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Association Of Tumor Necrosis Factor- Alpha (TNF-α) Polymorphism in Renal Failure Patients

Ayat Sadiq Saleh[1] **Ali Naeem Salman** **Layla Alhassan**

Ayat_salih20.bio@utq.edu.iq ali69na@gmail.com lyla.alhasan@utq.edu.iq

[1]Department of Biology, College Education for Pure Sciences, University of Thi-Qar

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Abstract:

Renal failure is a disorder in which the kidneys are unable to maintain the fluid, electrolyte, and pH balance of the extracellular fluids as well as remove metabolic waste products from the blood. The current study's objective was to investigate the relationship between the TNF-α promoter -264 C/T single nucleotide polymorphism (SNP) ,and glomerular filtration rate (GFR) in renal failure with and without diabetic mellitus patients compared to control. This study was conducted in the laboratories of Thi-Qar University's College of Education for Pure Sciences in collaboration with Al-Hussein Teaching Hospital, Nasiriyah General Hospital, and Thi-Qar Center for Nephrology and Dialysis in Thi-Qar Province from February 2022 to March 2023. The study included 40 renal failure with D.M patients, 40 patients without DM and 40 control. By using the PCR technique, the TNF-promoter gene polymorphism -264 C/T was identified, and genotyping was verified by direct sequencing. The current study found that there is a significant effect of *TNF-α* gene in renal failure patients with and without D.M compared to control. In addition, this study demonstrated an inverse correlation between effect of *TNF-alpha* gene on end-stage renal disease and decreased eGFR. The study concluded that there was a significant effect of *TNF- α* gene polymorphism is associated with markers of kidney disease severity and distant organ dysfunction among patients with RF. In addition, this study found correlation between effect of *TNF-alpha* gene on end-stage renal disease and decreased eGFR.

Keyword: Renal Failure, GFR, *TNF-α* gene, polymorphism-PCR

1-Introduction:

The disease known as renal failure occurs when the kidneys are unable to manage the fluid, electrolyte, and pH balance of the extracellular fluids and remove metabolic waste products from the blood. The underlying reason could be urologic problems with non-renal origin, systemic disease, or renal disease[1].

Acute or chronic disorders of the kidneys can cause renal failure. Acute renal failure develops suddenly and is frequently curable with the right diagnosis and care. In contrast, the kidneys' irreversible damage leads to chronic renal failure. It takes a while to develop, typically over a number of years [2]. Confusion, nausea, vomiting, exhaustion, and leg swelling are possible symptoms[1]. Both acute and chronic failure have consequences, including fluid overload, uremia, and high blood potassium levels. Other [3] negative effects of chronic failure include heart disease, hypertension, and anemia. [4]

Pro-inflammatory cytokines are produced as a result of ischemia-reperfusion injury and nephrotoxic insult in experimental settings, which alter the morphology, tubular epithelial cells and function of glomerular endothelium [5][6]. Additionally, Cytokines are able to cause damage to distant organs [7][8] Tumor necrosis factor (TNF), first termed by [9], was first recognized to cause apoptosis, or programmed cell death. At present, its believed that this molecule controls a number of significant cellular functions, including immune response, differentiation, proliferation, and growth [10] Numerous cell types, includes T cells, NK cells, neutrophils, macrophages, and monocytes, generate TNF-α. Between the HLA-B and HLA-DR genes on chromosome 6, in the class III region of the major histocompatibility complex, is the gene that encodes TNF-α [11]. TNF activates proinflammatory NF-B and MAP kinases, promotes cell survival, and binds to the target cells' TNFR1 and TNFR2 outer membrane-bound receptors [12]. The chemical stimulates phagocytes to take up and eliminate pathogens and cellular waste [13]. Additionally, it increases the vascular endothelium's expression of adhesion molecules to easier for immune cells to get to the sites of infection and tissue damage, especially for neutrophils and macrophages[14].

The genetic variations in the genes controlling TNF's production and effects, as well as the polymorphisms in the TNF locus itself, were associated to the functions that TNF-plays that appear to be incompatible[13]. It has been observed that increased production of TNF is caused by genetic changes at the TNF locus[15]. TNF-α levels that are, elevated in theblood have been linked to poor clinical outcomes in RF patients[16]. Previously, it has been discovered that functionally relevant polymorphisms in the TNF- (TNF-α) gene promoter region, which impact transcriptional activity[17], are linked to poor clinical outcomes in patients with critical illnesses, particularly those who require dialysis for RF [18]. In the present study, we examine the link between glomerular filtration rate markers and a functional polymorphism in the promoter region (position -264 bp) of the *TNF- α gene* in relation to the severity of kidney disease.

