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ORIGINAL RESEARCH

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The etiological effect and genetic risk of +252 A/G variant of TNF-β gene related to the susceptibility of urinary tract infection in a sample of Iraqi patients with type 2 diabetes: A case control study

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Abstract

Background and aim: Urinary tract infection (UTI) is the most common infection in type 2 diabetes patients. TNF-β is a cytokine with multiple functions in immunomodulatory and inflammatory mechanisms. The variation at position +252 A/G of TNF-β impacts both gene expression and plasma concentration of TNF-β proteins. The findings may shed light on the genetic factors that predispose diabetic patients in Iraq to UTIs.

Methods: A total of 200 individuals were divided into 100 patients with type 2 diabetes, categorized according to UTI, and 100 control subjects. Genetic analysis of +252 A/G of the TNF-β gene was carried out using the TaqMan probe allele discrimination method. The level of TNF-β was estimated by the ELISA technique.

Results: In the recessive model (GG vs. AA/AG) of TNF-β + 252 A/G in T2D/UTI patients compared to controls, a significant association $p = 0.029$ (OR: 2.8; CI 95% = 1.14–7.09): E = 15.6% was observed. Furthermore, in T2D patients without UTI, the dominant model AA versus AG/GG was associated with a preventive role P: 31.3% (OR: 0.4; CI 95% = 0.22–0.88) and a p value = (0.02). Overall, AG proportions showed a high level of TNF-β within the control group $p = 0.03$, while all proportions of the +252 A/G showed significant differences in TNF-β level between groups $p \leq 0.05$. Pearson's correlation analysis observed a link between TNF- levels, fasting plasma glucose (FPG), and HbA1c.

Conclusion: In T2D patients, the G allele may be linked to a higher probability of UTI, as well as an increased level of TNF-β in a genotype-dependent manner.

KEYWORDS

(rs909253) +252 A/G SNP, tumor necrosis factor beta (TNF-β), type 2 diabetic, urinary tract infection

1 | BACKGROUND

Urinary tract infections (UTIs) are still more prevalent, more severe, and have poorer outcomes in people who have type 2 diabetes. They are often more frequently accompanied by antibiotic-resistant.[1] Diabetes mellitus type 2 is a diverse set of illnesses defined by varying degrees of insulin resistance, decreased insulin secretion, and elevated glucose generation. People who have diabetes are more susceptible to infections due to their weakened immune systems. Diabetic patients have a higher risk of hospitalization, which increases the morbidity and mortality attributed to infection.[2,3] UTIs are much more intense, triggered by even more resistant pathogens, and associated with impaired outcomes than in persons without diabetes. Diabetic patients are more likely than nondiabetics to develop UTIs like asymptomatic bacteriuria (ASB) and some difficult aspects such as emphysematous pyelonephritis, renal abscesses, and renal papillary necrosis.[4,5] Diabetes type 2 is consider a risk factor not just for community-acquired UTI, but also for healthcare-associated UTI, catheter-associated UTI, and reoccurring UTI after a transplant of the kidney.[6,7] Furthermore, these patients are more likely to have extended-spectrum -lactamase-positive Enterobacteriaceae, fluoroquinolone-resistant uropathogens, carbapenem-resistant Enterobacteriaceae, and vancomycin-resistant Enterococci as the cause of their UTI.[8] Urinary tract infections make diabetic patients' blood sugar control challenging, trying to increase monitoring for blood sugar, lowering quality of life, and imposing substantial healthcare expenditures on the patient.[9] The TNF-β was previously known as Lymphotoxin-alpha (LT-α), another TNF superfamily member involved in immunoinflammatory responses, host immunity, and immune system improvement.[10] Following research, however, revealed differences between TNF-α and TNF-β, particularly in cell origin, secretion interactions, signal transduction pathways, and gene expression regulation, prompting the renaming of TNF-β to LT-α.[11]

The tumor necrosis factor (TNF) superfamily has 19 members and tumor necrosis factor beta (TNF-β) is encoded by TNF-β gene. TNF-β is a lymphocyte-produced pro-inflammatory cytokine that is structurally closely linked to the TNF-α.[12] The TNF-β gene is found in tandem with TNF-α gene on the long arm of chromosome 6 within a 7 kb locus in the MHC class III region (6p23-q12).[13]

A polymorphism at nucleotide position 252 within the first intron of the TNF-β gene (A252G) appears to affect both TNF-α and TNF-β expression levels, as well as a phorbol ester-responsive element.[14,15] This polymorphism has also been linked to a higher likelihood of establishing breast, gastrointestinal, bladder, and colon cancer.[16]

As a result, this variant (A + 252G) in the TNF-β gene was studied for its association with UTI susceptibility in patients with type 2 diabetes in the Iraqi population.

