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Effect of C677T Mutation of Methylene Tetrahydrofolate Reductase Gene as a Genetic Risk Factor in Iraqi Infertile Men

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Abstract: Male infertility in Iraq, particularly in the previous three decades diagnosed remarkably at a young age, due to a variety of variables, the most important are war and devastating civil wars, stress, lifestyle, smoking, dietary habits, and genetic susceptibility. The methylene tetrahydrofolate reductase (*MTHFR)* gene has received a lot of interest, mainly because the *MTHFR* C677T variant was found to be a risk factor for male infertility, especially in Severe Oligoasthenozoospermia patients. The study aimed to determine the prevalence of this crucial gene mutation across the country and the estimation of total endogenic human 8-hydroxyl-2'-deoxyguanosine concentrations as a specific marker of oxidative injury to sperm DNA. Semen analysis was done for 100 men. The analysis was accounted as recommended by WHO criteria. All DNA samples successfully extracted using the organic method. The genetic variation of the SNP *MTHFR* gene 677 C > T (A222V, rs1801133) was discovered using real-time polymerase chain reaction (RT-PCR) to detect different genotypes. Enzyme-linked immunosorbent assay was performed for measurements Hydroxydeoxyguanosine (8-OHdG) in semen samples, which is considered as a biomarker of cellular oxidative stress. The *MTHFR* C677T gene polymorphisms were found to be a risk factor for male factor infertility in the Iraqi population in this investigation. Furthermore, this study found a considerably greater amount of 8-OHdG in sperms from all infertile groups when compared to controls, implying that oxidative DNA damage has a major impact on sperm characteristics including concentration and progressive motility.

Keywords: *MTHFR* C677T, 8-OHdG, male infertility, sperm DNA oxidative stress.

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Introduction

Infertility is a global problem; affecting 15% of couples with unprotected intercourse and 70% of these cases are associated with the male factor (1). Male infertility is primarily due to defective spermatogenesis and the causes can be attributed in part to neurogenic factors, genital tumors, germ cell aplasia, defective sperm transport, Varicocele or environmental toxins; while modern diagnostics and a wide range of studies have clarified the pathogenesis of male infertility, about 50% of infertility cases are still unknown(2). Several studies have studied genetic mutations of genes that are connected to male infertility; one of them is Methylenetetrahydrofolate reductase (MTHFR), the *MTHFR* gene product is one of the most important enzymes in folate metabolism that is crucial for many cellular functions. This enzyme reduces 5-10-

methylenetetrahydrofolate to its biologically active 5-methyltetrahydrofolate form, and 5-methyltetrahydrofolate is then transferred to its methyl group to convert homocysteine into methionine. Subsequently, methionine provides the S-adenosylmethionine forming methyl group (3). It serves as a methyl donor for DNA synthesis, methylation processes, protein synthesis, and DNA methylation, methylation is necessary for maintaining the fertilized male gamete potential as well as the integrity of sperm DNA (4) *MTHFR* gene expression is highest in the testis compared to any other organ, indicating that *MTHFR* plays a key role in spermatogenesis regulation (5). A common single nucleotide polymorphism (SNP) is the C677 T polymorphism in the coding region of the human *MTHFR* gene which changes an alanine into a valine residue. Its polymorphic distribution is highly variable among different populations (6). This gene variant codes a heat-sensitive form of *MTHFR*, which decreases the activity of the enzyme by about 35% in the heterozygote (CT) and 70% in a mutant homozygote (TT) (7). This SNP considers as a missense mutation which leads to protein conformation alteration and thus enzyme activity is reduced (8). It has been documented that the homozygous C667 T in the *MTHFR* gene is associated with the risk of some human diseases, including certain cardiovascular disorders, cancers, and neural tube defects (9).

Evidence suggests that up to 80% of the pathology of infertility in men related to DNA damage in the sperm such as oxidative stress,which may also be a cause of DNA damage (10). Oxidative stress occurs when cells no longer have the antioxidant capacity to manage the excess production of reactive oxygen species (ROS) (11) DNA damage caused by ROS results in the generation of apurinic/apyrimidinic (AP) sites, single/double strand breaks, thymine glycol and clustered DNA lesions produced oxidatively (12). In this study, we suggest that *MTHFR* gene C677 T is linked to infertile Iraqi males and investigate the correlation between *MTHFR* gene and male polymorphism C677 T, and explored the elevated concentrations of oxidative base adduct 8-hydroxy-2'-deoxyguanosine (8-OH2dG) in patient semen samples.

