The Prevalence of TNF-α (-308 G/A) SNPs and Their Risks Among Kurdish Infertile Males in Erbil Province

Ahmed A Al-Naqshbandi[1], Suhaila N Darogha[1], Kalthum A Maulood[1], Tanya S. Salih[2]
1 Department of Biology, College of Education, Scientific Department, University of Salahaddin, Kurdistan Region, Iraq.
[2]Department of Medical Microbiology, College of Science, Cihan University-Erbil, Kurdistan Region, Iraq

Abstract—Background: **The role that cytokines play in the reproductive function is becoming more significant. Polymorphisms in the tumor necrosis factor-α (TNF-α) gene might be involved in male infertility. This study attempted to investigate how TNF-α gene polymorphisms affect the quality of infertile males' semen.**
Methods: **In the current study, the ELISA method was used to assess the serum TNF-α level in 40 fertile males and 144 patients who had been diagnosed with infertility. The genotyping of the TNF-α (-308 G/A) (rs1800629) SNPs gene was determined using the T-ARMS-PCR technique.**
Results: The serum levels of TNF-α in infertile groups increased significantly compared to the fertile ones. There was no association between genotypes in infertile males in comparison with a fertile group. While Occurrence of the GA or AA genotypes in TNF-α (–308) gene increase risk of male infertility, AA genotype in normozoospermic male {probability (p)= 0.025, relative risk (RR)= 14.88}, in oligozoospermic male, GA and AA genotypes (p= 0.55, RR= 3.86) and (p= 1.00, RR= 1.61), respectively.
Conclusions: Gene polymorphisms of TNF-α (-308 G/A) have no impact on male infertility. However, the existence of the A allele in TNF-α (-308 G/A) genotypes may increase the risk of male infertility in our population.

Keywords: TNF-α gene polymorphisms, Asthenozoospermia, Teratozoospermia, Oligozoospermia.

Introduction

Tumor necrosis factor-α is a multifunctional cytokine that has been involved in numerous cellular processes [1]. It's synthesized in the testes even in the absence of inflammatory or immunological activation stimuli and plays a crucial regulatory role in both the development and proper functioning of the testes [2]. There is a growing interest in the impact of TNF-α on human reproduction due to their ability to influence various aspects of reproductive physiology and regulation of fertility. Within the testes, germ cells which contain messenger RNA "mRNA" are responsible for the secretion of TNF-α, which is widely recognized as a crucial testicular paracrine factor and plays a significant role in the regulation of spermatogenesis [3, 4].

The TNF-α regulates testosterone activity by acting on the AR, consequently, it encourages cell survival during spermatogenesis [5]. It's one of the cytokines that is frequently detected in the male genital system, which increases the generation of reactive oxygen species "ROS" to cause sperm peroxidation and apoptosis [6]. It can also affect spermatogenesis and sperm performance by several functions, like lowering the generation of testosterone, and/or modifying the ratio of hormones in specific testes regions, and has a direct effect on spermatozoal motility [7].

The gene responsible for generating TNF-α is situated on chromosome 6p21.3, specifically inside the genomic region that encodes Human leukocyte antigen (HLA) is the idiom for the major histocompatibility complex (MHC) in humans. Between the HLA-DR class II and HLA-B class I genes is where this gene is located [8]. The promoter region of TNF-α analyzed for some SNPs at various positions "1031 T/C, 863 C/A, 857 C/T, 575 G/A, 376 G/A, 308 G/A, 238 G/A". Single nucleotide polymorphisms were shown to have an impact on TNF-α level, and various polymorphisms in the TNF-α gene cluster were connected to the altered synthesis of this substance [9]. The SNP located at position -308 in the promoter region of the TNF-α gene has been widely studied and is commonly linked to elevated transcription rates of the cytokine [10-12]. Higher expression of TNF-α is related with the -308 TNF-α allele. They noted that, as compared to individuals with normal sperm parameters, the distribution of the -308G/A allele was considerably greater in infertile males with testicular failure or with reduced motility of sperm [13]. One of the studies revealed a notable increase in the frequencies of TNF-α (-308G/A) allele substitution from G to A among the infertile patients compared to the fertile group. This substitution was found to be strongly linked with some conditions such as azoospermia, oligozoospermia, asthenozoospermia, and teratospermia [14, 15].