2 | METHODS

2.1 | Study design, participants, geographic area, and time periods

This work enrolled 200 participants of men and women from Baghdad province/Iraq. The study designed as a case–control experiment. One hundred patients with type 2 diabetes were involved and categorized according to UTI ($n = 50$ T2D with UTI) with a mean of age 50.3 ± 8.4 and ($n = 50$ T2D without UTI) with mean age 51.2 ± 7.7 along with 100 healthy control subjects with a mean of age 44.5 ± 8.4 available as blood donor, and none of them had a clinically evident of DM. The samples for this study were collected from the National Diabetes Center for Treatment and Research at Al-Mustansiriya University, Baghdad, Iraq, during the period from October 2021 to March 2022.

2.2 | Collection of data and evaluation of UTI

A layout questionnaire was used to collect information about age, body mass index (kg/m^2), and blood pressure from all subjects, just as it is done during routine examinations. The evaluation of UTI was made in compliance with the guidelines of the European Association of Urology,[17] and all 50 T2D patients with symptomatic UTI within 1 year were evaluated.

2.3 | Exclusion criteria

Type 1 diabetic patients, pregnancy, ischemic heart disease, hepatic disease; renal disorders, and use of an antimicrobial agent in the previous 10 days were all excluded.

2.4 | Ethics agreement and consent to participate

Participants proffered ethical written consent, and the research was endorsed by the ethics committees of the Biotechnology Research Center/Al-Nahrain University in Baghdad, Iraq, according to reference number (M.B.4.) as well as the Iraqi Ministry of Health's ethics committee for blood sampling.

2.5 | Biochemical and immunological detection

Five milliliters of fasting blood were drawn and divided into two tubes: 2 ml in a plain EDTA tube for DNA extraction and 3 ml in a gel tube for serological and biochemical detection. A commercial kit (Biolabo) was used to investigate the lipid profile, such as total cholesterol (TC) and triglyceride (TG), while low-density lipoprotein LDL was calculated using Friedewald's formula, fasting plasma

glucose (FPG) was measured using a Randox Laboratories, Ltd. kit, and blood urea was measured. Another aliquot of serum is used for TNF-β level cytokine assessment using the enzyme-linked immunosorbent assay (ELISA) technique, which employs a specific TNF-β human kit (ab119576/abcam) multiple steps standard assay/sandwich quantitative method with a range of 62.5–4000 pg/ml and a sensitivity of 5 pg/ml.

2.6 | Extraction and processing of (DNA)

The genomic DNA was isolated from stored aliquot of blood from 200 participants by a standard method using the proteinase K digestion of cells (ReliaPrep blood gDNA Miniprep system, Promega) according to a commercial kit protocol. A NanoDrop1000 Spectrophotometer (Gamma/Thailand) was used to estimate the concentration and purity of DNA; a good quality sample should have (1.8–2) A260/A280 ratio.

2.7 | Screening for the bi-allelic polymorphism of +252 A/G

The bi-allelic polymorphism rs909253 (previous name +252 A/G) in the first intron of the TNF-β gene was examined by the allelic discrimination method using TaqMan probes and a real-time PCR assay. The primers and fluorescent oligonucleotide probes were designed using the database of the National Center for Biotechnology Information (NCBI) on-line tool and produced by Alpha DNA Ltd. and stored at −20C. Primer, probe sequence, and condition of reaction were listed in Table 1.

The mixture of reaction includes 25 µl as a final volume contains: 12.5 µl Go *Taq* probe master mix (Promega), 1 µl each of primer; 0.5 µl of each probe with a concentration 10 pmol/µl; 4 µl of DNA sample and the DEPEC-treated water (prmega/USA) was used to complete the reaction solution.

