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Association between *GSTM1*, *GSTT1* Genes Variants and Some Physiological Parameters in Infertility Patients

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Abstract: Several studies refer to higher levels of oxidative stress are associated with infertility. The purpose of this study to know the genotype frequency of *GSTM1* and *GSTT1* genes in infertility patients and assest the relationship between the genes and some physiological parameters in infertility patients. Genotype (*GSTM1* and *GSTT1*) genes were determined by multiplex polymerase chain reaction (PCR). The result showed a significant increase in serum malondialdehyde (MDA), in unexplained Infertile (oligozoospermia and asthenozoospermia) groups compared fertile (Control) groups,. While,the result showed a significant decrease in serum glutathione (GSH) , in unexplained Infertile (oligozoospermia and asthenozoospermia) groups compared fertile(Control) groups, the percentage of genetic deletion in oligozoospermia samples as follows : The deletion of *GSTM1* ,*GSTT1* and null genotype (deletion of *GSTM*1 and *GSTT1*) are most common in infertility patients (oligozoospermia , asthenozoospermia) when compared to control group.. It could be concluded there use of measuring GSH and MDA activity to assess infertility patients as the first line deface. These parameters of the marker in infertility patients.

Keywords: Infertility, *GSTM1*, *GSTT1*, Malondialdehyde, Glutathione.

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Introduction

Infertility is a widespread problem worldwide. Infertility is the absence of pregnancy in the couple after a year to marry with an unplanned (1). It is a heavy burden on countless families, and affecting people both medically and psychosocially (2). In addition with important including psychological distress, social stigmatization, economic constraints, may lead to family separation (3). According to the World Health Organization (WHO) 72.4 million couples suffer from infertility worldwide (4). A male partner factor contributes to 40% of the cases of infertility (5). The cause may be related to a problem with the man, woman or both (6). Despite medical advances in the treatment of infertility, the problem could not be satisfactorily tackled so far for several reasons (7) in particular genetic causes (8).

Often this type of infertility is associated with a defect in some enzymes that play a key role in phase-II cellular detoxification and bioactivation reactions, and are generally considered to be ‘‘antioxidant’’ enzymes therefore, protection from oxidative stress is an important way to boost fertility (9).

Malondialdehyde (MDA) is the organic compound, it occurs naturally as an end product of lipid per oxidation (LPO) aftermath is a process where reactiveoxygen species degrade poly unsaturated lipids. The checking of MDA levels in different biological systems can be used as an important

pointer of lipid peroxidation both *in-vitro* and *in vivo* for various health disorderliness's (10, 11). Human sperm cells are particularly susceptible to oxidation of their plasma membranes in contrast with other cells due to the existence of a high level of polyunsaturated fatty acids (PUFA) in the membrane (Tavilaniet aGlutathione (GSH) is an important antioxidant in plants and animals . It is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals (12) : for example volatile organic solvents, physical agents chemical dusts and pesticides are implicated in infertility. (13).

This study was designed to detect. Study of Deletions Polymorphism of Glutathione S-transferases *GSTM1* and *GSTT1* genes in Iraqi patients with Oligozoospermia, Asthenozoospermia and fertile. Also of study the malondialdehyde (MDA) and glutathione in serum and seminal plasma of idiopathic male infertility men compare with fertile men.

Material and Methods

In this study, Patients have was selected according to clinical and laboratory examination. Men with oligozoospermia and asthenozoospermia were examined and diagnosed as related with infertility totally 50 blood samples were collected from oligozoospermic patients and infertility totally 50 blood samples were collected from asthenozoospermic patients, and 50 apparently healthy individuals (Control).

Blood Sampling, Venous blood samples (6ml) were collected from each for infertile men and fertile controls.

MDA Measurement in serum and seminal plasma

The serum obtained by putting the blood samples in tubes centrifuged at 5000 rpm for five minutes.

Seminal plasma was separated from spermatozoa by centrifugation at 3500 rpm for 15 minutes

The levels of MDA in serum were measured by the TBA method: Thiobarbituric acid assay-Lipid peroxidation is a complex process leading to the formation of various aldehydes including malonaldehyde (MDA) (14, 15).

