other antioxidant factors and unknown or unchecked polymorphisms in genes such as *GSTT1*, *GSTM3* and *GSTM5*. Although Alkan *et al.* [13] reported that seminal plasma superoxide dismutase, catalase and glutathione peroxidase activities in patients with idiopathic infertility were significantly lower than those in controls, it is extremely difficult to evaluate the contribution of each participating element to antioxidant activity, as a result of factors such as the multiplicity of antioxidants and overlap in their functions. In contrast, oxidative stress caused by excessive production of ROS has been associated with increased sperm damage and apoptosis [34], and this might account for the lower sperm concentration found in infertile individuals with the *GSTM1* null genotype. Further studies are required to investigate the possible contribution of such factors.

Because the level of GSH is potentially as important as the level of glutathione transferases in the rate of conjugation of different electrophiles with glutathione, the level of this cofactor and GST activity were also examined in seminal plasma and spermatozoa. Glutathione levels and GST activities remained unchanged between the *GSTM1* null and positive groups, suggesting that the observed effects in seminal plasma and spermatozoa might be the result of other antioxidant protection mechanisms.

Before a conclusion can be drawn from the results of the present study, it is important to bear in mind that our study had some limitations. First, we have not analyzed *GSTT1*, *GSTM3* or *GSTM5* genotype status, which all exhibit polymorphic expression. Furthermore, we did not assess GSTM1 protein levels in spermatozoa. Also, our findings were limited by the small sample size.

In conclusion, the results of the present study suggest that the seminal plasma and spermatozoa of idiopathic infertile men with *GSTM1* null genotype are more vulnerable to oxidative stress and damage. Therefore, polymorphism of *GSTM1* might play a role in the antioxidant capacity of spermatozoa in subjects with idiopathic male infertility.

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