Methods

Subjects selection: During this study period (February 2019–February 2020), in Kamal AL-Al-Sammarraie, Infertility Center./Ministry of Health, Iraq. 100 semen samples were collected from fertile and infertile men (different infertile phenotypes), aged from 22 to 46 years. The study included 25 subjects of a control group (normal parameter of semen analysis) and 75 subjects of infertile groups which were divided into 3 subgroups: Asthenozoospermia (A) (n=25), Oligoasthenozoospermia (OA) (n = 25), and severe Oligoasthenoteratozoospermia (SOA) (n =25). All participants received a questionnaire form include participant’s details regarding lifestyle habits like alcohol intake, smoking, dietary habits, folic acid intake, and occupation.

Semen collection: After 3 to 5 days of sexual abstinence freshly ejaculated human semen samples were collected after sperm fluid liquefaction at 37 ^{o}C for 30 min, a routine semen analysis was performed immediately, according to the World Health Organization's 2010

guidelines (13). Samples were centrifuged (5 min/12000 rpm) then the precipitate of semen cells was suspended with 750 µl of Tris EDTA (TE) buffer for DNA preservation in (deep freeze -80° C) until extraction processed, all samples were preserved.

DNA Extraction: Organic procedure (Phenol-chloroform extraction method) was carried out according to (14,15). The concentration and purity of DNA of all samples were evaluated using a NanoDrop spectrophotometer from Thermo scientific / USA.

Enzyme-linked immunosorbent assay(ELISA): To measure the Hydroxydeoxyguanosine (8-OHdG) in neat semen sample (cell pellet with seminal plasma), using an ELISA kit from (Cusabio, China) following the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate ELISA reader (Biobase/ Germany) considered as a biomarker of cellular oxidative stress. About 100 µl cell pellet with seminal plasma) were rinsed with 1X PBS, homogenized in 1 ml of 1X PBS, and stored -80°C until the test processed.

Genotyping: The C677T SNP (rs1801133) of the *MTHFR* gene was studied using TaqMan allelic discrimination assay by MTHFR 677 Real-Time PCR Kit Ready-to-Use from (SNP, Biotechnology). The kit system is designed for use with sequence-specific primers and probes, with each master mix often including internal control. Allelic discrimination Procedure follows the manufacturer's recommendations for qPCR reaction set-up and cycling

Conditions were mix 5 µl (100 ng/µl) DNA and 20 µl master mix for each sample, (reach to final volume 25ml). The cycling condition was set in initial denaturation 95 ºC for 3 minutes (enzyme activation) followed by denaturation 95 °C for 15 seconds, Annealing and extension 60 °C for 30 seconds,(30 cycles). Real-Time PCR (qRT–PCR) performed using the MIC-4 Real-time PCR System/Australia. Data analysis of CT value in allelic discrimination should be about $21 \leq CT \leq 26$.

Results

In the present study, patients groups were classified according to the different infertile phenotypes. The baseline distribution in general characteristics of infertile and fertile male groups was considered in all studied groups and was statistically performed and analyzed by ANOVA. There was no significant difference between the four studied groups regarding age, alcohol consumption, smoking status, type of infertility except in family history were highly significant ($p<0.002$). The microscopic semen parameters showed a highly significant difference between the infertile and healthy control groups (Table 1). The sperm microscopic seminal analysis of Severe Oligoasthenozoospermia (SOA) group was highly significant compared with all studied groups, and the samples were determined as an SOA when the sperm concentration of patients was less than 5 million/mL.

Table (1): The microscopic seminal parameters for the studied group.

Parameters	C group (n=25)	A group (n=25)	OA group (n=25)	SOA group (n=25)	*P* value
Sperm concentration (million/mL)	30.00±7.10 a	23.00±6.97 b	7.48±2.16 c	1.64±0.70 d	**0.00**
Progressive motility (%)	43.20±9.45 a	11.40±4.68 b	7.70±3.60 bc	6.00±2.04 c	**0.00**
Non-progressive motility (%)	27.20±6.13 a	20.80±6.06 b	13.95±6.46 c	7.20±2.53 d	**0.00**
Immotile (%)	29.60±8.88 a	67.80±8.04 b	77.20±11.92 c	86.80±3.78 d	**0.00**
Normal morphology (%)	67.60±4.35 a	42.40±12.1 b	20.83±9.28 c	7.80±3.25 d	**0.00**
Ejaculate volume (mL)	3.76±0.83 a	3.36±0.63 ab	3.32±0.47 ab	3.20±0.40 b	**0.01**
Round cells (per HPF)	1.40±0.64 a	1.64±0.70 a	1.56±0.80 a	1.40±0.50 a	0.50
DNA concentration (ng/µl)	328.92±120.25 a	334.88±142.55 a	107.56±39.07 b	104.24±38.49 b	**0.00**
8-OHdG levels (ng/µl)	100.40±20.51 a	264.14±91.79 b	315.08±95.57 c	333.67±82.07 c	**0.0001**

