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The Impact of *GSTM1*, *GSTT1*, and *GSTP1* Polymorphisms on Male Infertility in Basrah Province, Iraq

Ali Ahmed[1], Ayat Al-laaeiby[1*], Safaa Taha Yassen[2]
[1]*Biology Department, College of Science, University of Basrah, Basrah, Iraq*
[2] *University of Basrah\AL-Zahra Medical school*

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Abstract

Infertility is a global health issue presenting both biological and social implications for males and females. Current literature concerning male infertility has been linked to a reduction of glutathione levels in the seminal plasma of men. Despite a growing body of literature on global infertility, there is currently little genetic research investigating the association of genetic polymorphisms with male infertility in Iraq. This study aims to explore the association of Glutathione-s-transferase gene polymorphisms (*GSTM1*, *GSTT1,* and *GSTP1*) with Iraqi male infertility. Single and double gene test analyses were performed to study the differences between study groups. Genetic analysis of the target genes *GSTM1*, *GSTT1,* and *GSTP1* were carried out. Statistical analysis of single gene testing revealed that there was no significant difference between the control and infertile case groups, considering *GSTM1* null, *GSTT1* null, and *GSTP1* genotype. Further analysis of double genes revealed a significant difference between patients and the control group with the *GSTM1*(+)\CC (*P*-value=0.04; OR=3.2; 95%CI=1.1-8.7) or *GSTM1*(+)\TC+CC genotypes (OR:3.5;95%CI: 1.2-10.03; *P*-value=0.02; 95%CI=1.2-10.03). Collectively, the results of this study showed that single gene analysis of the *GSTM1* and *GSTT1* null genotypes showed no association with the risk of developing male infertility. However, the results revealed that the *GSTP1* genotype assumes a protective role when combined with the present *GSTM1* genotype.

Keywords: Glutathione S transferase, Infertility, Iraq, Male, Polymorphisms.

التاثير المشترك لتعدد اشكال جينات *GSTM1*, *GSTT1* and *GSTP1* على خصوبة الرجال في العراق

علي احمد[1], ايات اللعيبي[1*] , صفاء طه ياسين [2]
[1]قسم علوم الحياة, كلية العلوم, جامعة البصرة, البصرة, العراق
[2]كلية طب الزهراء, جامعة البصرة, البصرة, العراق

الخلاصة

يُعد العقم مشكلة صحية عالمية لها آثار بيولوجية واجتماعية على الذكور والإناث على حد سواء. اثبتت الابحاث المهتمه بدراسة العقم عند الذكور بانخفاض مستويات الجلوتاثيون في البلازما المنوية لدى الرجال. على الرغم من وجود مجموعة متزايدة من الدراسات المتعلقة بالعقم عند الذكور في العالم. إلا أنه لا يوجد

*Email: ayat.esmaeel@uobasrah.edu.iq

حالياً سوى القليل من الأبحاث الوراثية التي تبحث في ارتباط تعدد الأشكال الوراثية بعقم الذكور في العراق. ولذلك، تناولت الدراسة الحالية ارتباط تعدد الأشكال الجينية ل *GSTM1*و *GSTT1* و *GSTP1* لأنزيم الجلوتاثيون–ترانسفيراز وعلاقتها بالعقم عند الذكور في العراق. تم إجراء التحليل الجيني للجينات من خلال دراسة طفرة الحذف لكل من *GSTM1* و *GSTT1* والانماط الجينية ل *GSTP1* كشف التحليل الإحصائي لاختبار الجين الواحد عن عدم وجود فرق معنوي بين المرضى وعينات السيطرة للجينات الثلاثة السابقة. عند دراسة تاثير الجينات المزدوجة اظهرت النتائج فرق معنوي بين المرضى والمجموعة السيطرة مع الأنماط الجينية لـ*GSTM1*(+)\CC (*P*–value=0.04; OR=3.2; 95%CI=1.1–8.7) أو *GSTM1*(+)\TC+CC. (*P*–value=0.02; 95%CI=1.2–10.03; OR:3.5;95%CI: 1.2–10.03) استنتجت الدراسة عدم وجود ارتباط معنوي بين طفرة الجينات المفردة *GSTM1* و *GSTP1* *GSTT1*وخطر الإصابة بالعقم عند الذكور. في حين اشارة النتائج أن النمط الوراثي *GSTP1* يقوم بدور وقائي عندما يقترن بالنمط الوراثي *GSTM1* الطبيعي .

