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Detection of genetic in some antioxidant genes in the identification of infertility males

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Abstract
Male infertility has started to increase very rapidly around the world. This increase in infertility comes with an increase in environmental pollutants and lifestyle factors, in addition to some genetic disorders in people living in Iraq. The aim of this research was to find out the causes of undiagnosed infertility and the extent to which genetic disorders affect infertility between genetic polymorphisms in the locus of two genes, namely, NRF2, and GST of both types (GSTT1, GSTM). In methods two distinct test tubes were used to collect semen and venous blood samples from each subject, one used for physiological analysis of semen and the other EDTA tube used for DNA extraction. Genomic DNA was isolated from blood from all samples from patients. The DNA concentration and purity were measured by Bio drop, and the DNA concentration was validated for the PCR reaction, using agarose gel electrophoresis, DNA quality was determined and stained with safe red DNA, at -20 °C, all samples were stored until further use. The current study showed a significant increase in physiological tests in infertile patients compared with the control group. In this study of the NRF2 gene, GST, the results of the study showed that all the samples that were studied from among the control group and male infertile males carry the heterogeneous genotype (AG) of the genetic mutation that affects the gene NRF2 at the locus rs35652124, and the distribution of the mutant genotype is (GG) and normal genotype (AA) were (0%), while the percentage of heterogeneous genotype (AG) appeared (100%). The study showed the polymorphism of the (GST) gene in terms of presence and deletion, and the results showed that there was a presence of the (GSTT1 and GSTM1) genes by 35%, a loss of the GSTT1 gene by 30% and a loss of the GSTM1 gene by 35% in infertile men compared to the control group. The presence of high genes (GSTT1 and 1GSTM) was 55% and loss of GSTT1 gene and low GSTM1 gene was 25% and 20%, respectively, compared to the control group

Keywords: infertility, Detection, GST, NRF2

Introduction
The World Health Organization (WHO) defines infertility as the failure of a couple to conceive after one year of regular unprotected intercourse [1]. Male infertility refers to the inability of a man to cause a female to become pregnant, and accounts for 40-50% of human infertility cases, affecting approximately 7% of all men [2].
ROS play a role in sperm function and fertilization The literature describes both the physiological and pathological role of ROS in fertility A delicate balance between ROS essential for physiological activity and antioxidants to protect against cellular oxidative injury is essential for fertility, although ROS are essential for fertility Reactivity is essential for the normal physiological function of sperm, but excessive oxidative stress can cause an increased susceptibility to DNA damage, which can lead not only to infertility, but also recurrent pregnancy loss or genetic mutations that cause childhood diseases [3]. Due to the high susceptibility of the unsaturated fatty acid content of sperm to oxidative stress, significant membrane damage can occur [4]. Free radicals have the ability to directly damage sperm DNA by attacking the purine and pyrimidine bases [4]. Reactive oxygen species cause damage through single and double DNA breaks, cross-linking, and chromosomal rearrangements [5]. Most of the sperm genome (about 85%) is associated with the central nucleoprotamine that protects it from free radical attack [6].

Antioxidants and Sterility
Antioxidants are compounds that eliminate and prevent the formation of reactive oxygen radicals (ROS), or oppose their actions. The main antioxidants are vitamin A, vitamin E, beta-carotene, vitamin C and the mineral selenium. A number of nutritional treatments have been shown to improve sperm count and animal motility. Spermatozoa, including carnitine, arginine, zinc, selenium and vitamin 12B [7].

Antioxidants such as vitamin C, vitamin E, glutathione and coenzyme Q10 have also proven useful in treating male infertility [8]. To protect cells and organ systems against ROS, humans have developed a highly complex and complex antioxidant protection system to protect cells and organ systems from ROS [9].