Data were expressed as mean ± SD; Statistical analyses ANOVA followed by Post Hoc test (Tukey's test) for multiple comparisons. C: control; A: Asthenozoospermia; OA Oligoasthenozoospermia; SOA: Sever Oligoasthenozoospermia.

8-OHdG levels (ng/µl)

ELISA technique was performed for the estimation of total endogenic human 8-OHdG concentrations and quantitatively measured as a specific marker of oxidative stress to sperm DNA, DNA damage has also been employed as a test for intracellular ROS-induced oxidant stress. The result demonstrated that the sperm 8-OHdG concentration of OA and SOA group was highly significant (p= 0.0001) compared with a control group (Table 1). By reducing the data using computer software capable of creating a four-parameter logistic (4-PL) curve-fit, construct a regular curve (e.g. linear, semi-log, log / log, logistic 4 parameters) figure (1)

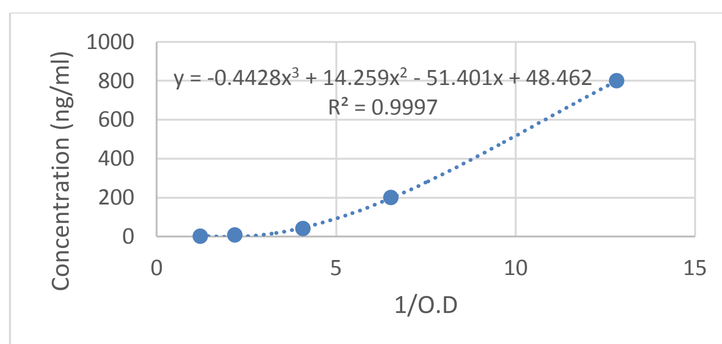

Figure (1): Standard curve of 8-OHdG creating a four-parameter logistic (4-PL) curve.

DNA molecular analysis

Total DNA was extracted successfully from all seminal fluid samples. The total DNA concentration of OA and SOA groups was significantly different ($p<0.05$) compared to the control group, but did not change significantly in the A group compared to the control group (Table 1).

MTHFR Gene polymorphism 677 C > T (A222V, rs1801133)

The genetic variation of the SNP *MTHFR* gene 677 C > T (A222V, rs1801133) was discovered using real-time polymerase chain reaction (RT-PCR) to detect different genotypes, presented with three genotypes CC or wild type, CT or hetero-genotype, TT or recessive genotype, with two alleles (C and T).

Analysis of Hardy-Weinberg Equilibrium (HWE) refers to the distribution of genotype frequencies in the control group(C), and asthenozoospermic group(A), it was compatible with HWE analysis, and the result revealed that there was no significant difference between observed and expected genotype frequencies of the SNP rs1801133 in both groups C (P-value= 0.27) and A (P-value = 0. 7) (Table2). Genetic Association of rs1801133 SNP with (A) group was adopted to analyze the association between alleles and genotypes of rs1801133 and risk of infertility. Logistic regression analysis revealed that the alleles or genotypes were associated with susceptibility to infertility in the present sample of Iraqi patients (Table 3). The heterozygous genotype, CT allele frequency was higher in patients in comparison with controls (52 vs. 36 %; p = 0.86). The OR of such difference was 2.88 (95% CI: (0.8-9.6). The homo-mutant genotype model, TT genotype frequency was increased significantly in patients than in control (16 vs. 0 %; OR = 17.4; 95% CI: 0.08-36.4; p = 0.033). The allelic model, T allele frequency was increased significantly in patients in comparison with controls (42 vs. 22 % O.R. = 3.4; 95% C.I.: 1.3-8.5; p = 0.0075) (Table 2b).

Table (2): Frequencies and percentages of *MTHFR* gene polymorphism C677T rs1801133 and their (HWE) in C group and A group.

