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Variants in the Antioxidant Gene (*catalase*) and its Correlation with Idiopathic Male Infertility in Iraqi Population

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Abstract: Infertility is a multifactorial and polygenic disease. A vast majority of infertility is still unexplained despite modern diagnostic techniques. Catalase (CAT), the key enzyme for high H2O2 elimination, constitutes, along with superoxide dismutase (SOD) and gluthatione peroxidase (GPX), the main enzymatic antioxidant system. Catalase enzyme plays an important role in seminal antioxidant defense and is present in seminal plasma at high concentrations. Low concentrations of catalase enzyme in seminal plasma have been related with male infertility. The aim of the study was to investigate the status of the antioxidant enzyme; catalase in clinically diagnosed infertile males and find the potential association of CAT gene variant in the promoter region (rs1001179 [−262C/T] and rs7943316[−21 A/T] and other CAT gene variant CAT rs1049982 [T/C], and CAT rs12270780 [G/A] and their association with the gene expression of the CAT gene. The study consisted of 100 clinically diagnosed infertile males (50 oligozoospermia and 50 asthenozoospermia) and 50 fertile volunteers. Polymerase chain reaction followed by sequencing were performed for genotyping of catalase variants. mRNA level of Catalase was analyzed by using semi quantitative technique. There was no significant difference in allele frequencies and genotype distribution for all SNPs. It was concluded that gene expression shows little increase in the level of CAT mRNA of fertile samples when compared to infertile samples.

Keywords: Idiopathic infertility, CAT, catalase variants, antioxidant enzyme.

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Introduction

Infertility is a failure to apprehend a child after 12 months of regular and unprotected sexual intercourse (1). Unfortunately, (30–40)% of infertility is still unexplained despite modern diagnostic techniques that acknowledged much more etymology of infertility. Male infertility is divided into different types on the basis of the concentration and motility of sperm cells (2). Oxidative stress is considered one of the factors for infertility. Antioxidant enzymes are the proteins that catalyze the reactive oxygen species (ROS), reactive nitrogen species (RNS), and their by-products into nontoxic molecules. Free radicals are continuously producing in cells and antioxidant enzymes remove them (3). Hence, antioxidants represent the main defense mechanism against cell damage by oxidative stress. Excessive ROS production in the body is the main cause

of several diseases. ROS disturb the cellular and molecular pathways while antioxidant enzymes protect them from damage (4). Catalase (CAT; EC 1.11.1.6) is a major antioxidant enzyme that is engaged in the clearance of ROS. CAT functions in the conversion of hydrogen peroxide to oxygen and water and lessens the toxic effects of hydrogen peroxide (5). The CAT gene is located at position 11p13 and encodes CAT. Polymorphism in this gene has been associated with a reduction in CAT level (6). Antioxidant genes are responsible for the normal functioning of spermatozoa and spermatogenesis. Any mutation in these genes can directly affect male fertility (7). Promotor of a gene is a vital component for transcriptional initiation. Any change in the sequence of a promotor can terminate or alter the gene function. Mutation or polymorphism in promotor genes can enhance or reduce messenger RNA level and consequently protein expression. Polymorphism in CAT gene promotors is predominantly linked with several disorders such as cancers, diabetes, obesity, depression, and other diseases accompanied by oxidative stress (8). Single-nucleotide polymorphism in the antioxidant enzyme gene is associated with the sperm DNA damage. CAT gene polymorphism has been linked with the risk of DNA fragmentation and male infertility (9). A functional polymorphism in antioxidant genes can cause oligospermia, oligoasthenospermia (10).

Materials and Methods

Subjects

This present study included 150 men as volunteers, who donated semen for analysis at the Kamal Al-Samurai Specialized Hospital for Infertility and IVF. The study was approved by the local Ethics committee of the Hospital. The study was conducted from April 2021 to February 2022. The patient parameters that were recorded and analyzed included age, height, weight, body mass index (BMI), alcohol status, medication, fertility history, and occupation. A professional andrology doctor interviewed all contributing males. After the initial screening, data were collected from all participants.

Inclusion Criteria

Patients included in this investigation were belonging couples living together with regular unprotected coitus for a reasonable period of time but not less than 1 year without conception and have defect semen analysis in sperm count or motility/

Exclusion Criteria

Patients who are azoospermics, Patients on any medication or antioxidant supplementation, past medication history, chronic illness and serious systemic diseases, Past medical history, testicular varicocele or hydrocele, Mumps after puberty, Males exposed to radiotherapy or chemotherapy and alcoholics drinkers.