Materials and Methods

The current research is based on a prospective case-control study. The participants enrolled in this study were from Rizgary Teaching Hospital, Shayi private clinical laboratory and Runahi IVF Center, and in Erbil City from 3rd September 2021 to 4th September 2022. The interview and the structured questionnaire were designed in the data collection process. Patient groups included 144 infertile males, whose ages ranged between 17-59 and categorized as normozoospermia,

DOI: http://doi.org/10.24086/biohs2024/paper.1436

oligozoospermia, and azoospermia, of which 52 (36.11%) were from Rizgary Teaching Hospital, 68 (47.22%) from Runahi IVF Center, and 24 (16.67%) from Shayi private clinical [16] ranged between 18-47 without a history of illnesses or reproductive disorders, of which 18 (45%) were from Rizgary Teaching Hospital, 12 (30%) from Runahi IVF Center, and 10 (25%) from Shayi private clinical laboratory.

Exclusion Criteria

Male infertiles with obstructive azoospermia, altered testosterone, FSH, and LH hormone levels, urogenital tract diseases, cryptorchidism, and other chronic illnesses not included in the study.

Ethical Considerations

The volunteer participants were properly informed about the goals of the study. and they guaranteed that the data would only be used for specific academic purposes. Additionally, we made sure that ethics would always come first in this research.

Seminal Fluid Analysis

The participants were informed and directed to get the seminal fluid by masturbation after 3-5 days of sexual abstinence. After incubation and liquefaction time, seminal fluid was investigated for macroscopically and microscopically analysis within an hour of ejaculation according to WHO guideline [16].

Blood Collection

Blood sampling was performed by withdrawing 7 ml of venous blood from each participant using a disposable syringe (venipuncture technique) and distributed into a labeled EDTA tube (2ml), then kept in a freezer at -20 °C for DNA extraction. The rest amount of blood (5ml) was putted in a gelatin tube, left to clot at room temperature then centrifuged at 6000 rpm for 5 minutes, the sera produced were dispensed into a labeled and sterile Eppendorf tube and preserved in a freezer at -20 °C to test the total quantity of TNF-α.

Estimation of serum TNF-α by ELISA

The serum TNF-α level was assessed using a commercial ELISA kit after the frozen serum had thawed at room temperature. The analysis was carried out at Salahadden University's Laboratory of Biotechnology, Education College.

DNA isolation and genotyping of TNF-α (-308 G/A) SNPs

The frozen blood in an EDTA tube was left to thaw at room environment to separate the DNA, and DNA extraction kit employed through implementing manufacturer instructions. DNA purity and amount determined through nano-drop by computing the absorbance at wavelengths 260-280 nm, that's considered from 10-97 ng, and the purity of extracted DNA was observed between 1.7 to 1.9.

By employing the T-ARMSPCR procedure, the genotype of the G and A allele of the TNF-α gene at position -308 was determined. Table (1) present the primers that were designed to target the TNF-α gene's SNPs, based on the [17] technique. The PCR reaction carried out using 2X Prime Taq Premix with a 20 µl reaction mixture "nuclease free water 5 µl, nuclease free water 5 µl, nuclease free water 5 µl, nuclease free water 5 µl, Taq master 10 µl, forward and reverse primer (outer, inner) 1 µl for each, DNA template 1 µl".

The PCR profile included 35 cycles in 2 phase after a 5 min initial melting at 94 °C. First phase included 10 cycles "denaturation: 15sec. at 94 °C, annealing: 50 sec. at 65 °C, extension: 40 sec. at 72 °C" and second phase included 25 cycles "denaturation: 20 sec. at 94 °C, annealing: 50 sec. at 59 °C, extension: 50 sec. at 72 °C" then holed at 20 °C for 10 min. Following PCR cycles, the products that were amplified were then examined for their size and visualized by 2 % agarose gel electrophoresis in which conducted for 50 min. "75 V/cm2 for 20 min. then 85 V/cm2 for 30 min.", stained with safe dye (Figure 1). Following electrophoresis, the genotype registered using the genotyping kit schedule (Table 1).

