Is There a Relation Between Testosterone or Estradiol Levels in Seminal Plasma and GSTM1 Polymorphism in Infertile Patients?

İnfertil Hastalarda GSTM1 Polimorfizmi ile Seminal Plazmadaki Testosteron veya Estradiyol Düzeyleri Arasında bir İlişki Var mıdır?

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Abstract
Purpose: Glutathione S-transferase M1 (GSTM1) enzyme has been suggested to serve as a steroid-binding protein by its ability to bind to testosterone and estradiol, two important hormones in seminiferous tubule that are essential for spermatogenesis. We investigated whether GSTM1 polymorphism was associated with blood and seminal plasma levels of testosterone and estradiol in subfertile and control subjects.
Material and Methods: The levels of total estradiol and testosterone were measured by using an electrochemiluminescence immunoassay in serum and seminal plasma from 103 individuals including 62 subfertile patients. GSTM1 polymorphism was examined using polymerase chain reaction.
Results: The estradiol and testosterone levels in seminal plasma were not significantly different in the control and subfertile subjects. No role for GSTM1 enzyme as a steroid-binding protein seemed likely as there was also no significant difference in seminal plasma estradiol and testosterone levels according to GSTM1 genotype. Using Spearman rank correlation analysis, significant positive correlations were found between seminal estradiol and serum estradiol in infertile males, and between seminal testosterone and serum testosterone in fertile males, independent of GSTM1 genotype.
Conclusion: The results suggest that GSTM1 polymorphism is not a genetic risk factor for seminal estradiol and testosterone levels in infertile males.

Key Words: **Estradiol; Glutathione S-transferase M1; Infertility, Male; Polymorphism, Genetic; Testosterone.**

Özet
Amaç: Glutatyon S-transferaz M1 (GSTM1) enziminin seminifer tübülde spermatogenesis için gerekli olan estradiyol ve testosteron hormonlarını bağlayabilen bir protein olduğu ileri sürülmüştür. Çalışmamızda kontrol ve infertil erkeklerde, seminal plazma testosteron ve estradiyol düzeyleri ile GSTM1 polimorfizmi arasında bir ilişkinin var olup olmadığı araştırıldı.
Gereç ve Yöntem: Altmışikisi infertil erkek olan toplam 103 erkek bireyden elde edilen serum ve seminal plazmadaki total estradiyol ve testosteron düzeylerinin ölçümü elektrokemilüminesans yöntemi ile yapıldı. GSTM1 genindeki polimorfizm, Polimeraz Zincir Reaksiyonu ile tespit edildi.
Bulgular: Seminal plazmadaki estradiyol ve testosteron düzeylerinde kontrol ve infertil gruplar arasında anlamlı bir farklılık gözlenmedi. Seminal plazma testosteron ve estradiyol düzeyleri GSTM1 genotipine göre karşılaştırıldığında gruplar arasında anlamlı bir farklılık bulunmadı. Spearman rank korelasyon analizine göre fertil erkeklerde seminal plazma ve serum testosteronları arasında, infertil erkeklerde de seminal plazma ve serum estradiyol düzeyleri arasında anlamlı pozitif bir korelasyon saptandı. Ancak bu ilişkiler GSTM1 genotipine göre bir değişme göstermedi.
Sonuç: Sonuçlarımız, GSTM1 polimorfizminin infertil erkeklerdeki seminal estradiyol ve testosteron düzeyleri için bir risk faktörü olmadığını düşündürmektedir.