Data Collection

Blood and seminal fluid samples were taken by masturbation from both infertile and fertile groups after 3-7 days of abstinence for seminal fluid profile analysis, followed by liquefaction for 30 minutes for macroscopic and microscopic evaluation, blood samples were used for DNA isolation for genotyping study, and seminal fluid samples were used for RNA isolation to evaluate the gene expression of *catalase* gene.

Genotyping

Nucleotide changes were determined by using polymerase chain reaction (PCR) and followed by sequencing technique analysis for CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A]. The primer sequences and the obtained fragment sizes are shown in (Table 1).

Table (1): Primers used in the study.

Primer name	Sequence	Annealing temp. (°C)	Product size (bp)
rs1001179-F	5`TGTAAAACGACGGCCAGTGCATCCATCCATCCTTTG`	60	1006
rs1001179-R	5`CAGGAAACAGCTATGACAGCTCTCTGCCTCTTTC3`		

RNA Extraction and Reverse Transcription

RNA was extracted from samples from the patients (oligozoospermia and asthenozoospermia) and control samples using the TRIzol™ Reagent according to the manufacturer´s guidelines. All the mRNA was reverse transcribed using the Using GoScript™ Reverse Transcription System Kit) according to the manufacturer's guidelines.

Semiquantitative Analysis of mRNA Expression

The obtained cDNA was amplified for a semiquantitative detection of the CAT mRNA using the specific primers F 5'-GTTACTCAGGTGCGGGCATTCTAT-3' and R 5'-GAAGTTCTTGACCGCTTTCTTCTG-3' and as an internal control, *β-globin* was amplified in different reactions using the specific primers F 5′ -GAGCCATCTATTGCTTACA-3′ and R 5′- CCAACTTCATCCACGTTC-3′. The PCR program was performed as follows: RT. Enzyme Activation (initial 37°C, 15 min) followed by denaturation (95°C, 5 min) and then 40 cycles of denaturation (95°C, 30 s), annealing (60ºC, 30s) and elongation (72°C, 30s).

Statistical Analysis

All data obtained from this study were analyzed using International Business Machines Statistical Package for the Social Sciences (IBM SPSS©) Statistics Version for Windows software package version 26 (2018) (SPSS Inc., USA). The results were analyzed statistically, and the values were expressed as Mean ± SD, statistical significance of the difference in mean of certain results was assessed by using the student's -test. The $P<0.05$, $P<0.001$ was considered to be statistically significant (11).

Results

DNA extraction by Geneaid DNA extraction kit was used in order to prepare pure DNA for PCR from blood samples. The results of DNA extraction showed that fresh blood samples yielded enough DNA concentration for PCR amplification. The quantification of DNA by Nanodrop revealed that the DNA concentration ranged between 5.5 – 9 ng /µl and the purity range was between 1.60 – 1.9. The detection of four SNPs in the catalase gene (*CAT* rs1001179 [−262C/T], *CAT* rs7943316[−21 A/T], *CAT* rs1049982 [T/C] and *CAT* rs12270780 [G/A] was achieved PCR technique followed by sequencing for PCR product .The PCR product was detected as 1006 bp band (Figure 1).

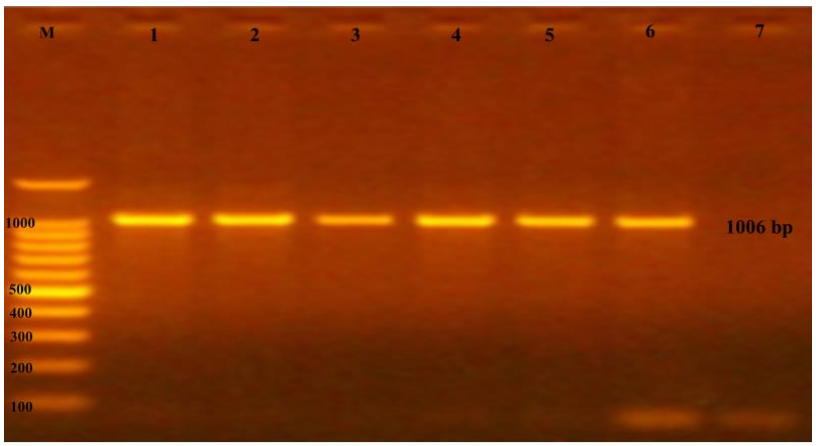

Figure (1): PCR products of catalase gene. using 1.5% agarose and ethidium bromide stain. M= 100 bp DNA ladder; lanes 1 – 6 show the PCR product of 1006bp, lane 7 is negative control.

