Egyptian Journal of Medical Microbiology Volume 33 / No.2 / April 2024 67-72 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Role of Tumor Necrosis Factor Alpha Gene Polymorphism (TNFα -308 G/A) and Prediction of Diabetic Nephropathy

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Key words:
TNF- α, type 2 diabetes, diabetic nephropathy, TNFα -308 G/A, Polymorphism

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ABSTRACT

Background: *Diabetic nephropathy, a chronic kidney disease resulting from diabetes mellitus microvascular complications, is thought to be the primary cause of end-stage renal failure. Gene mutations affecting the TNF gene can impact the generation of tumor necrosis factor-α (TNF-α), which has been associated with worse clinical outcomes in patients with diabetic nephropathy who are in severe condition.* ***Objective:*** *The purpose of this study was to assess the relationship between diabetic nephropathy and TNFα -308 G/A gene polymorphism in individuals with diabetes.* ***Methodology:*** *50 diabetic individuals were recruited in this case-control research and were split into two groups: 25 patients with nephropathy and 25 patients without nephropathy. As a typical control group, a third set of blood donors (n = 25) appeared to be in good condition. Patients' information was gathered through the use of an interview questionnaire. The test for gene polymorphism was PCR. The TNF α308 G/A gene's A allele has a positive, statistically significant correlation (P-value ~0.05) with DN.* ***Results:*** *The prevalence of DN among the study participants may be predicted by the presence of an A allele in the TNF α308 G/A gene with a statistically significant P-value (P-value < 0.05). The study participants' TNF α308 G/A gene patterns and albumin/creatinine ratio did not exhibit a statistically significant relationship.* ***Conclusion:*** *According to this study, having an A allele in the TNF α308 G/A gene may contribute to the pathophysiology of diabetic nephropathy and be a predictor of nephropathy in diabetic populations.*

INTRODUCTION

Diabetes is a chronic illness. Diabetes patients demand long-term determination and self-control, and their caregivers need to continuously comprehend humanity and maintain a cautious optimism to support the patients through both their ups and downs in life [1]. In seriously ill patients, kidney impairment is typically accompanied with a higher fatality rate, particularly in patients with diabetic nephropathy (DN)[2]. More than one-third diabetic patients have diabetic nephropathy, a lifelong kidney disease caused by microvascular effects of diabetes mellitus that is thought to be the primary cause of end-stage renal failure[3]. Thus, the identification of cell damage biomarkers that might indicate the course of DN has drawn attention in the scientific community recently[4].

Tumor necrosis factor alpha (TNF-α) is expressed as a transmembrane protein and is organized into stable homotrimers. The proteolytic cleavage of a cytokine by the metalloprotease TNF-α-converting enzyme (TACE)2 results in its release into the extracellular environment[2].Within the highly polymorphic major histocompatibility complex (MHC) region of the human genome, on chromosome 6 p 21.3[5], lies the highly polymorphic TNF-α gene. Multiple research studies have suggested that MHC is beneficial for the occurrence of DN, macrovascular disease, and diabetic retinopathy[6].

The possibility that genetic variations in cytokine and inflammatory modulators might lead to a poorer prognosis for diabetes patients has drawn a lot of attention[7]. Modifications to the transcriptional activity of the TNF-α gene can have a direct effect on TNF-α production, due to significant polymorphisms in its promoter region [8]. Thus, the purpose of this study was to evaluate the relationship between DN and the TNFα-308 G/A gene polymorphism in Egyptian diabetics.

METHODOLOGY

Between December 2022 and July 2023, this case-control research was carried out at the Suez Canal University Hospital's (SCUH) Clinical Pathology and

Internal Medicine Departments. This study included 50 diabetic patients divided into two cohorts: those with diabetes; diabetic patients with nephropathy (n=25); and diabetic patients without nephropathy (n=25). A third group of apparently healthy blood donors (n=25) served as a normal control group. Every participant gave written, informed consent, and on November 15, 2023, the Suez Canal University Faculty of Medicine's Ethics Committee approved the study (Research 5123#). The methods employed in this research follow the guidelines supplied by the Declaration of Helsinki. Patients of both sexes who were at least 20 years old and agreed to participate within our research were included. While pregnant women were excluded.