1. Introduction

Infertility is a health condition that indicates the inability of a couple to conceive a child, and it affects both females and males. The recent increase in research concerning the pathophysiology of infertility has rectified past social and medical prejudices regarding infertility, which were previously attributed solely to females[1]. Brugo-Olmedo *et al*. have placed specific parameters to define infertility as a disease of the reproductive system that disables couples from successfully fulfilling pregnancy following a reasonable time of sexual intercourse that does not involve any form of contraception [2]. According to Agarwal *et al*., a meta-analysis of global infertility cases revealed there is an inconsistency in the percentage of infertility cases globally, and it varies from one continent to the other [3]. The highest rate of prevalence of male infertility was recorded in Africa and Central and Eastern Europe [3]. Globally, infertility affects around 10-15% of couples [4]. A previous study conducted in Ramadi, located in western of Iraq, indicated the significant incidence of primary infertility [5]. In a study conducted by Alnajjar *et al*. in the north of Iraq, Kirkuk city, it was found that the majority of infertile males were between the ages of 26 and 35. These individuals had azoospermia with primary infertility [6]. According to Al-Jebouri, the incidence of infertility between 2003-2013 was higher than in 1980-1990, concluding that this rise may be connected to the stress experienced by people due to war [7].

Male infertility is considered to have a multifactorial aetiology, with a variety of factors contributing to its development, including, but not limited to, genetic, environmental, clinical, and lifestyle factors. Research conducted by Alahmar *et al*. into possible causes of DNA damage that results in male infertility reviews the association of Oxidative Stress (OS) to male infertility [8]. OS is defined as a chemical disturbance in the balance between Reactive Oxygen Species (ROS) production, such as free radicals and protective antioxidant defenses, resulting in cellular and tissue damage [9]. The antioxidant defense system protects cells by detoxifying ROS to prevent any damage[10]. This protective role involves both enzymes such as catalase, Superoxide Dismutase (SOD), and peroxidases as well as non-enzymatic systems, which includes the elimination of oxidants by vitamin C, vitamin E, and glutathione [11].

Glutathione is an antioxidant produced by the liver, containing non-protein thiol, and is composed of the amino acids glutamine, cysteine, and glycine [12]. Glutathione was obtained by purifying somatic and gamete cells, observing an association between male infertility and reduced levels of glutathione in the seminal plasma[13]. The protective role of glutathione in semen against ROS, which influences sperm motility and causes sperm DNA damage, was improved previously [10,14]. Glutathione plays a significant role in the process of

detoxifying xenobiotics by conjugating them with xenobiotics and converting them into a more soluble form, which can then be eliminated from the body[14].

The association of glutathione S transferase gene polymorphisms was investigated in different regions such as China, Egypt, and Iran [11,12,14,15,16]; however, the results were inconclusive. There is currently little research specific to infertility in males of Iraqi origin compared to global infertility data. Therefore, the aim of this study is to investigate the effect of glutathione S-transferase genes, *GSTM1*, *GSTT1,* and *GSTP1* on the development of male infertility. This will be achieved through conducting a statistical analysis of the primary research data obtained. It was hypothesized that genetic polymorphisms of *GSTM1*, *GSTT1*, and *GSTP1* genotypes are associated with the susceptibility of male infertility.

2. Materials and Methods

The methodology component of this research comprises two subdivisions. The first is the primary data collection and genetic analysis. The second is the statistical analysis of the data obtained.

2.1 Samples collection

The design of the study is case control, which involves comparing blood samples obtained from 50 males clinically diagnosed with infertile to 50 fertile males who serve as the control group. Blood samples were collected from both groups at Basrah Specialist Children and Women Hospital in Basrah province of Iraq from November 2020 through March 2021. Participants of cases and controls were selected randomly. The samples obtained were transferred to the laboratory in Ethylenediaminetetraacetic Acid (EDTA) tubes and stored at 20 C°. Formal consent in written agreement was obtained from all participants involved, and a thorough briefing of the study and its aims were provided. Participants were provided with a questionnaire to answer. All participants remained anonymised, which was in line with patient confidentiality and privacy. Ethical approval has been approved under the reference (7/54/573) date 27.1.2022.

2.2 Genomic DNA extraction

Genomic DNA was isolated using the Geneaid kit following the manufacturer's instructions (https://www.geneaid.com/Reagent-DNA-Extraction/GEB).

2.3 Polymerase Chain Reaction (PCR)

Multiplex PCR was used to amplify *GSTM1* and *GSTT1* genes using 5′-CTGCCCTACTTGATTGATGGG-3′ and 5′-TGGATTGTAGCAGATCATGC-3′ for *GSTM1* gene and 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGATCATGGCCAGCA-3 for *GSTT1* gene meanwhile human β-globin was used as internal control 5′-ACACAACTGTGTTCACTAGC-3′ and 5′-CAACTTCATCCACGTTCACC-3′. The PCR reaction mixture contained 12.5 µl of Green master mix (2X, Promegs), 1 µl of forward and reverse primer (10 µM,) 50 ng of genomic DNA, and the total volume made up to 25µl by nuclease-free water. PCR condition for amplifying target genes was three essential steps: initial denaturation at 95 ˚C for 5 min, followed by 35 cycles including denaturation at 95 ˚C for 45 sec., annealing at 58 (for multiplex PCR) and 60 °C (*GSTP1* gene fragments) ˚C for 45 sec and extension at 72 ˚C for 45 sec followed by one more cycle final extension at 72 ˚C for 10 min.