Association between Single Nucleotide Variants in Catalase Gene and Risk of Male Infertility

The detection of SNP −262C/T in the catalase gene was achieved by PCR technique followed by sequencing for PCR product. The result of sequencing showed another three variants (CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A]) in the PCR product resulted from the amplification of 1006 bp of catalase gene by designed primers.

There was no significant difference in allele distribution of the catalase gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C], and CAT rs12270780 [G/A] were examined in the idiopathic infertile male (oligozoospermia and asthenozoospermia) and control subjects. (Table 4-9) and (4-10). The frequency of an allele (C) at CAT [−262C/T] was 80%, 75% and75% in oligozoospermia , asthenozoospermia and controls respectively , and the frequency of an allele (T) was 20%, 25%, and 25% in oligozoospermia, asthenozoospermia, and control respectively; also the frequency of an allele (A) at CAT [−21 A/T] was 71% and 75% in oligozoospermia and asthenozoospermia and 67% in controls, and the frequency of an allele (T) was 29%, 25%, and 33% in oligozoospermia, asthenozoospermia, and control respectively

In CAT rs1049982 [T/C], the allele frequency of the allele (T) was 69% in oligozoospermia, and 85% in both asthenozoospermia, and controlgroups, while allele C was 31% in oligozoospermia, and in both asthenozoospermia, and control was 42%. In case of CAT rs12270780 [G/A] allele frequency of the allele (G) was 80% and 70% in oligozoospermia and asthenozoospermia and 75% in controls, and the frequency of allele (A) was 20%, 30% and 25% in oligozoospermia, asthenozoospermia, and control respectively. The genotype distribution of catalase gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A] were examined in the idiopathic infertile male (oligozoospermia and asthenozoospermia) and control subjects (Table 4-11) and (Table 4-12).

There was no significant difference in genotype distribution for all variants. For CAT [−262C/T] the frequency of the CC was 60 % and 50% in idiopathic infertile groups (oligozoospermia and asthenozoospermia) versus 50% in control group, while CT was 40% in oligozoospermia and 50% in both asthenozoospermia and control groups. TT was not found in all of the studied groups.

For CAT [−21 A/T] the genotype AA was 52%, 58% and 48%in idiopathic infertile groups (oligozoospermia and asthenozoospermia) and control group, respectively. The distribution of AT genotype was 38%, 34% and 38% in oligozoospermia, asthenozoospermia and control groups respectively; and the TT was 10%, 8% and 14% in oligozoospermia, asthenozoospermia and control groups respectively. For

CAT rs1049982 [T/C] the distribution of the TT was 50%, 42% and 36% in oligozoospermia, asthenozoospermia and control groups respectively; and TC genotype was 38%, 32% and 44% in oligozoospermia, asthenozoospermia and control groups respectively; and while the genotype TT 20% in control group, while in idiopathic infertile groups (oligozoospermia and asthenozoospermia) was 12% and 26% respectively.

For CAT rs12270780 [G/A] the genotype GG was 70%, 50% and 60% in oligozoospermia, asthenozoospermia and control groups respectively; and GA was 20%, 40% and 30% in oligozoospermia, asthenozoospermia and control groups respectively; while AA genotype was 10% in all groups.

The 2X2 contingency chi square test was used to assess the allele frequency comparisons.

Table (2): Allele distribution of catalase gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A] in oligospermia and control groups.

***CAT* rs 1001179 [262C/T]**					
Alleles	**Groups**			**OR**	**CI**
	Oligospermia	**Control**	**Chi-square**		
C	80(80%)	75(75%)	0.322(0.5700)	1.14	0.73-1.78
T	20(20%)	25(25%)	1.111(0.2918)	0.64	0.28-1.47
***CAT* rs7943316[−21 A/T]**					
A	71(71%)	67(67%)	2.2319(0.6301)	1.12	0.7-1.8
T	29(29%)	33(33%)	0.5161(0.4724)	0.77	0.38-1.56
CAT rs1049982 [T/C]					
T	69 (69%)	58(58%)	1.9055(0.1674)	1.42	0.86-2.32
C	31 (31%)	42(42%)	3.3151(0.6864)	0.54	(0.28-1.05)
CAT rs12270780 [G/A]					
G	80(80%)	75(75%)	0.322(0.5700)	1.14	0.73-1.78
A	20(20%)	25(25%)	1.111(0.2918)	0.64	0.28-1.47

Table (3): Allele distribution of catalase gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A]) in asthenospermia and control groups.