An interview questionnaire was employed to collect data from patients. A questionnaire included, age, sex, medical history, and any related complications. Physical examinations were conducted (blood pressure). The Body Mass Index (BMI) was determined using weight and height data. To perform genotyping, DNA extraction, and biochemical testing, six (6.0) milliliters of blood were drawn from each participant The lipid profile and fasting blood glucose level were measured using the fully automated auto-analyzer Cobas c 501 (Roche Diagnostics, Mannheim, Germany).

DNA extraction and genotyping:

Using a commercially available Spin-column method kit for DNA extraction (QIAamp®DNA Blood Mini Kit) (Avenue, Stanford, Valencia, CA, US), DNA was isolated from the blood leucocytes of every participant[9].

Amplification and detection for GLN223ARG gene:

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, as reported by Wilson and colleagues, used for genotyping the LepR polymorphism.[10].

Statistical analysis:

The SPSS software (Statistical Package for Social Science) version 26 was utilized to computerize and do statistical analysis on the gathered data. Using the Shapiro Walk test, data was examined for normal distribution. When applicable, data was shown using tables and graphs. Qualitative variables were represented by relative percentages and frequencies. The chi square test ($\chi 2$) was used when necessary to ascertain the disparity across the qualitative variables. The standard deviation and mean were employed to convey quantitative data. Mann Whitney test was used for variables that are non-parametric, the disparity between the quantitative variables in two groups was calculated using the Mann Whitney test. For non-parametric variables, the difference between the quantitative variables in three groups was calculated using the Kruskal Wallis test. To find out the way two variables were related, Spearman correlation analysis was employed. Simple linear regression for detecting independent predictors to dependent variable. Level of P-value < 0.05 indicates significant while, P≥ 0.05 indicates non-significant difference.

RESULTS

Sociodemographic and laboratory characteristics **(Table 1, 2)**

This case-control research was done to assess the relationship between DN and the TNFα-308 G/A gene polymorphism in Egyptian diabetics. In terms of gender, females had difference that is statistically significant more than male in the control group (P-value <0.05). P-value >0.05 did not possess a statistically significant variation in age between the groups. Diabetic group with albuminuria were with lower weight and higher Systolic blood pressure with difference statistically significant (P-value <0.05). While diabetic group without albuminuria had statistically significant difference higher diastolic blood pressure (P-value <0.05) (table1).

We noticed that serum Creatinine, Fasting Blood Sugar and HbA1c were higher among diabetic group with albuminuria and this difference is statistically significant (P- value <0.05), as regard Albumin / creatinine ratio is the well-established gold standard to diagnose diabetic nephropathy when albuminuria ≥ 30 mg. Albumin/ creatinine ratio was higher among diabetic group with albuminuria and this difference is statistically significant (P-value <0.05) (table2).

Table 1: General characteristics of the studied participants (n=75).

General characteristics		Diabetic with albuminuria (n=25)	Diabetic without albuminuria (n=25)	Healthy (n=25)	P-value
Age *(years)*	*Mean ±SD*	56.2±8.3	57.2±6.6	53.9±7.2	0.160
	Range	(30-66)	(35-66)	(35-66)	
	Median	60	58	53	
Gender *NO (%)*	*Male*	12(48)	17(68)	7(28)	**0.018***
	Female	13(52)	8(32)	18(72)	
Weight *(kg)*	*Mean ±SD*	72.4±8.5	80.4±5.2	80.5±4.7	**0.001***
	Range	(52-85)	(69-89)	(70-90)	
	Median	75	80	80	
BMI *(kg/m²)*	*Mean ±SD*	22.9±1.9	23.3±1.5	24.1±0.7	0.126
	Range	(19-24.9)	(20-24.9)	(21.5-24.9)	
	Median	23.8	23.6	24.2	
Systolic blood pressure *(mm/Hg)*	*Mean ±SD*	135±20	133±18	122±11	**0.025***
	Range	(100-180)	(100-170)	(100-140)	
	Median	130	130	120	
Diastolicblood pressure *(mm/Hg)*	*Mean ±SD*	78±18	84±13	77±7	0.096
	Range	(25-100)	(60-120)	(60-90)	
	Median	80	80	75	

