Tumor necrosis factor alpha polymorphisms and risk of male infertility

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Abstract

Background: Tumor necrosis factor-alpha is a multifunctional cytokine that controls spermatogenesis-related cellular functions. Objective: To investigate the association of TNF-a-308 gene Polymorphism with idiopathic male infertility by using PCR and ELISA. Methods: This case-control study was conducted during January to December 2021 was included 50 infertile males attending the Center of infertility in Wasit governorate compared with 50 fertile males as control group. From each participant blood and semen samples were collected according to the standard method. Tumor necrosis factor-alpha (TNF-a) 308 genotyping was done by using allele-specific PCR while TNF-a serum levels were measured by using sandwich ELISA method. Results: Serum level of TNF-a in patients was 27.63 pg/ml while in control group was 8.08 pg/ml with significant difference P<0.001, on the other hand there was none significant difference between both studied groups regarding seminal plasma concentration of TNF-a (p- value=0.193). The results found that Tumor Necrosis Factor –alph-308 A allele was with higher frequent among infertile males (70%) than fertile males. In addition present study showed higher rate of G allele of TNFR1 36 in patients (64%) as compared with control group (36%) with statistically significant difference (P=0.001). As well as the current work revealed higher serum TNF-a level in patients carrying AA genotype of TNF-a 308 comparying with patients have GG genotype but statistically not significant. In addition current study showed inverse correlation between seminal plasma TNF-a level with concentration of sperms. Aa well as there was increase in TNF-a serum levels in smoker patients as compared with non smokers but statistically not significant (P=0.60). Also there was lower sperm concentration and grade A of motility in smokers pateints as compared with non smokers with statistically significant difference. Conclusion: Seminal plasma TNF-a level may negatively affect sperm concentration, grading of motility and morphology, also the smoking habit may inversely affect sperm concentration and grade of motility.

Keyword: Infertility, TNF-a, smoking, polymorphism

Introduction

Male infertility is defined as the inability to conceive after a reasonable period of sexual activity without the use of contraception is caused by a complex interaction of factors such as endocrinal genetic and/or environmental factors. Hereditary infertility is common in idiopathic infertility. The mutations and genes linked to male infertility are well understood. Initially, single nucleotide polymorphisms were employed to search for genetic risk factors using a specific genes technique (1).

Many of the cytokines detected in seminal plasma were also found in the male reproductive tract. TNF- a is a cytokine regulating peptide that is produced and secreted by leukocytes and has been connected to growth and differentiation factors. Furthermore, TNF-'s seminal plasma levels have been shown to be inversely connected with progressive sperm motility in men with infertility that is unexplained. Said et al. found that when sperm are exposed to high TNF-a concentrations, they lose a significant amount of genomic and functional integrity (2).

Genetic factors like the single nucleotide polymorphisms have been found to influence TNFα levels, with various polymorphisms in the gene cluster of the TNF that is related to altered production. The infertile males with the change in the motility of the sperms or testicular failure had a considerably higher frequency of 308 allele than patients that have normal parameters of the sperm (19.40%). A study conducted by Zalata et al., linked a polymorphism of the single nucleotide in TNFα (-308) gene to an increase in the seminal caspase9 and a decrease in the numbers of the sperms, normal sperm morphology, acrosin activity, motility, and seminal alpha-glucosidase (3).

The aim of the study

-Assessing the relation between TNF-α polymorphism and their serum levels with male infertility.

Materials and Methods:

This case-control study was conducted during January to December 2021 was included 50 infertile males who visited the Waist Center for Infertility, blood samples and seminal plasma were taken from each one.

Inclusion criteria: Males with infertility for at least a one year (idiopathic infertility).

Exclusion criteria: included the age >50 years, vasectomy, azoospermia, hypogonadism, varicocele, diabetes, hypertension, chemotherapy, cancer, tuberculosis, HIV, alcohol or drug abuse, aberrant karyotype, and chronic genital infections.

Semen samples of cases were carried out according to WHO 2010 guidelines. After liquefaction of semen sample, equal volume of phosphate buffered saline was added for each sample and stored -20 C until assay.

Control group: The current study comprised (50) fertile males aged 20 to 50 years from the general population, they all had no therapy and had no other chronic or systemic disorders

Two ml of blood samples were collected from each participant. Allele-specific polymerase chain reaction (AS-PCR) was used to investigate the distribution of the guanine (G)-to-adenine (A) substitution at position -308 in the promoter region of the TNF-a gene. PCR primers were used to amplify an 836 base pair fragment of the TNF promoter region at position -308 (G-A) as shown in table (1).