CAT rs 1001179 [262C/T]					
Alleles	**Groups**			**OR**	**CI**
	Asthenospermia	**Control**	**Chi-square**		
C	75(75%)	75(75%)	----	----	----
T	25(25%)	25(25%)	----	----	----
***CAT* rs7943316[−21 A/T]**					
A	75(75%)	67(67%)	0.9014(0.3424)	1.25	0.79-2
T	25(25%)	33(33%)	2.2069(0.1373)	0.57	0.28-1.2
CAT rs1049982 [T/C]					
T	58 (58%)	58(58%)	----	----	----
C	42 (42%)	42(42%)	----	----	----
CAT rs12270780 [G/A]					
G	70(70%)	75(75%)	0.3448(0.5570)	0.87	0.55-1.38
A	30(30%)	25(25%)	0.9091(0.3403)	1.44	0.68-3.05

Table (4): Genotype distribution of *catalase* gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A Genotype distribution of catalase gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A] in oligospermia and control groups.

CAT rs 1001179 [262C/T]					
Alleles	**Groups**			**OR**	**CI**
	Oligospermia	**Control**	**Chi-square**		
CC	30 (60%)	25(50%)	0.9091(0.340)	1.44	0.68-3.05
CT	20 (40%)	25(50%)	1.111(0.2918)	0.64	0.28-1.47
TT	0 (00%)	0 (00%)	----	----	----
***CAT* rs7943316[−21 A/T]**					
AA	26 (52%)	24(48%)	0.16(0.6891)	1.17	0.54 2.57
AT	19 (38%)	19(38%)	----	----	----
TT	5(10%)	7 (14%)	0.6667(0.4142)	0.51	0.1-2.59
CAT rs1049982 [T/C]					
TT	25 (50%)	18(36%)	2.2791(0.1311)	1.93	0.82-4.45
TC	19 (38%)	22(44%)	0.439(0.5075)	0.75	0.31-1.78
CC	6 (12%)	10(20%)	2(0.1572)	0.36	0.091.51
CAT rs12270780 [G/A]					
GG	35 (70%)	30(60%)	0.7692(0.3804)	1.36	0.68,2.71
GA	10 (20%)	15(30%)	2(0.1572)	0.44	0.14,1.38
AA	5 (10%)	5 (10%)	----	----	----

Table (5): Genotype distribution of *catalase* gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A Genotype distribution of catalase gene (CAT rs1001179 [−262C/T], CAT rs7943316[−21 A/T], CAT rs1049982 [T/C] and CAT rs12270780 [G/A] in asthenospermia and control groups.

Alleles	Groups			OR	CI
	Asthenospermia	Control	Chi-square		
***CAT* rs 1001179 [262C/T]**					
CC	25 (50%)	25(50%)	----	----	----
CT	25 (50%)	25(50%)	----	----	----
TT	0 (00%)	0 (00%)	----	----	----
***CAT* rs7943316[−21 A/T]**					
AA	29 (58%)	24(48%)	0.9434(0.3314)	1.46	0.38-3.14
AT	17 (34%)	19(38%)	0.222(0.6373)	0.8	0.32-2.02
TT	4 (8%)	7 (14%)	1.6364(0.2008)	0.33	0.06-1.86
CAT rs1049982 [T/C]					
TT	21 (42%)	18 (36%)	0.4615(0.4969)	1.36	0.56-3.32
TC	16 (32%)	22(44%)	1.8947(0.1686)	0.53	0.21-1.31
CC	13 (26%)	10 (20%)	0.7826(0.3763)	1.69	0.53-5.42
CAT rs12270780 [G/A]					
GG	25 (50%)	30 (60%)	0.9091(0.3403)	0.69	0.33-1.47
GA	20 (40%)	15 (30%)	1.428(0.2319)	1.78	0.69-4.58
AA	5 (10%)	5 (10%)	----	----	----

Antioxidant Enzyme (catalase) mRNA Expression in Spermatozoa

Relative quantitative RT-PCR analysis was performed to determine the level of antioxidant genes mRNA expression in spermatozoa from men that differ in sperm count and motility (oligozoospermia and asthenospermia groups) and from fertile men with normozoospermia (control group).