*Kruskal Wallis Test ; Chi-square Test; *: Significant at p <0.05*

Table 2: Laboratory investigations of the studied participants (n=75).

Laboratory investigations		Diabetic with albuminuria (n=25)	Diabetic without albuminuria (n=25)	Healthy (n=25)	P-value
S. Creatinine *(mg)*	*Mean ±SD*	1.12±0.8	0.73±0.2	0.8±0.2	0.096
	Range	(0.5-4.18)	(0.5-1.18)	(0.51-1.2)	
	Median	0.8	0.7	76	
Alb./ crea.ratio *(mg/gm creat.)*	*Mean ±SD*	1214±2677	9.4±7.9	7.6±7	**0.0001***
	Range	(30.01-12318.84)	(0-28)	(0.6-28.5)	
	Median	230.76	6.73	5.2	
FastingBlood Suger *(mg/dl)*	*Mean ±SD*	155.3±37	153.6±32.5	89.4±8.9	**0.0001***
	Range	(98-231)	(116-233)	(70-105)	
	Median	146	143	90	
Hb A1c %	*Mean ±SD*	7.34±1.3	7.4±1.2	5±0.7	**0.0001***
	Range	(5.2-10.7)	(5.3-11)	(4-6.5)	
	Median	7.3	7.2	5	

*Kruskal Wallis Test, *p is significant at <0.05*

Genotyping for TNFα 308G/A gene polymorphism

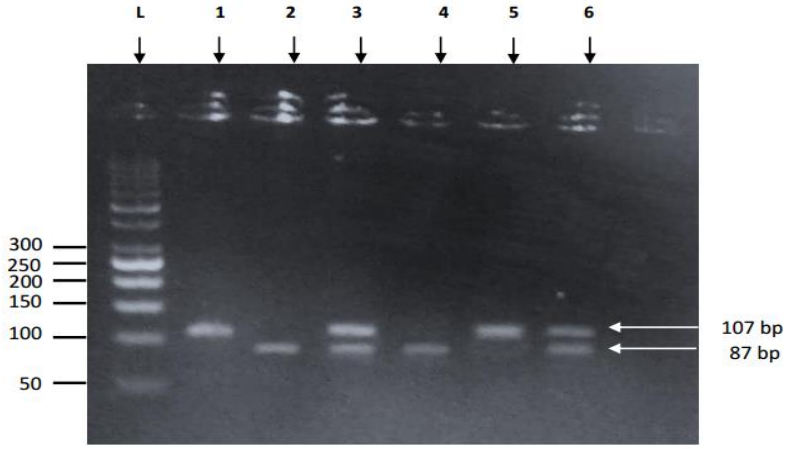

Fig. 1:Electrophoretic patterns of different TNFα 308 gene polymorphism (rs1800629G/A) genotypes. By using (REFLP) technique: photograph of a agarose gel showing the digested PCR product for TNFα.

Lane L: *DNA ladder;* ***Lane 1, 5:*** *Homozygous GG genotype (one band 107 bp),*
Lane 2, 4: *Homozygous AA genotype (one band 87 bp);* ***Lane 3, 6:*** *Heterozygous GA genotype (two patterns 107, 87 bp)*

The PCR product was treated by restriction enzyme (NcoI). The appearance of a one single band with assize (107bp) represent the existence of G allele, but if a single band with a size of 87 bp allele A presents , there were three genotypes GG, AG, AA with three patterns: Homozygous GG genotype (one band 107 bp), Homozygous AA genotype (one band 87 bp), Heterozygous GA genotype (two patterns 107, 87 bp) as shown in figure 1.