Table (1) Primers of TNF-α -308 Polymorphism.

Primer	Sequence 3′ ⟶ 5′	Allel
TNF F	CTGCATCCCCGTCTTTCTCC	Common
TNF R1	ATAGGTTTTGAGGGGCATCG	G
TNF R2	ATAGGTTTTGAGGGGCATCA	A

Serum level of TNF-a was measured by using Sunlong Biotech ELISA Kit.

Statistical analysis:

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 software and Microsoft Excel 2013. Numerical data were described as median and confidence intervals. While, categorical data were described as count and percentage. Mann-whiteny u test used for comparison between 2 groups. Chi-square test or Fisher exact test was used to describe the association between variables. Odd ratio was calculated to estimate the risk of gene polymorphism. The lower level of accepted statistical significant difference is equal or bellow to 0.05 (4).

Results:

Table (2) showed high statistical significant difference ($p \leq 0.001$) between cases and controls concerning serum level of TNF-alpha (pg/ml).

Table (2) Seminal TNF-a level in studied groups

	Study groups		
	Cases	Controls	P value
Seminal plasma TNF-alpha (pg/ml)	0.88 (0-11.17)	0.88 (0-23.51)	0.193^{NS}
Serum TNF-alpha (pg/ml)	27.63 (21.45-37.91)	8.08 (0-27.63)	<0.001**

NS: none statistical significance ($p>0.05$) *: significant difference ($p \leq 0.05$)
**: high statistical significant difference ($p \leq 0.001$)
All samples in this study were also categorized according smoking habit, the results indicate that 76% of patients were smoker while 24 % non smoker p –value =<0.001 (table-3)

Table (3) Smoking habit in studied groups.

	Study groups		P value
Smoking habit	Cases	Controls	
Smoker	38	7	<0.001**
%	76.00%	14.00%	
Non smoker	12	43	
%	24.00%	86.00%	

**: high statistical significant difference ($p \leq 0.001$)

Regarding TNF-308G>A SNP the results showed that AA homozygous alleles were dominant (50% in patients) followed by AG heterozygous allele constitute (40%) and the GG homozygous allele was the least one 10% while the AA in control (20%) , AG (16%) and GG (64%) with high statistical significant difference (p≤0.001) between patients and controls table (4).

Also there was highly significant difference between study groups concerning allele type with p-value =0.001.

Table (4) TNF-308 genotype in studied groups.

TNF-α -308 G > A		Study groups		P value	Risk estimation	
		Patients	Controls		Odd ratio	25-75 CI
Geno308	AA mutant	25	10	<0.001**		
		50.0%	20.0%			
	AG	20	8			
		40.0%	16.0%			
	GG wild	5	32			
		10.0%	64.0%			
Allele308	A	70	28	<0.001**	6	3.29 to 11.12
		70.0%	28.0%			
	G	30	72			
		30.0%	72.0%			

**: high statistical significant difference (p≤0.001)

Table (5) revealed significant association between seminal plasma TNF level and TNF-308 genotypes in patients group.

Table (5) Seminal plasma TNF-a level in patients according TNF-308 genotypes.

Seminal plasma TNF-alpha (pg/ml)		Patients
TNF-α -308 G > A	AA	0 (0-2.94)
	AG	2.94 (0-13.23)
	GG	11.17 (0-15.28)
P value		0.007*

TNF-α -308 G > A	A	0.88 (0-5)
	G	3.97 (0-15.28)
P value		0.006*

Table (6) showed no association between serum TNF-a level and TNF-308 genotypes in patients group.

Table (6) Serum TNF-a levels in patients group according TNF-308 genotypes.

Serum TNF-alpha (pg/ml)		Cases
TNF-α -308 G > A	AA	27.63 (21.45-37.91)
	AG	30.71 (21.46-39.97)
	GG	23.51 (21.45-23.51)
P value		0.913^{NS}
TNF-α -308 G > A	A	27.63 (21.45-37.91)
	G	25.57 (21.45-37.91)
P value		0.938^{NS}

Table (7) showed significant association between grade A motility with genotype AA and AG p-value =0.048.

Table (7) The association between TNF-a-308 genotype and seminal fluid analysis parameters.