2-Methdology of proposed methods:

This study was conducted in the laboratories of Thi-Qar University's College of Education for Pure Sciences in collaboration with Al-Hussein Teaching Hospital, Nasiriyah General Hospital, and Thi-Qar Center for Nephrology and Dialysis in Thi-Qar Province from February 2022 to March 2023. The study included (100) patients for both sex : (40 patients without Diabetes mellitus), (40 patients with Diabetes mellitus) who had their serum urea level and serum creatinine level checked by specialized physicians and pathologists ,and (20 healthy).Patients ranged in age from < 34 to ≥ 65 years old and distributed (5 groups) depending on the estimated (eGFR), which was established through serum creatinine testing. Hospitalized RF patients with various etiologies (hypertension, diabetics, hypertensive + diabetic interaction, Cardiovascular diseases and unclear etiology) had their blood samples and sera obtained. Two milliliters were put directly in a sterile tube containing EDTA for DNA extraction.

Glomerular Filtration Rate Estimation: The eGFR program was used to calculate creatinine in men with normal renal function with a correction for women, using an equation proposed by [19].

TNF- α Genotyping

Using a kit from Geneaid/Taiwan, genomic, DNA (gDNA) was isolated from Peripheral Blood Leukocytes. Aspectrophotometer (Nanodrop, THEERMO, USA) was used to measure the amount of DNA in accordance with the manufacturer's instructions. This device measures the amount of DNA (ng/microliter) and verifies its purity by reading the absorbance (260/ 280 n m). Polymerase Chain Reaction (PCR) ,amplification of DNA for *TNF- α* gene. The following cycling conditions were used: 94°C for 5 min; 35 cycles of (94°C, 30 sec), (59°C, 30 sec) , (72°C, 45 min); followed by 72°C for 7 min. Primers for the *TNF-α* gene were as follows;

Primers	Primer sequences
F	5'-TATGTGATGGACTCACCAGGT -3'
R	5'-CCTCTACATGGCCCTGTCTT -3'

All samples should have a proliferative fragment of 264 bp as a PCR accuracy check. The normal genotype (CC), as well as the mutant genotypes (CT), were included in the polymerase chain reaction (PCR) results that were anticipated for this investigation. Following PCR, the products were electrophoretically separated on an agarose gel, and bands were then seen using ultraviolet vision (Figure.1).

3-Statistical analysis: The results of the current study were analyzed using Chi-square test and F test were also used to study the frequency of genotypes for the *TNF-alpha* gene using the SPSS statistical analysis program.

4-Results and Discussion:

Demographic characteristics:

The frequency distribution of study groups by age was also displayed, and there was a statistically significant, differences among renal failure patients with, and without D.M and healthy subjects (P = 0.001),as in the illustrated table (1).

Table 1. distribution of study groups according to Age

Age groups	No. of patients With D.M 40	No. of patients Without D.M 40	No. of control 40	Total	*P*
Age (years)					
< 34, *n* (%)	2 (5.00%)	8 (20.00 %)	20 (50.00 %)	20 (20.00 %)	**0.001 ****
35-44, *n* (%)	4 (10.00%)	10 (25.00 %)	6 (20.00 %)	18 (18.00 %)	
45-56, *n* (%)	10 (24.00%)	10 (25.00%)	4 (10.00%)	22 (22.00%)	

57-64, *n* (%)	14 (35.00%)	2 (5.00%)	6 (10.00%)	18 (18.00%)	
≥ 65, *n* (%)	10 (25.30%)	10 (25.00 %)	4 (10.00 %)	22 (22.00 %)	

The frequency distribution of renal failure patients with diabetic mellitus , renal failure patients without diabetic mellitus and control subjects according to GFR was also shown, The frequency distribution of study groups was significantly different according to GFR (P= 0.001), as displayed in table 2.

Table 2. Distribution of study groups according to GFR

GFR				
sex	**Groups**	**Mean ± SD**	**N**	**P. Value**
male	Diabetes	15.8353 ± 4.05002	34	
	Non Diabetes	14.0812 ± 3.82330	26	
	control	101.0000 ± 8.44894	24	
	Total	31.3312 ± 34.26088	84	
female	Diabetes	15.2333 ± 4.42207	6	0.68
	Non Diabetes	13.8571 ± 3.20713	14	N.S
	control	104.3333 ± 8.01665	16	
	Total	35.0538 ± 38.98616	36	
Total	Diabetes	15.7450 ± 4.05383	40	
	Non Diabetes	14.0027 ± 3.57918	40	0.001 **
	control	102.0000 ± 8.25897	40	
	Total	32.2991 ± 35.38427	120	

SD= Standard Deviation , P= ANOVA test , N.S= no significant

Table (3): The results of the current study showed that there is relationship between the genotypes of the *TNF-α* gene and the incidence of RF. The results showed significant difference between patients and controls when genotype C\C and C/T (P=0.014), as in the illustrated figure (1).