The *GSTP1*Ile\Val polymorphism was investigated using the Polymerase Chain Reaction-Restriction Fragment Length Polymerase PCR-RFLP technique[16]. The amplification was carried out using the amplicon amplify with primer pairs 5′-GTAGTTTGCCCAAGGTCAAG-3 and 5′-AGCCACCTGAGGGGTAAG-3′. The PCR

products (50 infertile and 47 control) of *GSTP1*Ile\Val were sent for sequencing (https://dna.macrogen.com/), and the polymorphism was analysed. The sample sequences were digested virtually with *Alw26*I using the Snapgene software. The DNA sequence was submitted to the Snapgene software, and the restriction site of *Alw26*I was determined within the sequence. Three genotypes were characterised by the number of fragments identified. Individuals carrying the homozygous Ile/Ile showed two fragments; homozygous Val/Val showed three fragments, whereas heterozygous Ile/Val individuals were distinguished by the overlapping sequencing peaks.

2.4 Statistical analysis

The primary data results were analysed statistically using the Statistical Package for the Social Sciences (SPSS Statistics) software version 16 (IBM Corp., 2007). The normality of the data was investigated using the *p*-value, which was smaller than 0.05, indicating that the data were not normally distributed. Both Chi-squared and Fisher's exact test were used to determine the statistical significance of the results, using the P-values obtained at the 95% confidence interval. Odd ratio (OR) and 95% confidence intervals (CI) had investigated the strength of association of gene polymorphisms between cases and control.

The odds ratio provides a measure of the likelihood of infertility associated with specific gene polymorphisms in comparison to a control group, whereas the 95% CI offers a range in which the true odds ratio is expected to fall with 95% confidence. An OR greater than 1 suggests a positive association, while an OR less than 1 suggests a negative association.

3. **Results**

3.1 Donors characterization

Table 1 summarises the demographic characteristics of infertile participants and the control group. The mean age was 38.5±2.5 years (St.dv=1.05). No significant difference was observed between infertile participants and controls in terms of age groups, occupations, and academic levels.

Table 1: Demographic features of the study population

Characteristics	Patients(%)	Control(%)	P-value	OR	95%CI
Age groups					
18-24	6(12.0%)	6(12.0%)			
25-31	23(46%)	20(40.0%)			
32-38	11(22.0%)	18(36.0%)	0.7		
39-45	6(12.0%)	4(25.0%)			
46-52	2(2.7%)	1(2.0%)			
53-59	2(2.7%)	1(2.0%)			
Occupations					
Employed	18(36.0%)	15(30.0%)	0.6	1.3	0.56- 3.02
Unemployed	32(64.0%)	35(70.0%)			
Academic levels	Academic levels	Academic levels			
Illiterate	2(4.0%)	2(4.0%)			
Primary school	26(52%)	24(48.0%)	0.3		0.028-0.3
Secondary school	15(30.0%)	10(20.0%)			
Graduate	7(14.0%)	14(28.0%)			
Types of male infertility					
Azoospermia	18(36.0)				
Oligospermia	5(10.0)				
Asthenospermia	9(18.0)				
Oligoasthenospermia	15(30.0)				
Idiopathic infertility	3(6.0)				

***p*-value≤ 0.05 No significant differences**

3.2 The frequency of single GSTM1, GSTT1 and GSTP1 genes polymorphisms among infertile and fertility males

The distribution of *GSTM1*, *GSTT1,* and *GSTP1* genotypes amongst cases and control groups is presented in Table 2. The frequency of the *GSTM1* null genotype in the infertile participants (24%) was slightly lower than that of the control group (34%). The prevalence of the GSTT1 null genotype in the infertile participants showed only a little difference compared to the control groups, with rates of 8% and 4%, respectively. The frequency of the *GSTP1* genotype was investigated, and the results revealed that the wild-type TT (Ile\Ile) genotype is higher in the infertile cases, with (62%) compared to (44%) in the control group. The combination of the homozygous CC (Val\Val) and heterozygous TC (Ile\Val) were 38% and 55% in the infertile cases and control group, respectively. The statistical analysis observed no significant difference in the distribution of genes among the infertile participants and controls.

Table 2: The frequency of Glutathione S transferase genes (*GSTM1*, *GSTT1,* and *GSTP1*) among cases (infertile males) and control (fertile males) groups.