Genotype	C group (n=25)		*P* value	A group (n=25)		*P* value
	Observed n (%)	Expected n (%)		Observed n (%)	Expected n (%)	
CC	16(64.00%)	16.81(67.20%)	0.27	8(32.00%)	8.41(33.60%)	0.7
CT	9(36.00%)	7.38(29.50%)		13(52.00%)	12.18(48.70%)	
TT	0(0.00%)	0.81(3.20%)		4(16.00%)	4.41(17.60%)	

p: Two-tailed Fisher's exact probability. Significant p ≤0.05. distribution C: control group, A: asthenozoospermic group, HWE: Hardy-Weinberg Equilibrium

Table (3): Genotype and allele frequencies of *MTHFR* gene polymorphism C677T rs1801133 in the C group and A group.

Genotype/allele	n (%)		*P* value	Odd ratio	(95% CI)
	C group (n=25)	A group (n=25)			
CC	16(64.00%)	8(32.00%)	---	1.00	(Reference)
CT	9(36.00%)	13(52.00%)	0.86	2.88	(0.8-9.6)
TT	0(0.00%)	4(16.00%)	0.033	17.4	(0.08-36.4)
C	41(82.00%)	29(58.00%)	---	1.00	(Reference)
T	9(18.00%)	22(42.00%)	0.0075	3.4	(1.3-8.5)

OR: odd ratio, CI, confidence interval, C: control group, A: asthenozoospermic patients

The current study's findings show no significant difference between the expected and observed genotypes (p = 0.16) in Oligoasthenozoospermia groups that means the result is compatible with the HWE (Table 4). Three genetic models as mentioned (Table 5) were adopted to analyze the association between alleles and genotypes of rs1801133 and the risk of men's infertility. Under heterozygous genotype, there was no significant variation in the heterozygous genotype frequency CT in a comparison between patients and controls (36 vs. 36 %; p = 0.36). Although the OR of such difference was 1.77 (95% CI: 0.5-6.1). The homomutant genotype model, TT genotype frequency was increased significantly in patients than in control (28 vs. 0 %; OR = 26; 95% CI: 0.1-50.8; p = 0.031). This means that men caring for this genotype have a higher risk for infertility than CT genotype. In the allelic model, a significant variation in the homozygous genotype frequency of T allele frequency was increased significantly in patients in comparison with controls (23 vs. 18 % OR. = 3.88; 95% C.I.: 1.5-9.6; p = 0.0035).

Table (4): Frequencies and percentages of *MTHFR* gene polymorphism C677T rs1801133 and their Hardy-Weinberg equilibrium (HWE) in the C group and OA group.

Genotype	C group (n=25)		P value	OA group (n=25)		P value
	Observed n (%)	Expected n (%)		Observed n (%)	Expected n (%)	
CC	16(64.00%)	16.81(67.20%)	0.27	9(36.00%)	7.29(29.20%)	0.16
CT	9(36.00%)	7.38(29.50%)		9(36.00%)	12.42(49.70%)	
TT	0(0.00%)	0.81(3.20%)		7(28.00%)	5.29(21.20%)	

p: Two-tailed Fisher's exact probability. Significant p ≤0.05. Distribution C: control group; OA: Oligoasthenozoospermia.

Table (5): Genotype and allele frequencies of *MTHFR* gene polymorphism C677T rs1801133 in the C group and OA group.

Genotype/allele	n (%)		P value	Odd ratio	(95% CI)
	C group (n=25)	OA group (n=25)			
CC	16(64.00%)	9(36.00%)	---	1.00	(Reference)
CT	9(36.00%)	9(36.00%)	0.36	1.77	(0.5-6.1)
TT	0(0.00%)	7(28.00%)	0.031	26.0	(0.1-50.8)
C	41(82.00%)	27(54.00%)	---	1.00	(Reference)
T	9(18.00%)	23(46.00%)	0.0035	3.88	(1.5-9.6)

OR: odd ratio, CI, confidence interval, C: control group, OA: Oligoasthenozoospermia.

Whereas in the severe oligoasthenozoospermia patients group, there was significant heterogeneity between expected and observed genotypes, which inconsistent the Hardy-Weinberg equilibrium (Table 6). Mutations, natural selection, nonrandom mating, genetic drift, and gene flow are among the causes that can disrupt the Hardy-Weinberg balance (16). Also, association studies with higher sample size and hence research power is required to discover a small risk linked with these polymorphisms (17). Standard power calculations in whole-genome scans reveal that up to 1000 participants are needed to detect substantial gene main effects (18).