2.8 | Data analysis

Data from the current study were analyzed via SPSS 28.0.1.1 (15) software. Descriptive statistics were performed, and the continuous data were presented with mean ± standard deviation (SD), minmax, median, and confidence level (95%). The comparison of demographic, biochemical, and immunological results between studied groups was done using ANOVA analyses, followed by a post hoc test and χ^2 for categorial variable. The confidence interval of 95%, the odd ratio (OR), and Fisher's exact test of allele frequency were calculated to assess the risk of the single-nucleotide polymorphism (SNP). The Hardy-Weinberg Equilibrium (HWE) was calculated by the χ^2 test to observe the deviation of genotypes and alleles using online program software (https://www.had2know.org/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html). The Spearman coefficient analysis was used for positive and negative correlations. A statistically meaningful result was demonstrated by $p \leq 0.05$.

3 | RESULTS

3.1 | Demographic and clinical characteristics of the study groups

Table 2 summarizes the data on morphometric and clinical parameters for all groups studied. Not surprisingly, the blood level (mean ± SD) of FPG, TG, TC, LDL, HbA1c, TNF-β, and blood urea were significantly higher in T2D with UTI and without UTI than in control

TABLE 1 Allelic discrimination conditions of TNF-β +252 A/G polymorphism.

Bi-allelic SNP ID	Primer's sequence	Reaction condition	Region/location
TNF-β (rs909253) A + 252 G	F-5CCATCTGTCTCATT-3	Initial denaturation 95 C° for 5 min of 1 cycle	Intron variant at +252
		5 cycles for primers ↓	
	R-5AAGGTGAGCAGA-3	Denaturation at 95 C° for 25 s	
	Probe's sequence		
	5-CCATGATTCC-3	Annealing at 58 C° for 35 s	
	5- GCCATGGTTCC-3	Extension at 72 C° for 45 s	
	Note: Taqman probes contain a reporter dye (FAM and VIC) at the 5 end and a quencher dye (BHQ) at 3 end	40 cycles for probes ↓	
		Denaturation at 95 C° for 30 s	
		Annealing at 58 C° for 35 s	
		Extension at 72 C° for 20 s	

Abbreviations: SNP, single nucleotide polymorphism; TNF-β, tumor necrosis factor beta.

TABLE 2 The data of morphometric, biochemical, and immunological tests.

Parameters	Control (*n* = 100)	T2D with UTI (*n* = 50)	T2D without UTI (*n* = 50)	*p* Value
Age[a]				0.321
(Mean ± SD)	44.5 ± 8.4	50.3 ± 8.4	51.2 ± 7.7	
Median	44	50	52	
Min–max	31–60	28–70	42–65	
Confidence level (95.0%)	3.9	7.3	5.9	
Gender[b]				**0.013***
Male%	67/100 (67%)	18/50 (36%)	30/50 (60%)	
Female%	33/100 (33%)	32/50 (64%)	20/50 (40%)	
Total	100	50	50	
BMI[a]				0.59
(Mean ± SD)	25.5 ± 3.9	26.5 ± 3.3	26.9 ± 4	
Median	27.4	26.3	28.4	
Min–max	18.6–30.4	21.9–33	20.9–32.4	
Confidence level (95.0%)	2.2	1.9	2.3	
Ss Bp (mmHg)[a]				0.27
(Mean ± SD)	11.9 ± 1.1	13 ± 2	12.5 ± 1.8	
Median	12	12.5	12	
Min–max	10–15	10–18	10–17	
Confidence level (95.0%)	0.6	1.1	1.08	
Ds Bp (mmHg)[a]				0.88
(Mean ± SD)	7.7 ± 0.8	7.9 ± 0.9	7.7 ± 0.8	
Median	8	8	8	
Min–max	7–10	6–9	7–10	
Confidence level (95.0%)	0.51	0.52	0.46	
FPG (mg/ml)[a]				**0.0001***
(Mean ± SD)	94.2 ± 7.4	251.6 ± 71.5	247.1 ± 91.6	
Median	95	234	226	
Min–max	83–109	178–384	110–400	
Confidence level (95.0%)	4.47	43.2	55.4	
TG (mg/ml)[a]				**0.05***
(Mean ± SD)	143.7 ± 21.1	174.3 ± 35.4	160.8 ± 37.8	
Median	147.01	183	155.5	
Min–max	99.4–180.1	117–231	101.6–225	
Confidence level (95.0%)	12.1	20.4	21.8	
TC (mg/ml)[a]				**0.007***
(Mean ± SD)	130.9 ± 25.6	268.3 ± 96.2	267.1 ± 107.9	
Median	129.6	279.5	291.5	
Min–max	95.2–188.1	99.5–428	100–402	
Confidence level (95.0%)	14.8	55.5	62.3	