Anahtar Kelimeler: **Erkek infertilitesi; Estradiyol; Genetik polimorfizm; Glutatyon S-transferaz M1; Testosteron.**

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Introduction

Glutathione S-transferase (GST; EC 2.5.1.18) which is one of the Phase II detoxification enzymes are izoenzyme family that play a role in various electrolyphic metabolite conjugation formed during oxidative reactions. With this function, GST isoenzymes have important functions against carcinogens, antitumor drugs, environmental pollutants and oxidative stress products. Since GST binds to various ligants such as bile acids, steroid hormones and neurotransmitter, it also plays as a carrier protein in the organism (1-5). Cytoplasm GST isoenzymes are divided into seven different classes and classified as alpha, mü, pi, teta, sigma, kappa and zeta. These isoenzymes may be specific towards different or same substrates. Polymorphism of α (alpha), μ (mü), π (pi) and θ (teta) classes are defined. Among these, there is polymorphism of deletion where the gene is completely lost in GSTM1 isoenzyme (a member of GSTμ) and GSTT1 isoenzyme (a member of GSTθ). It is known that people who have homozygote deletion for these genes (null genotype), do not have enzymatic activity for related enzymes. It was shown that approximately half of these have homozygote null genotype for this gene in various populations (6). It is repoted that people with homozygote deletion for GSTM1 gene have more tendency towards diseases related with oxidative stress (lung, bladder, breast, colorectal, ovarium and skin cancer) in many epidemiological studies (7-10). Also there are studies showing that gene polymorphism may be related to male infertility (11, 12). In these studies, it was claimed that the cause of increasing tendency to oxidative diseases may be due to people with null genotype not having GSTM1 enzyme which needs for detoxification of oxidative stress products.

Seminiferous testicular fluid (STF) contain enzymatically active seminifer GST enzymes (13). Mukherjee et al showed that GSTM1 in STF was bound with testosterone and estradiol (14). These researchers also claimed GSTM1, with catalytic function and binging of sex steroid hormone, was one of the multiple function proteins in seminifer tubule compartment. It is widely accepted that steroid binding proteins such as androgen binding protein (ABP) and sex hormone binding globulin (SHBG) are required to sustain the optimal level of steroids in tubular compartment (15-20). In the light of this information, it is thought that there may be relationship between genetic polymorphism in GSTM1 and testosterone or estradiol concentrations in seminal plasma. If there is such a relationship, it can be considered that GSTM1 genotype may be a risk factor in the pathogenesis of male infertility. For this reason, a relationship amongst testosterone or estradiol concentrations in seminal plasma or serum, GSTM1 deletion polymorphism and male infertility was studied.

Materials and Methods

Fertile control group was composed of 41 voluntary individuals with normal semen analysis and infertile patient group was composed of 62 individuals. Semen analysis was evaluated in accordance with the guide of World Health Organization (WHO) (21). The approval was obtained from the Ethics Committee of Istanbul University, Cerrahpasa Medical Faculty. All individuals included in the study were informed about the study. Volume, pH, sperm concentration, motility and morphology were evaluated for semen analysis. Total testosterone and estradiol concentrations in serum or seminal plasma were measured using Roche MODULAR ANALYTICS E170 system, according to electrochemiluminescence method (ECLIA).

DNA isolation from leukocytes was studied according to methods of Miller and his colleagues in blood samples with EDTA (22). Primary series used for GSTM1 gene region were as follows: GSTM1: (G5-5' GAA CTC CCT GAA AAG CTA AAG C 3');G6-5' GTT GGG CTC AAA TAT ACG GTG G 3'), β globin gene primary series: (GH 20-5' GAA GAG CCA AGG ACA GGT AC 3'; PCO 4-5' GAA CTT CAT CCA CGT TCA CC 3'). GSTM1 gene was increased with polymerase chain reaction (PCR) in genomic DNA samples of individuals. In order to increase GSTM1 gene region, a PCR program of 35 cycles (initial denaturation for 5 minute at 95ºC, denaturation for 1 minute at 94ºC, connection for 1 minute at 55ºC and extension for 1 minute at 72ºC) had been used. Amplification products were displayed under 2% agarose gel electrophoreses including etidium bromide after it is run for 30 minutes in 120 V under UV light in transilluminator. Lack of 219 base double bands is determined in homozygotes individuals with GSTM1 gene deletion. For control purpose, ß globin gene in 268 base double length is proliferated (23).