Genotype		Cases(%)	Control(%)	P-value	OR	95%CI
GSTM1	Present (+)	38(76)	33(66.0)	0.27	1.6	0.68-3.909
	Null(-)	12(24)	17(34.0)			
GSTT1	Present (+)	46(92)	48(96.0)	0.6	0.4	0.084-2.74
	Null(-)	4(8)	2(4.0)			
GSTP1	TT	31 (62)	21 (44)			
	CC	3 (6)	8(17)	0.1	2.9	0.78- 11.07
	TC	16 (32)	18(38)	0.2	1.77	0.7-4.27
	CC or TC	19(38)	26(55%)	0.87	2.02	0.89-4.5

Statistically significant (P<0.05)

GSTP1 TT (Ile\Ile)

GSTP1 CC (Val\Val) C)

GSTP1 TC sequences (Ile\Val)

GSTM1 Glutathion S-transferase mu 1

GSTT1 Glutathion S-transferase Theta 1

GSTP1 Glutathion S-transferase pi 1

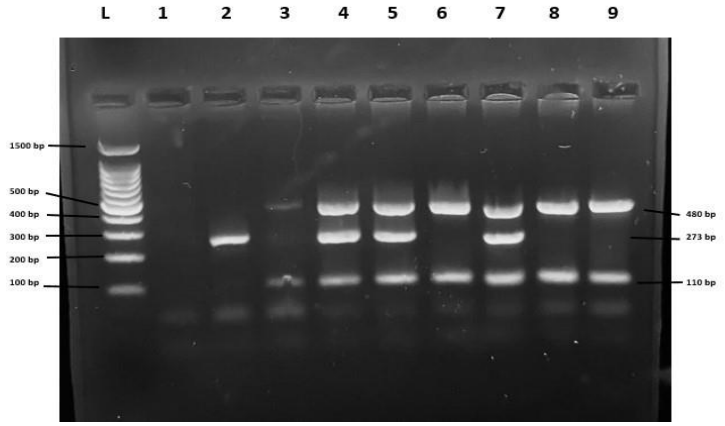

Figure 1: PCR product of *GSTM1* and *GSTT1* genes separated on gel electrophoresis stained with ethidium bromide. Lane L is a 100 bp ladder; Lane 1 represents control; Lane 2 is GSTT1 null genotype; Lane 3-5,7 are normal individuals with three bands, while Lane 8&9 are samples with null *GSTM1*.

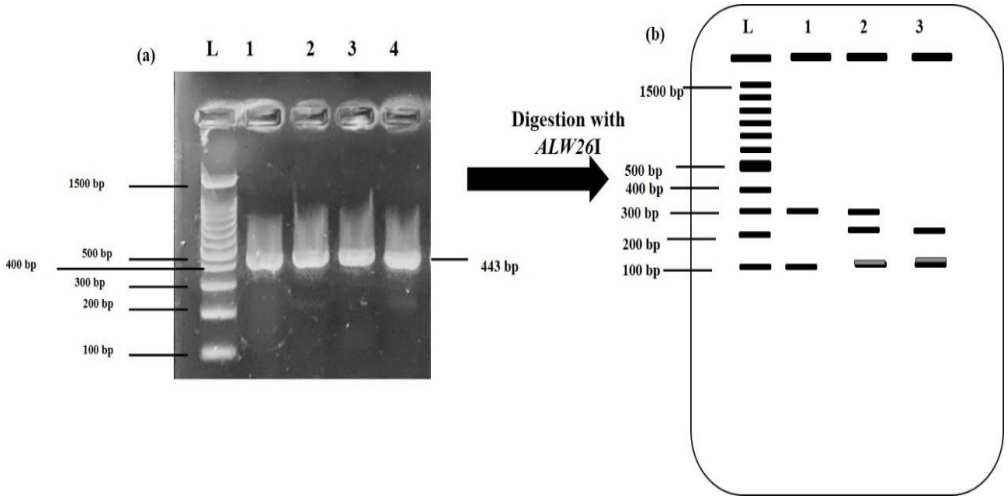

Figure 2: Gel electrophoresis of the *GSTP1* gene. a) PCR products were separated on agarose gel stained with ethidium bromide. Lane L is the ladder, while lane 1,2,3,4 represents amplified fragments size 443bp. b) The estimated size of *GSTP1* sequences subjected to virtual digestion by *ALW26*I restriction enzyme using the Snap-gene software.