TABLE 2 (Continued)

Parameters	Control (n = 100)	T2D with UTI (n = 50)	T2D without UTI (n = 50)	p Value
LDL (mg/ml)[a]				**0.006***
(Mean ± SD)	62.8 ± 22.4	255.4 ± 86	281.1 ± 49.7	
Median	56.3	240.5	267	
Min–max	34–103	95–387	229–377	
Confidence level (95.0%)	16.06	35.5	35.5	
HbA1c[a]				**0.002***
(Mean ± SD)	5.5 ± 0.5	7.9 ± 1.2	8.03 ± 1.6	
Median	5.6	7.9	7.7	
Min–max	4.6–6.5	6.4–9.7	6.1–11.2	
Confidence level (95.0%)	0.3	0.81	1.08	
TNF-β (pg/ml)[a]				**0.004[a]***
(Mean ± SD)	263.9 ± 120.2	749.9 ± 332.2	612.07 ± 275.8	
Median	238.5	851	603	
Min–max	120–582	198–1246	196–1087	
Confidence level (95.0%)	69.4	191.8	159.2	
B. Urea (mg/dl)[a]				**0.004***
(Mean ± SD)	24.7 ± 3.5	37.4 ± 3.3	30.3 ± 2.8	
Median	24.8	38.3	30.9	
Min–max	19.7–30.1	29.8–41.2	25.1–35.1	
Confidence level (95%)	2.2	2.1	1.8	

Note: Values were presented as (mean ± SD), Median, min–max, and confidence level (95%).

Abbreviations: BMI, body mass index; B. urea, blood urea; Ds Bp, diastolic blood pressure; FPG; fasting plasma glucose; HbA1c, glycated hemoglobin; LDL, low density lipoprotein; Ss Bp, systolic blood pressure; TC, total cholesterol; TG, triglyceride; TNF-β, tumor necrosis factor beta; T2D, type 2 diabetes.

[a]By one-way analysis of variance (ANOVA); post hoc test was used for comparison between groups.

[b]By χ^2 analysis for categorial variable.

*Significant at level ($p \leq 0.05$).

("$p \leq 0.05$"). Moreover, gender distribution showed a significant result ($p = 0.013$) between groups.

3.2 | Impact the biallelic and genetic models of the TNF- beta +252 A/G polymorphism on UTI susceptible

To assess the impact of genetic model and alleles distribution of TNF-β +252 A/G SNP, Tables 3 and 4 illustrated the results. The control group seemed to have more frequent of AA homozygous and AG heterozygous genotypes than the T2D/UTI group, but the mutant GG genotype was more common in the T2D/UTI group. The recessive model GG versus AA/AG was observed to have a significant association with T2D/UTI patients when compared to controls ($p = 0.029$); OR:2.84; 95%CI: [1.14–7.09]; and consistence with an etiological effect E:15.6% in population. At the same time, the dominant model AA versus AG/GG had significant differences $p = 0.025$ with a calculated OR:0.44; 95% CI: [0.22–0.88] and found to have a preventive effect (P:31.3% between T2D without UTI patients and controls). In this manner, the AA, AG, and GG proportions were more frequent in control than T2D/without UTI. In particular, individuals who carried GG genotype more prone to risk and susceptible of UTI.