Scientific basis (Principle). Principle, MDA formed from the oxidation of polyunsaturated fatty acids was identified as the product of Lipid peroxidation (LPO) that react with thiobarbituric acid (TBA), in coexisting trichloroacetic acid (TCA). the method of determination of MDA is based on the colorimetric reaction with thiobarbituric acid (TBA) forming pink color product, which can be measured by spectrophotometer at a wavelength of 532 nm.

GSH Measurement in serum and seminal plasma

Serum reduced glutathione (GSH) was measured by thiol concentration according to Ellman's method (16).

Scientific basis (Principle). This methods depend on the reaction of 5, 5-Dithio bis (2-nitrobenzioc acid) (DTNB) with aliphatic thiol compounds at pH8.0 to produce one mole of p-nitrothiol phenol anion permolethiol. Since this anion highly colored (ε=13600 at 412 nm), it can be used to measure the thiol concentration. Genetic analysis: The gstm1 and gstt1 genotypes were analyzed by multiplex PCR according to the protocol of Arand (17).

using a specific primer designed by NCBI .The primer was custom synthesized at Add Bio/ Korea Company as a lyophilized product. The following primers were used: *GSTM1*: F- (5-GAA CTC CCT GAA AAG CTA AAGC-3) R- (5-GTT GGG CTC AAA TAT ACG GTG G-3).GSTT1: F- (5-TTC CTT ACT GGT CCT CAC ATC TC-3) R- (5-TCA CCG GAT CAT GGC CAG CA-3) Albumin : F- (5-GCC CTC TGC TAA CAA GTC CTAC-3) R- (5-GCC CTA AAA AGA AAA TCG CCA ATC-3) (17).

The amplification reactions were carried out in a volume of 50 µl containing (25ng) DNA;10 mMTris-HCl; 50 mMKCl ; 1.5 mM MgCl2;200 µM (each) dATP, dCTP,dGTP and dTTP (Geneaid); each primer was at 20 pM and 2.5 unit of Taq polymerase (Geneaid). The amplification was carried out as: Initial denaturation at 95 oC for 3 min, 30 cycles in thermocycler (PCR Biorad T-100 Thermal cycler ,Biolinx/ India) as follow : 94 °C for 1 min.; 61°C for 1 min ; 72 °C for 1 min. and 5 min final extension for last cycle. The PCR products were analyzed on 2%Agarose gel electrophoresis to detect the absence or presences of these genes. Albumin gene used as internal control.

Statistical Analysis

The Statistical Analysis System-SAS (18) program was used to detect the effect of difference factors in study parameters. Least significant difference LSD test (Analysis of Variation-ANOVA) was used to significant compare between percentage (0.001 probabilities) this study.

Results

The internal control amplified Albumin fragment was 350 bp in length, whereas presence of the gstm 1 and gstt1 genes were identified by 215 and 480 bp fragments, respectively. Although these assays didnot distinguish between heterozygote and homozygote positive genotypes, they conclusively identify the null genotypes (Figure 1).