The homomutant genotype model, TT genotype frequency was increased significantly in patients than in control (32 vs. 0 %; OR = 24.3% CI: 0.1-46.5; p = 0.033). This means that men caring for this genotype have a higher risk for infertility than CT genotype. In the allelic model, a significant variation in the homozygous genotype frequency of T allele frequency was increased significantly in patients in comparison with controls (44 vs. 18 % OR. = 3.57; 95% C.I.: 1.4-8.9; p = 0.0062) (Table 7).

Table (6): Frequencies and percentages of *MTHFR* gene polymorphism C677T rs1801133 and their Hardy-Weinberg equilibrium (HWE) in the C group and SOA group.

Genotype	C group (n=25)		*P* value	SOA group (n=25)		*P* value
	Observed n (%)	Expected n (%)		Observed n (%)	Expected n (%)	
CC	16(64.00%)	16.81(67.20%)	0.27	11(44.00%)	7.84(31.40%)	0.01
CT	9(36.00%)	7.38(29.50%)		6(24.00%)	12.32(49.30%)	
TT	0(0.00%)	0.81(3.20%)		8(32.00%)	4.84(19.40%)	

p: Two-tailed Fisher's exact probability. Significant p ≤0.05. Distribution C: control group; SOA: Sever Oligoasthenozoospermia

Table (7): Genotype and allele frequencies of *MTHFR* gene polymorphism C677T rs1801133 in the C group and SOA group.

Genotype/allele	n (%)		P value	Odd ratio	(95% CI)
	C group (n=25)	OA group (n=25)			
CC	16(64.00%)	11(44.00%)	---	1.00	(Reference)
CT	9(36.00%)	6(24.00%)	0.96	0.96	(0.2-3.5)
TT	0(0.00%)	8(32.00%)	0.033	24.3	(0.1-46.5)
C	41(82.00%)	28(56.00%)	---	1.00	(Reference)
T	9(18.00%)	22(44.00%)	0.0062	3.57	(1.4-8.9)

OR: odd ratio, CI, confidence interval, C: control group, SOA: Sever Oligoasthenozoospermia.

To further investigate the diagnostic value of sperm 8-OHdG levels (ng/µl) in all groups, ROC curves were constructed. According to the ROC curve, the area under the curve (AUC) regarding infertile groups were :[(A) (0.98), (OA) (0.99) and (SOA) (0.99)], sensitivity was :[(A) (92.00%), (OA) (96.00%) and (SOA) (96.00%)], and a specificity of 99.99% (Table 9).

Table (8): Association between *MTHFR* gene polymorphism C677T rs1801133 and 8OHdG level in the studied groups.

Groups	Genotypes			*P* value
	CC	CT	TT	
C group	n=16	n=9	n=0	0.60
8OHdG (ng/ml)	98.75±22.47	103.33±17.32	----	
A group	n=8	n=13	n=4	0.30
8OHdG (ng/ml)	280.18±94.29[a]	226.21±77.87[a]	292.30±101.46[a]	
OA group	n=9	n=9	n=7	0.02
8OHdG (ng/ml)	213.01±49.51[a]	311.62±112.67[b]	319.33±91.30[b]	
SOA group	n=11	n=6	n=8	0.25
8OHdG (ng/ml)	271.25±79.34[a]	349.58±69.29[a]	339.04±97.14[a]	

Data were expressed as mean ± SD; Statistical analyses were performed by ANOVA followed by Post Hoc test (Tukey's test) for multiple comparisons. ANOVA significance test (2-tailed); a,,b,c and d:(Similar small letters indicate non-significant differences) C: control; A: Asthenozoospermia; OA Oligoasthenozoospermia; SOA: Sever Oligoasthenozoospermia.

Table (9): Receiver Operating Characteristic curve data of the studied infertile groups concerning the value of sperm 8-OHdG levels (ng/µl).

	A	OA	SOA
AUC	0.98	0.99	0.99
***P* value**	0.00	0.00	0.00
CV	127.52 (ng/ml)	146.05 (ng/ml)	184.17 (ng/ml)
Specificity	88.00%	99.00%	96.00%
Sensitivity	92.00%	96.00%	96.00%
PPV	88.50%	100.00%	96.00%
NPV	91.70%	96.20%	96.00%
PLR	7.67	Infinity	24.00
NLR	0.09	0.04	0.04
AC	90.00%	98.00%	96.00%

AC, accuracy; AUC, area under curve; CV, cut off value; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio ; PPV, positive predictive value; ; A: Asthenozoospermia; OA Oligoasthenozoospermia; SOA: Sever Oligoasthenozoospermia.