TABLE 1: DEVELOPED PRIMER SEQUENCES FOR TNF-A (-308 G/A) GENOTYPING IDENTIFICATION

Gene polymorphism	Minor allele	Primer name	Primer sequence (5-3)	allele	Amplicons size (bp)	Product size (bp)	Genotype
TNF-α (-308G/A) (rs1800629)	A	Forward outer	AGGACTCAGCTTTCCGAAGCCCCTCCCA		304	Normal homozygote: 162/304	GG
		Rivers outer	TTCTGTCTCGGTTTCTTCTCCATCGCGG		304	Heterozygote: 162/197/304	GA
		Forward inner	GTAGGACCCTGGAGGCTGAACCCCGTACT	G	162	Mutant homozygote: 197/304	AA
		Rivers inner	GGAGGCAATAGGTTTTGAGGCGCAGGG	A	197		

Figure 1: Agarose gel electrophoresis demonstrates PCR results for the TNF-α (-308 G/A) SNPs. Lane 1: DNA ladder (100 bp), Lane 8: GG genotype, Lane 10: AA genotype, Lane 12: GA genotype

Statistical Analysis

The GraphPad Prism version 9.0 was used for entire statistical analysis. The relationship between genotypes and infertility was assessed by calculating the relative risk (RR) and 95% confidence intervals (CI). The Hardy-Weinberg equilibrium (HWE) utilized to compare the diplotypes distributions of fertile and infertile cohorts. A P-value of <0.05 deemed as statistically significant.

DOI: http://doi.org/10.24086/biohs2024/paper.1436

RESULTS AND DISCUSSION

Evaluation of Serum TNF-α and their Correlation with Seminogram

Figure (2) displays that there was significant variation of serum TNF-α concentration between fertile and infertile males. Fertile males had mean serum TNF-α concentrations of 167.3±27.56 pg/ml. In infertile groups, TNF-α serum levels were 380.5±324.1pg/ml in normozoospermia, 326.4±253.7 pg/ml in oligozoospermia, and 322.1±317.1 pg/ml in azoospermia. Comparing the fertile and infertile participants, there was significant variation in the serum concentrations of TNF-α. There were differences between the infertile groups, but they were not statistically significant.

The biological implications of TNF-α are various and have the potential to interfere with or disturb reproductive functions. The outcome of the current investigation indicated a statistically significant rise in the concentrations of TNF-α in the infertile males compared to the fertile males. However, there were no significant changes seen when comparing the TNF-α concentration in the serum of different infertile participants. The outcomes of TNF-α are in line with a previous study, which indicated a significantly raised concentration of TNF-α in infertile males compared to controls [18]. The TNF-α is a pro-inflammatory cytokine and might elevate in a variety of non-specific conditions and infertility.

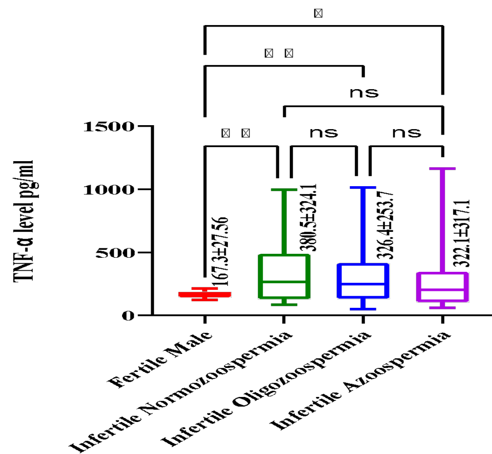


Figure 2: Level of serum TNF-α among fertile and infertile males

The TNF-α serum levels in normozoospermic and oligozoospermic infertile males were non-statistically negatively linked with spermatozoa concentration, spermatozoa count, and spermatozoa motility. Non-significant positive association of TNF-α serum level with immotile sperm was found. Non-significantly negative correlation was found between serum level of TNF-α and normal sperm morphology and positively with abnormal sperm morphology in normozoospermic and oligozoospermic infertile males (Table 2).