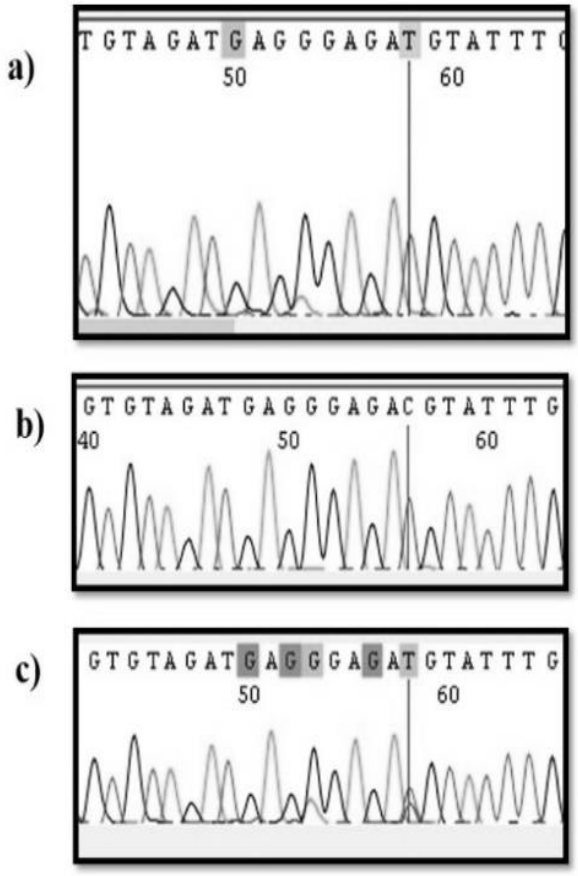

Figure 3: Sequencing of *GSTP1* gene amplified fragments of cases and control groups. A) *GSTP1* TT sequences (Ile\Ile) B) *GSTP1* CC sequences (Val\Val) C) *GSTP1* TC sequences (Ile\Val).

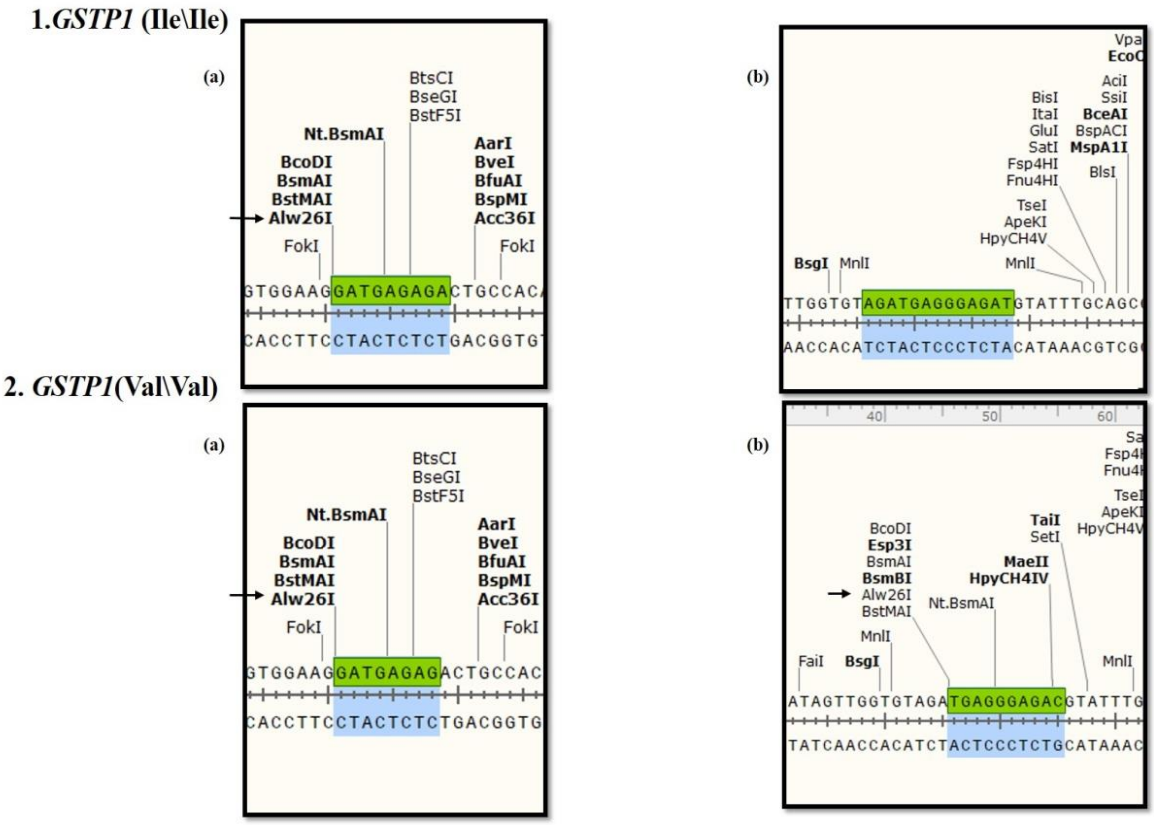

Figure 4: The restriction sites in the sequences of different genotypes determined by the Snap gene software. 1) Image A shows the restriction enzyme *ALW26*I cut the sequence (arrow points *ALW26*I) in one site showing Ile\Ile homozygous genotype, while image B shows no restriction site (T-T). 2) Images A and B show the restriction enzyme *ALW26*I digested the sequence (arrow points *ALW26*I) at two sites showing Val\Val homozygous genotype (C-C).