Total RNA extraction was accomplished with a purity range of 1.75 to 1.95. The first stage in RT-qPCR was the synthesis of cDNA, which was followed by the amplification of the target genes. Analysis of the double Ct was used to calculate the expression of the *CAT* gene, with β-globin serving as the reference gene. The amplification was recorded as having a Ct value (Figure 2).

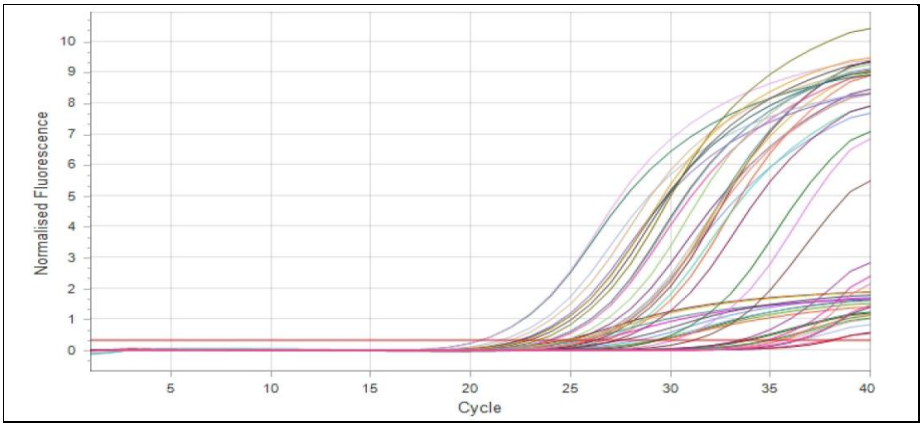

Figure (2): Amplification plots for catalase expression obtained by RT PCR.

Melt Curve Analysis

Melt curve analysis was carried out to check the annealing of the primers to establish the reaction's specificity for *CAT*. The accuracy of the results was ensured by a sharp, single peak that showed only one PCR product (Figure 3 The important result in (Table 6) clarified that CAT gene has relatively high expression in asthenospermia (1.120) in comparison with controls and oligozoospermia (0.982 and 1, respectively). In spite of there is no significant differences in the folding of CAT gene expression but this slight increase in level of CAT mRNA may be because of the presences of multiple transcription start points at CAT gene.

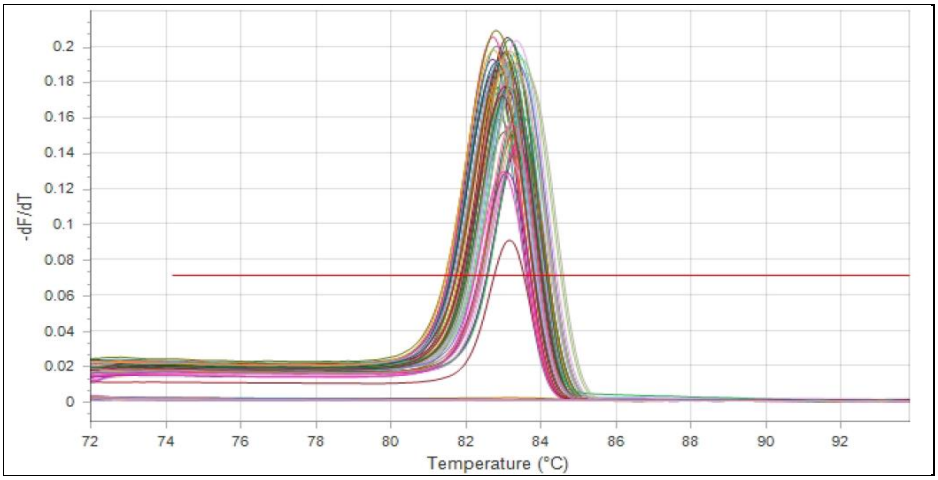

Figure (3): The catalase expression melting curve.

Table (6): The gene expression calculation levels in different stages (Ct value, ΔCt, ΔΔCt and folding) for CAT gene.