TABLE 2: CORRELATION OF SERUM TNF-A LEVEL WITH INFERTILE MALES SEMEN CHARACTERISTICS

Parameters		Infertile men groups					
		Normozoospermia		Oligozoospermia		Azoospermia	
		Pearson correlation	p. value	Pearson correlation	p. value	Pearson correlation	p. value
Volume		-0.597	0.011	0.032	0.784	0.015	0.976
Sperm Concentration		-0.027	0.890	-0.006	0.958	NA	NA
Sperm Count		-0.283	0.145	-0.051	0.663	NA	NA
Motility	Active	-0.205	0.296	-0.157	0.177	NA	NA
	Slow	-0.252	0.196	-0.031	0.788	NA	NA
	Non-progresive	-0.189	0336	-0.023	0.846	NA	NA
	Immotile	0.246	0.207	0.096	0.408	NA	NA
Morphology	Normal morphology	-0.032	0.872	-0.027	0.818	NA	NA
	Abnormal morphology	0.032	0.872	0.027	0.818	NA	NA

NA: Not applicable

The correlation between TNF-α and male infertility, as well as its impact on sperm characteristics, remains a subject of debate. There are a limited reports indicating a negative association between TNF-α and seminogram. The relationship of serum TNF-α level with spermatozoal parameters in our study revealed that TNF-α has no significant impact on sperm features, which is in the line with the previous results [7], and disagrees with the findings of others who found TNF-α negatively correlated with sperm count, motility, and morphology [19, 20]. The probable mechanism through which TNF-α exerts adverse effects on spermatozoa is through the mediation of ROS, as TNF-α has the capability to induce ROS generation in spermatozoa. Consequently, there may be an occurrence of sperm membrane peroxidation associated with ROS [21]. Apoptosis has been proposed as a potential technique behind the manifestation of toxic effects induced by TNF-α. Upon binding to its receptor, TNF-α triggers the activation of several intracellular signaling molecules, including TNF-α receptor-associated factor (TRAF), TNF-receptor-associated death domain (TRADD), and receptor-interacting protein kinase 1 (RIPK1). Subsequently, the activation of caspase-2 and caspase-8 occurs, leading to the ultimate activation of effector caspase-3, which eventually results in cellular death [22, 23]. Depending on the previous mechanisms of TNF-α actions, the only explanation for the absence of relationship between elevated TNF-α and semen quality in our study might be modulating TNF-receptors (TNFRs).

Associations of TNF-α (-308 G/A) Genotypes Distributions and Alleles Frequencies in Infertile Males

The genotypes and allelic frequencies of the TNF-α (-308 G/A) rs1800629 SNPs in the infertile male subgroups as well as in fertile males are presented in Table (3). Three genotypes (GG, GA, and AA) of TNF- α (-308) are present within the study participants. In infertile subgroups, the genotype frequencies in normozoospermia were 24 (85.71%), 0 (0%), and 4 (14.29%); oligozoospermia were 72 (94.74%), 3 (3.95%), and 1 (1.31%); azoospermia were 40 (100%), 0 (0%), and 0 (0%), respectively. The frequency of the G and A alleles in normozoospermia were 48 (85.71%) and 8 (14.29%); in oligozoospermia there were 147 (96.71%) and 5 (3.29%); in azoospermia there were 80 (100%) and 0 (0%); and in fertile males, there were 80 (100%) and 0 (0%), respectively. The frequencies of mutated homozygous AA genotypes of TNF-α differed significantly in normozoospermic and non-

DOI: http://doi.org/10.24086/biohs2024/paper.1436

significantly in oligozoospermic infertile males (RR:14.88, p=0.025 and RR:1.61, p=1.00, respectively). In oligozoospemia, the frequencies of heterozygous GA genotypes of TNF-α differed non-significantly (RR:3.86, p=0.55). Regarding the azoospermia group, no mutation in this genome was detected. Infertile participants with normozoospermia significantly differ and oligozoospermia did not significantly differ from the fertile group according to an analysis of the allele frequencies for genotypes at TNF-α (-308 G/A) carrying the A allele (RR:28.22, p=0.0001 and RR:6.0, p=0.167, respectively). The recessive model of TNF-α (AA+GA) vs. GG, in normozoospermic groups, exhibited significant relation to male infertility, while in oligozoospermic group, exhibited no relation to male infertility (RR:14.88, p=0.025 and RR=5.03, p=0.297), respectively.