	TNF-α -308 G > A						
	Genotype			P value	Allele		P value
	AA	AG/GA	GG		A allele	G allele	
Sperm concentration (million/ml)	35 (5-40)	37.5 (25-45)	40 (30-47)	0.180^{NS}	35 (25-40)	40 (25-45)	0.195^{NS}
Grade A %	20 (15-20)	20 (15-20)	15 (15-15)	0.048*	20 (15-20)	15 (15-20)	0.690^{NS}
Grade B %	20 (20-20)	20 (15-22.5)	20 (15-30)	0.460^{NS}	20 (15-20)	20 (15-25)	0.950^{NS}
Grade C %	20 (15-30)	20 (15-20)	15 (10-20)	0.111^{NS}	20 (15-30)	20 (15-20)	0.142^{NS}

Grade D %	40 (30-45)	40 (32.5-50)	40 (35-55)	0.563^{NS}	40 (30-45)	40 (35-55)	0.438^{NS}
Abnormal morphology %	35 (30-40)	32.5 (30-40)	35 (30-40)	0.396^{NS}	35 (30-40)	35 (30-40)	0.550^{NS}

Table (8) showed inverse correlation between seminal plasma TNF-a with concentration of sperm.

Table (8) The correlation between serum and seminal level of TNF-a with seminal fluid analysis parameters .

		Seminal plasma TNF-alpha (pg/ml)	Serum TNF-alpha (pg/ml)
Sperm concentration (million/ml)	R	-0.226*	-0.167
	P	0.024	0.096
Grade A %	R	0.177	-0.089
	P	0.078	0.379
Grade B %	R	-0.290**	-0.132
	P	0.003	0.191
Grade C %	R	-0.032	-0.047
	P	0.751	0.642
Grade D %	R	0.111	0.142
	P	0.273	0.159
Normal morphology %	R	-0.017	-0.017
	P	0.864	0.864
Abnormal morphology %	R	0.017	0.017
	P	0.864	0.864
Seminal plasma TNF-alpha (pg/ml)	R	1	-0.129
	P		0.2
Serum TNF-alpha (pg/ml)	R	-0.129	1
	P	0.2	
Seminal plasma TNFR (pg/ml)	R	-0.108	-0.103
	P	0.283	0.307
Serum TNFR (pg/ml)	R	0.166	-0.085
	P	0.1	0.401

Table (9) showed higher level of TNF-a in serum of smoker patients in comparision with non smokers but statistically not significant

Table (9) Correlation between smoking habit and serum and plasma levels

of tumor necrosis factor- alpha and tumor necrosis factor receptor 1

		Case		P value
		Smoker N=76	Non smoker N=24	
Seminal plasma TNF-alpha (pg/ml)	Median	0.88	1.91	0.436
	Percentile 25	0.00	0.00	
	Percentile 75	11.17	9.12	
	Percentile 25	0.00	0.00	
	Percentile 75	9.73	15.60	
	Percentile 25	0.00	0.21	
	Percentile 75	1.67	29.02	
Serum TNF-alpha (pg/ml)	Median	28.66	24.54	0.060
	Percentile 25	21.45	13.23	
	Percentile 75	39.97	33.80	
	Percentile 25	40.41	32.74	
	Percentile 75	63.86	66.12	
	Percentile 25	0.00	0.00	
	Percentile 75	1.03	0.45	

Table (10) showed the association between smoking habit end seminal analysis parameters, there were significant association between sperm concentration and motility grading with smoking habit.

Table (10) Association between smoking and semen parameters

		Case		P value
		Smoker	Non smoker	
Sperm concentration (million/ml)	Median	35.00	40.00	0.048
	Percentile 25	25.00	27.50	
	Percentile 75	40.00	46.00	

Grade A %	Median	15.00	20.00	0.022
	Percentile 25	15.00	15.00	
	Percentile 75	20.00	27.50	
Grade B %	Median	20.00	20.00	1.000
	Percentile 25	15.00	15.00	
	Percentile 75	25.00	22.50	
Grade C %	Median	20.00	20.00	0.641
	Percentile 25	15.00	15.00	
	Percentile 75	25.00	27.50	
Grade D %	Median	40.00	40.00	0.146
	Percentile 25	35.00	30.00	
	Percentile 75	50.00	45.00	
Normal morphology %	Median	65.00	65.00	0.000
	Percentile 25	60.00	65.00	
	Percentile 75	70.00	72.50	
Abnormal morphology %	Median	35.00	35.00	0.000
	Percentile 25	30.00	27.50	
	Percentile 75	40.00	35.00	

Discussion

This study was conducted exclusively in Iraqi Wasit Governorate, where many young people suffer from infertility, and it was from here current research shed light on this medical problem by studying genetically of some immune marker. Only samples from the Wasit Governorate's Infertility Center were collected. The samples were collected in accordance with strict guidelines. In this study, 50 samples from infertile male were compared to 50 samples from fertile male. The results revealed no significant difference in age between the two groups, $p = 0.371$. There was a significant difference in semen concentration, $p = < 0.001$, and motility with $p = 0.001$ between fertile and infertile males.