3.3 | Accuracy of Hardy–Weinberg equilibrium (HWE) among study participants for three genotypes

TNF-β +252 A/G SNP was founded in three proportions: AA, AG, and GG, categorized with two alleles: (A) as a wild or major allele and (G) as a mutant or minor allele. In Table 5, the calculated HWE frequency

TABLE 3 Alleles and genetic models for TNF-β +252 A/G in control and T2D with UTI patients.

Allele/Genetic models	Control *N* = 100	T2D/UTI *N* = 50	OR	(95% CI) (lower–upper)	*p* Value	Impact
TNF-β (rs909253) +252A/G						
A (major allele)	146	66	0.72	(0.43–1.20)	0.22	P: 20.6%
G (minor allele)	54	34	1.39	(0.83–2.33)	0.22	E: 9.6%
Dominant model						
AA	56	28	1	(0.51–1.97)	1	E:0.0%
AG/GG	44	22				
Recessive model						
GG	10	12	2.84	(1.14–7.09)	**0.029***	E:15.6%
AA/AG	90	38				
Codominant/AG	34	10	0.49	(0.22–1.08)	0.089	P:17.5%

Abbreviations: E, etiological factor; p, Fisher's Exact Test; P, preventive factor; OR, odd ratio; T2D, type 2 diabetes; 95% CI, confidence interval.
*Significance at $p \leq 0.05$ level.

TABLE 4 Alleles and genetic models for TNF-β +252 A/G in control and T2D without UTI patients.

Allele/Genetic models	Control *N* = 100	T2D without UTI *N* = 50	OR	(95% CI) (lower– upper)	*p* Value	Impact
TNF-β (rs909253) +252A/G						
A (major allele)	146	59	0.72	(0.43–1.20)	0.22	P:20.6%
G (minor allele)	54	41	1.39	(0.83–2.33)	0.22	E: 9.6%
Dominant model						
AA	56	18	0.44	(0.22–0.88)	**0.025***	P:31.3%
AG/GG	44	32				
Recessive model						
GG	10	9	1.98	(0.75–5.19)	0.196	E: 8.9%
AA/AG	90	41				
Codominant/AG	34	23	1.65	(0.83–3.29)	0.15	E: 18.2%

Abbreviations: E, etiological factor; p, Fisher's Exact Test; P, preventive factor; OR, odd ratio; T2D, type 2 diabetes; 95% CI, confidence interval.
*significance at $p \leq 0.05$.

is based on observed values in the healthy control group. Meanwhile, the control group was identified as following Hardy-Weinberg equilibrium ("$p > 0.05$").

3.4 | Expression of TNF-β level in T2D with/without UTI patients, control and various genotypes of +252 A/G SNP

Serum levels of the TNF-β were distributed based on the TNF- +252 A/G polymorphism in T2D patients those with and without UTI, and healthy control groups. As previously shown in Table 2, the average of serum TNF-β level of the patient groups was markedly higher than in the control group (749.9 ± 332.2, 612.07 ± 275.8 vs. 263.9 ± 120.2); ($p = 0.004$). On basis to genotypes of the current TNF-β SNP, we compared the serum level of TNF-β between different proportions within groups. The findings revealed a highly significant level of TNF-β in the AG proportion inside the control group [CL (95%: 135.8]; ($p = 0.03$). As matter of fact, no statistically association of TNF-β plasma level through different genotypes of +252 A/G in T2D with/without UTI groups. Furthermore, the expression of TNF-β levels was analyzed and reported as highly significant between groups in the same proportion of +252 A/G, ("$p \leq 0.05$"). Interestingly, the AA, AG, and GG genotypes showed significant associations with the high level of TNF-β in the T2D/UTI group. All these findings are summarized in (Table 6 and Figure 1A,B).

TABLE 5 Hardy–Weinberg Equilibrium (HWE)/observed and expected values according to TNF-β +252 A/G genotypes in control group.

Group	Genotypes/Alleles distribution	Observed	Expected	HWE frequency *n* (%)	*p* Value	χ^2	Probability
Control *n* = 100	AA	56	53.2	(53.2%)	0.16	1.8	0.388
	AG	34	39.4	(39.4%)			
	GG	10	7.2	(7.2%)			
	A	146		73%			
	G	54		27%			

Abbreviations: TNF-β, tumor necrosis factor; χ^2, chi squared.