Antioxidants are present in semen in both sperm and seminal plasma, but they are abundant in seminal plasma, because the amount of sperm cytoplasm is low, making the antioxidant defense activity limited [9]. The seminal plasma is enriched with enzymatic antioxidants, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione S-transferase (GST), and non-enzymatic antioxidants such as glutathione. Vitamin A, Vitamin C, Vitamin E, Coenzyme Q10 and others. Deficiencies in the enzymatic or non-enzymatic antioxidant systems in seminal plasma are widely associated with male infertility, where the absence of any of these systems leads to the accumulation of excessive levels of reactive oxygen species, This leads to impairment of both the structural and functional integrity of the sperm [10].

Materials and methods

Case Study

The current study included (130) samples collected from men with undiagnosed infertility problems and (20) samples from men without any infertility problems that were counted as a control group.

Collection of Blood sample

(5.0) ml of semen was taken from the men under study and divided into two parts and placed in Eppendorf tubes, the first part was used in conducting physiological tests, and the second part was centrifuged for ten (10) ten minutes at one temperature. Speed (3000) r/min. For the purpose of obtaining semen plasma on which biochemical tests were performed. (2.0) ml of venous blood was drawn and placed in tubes containing the anticoagulant substance EDTA, which was used to extract genomic DNA.

DNA Extraction

DNA was extracted from the blood of all the samples included in the study, which are (140) samples, using the method modified by the researchers [11].

(ARMS-PCR) Amplification-Refractory-Mutation System

The DNA concentration in all study samples was adjusted by dilution by TE buffer solution to obtain the required concentration for PCR reactions and it was (25) ng/microliter for each sample. Four primers are added to each reaction, the first and second initiator of the main bundle, the third initiator of the natural allele, and the fourth initiator of the mutant allele. The result is two or more packets that express the result of the interaction.

The master reaction mixture was prepared for each PCR reaction by mixing the DNA sample and the special primers (4 primers specially designed to determine the genetic variation of the gene in question, added together to a mixture for a reaction with determining the optimum temperature for the binding of the primers in the PCR reaction) for each mutation with the components of the Master -mix inside a 0.2-capacity Alpendorf tube supplied by the English company Biolaps. The reaction volume was fixed to 20 microliters with distilled water. The mixture was discarded in the Microfuge for a period between (5-3) seconds to ensure that the reaction components were mixed, then the reaction tubes were inserted into the polymer device Thermocycler for the purpose of conducting the replication reaction using the special program for each reaction, then the sample was loaded into the pits of the previously prepared agarose gel at a concentration of 2% with the addition of the volumetric guide DNA prepared from Biolaps Company in one of the pits, after that the samples are transferred by running the Electrophoresis device for a period of time. It ranges between (70-60) minutes, after which the gel is photographed using a UV trans-illumination device.

Physiological Test

A number of physiological tests confirmed by the World Health Organization were measured by Computer Assisted Semen Analysis (CASA) of Taiwanese origin.

Biochemical test

The level of free oxygen radicals Reactive Oxygen Species was measured based on ELISA technology, as several tests prepared by SUNLONG were used.

Genotyping

Polymorphism of Nrf2 rs35652124 by ARMS-PCR:

100 ng of template was amplified using ARMS-PCR system, the sequ ence of primers used are as follows:

Primer	Sequence of Primer
Forward outer primer	5- GGGGTTCCCGTTTTTCTCCC -3
Revers outer primer	5-GCAGTCACCCTGAACGCCCT-3'
Forward inner primer	5- GACACGTGGGAGTTCAGAGGG-3
Revers inner primer	5- CTTTTATCTCACTTTACCGCCCGAG -3

Polymorphism of GST rs35652124 by ARMS-PCR:

100 ng of template was amplified using ARMS-PCR system, the sequence of primers used are as follows:

Primer	Sequence of Primer
GSTT1 Forward primer	5-TTCCTTACTGGTCCTCACATCTC-3
GSTT1 Revers primer	5-TCACCGGATCATGGCCAGCA-3
GSTT1 Forward primer	5-CTGCCCTACTTGATTGATGGG-3
GSTT1 Revers primer	5-CTGGATTGTAGCAGATCATGC-3