TABLE 3: THE GENOTYPES AND ALLELE DISTRIBUTIONS OF TNF-A -308 G/A IN THE FERTILE AND INFERTILE MALES

Seminogram categories	TNF-a (-308 G/A) (rs1800629)	Infertile male frequency	Fertile male frequency	Relative Risk (RR)	Etiology or Preventive Fraction	Exact Fishers Probability (P)	95% Confidence Intervals (CI)
Normozoospermia	Genotype						
	GG	24 (85.71%)	40 (100%)	NA			
	GA	0 (0%)	0 (0%)	NA			
	AA	4 (14.29%)	0 (0%)	14.88	0.14	0.025	0.80-277.61
	(GG+GA) vs AA	24 (85.71%)	40 (100%)	NA			
	(AA+GA) vs GG	4 (14.29%)	0 (0%)	14.88	0.14	0.025	0.80-277.61
	Allele						
	G allele	48 (85.71%)	80 (100%)	NA			
	A allele	8 (14.29%)	0 (0%)	28.22	14.4	0.001	1.62-490.76
Oligozoospermia	Genotype						
	GG	72 (94.74%)	40 (100%)	NA			
	GA	3 (3.95%)	0 (0%)	3.86	0.03	0.55	0.20-73.95
	AA	1 (1.31%)	0 (0%)	1.61	0.007	1.000	0.07-39.02
	(GG+GA) vs AA	75 (98.68%)	40 (100%)	NA			
	(AA+GA) vs GG	4 (5.26%)	0 (0%)	5.03	0.04	0.297	0.27-92.53
	Allele						
	G allele	147 (96.71%)	80 (100%)	NA			
	A allele	5 (3.29%)	0 (0%)	6.00	0.03	0.167	0.33-108.07
Azoospermia	Genotype						
	GG	40 (100%)	40 (100%)	NA			
	GA	0 (0%)	0 (0%)	NA			
	AA	0 (0%)	0 (0%)	NA			
	(GG+GA) vs AA	40 (100%)	40 (100%)	NA			
	(AA+GA) vs GG	0 (0%)	0 (0%)	NA			
	Allele						
	G allele	80 (100%)	80 (100%)	NA			
	A allele	0 (0%)	0 (0%)	NA			
NA: Not applicable							

DOI: http://doi.org/10.24086/biohs2024/paper.1436

The frequency of each genotype was evaluated by HWE in the groups of fertile and infertile participants. In the normozoospermic group, the occurrence of the GG and AA genotypes fluctuated between what was predicted and what was observed, with 24 (85.71%), 20.57 (73.46%), and 4 (14.29%), 0.57 (2.04%), respectively. While the occurrence of the GG, GA, and AA genotypes ranged between what was predicted and what was actually observed in the oligozoospermic group, they were respectively represented by 72 (94.74%), 71.08 (93.53%); 3 (3.95%), 4.84 (6.37%), and 1 (1.31%), 0.08 (0.10%). It was statistically significant when comparing the observed and expected genotype frequency values, demonstrating that this cohort's distribution is not below HWE (Table 4).

TABLE 4: DISTRIBUTIONS OF GENOTYPES AND ALLELE OF TNF-A -308 G/A IN THE FERTILE AND INFERTILE MALES

Case Categories		TNF-a gene at position -308 G/A (rs1800629)					
		Genotypes			HWE p. value	Alleles	
		GG	GA	AA		G	A
Infertile Normozoospermia	Observed	24 (85.71%)	0 (0%)	4 (14.29%)	0.000	48	8
	Expected	20.57 (73.46%)	6.86 (24.5%)	0.57 (2.04%)		NA	
Infertile Oligozoospermia	Observed	72 (94.74%)	3 (3.95%)	1 (1.31%)	0.004	147	5
	Expected	71.08 (93.53%)	4.84 (6.37%)	0.08 (0.10%)		NA	
Infertile Azoospermia	Observed	40 (100%)	0 (0%)	0 (0%)	NA	80	0
	Expected	40 (100%)	0 (0%)	0 (0%)		NA	
Fertile male	Observed	40 (100%)	0 (0%)	0 (0%)	NA	80	0
	Expected	40 (100%)	0 (0%)	0 (0%)		NA	
NA: Not applicable							