TABLE 6 Association between TNF-β level and genotypes of +252 A/G in study groups.

Groups	TNF-β level pg/ml +252/AA	+252/AG	+252/GG	*p* Value
Control	194.3 ± 67.9	348.8 ± 129.4	270.5 ± 59.9	**0.03***
Confidence level (95%)	71.3	135.8	62.8	
T2D with UTI	879.6 ± 52.8	1047.8 ± 104.9	794.8 ± 258.6	0.23
Confidence level (95%)	131.1	260.5	642.2	
T2D without UTI	503 ± 106	730.6 ± 197.4	768.6 ± 153.8	0.135
Confidence level (95%)	952.9	490.6	382.2	
p Value	**0.0001***	**0.002***	**0.002***	

Note: One-way analysis of variance (ANOVA); post hoc test was used for comparison between groups.
Abbreviations: TNF-β, tumor necrosis factor; T2D, type 2 diabetes; UTI, urinary tract infection.
*significance at $p \leq 0.05$.

3.5 | Spearman's correlation of TNF-β in patient's groups

To evaluate the link between the level of TNF-β and the biochemical variables, we have demonstrated Spearman's correlation analysis. It appeared that the level of TNF-β showed a positive correlation with all parameters, but only FBS (r2 = 0.608) and HbA1c (r2 = 0.657) were reached at a significant level ("$p \leq 0.05$"); among the T2D with UTI group. TNF-β levels, on the other hand, had a negative correlation with all biochemical parameters except B. urea and did not reach a significant level in T2D without UTI (Table 7).

4 | DISCUSSION

TNF-β polymorphism 252 A/G was already identified at location +252 in the first intron of the TNF-β gene, with one allele containing guanine (G) and the other allele containing adenine (A). The existence of G at this locus defines mutated allele, which is a less common allele accompanied with increased TNF-β level.[14] In this work, we studied the +252 A/G gene polymorphism and how it's associated with the level of TNF-β and the development of UTI in Iraqi patients suffering from type 2 diabetes. Diabetes mellitus is distinguished by changeable in insulin resistance, impaired of insulin action, and increased glucose production. Moreover, UTIs are usual in type 2 diabetes and are associated with worse results than individuals who do not have diabetes.[18,19] Various impairments in the immune system contribute to the pathogenesis of urinary tract infections (UTI), and diabetics are more likely than nondiabetics to develop all types of UTI, like asymptomatic bacteriuria, fungal cystitis, emphysematous cystitis, bacterial pyelonephritis, and perinephric abscess).[20]

Similarly, the current study's data, as shown in Table 2, suggested an increase in the prevalence of serum levels of FBS, lipid profile, HbA1c, B. urea, and TNF-β in T2D with and without UTI patients when compared to the control group, with a notable difference at level ($p \leq 0.05$) and more pronounced distinction in the T2D with UTI group. Type 2 diabetic pathogenesis has been linked to a number of factors, the most important of which are genetic and environmental factors. The study by[21] suggested that the increased levels of FBG, and HbA1c were significantly higher in patients with type 2 diabetes compared to healthy controls. The progression of inflammatory and immune-mediated T2D as a result of cytokines, including TNF-β, has additionally been noted as a factor in the development of diabetic and susceptible states.[22] Aside from the classic complications of the disease, diabetes has been linked to decreased T cell response, neutrophil activity, and humoral immunity ailments and tends to increase susceptibility to infections.[23]

Cytokine genetic variations may modify the transcription of these proteins, their levels of synthesis, and their potential role in causing disease susceptibility or resistance.[24] Several polymorphisms