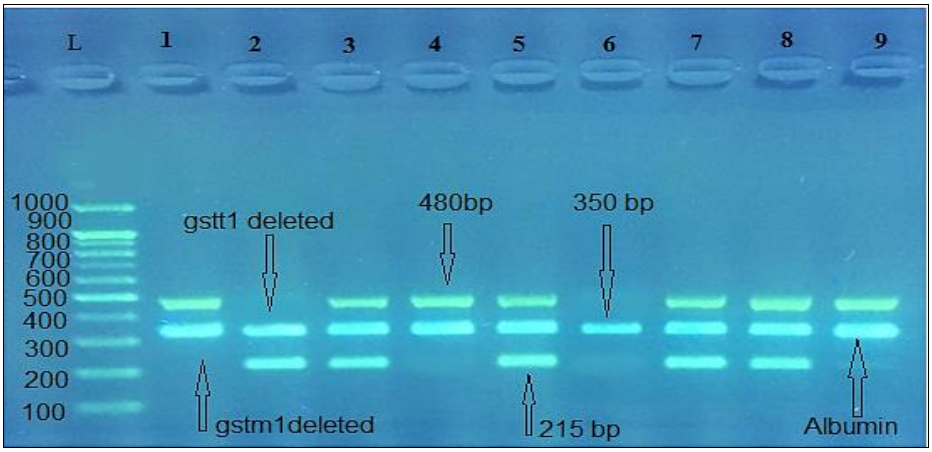

Figure (1): Electrophoresis of PCR products on agarose gel 1% (100 volt / 1 hr) . All samples belong to patients, Lane 1,4,9 : gstm1 deletion , Lane 2 : *gstt1* deletion, Lane 3 ,5,7,8: Normal genotype, Lane 6 : Null genotype , Lane L : DNA ladder (100 -1000 bp).

The results covered 100 cases of patients diagnosed as infertility patients (oligozoospermia,asthenozoospermia) and a 50 samples of controls .In the present study, observe significant different crude rates of the *GSTM1*,

GSTT1 and null genotype in infertility patients. Data of patients were distributed according to selected characteristics as major risk factors for infertility while control samples subjected to some of these criteria except for affected side as they were apparently healthy men.

The percentage of genetic deletion in oligozoospermia samples was 72% distributed as follows *:* *gstm1* deletion (38 %) , 16 % gsst1 were ,null genotype 18 % and 28 % were normal .While it were the percentage of genetic deletion in asthenozoospermia samples was 68 % distributed as follows: gstm1 deletion (34 %) , 14 % gsst1 were ,null genotype 20 % and 32 % were normal . For control samples, the percentage was: 84% normal, 8 % gstm1 deletion, 6 % *gstt1* deletion and 2 % null genotype. The gstt1 had a high percentage of deletion than gstm1 and the null genotype. (Table 1).

Table (1): Distribution of GSTM1 and GSTT1 genotypes among control subjects with infertility.

Group	Total	gstm1 deletion	gstt1 deletion	Null genotype	Normal
Oligozoospermia	50	19 (38 %)	8 (16 %)	9 (18 %)	14 (28%)
Total& %	50	NO 36 & 72 %			NO 14 & 28%
Asthenozoospermia	50	17 (34 %)	7(14 %)	10(20%)	16 (32 %)
Total& %		NO 34 & 68 %			NO 16 & 32%
Control	50	4(8 %)	3(6 %)	1 (2 %)	42 (84 %)
Total& %		NO 8 & 16 %			NO 42 & 84%

MDA and GSH level of serum

The result of the table (2) showed a significant increase in serum malondialdehyde (MDA), in unexplained Infertile (oligozoospermia and asthenozoospermia) groups compared to fertile (Control) group (7.12 ± 0.09, 6.86 ± 0.07,3.05 ± 0.05,respectively). While, the result of GSH level showed a significant decrease in unexplained Infertile groups compared to fertile (Control) groups (6.00 ± 0.05,6.11 ± 0.04, 9.71 ± 0.08, respectively).

Table (2): The level of serum MDA and GSH in studies groups.

Group	Mean ± SE	
	MDA (µmol/l)	**GSH (µmol/l)**
Control	3.05 ± 0.05 c	9.71 ± 0.08 a
Oligozoospermia	7.12 ± 0.09 a	6.00 ± 0.05 b
Asthenozoospermia	6.86 ± 0.07 b	6.11 ± 0.04 b
LSD value	0.242 **	0.173 **
P-value	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

MDA and GSH level of semen

The result of table (3) showed a significant increase in seminal plasma Malondialdeyde(MDA) in oligozoospermia and asthenozoospermia groups compared to fertile (Control) group (6.23 ± 0.05, 6.10 ± 0.04, 3.08 ± 0.06,respectively).

While,the result showed a significant decrease in serum

glutathione (GSH), oligozoospermia and asthenozoospermia groups compared to fertile(Control) group (7.19 ± 0.07, 6.93 ± 0.07, 11.13 ± 0.08,respectively).