The importance of TNF-α genetic polymorphisms in the epidemiology and etiology of male infertility is a subject of dispute. Some research imply a connection between gene polymorphisms in the TNF-α -308 gene and male infertility [14, 15]. Other researchers have figured that there is no observed link of male infertility and TNF-α polymorphism at position -308 [24, 25]. The findings of our study indicate that there is no connection between genetic variation in the promoter of the TNF-α -308 gene and the possibility of male infertility. So, we did not figured a significant difference in the variant genotype frequency of TNF-α -308G/A between fertile and infertile subgroups. However, we propose that the presence of the A allele is correlated with raised susceptibility to male infertility.

Evaluation of Serum TNF-α Level with Different TNF-α (-308 G/A) Genotypes

The results of TNF-α concentration related to TNF-α (-308 G/A) genotypes are displayed in Figure (3). In fertile males carrying GG, GA, and AA genotypes, TNF-α levels were 167.27±27.56 pg/ml, 0.00±0.00 pg/ml, and 0.00±0.00 pg/ml, respectively. In infertile males carrying GG, GA, or AA genotypes, TNF-α levels in normozoospermia were 316.03±264.7 pg/ml, 0.00±0.00 pg/ml, and 767.17±419.1 pg/ml; in oligozoospermia, they were 290.65±207.7 pg/ml, 956.24±68.66 pg/ml, and 1014.63±0.00 pg/ml; in azoospermia were 322.12±317.1 pg/ml, 0.00±0.00pg/ml, and 0.00±0.00pg/ml, respectively. Significant elevation of serum TNF-α concentration in mutated homo (AA) and hetero (GA) zygotes regarding TNF-α (-308 G/A) genotypes was figured compared to the non-mutated genotype (GG) in normozoospermia and oligozoospermia males.

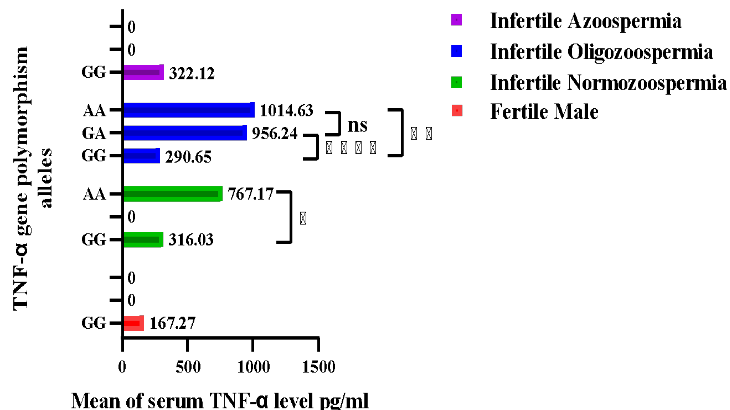


Figure 3: Serum TNF-α level according to TNF-α (-308 G/A) genotypes among fertile and infertile males

Genetic variations within the TNF-α gene have the potential to modify the production of TNF-α [13]. The -308 G/A SNP located in the promoter region of the TNF-α gene associated with an elevation in promoter activity, resulting in an elevated synthesis of TNF-α [26]. The notable finding in our study, when examining the infertile study group, in normozoospermia only homozygous AA exhibited higher serum TNF-α levels compared to wild-type GG, while in oligozoospermia both AA and AG exhibited elevated levels of TNF-α compared to GG. Additionally, the occurrence of A allele was observed with an raised level expression of TNF-α, which aligns with previous research findings [27].