Statistical analysis was evaluated by using SPSS 11.5 program for Windows. Student's t-test was used to compare group means for independent samples and $p<0.05$ was accepted as statistically significant difference. All data were shown as mean ± standard deviation (SD).

Presence of a relationship between concentration of testosterone or estradiol in seminal plasma and in serum was controled using Spearman rank correlation test.

Results

The characteristics of the population for the infertile and fertile male individuals and semen analysis values are shown in Table I. Number of sperms in infertile group (t=10.926; p<0.001), sperm motility (t=4.058; p<0.001) and sperm morphology (t=4.580; p<0.001) were found to be significantly lower than those of the fertile group. Infertile and fertile individuals were divided into two groups according to presence of GSTM1 allel (homozygote for GSTM1 allel). Number of sperms were found to be significantly lower in infertile individuals without GSTM1 allel than individuals with infertile GSTM1 allel (t=3.081; p<0.01), individuals with fertile GSTM1 allel (t=9.862; p<0.001) and without fertile GSTM1 allel (t=7.313; p<0.001). Sperm morphology was significantly lower in infertile individuals without GSTM1 allel than infertile individuals with GSTM1 allel (t=4.210; p<0.001), fertile individuals with GSTM1 allel (t=6.892; p<0.001) and fertile individuals witout GSTM1 allel (t=4.253; p<0.001). Sperm motility was significantly less in infertile individuals without GSTM1 allel than fertile individuals with GSTM1 allel (t=4.743; p<0.001) and without GSTM1 allel (t=2.567; p<0.05).

When total testosterone or estradiol concentration in seminal plasma or serum were compared separately, no statistically significant difference was found between infertile and fertile individuals. When infertile and fertile individuals were divided according to presence of GSTM1 allel, testosterone or estradiol concentrations in seminal plasma or serum and the rate of testosterone in seminal plasma to testosterone in serum and the rate of estradiol in serum to estradiol in seminal plasma were also not found to be statistically different (Table II).

Table I. Population features and semen parameters, in infertile and control groups according to GSTM1 genotype.

Genotype	n	Age (Year)	No sex period (day)	Sperm number ($x10^6$/ml)	Sperm motility (%)	Sperm morfology (%)
Infertil Group	62	38.98±6.66	3.68±0.76	19.79±11.70 [a]	46.44±8.32[a]	43.90±6.87[a]
GSTM1+	28	40.00±5.31	3.82±0.77	24.64±12.70	48.64±7.89	47.50±6.22
GSTM1null	34	38.15±7.58	3.56±0.75	15.79±9.20[b,c,d]	44.62±8.33[c,d]	40.94±5.97[b,c,d]
Control Group	41	40.37±8.86	3.88±0.81	65.34±24.94	53.41±8.69	49.20±4.85
GSTM1+	23	41.70±9.00	3.91±0.85	64.96±22.68	55.04±8. 01	50.00±3.95
GSTM1null	18	38.67±8.64	3.83±0.79	65.83±28.25	51.33 ±9.30	48.17±5.75

Significantl differ [a] from control group (Student's t-test; p<0.001); [b] infertile GSTM1+ group (p<0.01); [c] fertile GSTM1null genotype group (p<0,001) and [d] fertile GSTM1+ group (p<0,001).

Table II. Genotype estradiol and testosterone concentrations of infertile and control groups in serum and seminal plasma

Genotype	n	Estradiol in Serum (pg/ml)	Estradiol in Seminal Plasma (pg/ml)	(Seminal plasma/ serum) estradiol	Serum testosteron (ng/ml)	Seminal plasma testosteron (ng/ml)	(Seminal plasma/ serum) testosteron
Infertil Group	62	32.48±11.23	53.56±25.12	1.74	5.04±1.90	3.61±2.20	0.76
GSTM1 +	28	31.92±12.17	54.40±27.36	1.79	4.59±1.56	3.16±2.11	0.72
GSTM1 null	34	32.95±10.57	52.87±23.51	1.69	5.42±2.09	3.98±2.23	0.81
Control Group	41	32.62±9.53	54.52±27.28	1.80	4.53±1.89	3.25±2.01	0.73
GSTM1 +	23	31.27±9.26	49.82±21.93	1.73	4.63±2.05	3.39±2.05	0.76
GSTM1 null	18	34.35±9.86	60.51±32.57	1.91	4.40±1.73	3.08±2.01	0.07