Table (3): The level of semen MDA and GSH in studies groups.

Group	Mean ± SE	
	MDA (µmol/l)	GSH (µmol/l)
Control	3.08 ± 0.06 b	11.13 ± 0.08 a
Oligozoospermia	6.23 ± 0.05 a	7.19 ± 0.07 b
Asthenozoospermia	6.10 ± 0.04 a	6.93 ± 0.07 c
LSD value	0.145 **	0.227 **
P-value	0.0001	0.0001

Means having with the different letters in same column differed significantly. ** (P≤0.01).

Discussion

Genetic variations within selected GST genes may influence the infertility risk due to exposure to carcinogen compounds toxic may lead to decreasing the ability to detoxify them (19). This increased risk may be partially attributed to exposure to pollution infertility and altered the capacity to metabolically detoxify hazardous compounds (20).

Numerous previous studies have investigated the role of these GST polymorphisms in relation to infertility susceptibility. Or any significant single gene effects among *GSTM1* and *GSTT1* on infertility risk among all subjects (21).

Safarinejad, *et al* (22) reported a moderate increase in the infertility risk associated with the *GSTM1* gene deletion for Caucasian men However, *GSTM1* and *GSTT1* deletion were significantly associated with infertility (22). This result agree with (23) which stress edthe amounting body of evidence that links infertility and cigarette use, deletion some gene was associated with increase infertility Other study, which they found that deletion gene is associated with a higher risk of infertility (24,25,26).

Increased MDA concentration in idopathic Infertile coald be due to increased production of reactive oxygen species and hence more lipoxidation products. Increased levels of MDA associate with a variety of acute and chronic pathophysiological processes in human beings that related to increase of free radical generation occurs in states of tissue hypoxia, hyperoxygenation, various Infection, chronic diseases and metabolic disorders (27). The lower level of serum GSH in oligozoospermia and asthenozoospermia found to be associated with the increase in lipid peroxidation, and free radicals generation for many causes which sure decreases the levels of antioxidants in the serum as a results of their constant destruction during the neutralization of these free radicals (28) which indicates that the antioxidant defense system was impaired in oligozoospermia and asthenozoospermia subjects, compared with control. on the other hand, it is suggested that the increase in the levels of MDA in the serum is linked to the increase in the levels of exogenous free radicals generated from pollution such as cigarette smoke that ultimately leads to lipid peroxidation (29). Many studies have reported a decreased in GSH concentration in groups oligozoospermia and asthenozoospermia may be return to the decreased in NDPH Co–enzyme which consider as reduction substance

and essential in build Glutathione or as cataleptic substance for Glutathione Reductase enzyme which work to return Glutathione from GSSH form to GSH (30,31).

Several Studies suggested that detection of MDA concentrations in seminal plasma has an indicative considered on the diagnosis of male infertility induced by overproduction of reactive oxygen species in male reproductive system (32).

Many studies have reported a decreased in GSH concentration in groups oligozoospermia and asthenozoospermia when compared with fertile (control) groups. Also, the results were agreed with other studies who study sterility and its relationship with oxidative enzymes (33, 34).

Conclusion

The deletion of *GSTM1*, *GSTT1* and null genotype (deletion of *GSTM1* and *GSTT1*) were most common in infertility patients (oligozoospermia, asthenozoospermia) when compared with control group.

Present study demonstrates there is increased oxidative stress in Serum and seminal plasma in idiopathic infertile male compared to fertile male. This study also demonstrates the increased antioxidant level malondialdehyde (MDA) and decreased level glutathione (GSH) in idiopathic infertile male compared to fertile. Thus, malondialdehyde and glutathione can serve as an important marker may indicate the prognosis or severity of infertility.

This study found a relationship between GSH and MDA levels of seminal plasma with semen quality. Therefore, MDA and GSH might have some fertility enhancing role by reducing lipid per-oxidation. It could therefore be proposed that the concentration of reduced glutathione, a non-enzymatic antioxidant of seminal plasma, could be used as a chemical parameter to assess male fertility.

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