Current study revealed significant higher serum concentration of TNF-a in patients as compared with fertile males. Said et al showed that exposing sperm to high levels of TNF-a in serum causes a significant loss of functional and genomic integrity (5). TNF-a levels in seminal plasma were found to be inversely associated with sperm motility progression (6).

Shukla K et al revealed that TNF-a genotype substitutions were shown to have a high level of correlation with infertile males, which was corroborated by allele and genotype meta-analysis, establishing it as a risk factor (7).

Kurz et al. Found no association of TNF-a serum level with sperm motility and concentration in Australian population (8).

Lazaros et al also revealed no assocaition between TNF-a level with parameters of seminal fluid analysis (9).

Several cytokines influence testicular function directly. TNF-a is created by the human body even when there is no inflammation or immunological activation, the testis can become inflamed. TNF-a appears to have an important role (10).

This cytokine helps the body fight infection quickly. After the identification of the TNF- polymorphisms, several studies investigated the genetic effect of these polymorphisms on the susceptibility to different human diseases such as infertility. The association between TNF- gene polymorphisms and susceptibility to male infertility was also raised and reported in different populations.

According to the findings of the genotype AA allele was dominant in 50% of the patients, compared to 20% in the control, followed by the heterozygous genotype AG in 40% of the patients and 16% in the control on the other hand the genotype GG allele was 10 % in patients, and frequency of the G allele in the controls was 64 % and the frequency of A allele is 70 % in the patient compared with the control 28 % and the frequency of the G allele is 30 % in the patient while 72% in the control, representing a high significant difference between the two groups P = 0.001.

In this study the concentration of TNF -a in seminal fluid was 0.88 pg./ml with arrange from o.11 pg./ml to 17 pg/ml. The level of TNF-a in seminal fluid had no statistically significant relationship with general parameters such as the age of the males. Seminal plasma TNF levels are related to sperm count, progressive motility, , and total number of motile sperm. In addition the 308A allele appears to association with increase TNF production but statistical not significant, suggesting that TNF medications could play role in male infertility. Biological response modifiers (which neutralize TNF and/or limit its signal transduction downstream) could potentially aid infertile patients, , specifically, those with diminished sperm production (i.e., primary testicular dysfunction) or decreased sperm motility (12).

In the Egyptian population, Zalata et al. (3) found that fertile males had a higher frequency of TNF -308 GG genotypes than infertile males. In the Indian population, the frequency of the AA genotype was consistently higher in infertile individuals than in fertile ones and infertile males had higher levels of apoptosis and necrosis. Shukla et al. (13) observed the substitution levels from G to A in the TNF- gene were considerably greater in infertile people compared to healthy fertile controls in an Indian community.

This is similar to the current results which showed that the GG homozygous in the patient was 10%, compared to 64% in the control, and genotype AA is high in the patient by 50% to 20% in the control. Also there was highly significant difference between study groups concerning allele type with p-value =0.001.

In this study the infertile smokers had a higher TNF- level in their blood than nonsmokers but statistically not significant.

There was a link between smoking and TNF-a levels in the blood. However, serum levels of TNF-a were found to be higher in smokers than in nonsmokers, but not significant statistically (p- value =0.060).

Nicotine has already been shown to stimulate TNF-a secretion at low concentrations (14). A number of studies have demonstrated the effect of smoking on TNF-A production.

Healthy person's peripheral blood Nicotine was found to increase TNF-secretion at low doses, but it inhibited TNF-production at levels comparable to those found in smokers' plasma (15).

Wang and colleagues revealed that nicotine, a component of cigarettes, causes release TNF-; additionally, the TNF- released causes the expression of adhesion molecules selectin on endothelial cells, resulting in an increase in monocyte adherence to the endothelial cells (16).

The finding that the ratio of TNF-/TNFR produced from activated T cells was higher in smokers was especially interesting. There were also significant correlations between the TNF-/TNFR ratios and the degree of smoking. This is due to an increase in TNF- release with increased smoking severity compared to little or no change in TNFR release. After stopping smoking, this change in the balance of TNF- and TNF receptor release appears to be maintained (17).

In conclusion: Increased seminal plasma of TNF-a negatively affects the parameters of spermatozoa in infertile men, could be affected by the TNF- G308A variant allele. In addition there was increase in TNF-a level in smoker patients compared to non-smoker and the TNF-a level is linked to sperm motility and concentration

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