Groups	β-globin Ct (mean±SD)	CAT Ct (mean±SD)	ΔCt (mean)	ΔCt Calibrator	ΔΔCt (mean)	2^-ΔΔct	Experimental / control	Fold of gene expression
Oligospermia	24.13 ± 4.41	27.06 ± 3.34	2.93	8.939	-6.01	168.82	168.82 / 171.93	**0.982**
Asthenospermia	23.48 ± 3.35	24.96 ± 3.15	1.48	8.939	-7.46	192.65	192.65 / 171.93	**1.120**
Control	25.86 ± 3.83	28.13 ± 4.50	2.27	8.939	-6.67	171.93	171.93 / 171.93	**1**
p-value								**0.757** NS
LSD								**0.423**

Correlation between SNPs in the Promoter of *Catalase* and its Gene Expression in Spermatozoa

The result of table 6 showed no significant correlation between the genotype of SNPs [-262C/T] and [−21 A/T] with gene expression of catalase. On the other hand, there is significant correlation (P≤0.001) between the genotype CT and CC of CAT SNPs [-262C/T] in oligozoospermia, asthenozoospermia and control groups.

When results compared between genotypes, the CT genotype was correlated with a high rate of gene expression of catalase and the lowest for the CC genotype in all groups of the study. in the case of SNP [−21 A/T] there is no significant correlation between the AA, AT, and TT genotypes and the rate of gene expression of the catalase gene.

Table (7): Correlation between *CAT* (rs1001179[-262C/T], rs7943316[−21 A/T]) and *CAT* mRNA level.

CAT rs 1001179 [262C/T]				
Genotype	**Groups**			**p- value**
	Oligospermia Folding (mean±SD)	**Asthenospermia Folding (mean±SD)**	**Control (mean±SD)**	
CC	0.633 ± 0.588	0.465 ± 0.178	0.633 ± 0.588	0.449
CT	1.370 ± 0.777	1.22 ± 0.361	1.370 ± 0.777	0.605
p-value	0.037**	0.002*	0.037**	
***CAT* rs7943316[−21 A/T]**				
AA	1.190 ± 1.193	1.155 ± 0.787	1.190 ± 1.193	0.974
AT	0.954 ± 0.695	1.104 ± 0.338	0.954 ± 0.695	0.453
TT	0.925 ± 0.796	1.189 ± 0.845	0.925 ± 0.796	0.660
p-value	0.887	0.951	0.887	

***(P≤0.05). Different capital letters show significant differences (P<0.05) between rows.**

Discussion

Catalase enzyme (CAT), coded in chromosome 11p3, is the principal enzyme in the detoxification of H_2O_2 to H_2O. H_2O_2 plays an outstanding role in male infertility as it causes lipid peroxidation which reduces the fluidity of the membrane thus causing motility reduction and poor oocyte-sperm membrane fusion. H_2O_2 in seminal plasma can appear directly as a consequence of the activity of leukocytes or indirectly as a product of the detoxification of ROS by superoxide dismutase(12).

In our study we found no significant difference in allele frequencies and genotype distribution for all SNPs. The result of this study was in agreement with Karam *et al*.,(13) who found that there were no significant differences in the genotypes or effect models between fertile and infertile groups (P > 0.05) of the *CAT* rs1001179 [262C/T] in Iranian population (13); also Bousnane *et al.* (14) show that the frequencies of both alleles and the different genotypes in the infertile cases and the controls were not statistically different (p>0.05) in Algeria population (14).

In Moscow, Ivanovna *et al.* (15) found that 50 % of patients with infertility have local mutations of the gene *CAT*, and about 2/3 of the patients were registered with the heterozygous genotype (262CT), and 10 % of the men showed the genotype homozygous for the defective allele (262TT). It is interesting to note that in 1/3 of men with normozoospermia, they also found local *CAT* gene mutations in both heterozygous and homozygous types (15).

On the other hand, these results were disagreeing with results confirmed in other studies carried out by the group of authors. In a case-control study involving 313 infertile patients and 80 fertile donors, there were statistically significant differences between fertile donors and infertile patients with the

CAT C-262 T SNP. The CC genotype was associated with a twofold increase in the risk of infertility (P = 0.001), while the CT genotype is protective (P = 0.001) (16).

The findings of Sadia *et al*. (17) demonstrated that polymorphism in this CAT gene is susceptible to male infertility. The dominant model for the CAT variation has demonstrated statistical significance (p = 0.000), and both the A and T alleles contribute to illness vulnerability that results in infertility (17).