Table III. Spearman rank correlation (r) values of estradiol concentrations between seminal plasma and serum or testosterone concentrations between seminal plasma and serum.

Genotype	n	Testosteron	Estradiol
Infertil Group	62	0.310	0.403**
GSTM1 +	28	0.312	0.397*
GSTM1 null	34	0.222	0.447**
Control Group	41	0.622**	0.086
GSTM1 +	23	0.547**	0.069
GSTM1 null	18	0.720**	0.123

*P<0.05; **P<0.01

Discussion

STF are important for life and maturity of germ cells. Proteins are needed in order to bind and transferred steroids in sertoli cells and STF (16). ABP and SHBG are secretive glycoprotein, binding testosterone and estradiol with a high affinity. It is accepted that ABP is important for keeping high level of testotesteron in tubular compartment. Along with this, it was determined that STF had GST isoenzymes and these isoenzymes were synthesized and released by Sertoli cells (24-28). It was shown that GSTM1 obtained from rat STS binds testosterone and estradiol (14).

There are studies claiming that GSTM1 gen polymorphism is related with infertility in males (12, 29). In these studies, it was claimed that individuals with null genotype without GST enzyme could not conjugate specific metabolites of enzyme and that products such as hydroxynonenal formed due to oxidative stress could not be detoxified in testicles. Therefore those individuals can have more tendency to infertility.

It was reported that testosterone level was lower and estradiol level was higher in seminal plasma when compared to blood (30). However, no correlation was found between blood and seminal plasma hormone levels in fertile males (31). It can be expected that concentrations of sex steroids are affected in individuals without GSTM1 gene allel because absence of GST enzyme in STF may cause insufficient transport of testosterone and estradiol to the cells. In that condition, the balance between seminal plasma testosterone or estradiol concentrations and their concentrations in the blood may be changed. Since both testosterone and estradiol are important in spermatogenesis GSTM1 null genotype may be a risk factor for male infertility.

In the present study when seminal plasma testosterone or estradiol level was compared between GSTM1+ and GSTM1 null genotypes in fertile and infertile male separately, no statistically significant difference was observed. These results may show that GSTM1 enzyme does not play a role in determining testosterone or estradiol levels in seminal plasma. Even though there is a positive relationship between serum and seminal plasma testosterone levels in infertile males and between serum and seminal plasma estradiol levels in fertile male, GSTM1 polymorphism had no effect on these correlations. Therefore, GST enzyme does not seem to have an important impact on steroid hormone levels. It is known that there was a positive relationship between seminal plasma and serum testosterone levels in infertile male (32), however there was no study that investigated the relationship between blood and seminal plasma testosterone and estradiol concentrations and GSTM1 polymorphism.

The fact that GSTM1 null genotype have no effect on seminal plasma testosterone and estradiol levels can be explained with compensatory increase in levels of other steroid binding proteins in absence of GSTM1 enzyme. However, in the present study, ABP, SHBG and free testosterone, free estradiol levels were not measured. Furtehmore we thought that the number of samples in groups was insufficient to reveal the little differences in hormone levels.

Consequently, the findings of the present study suggest that there is no relationship between testosterone or estradiol concentration in seminal plasma or serum and GSTM1 deletion polymorphism in fertile male. Even though GSTM1 gene polymorphism does not seem to be a genetic risk factor for seminal testosterone and estradiol in infertile males, further studies are needed in order to understand the role of GSTM1 in STF.

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