PCR was performed in a final 20 µl volume, with a master mix of bio-laps [1] and the condition of PCR technique in the table are as follows:

Result and Discussion

Determination of genetic variation of the NRF2 gene at (rs35652124)

The results showed, as shown in Figures (***1***) that all the samples that were studied from among the control group and male infertile males that they carry the reciprocal genotype for the genetic mutation that affects the gene NRF2 at the locus rs35652124, as it turns out when observing Figure (***1***) The result of the PCR reaction is bp in size 318, 213, 146, which indicates the emergence of genetic variation for the gene and for all (AG) genotypes with percentages of 100% as shown in Table (1):

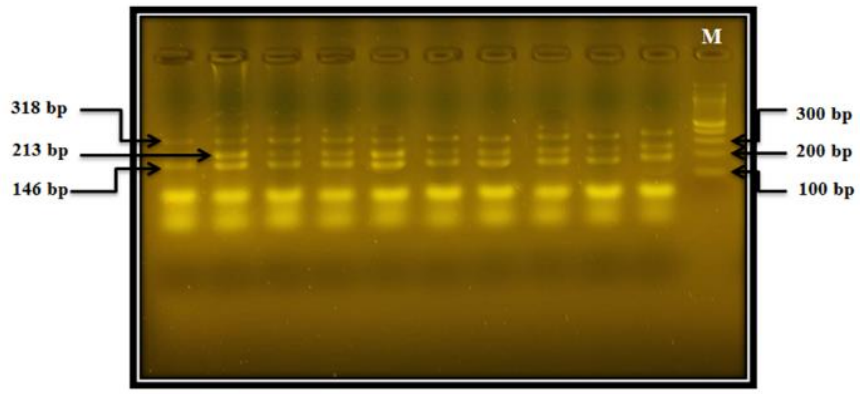

Fig 1

Figure (1): shows the result of the PCR reaction of the genetic variation of the NRF2 gene at (rs35652124) which was migrated in 2% agarose gel. As M represents the volumetric index and all samples are carriers of the heterozygous genotype (AG), the reaction product contains three bundles of 318 bp in size for the main gene, and the 213 bp bundle represents the normal allele, and the bundle bp146 represents the mutant allele, which was migrated in agarose gel at a concentration of 2%.

Table 1: Shows the distribution of the genotype and the allelic level of the NRF2 gene at the locus (rs35652124) between the control group and the infertile men, knowing that the A allele is the normal allele and the G allele is the mutant allele

Genotypes	Patients NO.(20)		Control NO.(20)		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
AA	0	0	0	0	1.0	1.0	0.5044 to 1.9824
AG	75	100	21	100			
GG	***0***	0	0	0			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
A	75	50	21	50	1.0	1.0	0.5044 to 1.9824

Table (1) shows the level of allelic observation and the genotype of the NRF2 gene at the locus (rs35652124). The results of the study for the group of men with undiagnosed infertility showed that the distribution of the mutant genotype (GG) and the normal genotype (AA) were (0%) As for the heterogeneous genotype (AG) only there is a percentage of (100%) compared to the control group in which the percentage of the mutant genotype (GG) and the normal genotype (AA) was also at (0%), while the heterogeneous genotype Presence (100%).

As for the level of allelic viewing, the study showed that the mutant allele in the group of infertile men was (50%) and the normal allele (50%) compared to the control group, where the percentage of viewing for the mutant and normal allele was (50%).

Several previous studies, including the study [12], indicated that NRF2 is the main enzyme in the defense of antioxidants, as it is a nuclear transcription factor that can stimulate oxidative enzymes through antioxidant receptors in response to oxidative stress and mediate In histological activation of effector genes and modulation of defense mechanisms in vivo against oxidative damage, as indicated by another study on male sterility, erythropoietic factor II (Nrf2) plays an important role in preventing the development of oxidative stress in spermatogenesis [13]. also demonstrated a strong relationship between the functional inconsistency in the Nrf2 promoter gene and abnormal spermatogenesis in humans.