3.3 The frequency of combined polymorphisms of GSTM1, GSTT1 and GSTP1 genes among infertile and fertility males

Table 3 displays the effect of the combined genes, presenting four possibilities that arise from the gene combination. The frequency of presence for the genes *GSTM1* and *GSTT1*(+) in the infertile cases and control group was 73.9% and 62.6%, respectively. Neither the control nor infertile cases presented an absence of both GSTM1(-) and GSTT1(-) genes. The frequency of individuals who carried the *GSTM1*(+) and *GSTT1* null genotype was slightly higher in the infertile cases compared to the control group. 26.1% and 35.4% of infertile cases and controls had *GSTM1* null genotype and *GSTT1*. No statistically significant difference was observed after studying the gene combinations among infertile males and controls.

Table 3: The distribution of combined genotype of *GSTM1* and *GSTT1* genotype amongst infertile cases and control group.

Genotype	Cases (%)	Controls (%)	*P*-value	OR	95% CI
GSTM1 & *GSTT1*(+)	34(73.9)	31(62.6)			
GSTM1(+) & *GSTT1*(-)	12(26.1)	17(35.4)	0.3	0.54	0.09-3.2
GSTM1(-) & *GSTT1*(+)	4(100)	2(100)	0.5	1.5	0.64-3.76
GSTM1(-) & *GSTT1*(-)	0(0%)	0(0%)	-	-	-

Statistically significant (P<0.05)

***GSTM1(+)* & *GSTT1*(+)= Presence no deletion**

***GSTM1*(-) &*GSTT1*(-)= Null genotypes**

3.4 The influence of double GST genotype combination on infertile case and control groups

Table 4 shows the frequency of the combined genotypes *GSTP1* and *GSTM1*. The frequency of *GSTP1* wild type (TT) and *GSTM1* (+) was higher amongst the infertile cases than the control group, with rates of 87.1% and 43.3%, respectively. The frequency of infertile cases carrying the GSTP1 wild type (TT) and null GSTM1 genotype was (33.3%), i.e., lower than that of the control group, which was 47.1%. Combining *GSTP1* (CC) with *GSTM1*(+), the risk was significantly increased (*P*-value=0.04; OR=3.2). The frequency amongst the infertile participants was lower than the fertile group carrying *GSTM1* (+) and *GSTP1*(TC) or (CC) with significant differences (P-value=0.02; OR=3.2 95%CI=1.1-8.7.

Table 4: The distribution of combined genotype of *GSTP1* and *GSTM1* genotype amongst infertile cases and control group.

Genotype	Cases (%)	Control (%)	*P*-value	OR	95% CI
GSTM1(+)\TT	27(87.1)	13(43.3)			
GSTM1(+)\CC	1(2.6)	4(13.3)	0.04*	3.2	1.1-8.7
GSTM1(+)\TC	10(26.3)	13(33.8)	0.06	2.7	0.93-7.77
GSTM1(+)\CC+TC	11(28.9)	17(56.7)	0.02*	3.2	1.1-8.7
GSTM1(-)\TT	4(33.3)	8(47.1)	0.5		
GSTM1(-)\CC	2(16.7)	4(23.5)	1	1	0.12-7.99
GSTM1(-)\TC	6(50)	5(29.4)	0.3	0.4	0.07-2.25
GSTM1(-)\CC+TC	8(66.7)	9(52.9)	0.7	0.5	0.12-2.6

***Statistically significant (P<0.05)**

The combination of the *GSTT1* and *GSTP1* genotypes was investigated, and the frequency of *GSTT1* present and *GSTP1* wild type (TT) was 63% in the infertile cases and 43.5% in the control group (Table 5). The frequency of *GSTT1* present and *GSTP1*/TC was rare in infertile cases and the control group (30.4% and 39.1%, respectively). The results revealed that the combined *GSTT1* and *GSTP1* genotypes showed no significant difference between the two groups.

Table 5: The distribution of the combined genotypes *GSTP1* and *GSST1* amongst infertile cases and the control group.