Association of Genotypes of TNF-α (-308 G/A) with Semen Quality in Infertile Males

Azoospermia is an instance in which there is no spermatozoa in the semen. The connection between TNF-α (-308 G/A) gene polymorphisms and the seminogram lacked to show in Figures 4-6.

In our investigation, we observed a total of four instances of TNF-α (-308 G/A) gene polymorphisms in males with normozoospermia and an equal number of occurrences in oligozoospermic infertile males. Therefore, the results related to the association between TNF-α (-308 G/A) gene polymorphisms and the seminogram failed to exhibit a statistically significant impact on the features of sperm quality. The mutated homozygous AA genotype exhibited a statistically non-significant increase in spermatozoa concentration compared to the GG genotype in normozoospermic infertile males (35.50±10.97, 34.71±0.00). Similarly, in oligozoospermic infertile males, the mutated homozygous AA genotype showed higher sperm concentration when compared to the GA and GG genotypes (8.00±0.00, 5.33±2.31, 3.01±3.07), respectively. In addition, the mutated homozygous AA genotype in normozoospermic infertile males resulted in a statistically non-significant increase in sperm counts (117.75±45.9) compared to the GG genotype (103.15±73.11). Similarly, in oligozoospermic infertile males, the mutated AA genotype exhibited a statistically non-significant increase in

DOI: http://doi.org/10.24086/biohs2024/paper.1436

sperm count compared to the GA and GG genotypes (40.00±0.00, 18.67±8.08, 10.86±12.37) per ejaculation, respectively (Figure 4: A, B).

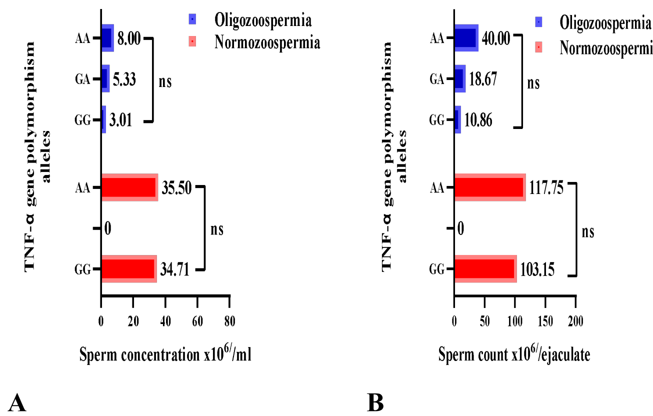
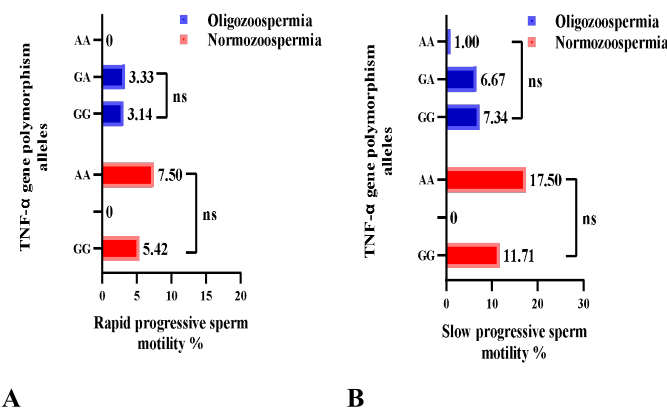


Figure 4: A: Sperm concentration, B: Sperm count according to TNF-α (-308 G/A) gene polymorphisms among infertile males

Regarding the correlation of TNF-α (-308 G/A) gene polymorphisms and sperm motility traits, normozoospermic infertile males with AA genotypes exhibited a decreased proportion of immotile sperm in comparison to individuals with GG genotypes (67.50±14.43, 79.54±20.65). Conversely, a higher percentage of slow progressive and non-progressive motile sperm (17.50±8.66, 11.71±9.80) and (7.50±2.88, 3.33±2.41), respectively. In males with oligozoospermia, individuals with the AA genotype had a higher proportion of immotile sperm in comparison to those with GG genotypes (98.00±0.00, 85.38±12.31), respectively. The statistical analysis indicated there was no significant connection between sperm motility and genotypes within the infertile groups (Figure 5: A, B, C, D).