Table 3 shows that Allele A was more common in the group of diabetics with albuminuria (52%) in comparison to the groups of diabetics without albuminuria (40%) and the healthy group (24%); nevertheless, the frequency distribution of allele A has no statistically significant difference (P-value >0.05). Distribution of the multiple TNFα 308G/A genotypes: The diabetic group with albuminuria revealed that 48% (n=12) were homozygous GG, 36% (n=9) were heterozygous AG, and 16% (n=4) were homozygous AA. On the other hand, the diabetic group without albuminuria revealed that 60% (n=15) were homozygous GG, 36% (n=9) were heterozygous AG, and 4% (n=1) was homozygous AA. The control group also revealed that 76% (n=19) were homozygous GG, 20% (n=5) were heterozygous AG, and 4% (n=1) was homozygous AA).

Allele A was more common in the group of diabetics with albuminuria (52%) in comparison with the groups of diabetics without albuminuria (40%) and the healthy group (24%); nevertheless, the frequency distribution of allele A has no statistical significance (P-value >0.05).

Table 3: Frequency of TNF α308 gene patterns of the studied participants (n=75).

TNF α308 gene patterns *Freq. (%)*	**Diabetic with albuminuria (n=25) NO (%)**	**Diabetic without albuminuria (n=25) NO (%)**	**Healthy(n=25) NO (%)**	**P-value**
GG	12 (48%)	15 (60%)	19 (76%)	0.239
AA	4 (16%)	1 (4%)	1(4%)	
GA	9 (36%)	9 (36%)	5 (20%)	
Non-A allele	12 (48%)	15 (60%)	19 (76%)	0.145
A allele	13 (52%)	10 (40%)	6 (24%)	

*Chi-square Test;*p is significant at <0.05*

The TNF α308 G/A gene's A allele shows positively statistically significant correlation (P-value < 0.05) with DN among the subjects under study as shown in **Table 4.**

Table 4: Spearman's correlation between A allele of TNF α308 G/Agene and nephropathy among the studied participants (n=75).

Cases	**A allele of TNF α308 G/A gene**	
Spearman's rho	*Correlation Coefficient(r)*	0.235
	P-value	**0.043***

Spearman Correlation: **p is significant at <0.05*

The development of DN among the study participants may be predicted by the presence of an A allele in the TNF α308 G/A gene with a statistically significant P-value (P-value < 0.05) **table 5.**

Table 5: Simple linear regression analysis using A allele TNF α308 G/A gene for predicting nephropathy among the studied participants (n=75).

StandardizedCoefficients	**P-value**	**95.0% Confidence Interval for B**	
Beta		**Lower Bound**	**Upper Bound**
0.235	**0.043***	0.013	0.774

** Linear regression:*p is significant at <0.05*

Among the subjects in our research, there was no statistically significant correlation found between the albumin/creatinine ratio and TNF α308 G/A gene patterns (P-value > 0.05) as shown in **table 6.**

Table 6: Comparison of Alb./ crea. ratio among TNF α308 G/Agene patterns of the studied participants (n=75).

Alb./ crea.ratio *(mg/gm creat.)*	**TNF α308 G/A gene patterns**			**P-value**
	GG (n=46)	**GA (n=23)**	**AA (n=6)**	
Mean ±SD	565±2053	107±211	385±681	0.135
Range	(0.55-12319)	(0-850)	(0-1738)	
Median	8.805	15.2	62.385	

*Kruskal Wallis Test *p is significant at <0.05*

Table 7 revealed thatv The study participants' albumin/creatinine ratio and A allele presence in the TNF α308 G/A gene has no statistically significant relationship (P-value >0.05).

Table 7: Relation of Alb./ crea. ratio and presence of A allele inTNF α308 G/A gene of the studied participants (n=75).