Lourdhu *et al*. (12) studied (-21 A/T) gene in the South Indian population and found a higher frequency of AT/TT genotype in the population and suggested a high prevalence of mutant genotype may increase the susceptibility to oxidative stress-associated diseases. Prevalence of this genotype (TT) has been found to alter in different populations, least in the Chinese population (12). Jianrong *et al*. (18) found that there were no significant differences in the genotypes and allele frequencies of *CAT* rs12270780 [G/A] between idiopathic nephrotic syndrome (INS) and control subjects (18).

Various SNPs have been identified in exons, introns, promoters and 5' and 3' untranslated regions. Polymorphisms (SNPs) in the promoter region of *CAT* gene are: -262C/T (rs1001179), -21A/T A/T (rs7943316) and *CAT* rs1049982 [T/C] (19). While *CAT* rs12270780 [G/A] located in intron (20).

Many sequences existing in the promotor region help in regulation and impact gene expression. It is believed that the polymorphism of *CAT* -21 A/T and −262C/T renders a reduction in the antioxidant capability of CAT and could be a risk factor for disease. rs7943316 SNP and 1001179 SNP have an association with the promotor region of the CAT gene. Any mutation or polymorphism in this sequence can induce overexpression or under-expression of the gene that can be lethal or have a deleterious effect. Polymorphisms of these genes (-21 A/T) and (-262C/T) are associated with an increased risk of idiopathic male infertility and also obesity, breast cancer, and other diseases (21).

The result of the gene expression is consistent with the findings of Linschooten *et al*. (22), who discovered that the expression of the anti-oxidant enzyme catalase was increased in infertile men, particularly smokers, but the difference did not reach statistical significance (p = 0.22) (22).

The results demonstrate that antioxidants may play a critical role in the pathogenesis of male infertility. Further screening for functional polymorphism in selected antioxidants genes of the population may resolve the highly prevailing idiopathic infertility cases. So, this might be an explanation for the increased oxidative stress in men living in Baghdad province, which increases the risk of infertility. Therefore, it is not far from the mind that the interaction of these environmental factors with particular genotypes of antioxidant gene polymorphisms can increase oxidative stress and the risk of sperm DNA damage (23).

Promoter region playing a major role in the regulation of gene expression. Presence of polymorphism in the promoter region modifies binding sites of transcription factors (TFs) which alters the gene expression level. Affecting the gene expression level by functional promoter polymorphisms associated with diseases. Humans possess the most genetic polymorphism

in the form of SNPs (single nucleotide polymorphisms) and they can affect gene function if they are located within or near a gene (24).

One of the most common polymorphisms, -21A/T (rs rs7943316) SNP located near a transcription start site (TSS). 21A/T polymorphism is located in a sensitive position and hence may alter the binding site of TFs. Misregulation of TFs deregulates the regulatory region where polymorphism resides. This ultimately disturbs the mRNA and protein levels in the disease condition. On the other hand, the result agrees with the finding of Garcia-Rodriguez *et al*. (16) whose result of the in-silico analysis of the effect of the CAT C-262T SNP over the binding of transcriptional factors. They discovered five transcriptional factors that bind to the gene's promoter in the region of the SNP without regard to the allele. Additionally, they found eight activating TFs that bind only to the T allele, compared to only two for the C allele (16).

In conclusion, our results show that for the CAT C-262T polymorphism the T allele confers a variant with higher expression levels than the C allele and, at least in seminal plasma. The result agreed with those of Garca Rodrguez *et al*., (16) in their insilico analysis of the effect of the CAT C-262 T SNP on transcriptional factor binding. They obtained 5 transcriptional factors that bind to the promoter of the gene in the SNP region regardless of the allele. Furthermore, they found 8 activating TFs that specifically bind to the T allele, whereas we only found 2 for the C allele. Also, their western gels result agreed with our result that CT genotype had the highest values of gene expression while the CC genotype had the lowest (16).

The result is concordant with those obtained by Forsberg *et al*., (25) who used the MatInspector program. They also support our theory that the T allele is a variant of the enzyme with a higher transcriptional rate. Interestingly, nuclear erythroid 2-related factor 2 (NRF2), a well-known activator in oxidative stress situations via Antioxidant Response Element, was one of the specific activators for antioxidant the T allele response element (ARE)(25).

Catalase expression in response to oxidative stress has been shown to be mediated by the NRF2-ARE signaling pathway (26).

Conclusion

Our results demonstrated that there is no correlation between catalase variant and its gene expression with idiopathic male infertility in Iraqi population. We predict that further studies with stricter controls will elucidate this pattern with high statistical significance.

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