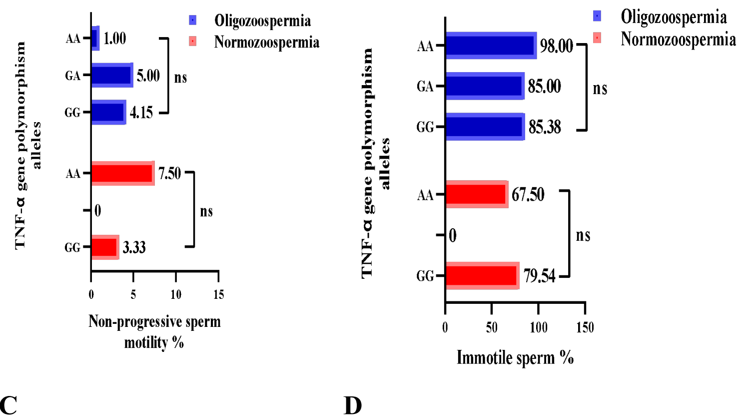


Figure 5: Sperm motility "A: rapid progressive, B: slow progressive, C: non-progressive, D: immotile" according to TNF-α (-308 G/A) gene polymorphisms among infertile males

The impact of the TNF-α gene on sperm morphology was also demonstrated. Males with the AA genotype who are normozoospermic and infertile exhibited a significantly higher proportion of abnormal sperm morphology (97.50±2.88) compared to individuals with GG genotypes (95.13±8.81). Males diagnosed with infertility and oligozoospermia, possessing AA and GA genotypes, had a higher proportion of abnormal spermatozoa morphology in comparison to those with GG genotypes (100.00±0.00, 99.33±1.15, 97.40±2.88), respectively (Figure 6: B).

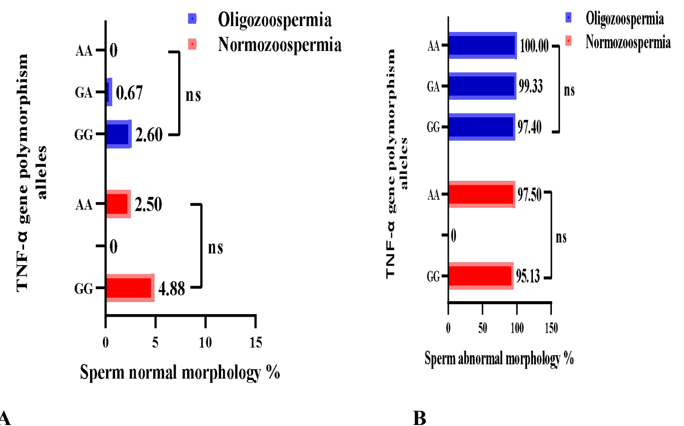


Figure 6: Sperm morphology "A: normal, B: abnormal" according to TNF-α (-308 G/A) gene polymorphisms among infertile males

The researchers found that peoples with TNF-α (-308G/A) genotypes exhibited a negative association with semen parameters. Specifically, these genotypes were found to significantly lower spermatozoa count, spermatozoa motility, and normal spermatozoa morphology when compared to individuals with the TNF-α GG genotype [25, 28]. The findings of the present study differed from prior studies about the correlation between the TNF-α (-308G/A) SNP and sperm characteristics, and our investigation did not identify any

DOI: http://doi.org/10.24086/biohs2024/paper.1436

association between the -308G/A SNP and abnormal semen parameters, except in sperm morphology, we found out that gene variation associated with abnormal sperm morphology.

Conclusion

The current study does not support the role of the -308 G/A TNF-α gene polymorphisms as indicators of risk for male infertility in the Kurdish male community. Despite that the existence of A allele in the TNF-α gene position -308 might contribute to male infertility.

Acknowledgment

The authors are grateful to the participants that's enrolled in current research as volunteers. Also, the Biotechnology Laboratory of the Biology Department, College of Education, Salahaddin University for collaborating in conducting all laboratory experiments.

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DOI: http://doi.org/10.24086/biohs2024/paper.1436