Alb./ crea. Ratio *(mg/gm creat.)*	**Presence of A allele in TNF α308 G/A gene**		**P-value**
	A allele (n=29)	**Non A allele (n=46)**	
Mean ±SD	164±362	565±2053	0.213
Range	(0-1738)	(0.55-12319)	
Median	20.7	8.8	

*Mann-Whitney Test *p is significant at <0.05*

DISCUSSION

The main conclusions of the study indicated that the presence of the TNF α308 G/A gene's A allele may be a reliable indicator of the beginning of diabetic nephropathy. The expression level of allele A was higher in the diabetic group with albuminuria (52%) compared to the diabetic group without albuminuria (40%) and the controls (24%). However, the difference's frequency distribution was not of statistical importance (P-value > 0.05). Additionally, among the individuals under study, the presence of the A allele in the TNF α308 G/A gene has a positive statistically significant correlation with DN (P-value <0.05). Since inflammatory processes have an impact on the onset and progression of renal affection owing to diabetes mellitus, positive relationship presents between the production of cytokines in the kidney and diabetic nephropathy.

Our results were supported by Rizvi and associates, who showed that inherited risk alleles of the TNF-α gene increase the development risk of diabetic nephropathy[11]. This was further demonstrated by Hameed and associates [12,13], who discovered a substantial correlation between DN development and the TNF-α gene's -308 G/A polymorphism. Tuglular et al. [14] reported a higher frequency of the AA genotype among individuals with end-stage renal diseases compared to healthy controls (p<0.001), Furthermore, the occurrence for allele A was greater in patients compared to controls, with no substantial change. This suggests that inflammation has a role in diabetic nephropathy and may be influenced by genetic factors.

This research included three age-matched groups and discovered that the albuminuric diabetic group had greater systolic blood pressure and a less weight, with a statistically significant difference (P-value <0.05). While diabetic group without albuminuria had statistically significant difference higher diastolic blood pressure (P-value <0.05). Wang et al. (2005) also reported that blood pressure systolic and diastolic were higher among diabetic nephropathy with macroalbuminuria and microalbuminuria in comparison with healthy controls (p< 0.001)[15].

According to this study, albumin creatinine ratio was higher among diabetic group with albuminuria and this difference has statical significance (P-value <0.05). Furthermore, Wang et al. [15] came to the same conclusions, stating that macroalbuminuric patients had higher ACR levels more than microalbuminuric patients when compared to healthy controls (137.04, 7.43 vs. 0.89, p <0.001). Similar findings were found by El-Edel et al. [16] (2020), who discovered a significant difference (p<0.001) in the ACR between diabetics with kidney disease and those without renal disease in comparison with those healthy controls (p< 0.001).

The study showed no significant correlation between albumin/creatinine ratio and TNF α308 G/A gene patterns in participants (P-value > 0.05). El-Edel et al.,[16] reported a substantial positive association between TNF α308 G/A and albumin creatinine ratio, which opposes the findings of this study. Variations in the study designs and methodologies might account for this variance. In summary, our work showed that the A allele of the TNF α308 G/A gene may predict the occurrence of nephropathy in diabetic populations and may have an influence in the pathogenesis of diabetic nephropathy.

CONCLUSION

In summary, our research demonstrates that the presence of the A allele of the TNF α308 G/A gene may be associated with the pathogenesis of diabetic nephropathy and may predict the occurrence of nephropathy in diabetic populations.

Conflicts of Interest: There is no conflict of interest regarding the publication of this paper.

Financial Disclosures: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Acknowledgments: The authors want to thank everyone who contributed to the success this study.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

Author contributions: All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Ethics approval and consent to participate: Approval was obtained from ethical committee of Faculty of Medicine, Suez Canal University (Research 5123#)

Consent for publication: The procedures used in this study adhere to the tents of the Declaration of Helsinki. Written, informed consent was obtained from each patient included in the study.

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