Genotype	Cases (%)	Control(%)	***P*-value**	OR	95% CI
GSTT1(+)\TT	29(63)	20(43.5)			
GSTT1(+)\CC	3(6.5)	8(17.4)	0.06	3.8	0.9-16.38
GSTT1(+)\TC	14(30.4)	18(39.1)	0.79	0.8	0.3- 2.18
GSTT1(+)\CC+TC	17(89.5)	26(100)	0.9	1.05	0.45-2.4
GSTT1(-)\TT	2(50)	1(100)			
GSTT1(-)\TC	2(50)	0(0)	0.5	0.3	0.008-12.8
GSTT1(-)\CC+TC	2(10.5)	0(0)	1	0.6	0.3-1.4

Statistically significant (P<0.05)

3.5 The influence of triple GST genotype combination amongst infertile cases and control groups

Table 6 presents the frequency of the *GSTM1* and *GSTT1* genotypes combined with the *GSTP1* genotype. The frequency of individuals with the presence of both *GSTM1* and *GSTT1*

and TC+CC (homozygous Val\Val and heterozygous Ile\Val) was higher in the control group 58.6% than in the infertile cases, 28.6%, showing a statistically significant between infertile and control groups (OR:3.5;95%CI: 1.2-10.03; *P*-value=0.02).

Table 6: Combined effect of triple GST genotypes among cases and control groups.

Genotype	Cases	Control	P-value	OR	95%CI
GSTM1(+)&*GSTT1*(+)\TT	25(71.4)	12(41.4)			
GSTM1(+)&*GSTT1*(+)\TC+CC	10(28.6)	17(58.6)	0.02*	3.5	1.2-10.03
GSTM1(+)&*GSTT1*(-)\TT	2(28.6)	1(100)			
GSTM1(+)&*GSTT1*(-)\TC+CC	1(100)	0(0)	1	0.66	0.3-1.48
GSTM1(-)&*GSTT1*(+)\TT	4(33.3)	8(47.1)			
GSTM1(-)&*GSTT1*(+)\TC+CC	8(66.7)	9(52.9)	0.7	0.56	0.12-2.6

***Statistically significant (P<0.05)**

4. Discussion

This study investigated the association of glutathione-S-transferase gene polymorphisms with male infertility in Iraqi individuals from Basrah province. Despite global studies examining the association of glutathione to the development of male infertility [16, 17,18], there is only a few previous research on the effect of glutathione-S-transferase polymorphisms (GSTM1, GSTT1, and GSTP1) genes on infertility in Iraqi males.

In this study, the frequency of the *GSTM1* and *GSTT1* null genotypes was 34% and 4% in the control group, respectively, while 24% of infertile males lacked the *GSTM1* and only 8% with the GSTT1 null genotype. Recently, Al-Mashadani *et al*. found that 35% of infertile males with the null *GSTM1* genotype and 20% lack *GSTT1*[19]. A previous study conducted by Al-awadi *et al*. determined the frequency of the *GSTM1* and *GSTT1* null genotypes to be 13.1% and 6.9%, respectively, amongst their participants[20]. Differences between the frequencies obtained in this study and those of Al-awadi *et al*. may be attributed to the regional differences of the participants involved. Al-awadi *et al*. included participants from central and southern Iraq, whereas the entirety of this study's participants were from Basrah, the southern province. In a study conducted using participants of Mexican origin, the frequency of the *GSTM1* null genotype was 42.6% compared with the 9.3% frequency of the *GSTT1* null genotype[21]. In their study, Hatagima *et al.* reported the frequency of *GSTM1* null genotype in Europeans and Africans to range between 42-60% and 16-36%, respectively, whilst being 42-54% amongst Asians[22]. The distribution of *GSTT1* null genotype in Caucasians and Asians was 13-26% and 35-52%, respectively [22].

The results of this study revealed that the frequency of the *GSTM1* null genotype in the control group was higher than that of the infertile group, with rates of 34% and 24%, respectively. This is in comparison to the *GST11* null genotype, which was lower in the control group, 4%, compared to 8% in the infertile group. Despite this difference, statistical analysis has revealed the results to bear no statistical difference between the infertile and control groups regarding the frequencies of the *GSTM1* and *GSTT1* null genotypes. Xiog *et al*. found that the distribution of the *GSTM1* null genotype in the infertile males and the controls was 51.6% and 50.9%, respectively, whilst the *GSTT1* null genotype frequency was 52% amongst the infertile groups compared to 47% in the control group[16]. The results of this study are, therefore, congruent to that of the current literature relating to the statistical insignificance of developing male infertility as a result of *GSTM1* and *GSTT1* null polymorphisms[5, 23]. Furthermore, Roshdy *et al*. conducted a study on Egyptian males in

which they found the presence of the *GSTM1* null genotype to have no significant difference between fertile and infertile males [17]. In contrast to the findings of our results, the *GSTM1* and *GSTT1* polymorphisms were found to be significantly associated with male infertility in studies conducted by Wu and Safarinejad *et al*..[22,14]