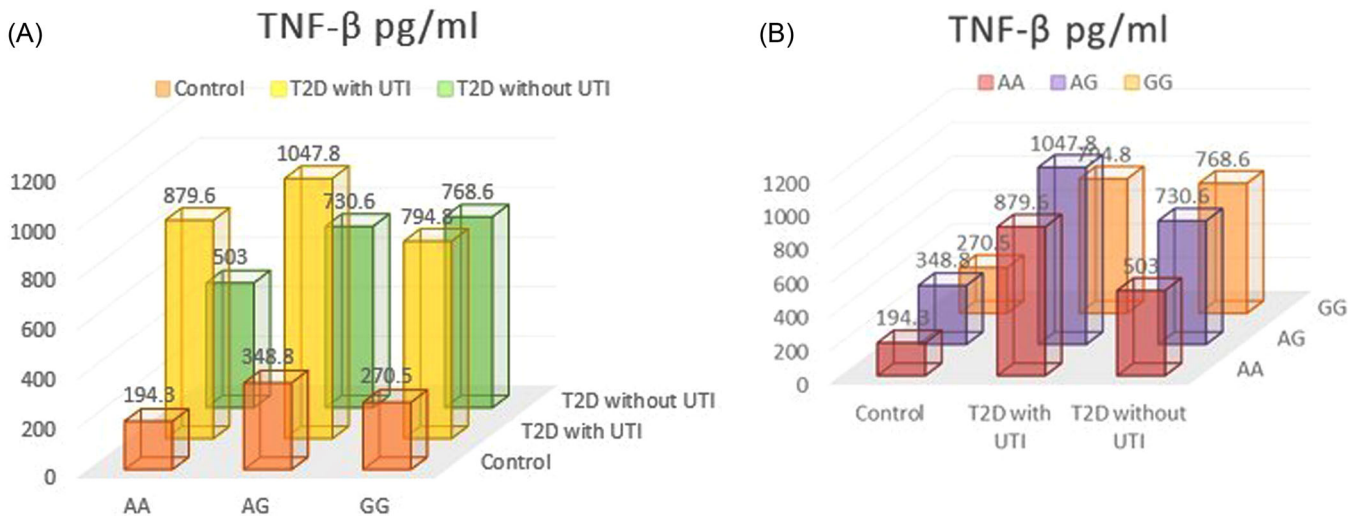

FIGURE 1 (A) Serum level of TNF-beta according to AA, AG and GG of (rs909253) +252 A/G in control, T2D with UTI patients, and T2D without UTI. (B) Serum level of TNF-beta according to groups with the same genotype. UTI, urinary tract infection.

TABLE 7 Correlation of TNF-β serum level with different biochemical variables among patients of T2D with/without UTI groups.

	TNF-β (pg/ml)							
	T2D with UTI				T2D without UTI			
			95% confidence interval (2-tialed)[a,b]				95% confidence interval (2-tialed)[a,b]	
Variables	Spearman's rho	*p* Value	Lower	Upper	Spearman's rho	*p* Value	Lower	Upper
FBS (mg/ml)	0.608	**0.036***	−0.881	−0.034	−0.043	0.893	−0.602	0.544
TG (mg/ml)	0.189	0.556	−0.447	0.698	−0.017	0.957	−0.585	0.562
TC (mg/ml)	0.392	0.208	−0.253	0.796	−0.138	0.668	−0.660	0.473
LDL (mg/ml)	0.119	0.713	−0.0503	0.660	−0.44	0.904	−0.656	0.602
HbA1c	0.657	**0.02***	−0.898	−0.115	−0.33	0.923	−0.621	0.578
B. urea (mg/dl)	0.217	0.499	−0.424	0.713	0.192	0.549	−0.429	0.690

Abbreviations: TNF-β, tumor necrosis factor; T2D, type 2 diabetes; UTI, urinary tract infection.

[a]Estimation is based on Fisher's r-to-z transformation.

[b]Estimation of Stander Error is based on the formula proposed by Fieller, Hartley, and Pearson.

*Significant correlation at $p \leq 0.05$ level.

exist in the TNF-β gene, and the presence of the +252 A/G variation, which is linked to TNF-β overexpression and plays a particular role in diabetes mellitus susceptibility to disease.[25,26]

In terms of the genetic models and allele distribution of the TNF-β +252 A/G polymorphism, our data showed that the recessive model (GG vs. AA/AG) showed a more than 2-fold susceptibility to UTI in T2D patients when compared to controls ($p = 0.029$); (OR: 2.84; 95% CI: [1.14–7.09] and has an attributable function (E = 15.6%). Taken together, these findings indicate that the G allele makes patients with T2D more susceptible to UTIs. Furthermore, the control group had a significantly higher prevalence of the dominant genotype AA vs. AG/GG than the T2D/UTI group OR: 0.44; [CI: 0.22–0.88] at the level of $p = 0.025$, and subsequent evidence that the A allele was a protective allele (P: 31.3%) in the Iraqi healthy population. Therefore; this observation results are the first to find a link between the TNF-β +252 A/G gene polymorphism and the occurrence of UTIs.