The results of the study also showed that the value of the odds level OR was (1.0) for the mutant genotype, which in turn is a risk factor and cause of undiagnosed infertility cases in the study samples.

Determination of the presence and deletion of the genetic polymorphisms

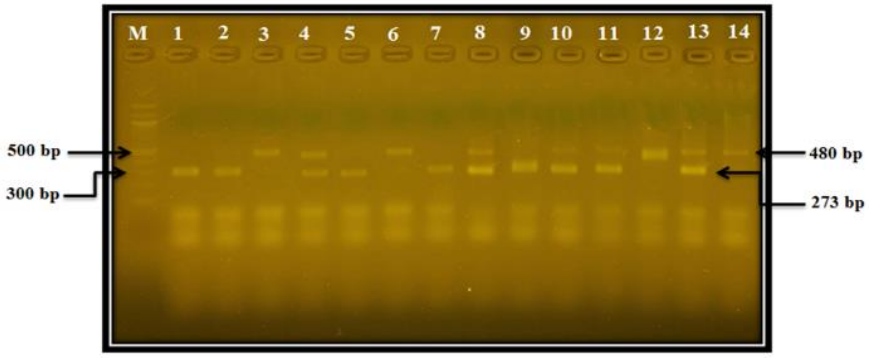

Fig 2

(GSTT1, GSTM1) of the GST gene

The results showed, as shown in Figures (2), that there is a relationship between infertility cases and the deletion of the GST gene at site A12457C. When observing Figure (2), it becomes clear that the result of the PCR reaction is 273 bp in size due to the presence of the GSTM1 gene as well as the product of the reaction 480 bp for the presence of the GSTT1 gene polymorphism, which indicates the direct effect of the presence and deletion of the GST gene polymorphisms in male infertility cases as shown:

Figure (2): shows the PCR product of the gene (GST) in its two forms (GSTM1, GSTT1), which were migrated in 2% agarose gel. As M represents the volumetric index and samples (3,6,12,14) represent samples carrying the genotype (GSTT1) with the reaction product bp 480, either samples (1,2,5,7,9) represent samples carrying the genotype (GSTM1). With the reaction product bp 273, while samples (4,8,10,11,13) represent the samples carrying both genotypes of the GST gene with the product of the PCR reaction with the product of the reaction containing two packages, the first with a size of bp of 480 for GSTT1 and the second package bp of 273 for GSTM1.

After the results of the PCR reaction were carried over to the groups on which the study was conducted, the bundles resulting from the replication appeared for all samples belonging to the GSTT1 and GSTM genetic forms, as shown in Figure (2). GSTT1 and GSTM), but if the 480 bp bundle appears only, it means the presence of the GSTT1 gene and the loss of the GSTM gene in the sample, but if the 273 bp bundle appears, it means the presence of the GSTM gene and the loss of the GSTT1 gene.

Table 2

genotype	patients	control
GSTT1, GSTM1 natural	%35	%55
GSTT1 and lost TO GSTM1	%35	%25
GSTM1and lost TO GSTT1	%30	%20
GSTT1 lost TO GSTM1 and	%0	%0

Table (2): shows the percentage of the presence of polymorphisms of genes (GSTT1 and GSTM1) in the study samples. The results showed, as shown in Table (4-3), the presence of the genes (GSTT1 and GSTM1) by 35%, the loss of the GSTT1 gene by 30% and the loss of the GSTM1 gene By 35% in infertile men compared to the control group, the presence of the genes (GSTT1 and GSTM1) was high, 55%, and the loss of the GSTT1 gene and the GSTM1 gene was low by 25% and 20%, respectively, compared to the control group. The occurrence of mutations that lead to the deletion and loss of these genes leads to the loss of the function of these genes and thus the absence of the enzymes encoded for these genes, which increases the incidence of many dangerous diseases as well as a defect in the antioxidant function, which plays an important role in increasing the production of ROS inside cells and from Then it is considered one of the causes of male infertility.

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