Discussion

Studies must be performed to confirm the role of the *MTHFR* gene in Iraqi infertile patients, although it seems likely that the *MTHFR* gene is involved due to its influence on spermatogenesis. The current study of *MTHFR* gene genotypes and allele frequencies in all patients groups and control group revealed that there was a significant variation between these frequencies, there was a positive correlation (etiological factor) between TT genotype (222Val/Val) with infertility, this means that men who caring these genotype have a higher risk for infertility than heterozygous CT genotypes. The homozygous C667 T mutation in the *MTHFR* gene has been linked with an increased risk of several human diseases, including certain cardiovascular disorders (19), cancers (20), and neural tube defects (21). This SNP C677T rs1801133 was first studied by Bezold *et al*. (22) by comparing 255 patients seeking fertility evaluation to 200 controls; they discovered a significantly different distribution (homozygous genotype) of this SNP in connection to male fertility (22). The gene *MTHFR* appears to have an important association within male infertility in many populations as in (23) a comprehensive Indian populations study that examining the 677C>T polymorphism in male infertility revealed that infertile individuals exhibited a significantly higher frequency of mutant (TT) genotype than fertile controls, indicating a relationship between the 677C>T polymorphism and male infertility.

Many meta-analyses studies revealed significant associations between polymorphism and male fertility for C677T variant in the *MTHFR* (24,25). Authors concluded that the MTHFR C677T polymorphism is capable of causing male infertility susceptibility in Asians (26) also some studies demonstrated that this polymorphism is associated with infertility in African, South, and East Asian men, but these results were not demonstrated in the European populations as Polish population that was studied (27,28,29,30) These inconsistent findings from different populations imply that the role of C677T in male infertility susceptibility a likely polygenetic characteristic influenced by gene–racial/ethnic interactions, Gene–nutrient/environmental interactions (31) as well as lifestyle, regional and occupational factors have been found to influence the impact of these *MTHFR* genetic variations and explain population diversity (32).

Three-dimensional structure of MTHFR protein analyzed and the data shows that 677C>T mutation is deleterious for mRNA structure. In addition, a comparison of normal and mutant mRNA indicated that the global mutant has two fewer loops in its secondary structure than the normal type (33).

The Association between *MTHFR* C677T genotypes with levels 8-hydroxy-2’-deoxyguanosine (8-OHdG) revealed an evaluation of the levels of seminal oxidative stress biomarkers 8-OHdG and compared between CC (wild-type), CT (heterozygous mutant), and TT (homo-mutant) genotypes of *MTHFR*C677T in sperm DNA of studied groups. The finding of the current study show in table (8) demonstrated a significantly higher level of 8-OHdG in A, OA, and SOA groups compared to control. The levels between genotypes in each group were not significant except in

Oligoasthenozoospermia patient group was highly significant ($p<0.05$). To compare the effect of levels 8-OHdG and MTHFR C677T genotypes in infertile men, greater sample sizes and well-designed studies are required. Larger sample size could lead to more accurate statistical results and more evidence to back up the improvement in secondary outcomes.

Statistical analyses ANOVA test revealed that the level of 8-OHdG in TT and CT genotypes was highly significant than other CC, interestingly the level of 8-OHdG in sever Oligoasthenozoospermia group was non-significant between all genotypes and high in all genotypes even in CC wild genotype. Mutations may accumulate in both sperm DNA (germline) and zygote DNA as a result of the DNA damage caused by mutagenic oxidative base adducts. Because the majority of mutations occur during cell replication (spermatogenesis), sperm is more sensitive to mutation accumulation than oocytes (34). Oxidative Sperm DNA damage (SDD) maybe lead to loss of sperm DNA integrity and thus preventing accurate genetic information transmission to offspring, which can result in childhood morbidity or even cancer. Furthermore, unhealthy lifestyle and social behaviors have an adverse influence on paternal genome integrity, as evidenced by oxidative sperm DNA damage, particularly in fathers, because sperm is extremely sensitive to oxidative damage due to low cytosolic anti-oxidants (35).

Conclusion

The present study reveals that the *MTHFR* C677T gene polymorphisms as a risk factor for male infertility in a sample of Iraqi population. In addition, this study significantly higher level of 8-OHdG in sperms of all infertile groups compared to control, the result indicated that oxidative DNA damage has a significant impact on sperm parameters such as concentration and progressive motility.

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