According to previous studies documented by,[27] the G allele of this SNP has a fundamental or supporting role and correlates with various conditions such as rheumatoid arthritis, Graves' disease, idiopathic membranous glomerulonephritis, IgA nephropathy, asthma diathesis, SLE with nephritis, and systemic sclerosis. Furthermore, TNF-β (G) allele has been linked to sepsis susceptibility and mortality.[28] Based on the findings of,[13] the GA proportion of TNF-β +252 A/G SNP was found to be positively related to the risk of oral lichen planus disease, whereas the AA proportion was found to be protective. The findings of[29] investigated the current TNF-β SNP and associated with temporomandibular disorders (TMD) susceptibility in

a Turkish population sample, and the AG heterozygous genotype was significantly higher in patients when compared to controls, whereas the AA genotype was significantly over-represented in controls ($p = 0.01$).

Hence, the distribution of the genotypes in controls was stable and followed the HWE ("$p > 0.05$"). Notably, the analysis by[27] found no deviation from HWE for the TNF-β +252 A/G variant in either the patient or control groups.

It should be noted that the three proportions of this polymorphism were significantly related to a high level of TNF-β, specifically in the AG genotype (1047.8 ± 104.9) of the T2D/UTI group (a hyperproducer of TNF-β). However, the results reported a high prevalence level of TNF-β in AG proportion 348.8 ± 129.4 within the control group. An important finding of[30] that the genetic polymorphism +252 A/G of TNF-β influences the expression of the level of TNF-β cytokine, and this polymorphism is associated with the risk of many diseases such as RA, SLE, sepsis, pancreatic, and breast cancer.

Other studies[14,31,32] concluded that the less common G allele of the +252 A/G genetic variant appears to be linked and correlated with elevated levels of TNF-β expression at the mRNA and protein levels. It has been noted that the individuals with the G allele produce a lot of TNF-β, whereas those with the A allele produce very little.[33] In this study, the results indicated the significant positive association between TNF-β with FBS and HbA1c in the T2D/UTI patients. Cytokines are well known for their role in promoting acute-phase protein production. Tumor necrosis factor-a (TNF-α) and lymphotoxin-a (LTA), formerly TNF-β, are found in insulin resistance; thus, high levels of cytokine production may predict the progression of type 2 diabetes by reducing insulin sensitivity, therefore, Obesity and hyperglycemia both cause clinically significant increases in pro-inflammatory cytokines.[34,35] In contrast to the findings of the current insights report,[36] there was no significant correlation between TNF and HbA1C in T2DM. There is convincing evidence that inflammatory processes obstruct basal metabolism and disrupt insulin signaling, resulting in higher expression of pro-inflammatory cytokines. These cytokines have the ability to bind to cell membrane receptors, causing inflammation and exacerbating glucose intolerance.[37]

4.1 | Limitations of the current work

Our study has some drawbacks. The small sample size of participants. There are no recent articles on the study topic. The present study has not been funded.

5 | CONCLUSION

Finally, there is mounting evidence that the G allele of the TNF-β +252 SNP is linked to UTI susceptibility in Iraqi T2D patients. Furthermore, the circulating level of TNF-β was higher in the AG heterozygous genotype. In the present study, we concluded that the +252 A/G variation is a genetic risk factor for UTI in T2D individuals. However, more investigations with various ethnic populations and a larger number of subjects are required to obtain more indications.

AUTHOR CONTRIBUTIONS

Shaimaa Y. Abdulfattah: Formal analysis; methodology; software; writing – review & editing. **Farah T. Samawi**: Data curation; formal analysis; investigation; methodology; project administration; software; writing – original draft; writing – review & editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The information that justifies the current study's findings are available from the relevant author (shaimaay26@gmail.com) upon reasonable request.

TRANSPARENCY STATEMENT

The lead author Shaimaa Y. Abdulfattah affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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