The frequency of the wild-type homozygous (Ile\Ile ;TT) genotype was 62% in the infertile group, whereas 44% was reported to be in the control group. The frequency of homozygous and heterozygous (CC+TC) was higher in the control group than in the infertile group, being 55% and 38%, respectively. There is no significant association between *GSTP1* genotype and the risk of developing male infertility. In a study conducted by Al-Rubae *et al*. the results determined that 54.55% of individuals carry the *GSTP1* (Ile/Ile) genotype, 44.55% with *GSTP1* (Ile/Val) and 0.91% with the *GSTP1* (Val/Val) genotype [25]. A study, conducted by Al-Rubae *et al*., involved both male and female participants from central Iraq [25]. Further literature supports the findings of this study, including research conducted on the Iranian population by Safarinejed *et al*. [24]. The results of their study showed that the frequency of wild-type Ile\Ile is higher amongst infertile cases (61.5%) than control cases (51.8%), and the combined genotype (homozygous Val\Val and heterozygous Ile\Val) was higher in the control group (48.2%) than the infertile group (38.6%) [24]. The findings of this study revealed that there is no significant association between the presence of the *GSTP1* genotype and the risk of developing male infertility. In concordance with previously conducted meta-analysis studies, our results stated there is no association between *GSTP1* Ile105Val polymorphism and male infertility [26]. Unlike Xiong *et al*., *GSTP1* Ile105Val polymorphism is associated with male infertility[27]. This study highlighted the double and triple combined effect of the GST genes. The results revealed that there is a significant association in patients carrying *GSTM1*(+)\CC or GSTM1(+)\CC+TC. Similarly the risk was increased more than three-fold in the patients carrying GSTM1(+) & GSTT1(+)\TC+CC genotype. Safarinejad *et al*. suggested that *GSTP1* heterozygous Ile\Val genotype assumes a protective role. The protective role of the *GSTP1*(Val\Val) genotype was suggested to be in patients with bronchial asthma[28].
However, caution must be taken when analyzing these data because of the small sample size and the limited number of polymorphisms that were included in this study. So, to make a decision on whether to accept or reject this suggestion, it is necessary to conduct genomic-based studies using larger sample sizes.

The above genes were selected due to their role in detoxification. The GSTs catalyse the metabolism of xenobiotics[29,30]. GST genes detoxify specific substrates and are involved in various mechanisms of biological detoxification [30]. The inconclusive data may be related to variations in race, differences in study design and analysis, and disparities in geographical origin.

The complexity of spermatogenesis requires the involvement of many genes, such as *PRM2*[30,31]. The interaction of GST genes may influence the process of sperm production. Eight identified genes, GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT, and GSTZ, encoded eight classes of GSTs, including alpha, mu, kappa, omega, pi, sigma, theta, and zeta, respectively [29,31]. Moreover, the different types of male infertility may also affect the results of the study. A previous study suggested the overexpression of the *GSTM2* gene compensates for the lack of *GSTM1* activity[32]. It could be suggested that the interactions among genes may compensate for the role of other genes, so the impact of *GSTP1* becomes evident when the results are combined with the *GSTM1* gene.

In conducting this study, a few limitations arose due to the global COVID-19 virus outbreak. This ultimately resulted in the size of the sample for both the control and infertile groups being considerably small in comparison to that of existing research surrounding male infertility. It is recommended that further studies be conducted in a similar manner with larger sample sizes and the inclusion of another gene group.

Further limitations include a lack of generalisability of the results due to the entire participant population being from the province of Basrah, Iraq. This disables the results from being extrapolated and used elsewhere in the literature. In future studies, I would suggest interrogating study groups information, such as the family history of infertility and whether the infertility is primary or secondary. Finally, the authors countered difficulties in obtaining *ALW*26I restriction enzyme and performed the PCR-RFLP analysis practically in the lab owing to the Causal circumstance of Coronavirus pandemic quarantines.

Conclusion

This study suggested that there is an association between glutathione genes and male infertility. Studying the mechanism of infertility in males may contribute to developing a new treatment to address the problem. Studying the mechanism of infertility in males may contribute to developing a new treatment to address the problem. This study focused specifically on the population of southern Iraq, as few genetic studies have addressed male infertility in Iraqi society. This study concluded that there is no statistically significant association between male infertility and GST genes. Also, the study highlighted the influence of the combination genotype of GST genes in male infertility. In the future, more attention should be paid to investigating genetic factors and their association with male infertility.

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Conflict of interest

There were no conflicts of interest throughout the conduction of this study, so no hidden agendas that would affect the results.

Authors Contributions

This study was designed and conceived by Dr. Ayat Al-laaeiby. All the lab work had been carried out by Ali Ahmed. Dr. Ayat analysed the sequence and results statistically. Dr. Ayat wrote the paper to complete the write-up of the study. Participants, both patients and controls, were diagnosed under the supervision of Dr.Safaa Taha Yassen, the physician.

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