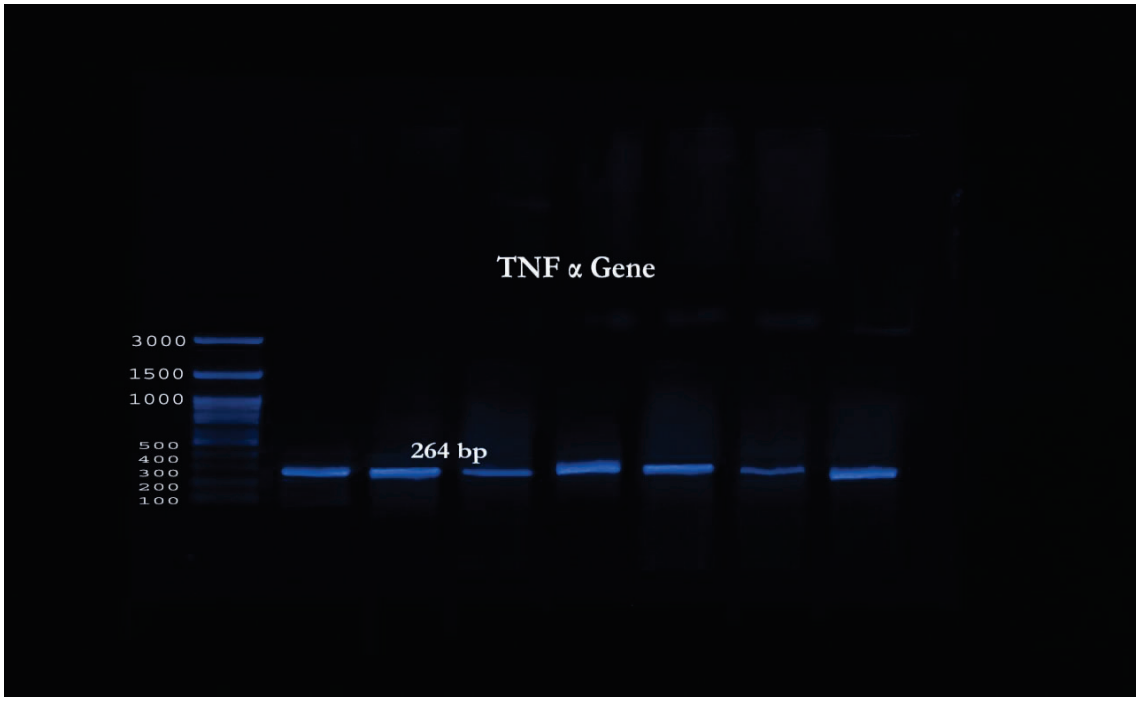

Figure 1. Agarose gel electrophoresis image that showed the PCR product analysis of *TNF-alpha gene*

Table 3. The frequency of *TNF-alpha* gene polymorphism in the study groups.

Mutations		Patients With D.M n=40		Patients Without D.M n=40		Control n=20		*Chi Square (X^2) P. value*
		Polym.	Frequency %	Polym.	Frequency %	Polym.	Frequency %	
TNF-alpha **243.C>T**	CC	22	55	34	85	14	70	**X^2 8.57 * P (0.014)**
	CT	18	45	6	15	6	30	
	-	-	-	-	-	-	-	

The result of current study showed the allelic frequency of TNF-α C was also significantly different in cases than in controls for -264 bp ,where (P= 0.029) ,as illustrated in Table (4).

Table 4. *TNF-alpha* gene polymorphism allele frequency in the study groups.

Mutations		Patients with D.M n=80		Patients without D.M n=80		Control n=40		*Chi Square (X^2) P value*
		Allele	Frequency %	Allele	Frequency %	Allele	Frequency %	
TNF-alpha 243.C>T	C	62	77.5	74	92.5	34	85	X^2 7.05 * P (0.029)
	T	18	22.5	6	7.5	6	15	

The current study showed high significant differences for GFR and RF occurrence. There were high significant differences in the genotype C/C and C/T of TNF-α (P= 0.001), while genotypes of TNF-R2 also shows high significant differences (P=0.001) , as seen in Table (5).

Table 5. The effect of *TNF-alpha* gene on GFR in RF patients

Mutations		GFR				*P. value*
		Patients With D.M n=40	Patients Without D.M n=40	Control n=20	*Mean*	
TNF-α 243. C>T	CC	15.00 ± 2.52	13.57 ± 3.68	102.28 ± 8.92	31.76 ± 35.84	0.337
	CT	16.64 ±4.31	16.43 ± 1.39	101.33 ± 7.17	33.54 ± 34.84	
	Mean	15.74 ± 4.05	14.00 ± 3.57	102.00 ± 8.25	31.29 ± 35.38	0.001**
	A =Groups (0.001**) , B = Polymorphisms (N.S), A*B (N.S)					

5-Discussion:

The results of the current study were consistent with the results of the study conducted by the previous studies[20] [21[22] ,Where it was found in the study that the age group (45-56),(≥ 65) years recorded the highest category of kidney failure, with a rate of 22%.chronic kidney disease (CKD) is common in the elderly, and this is what prompted professional organizations to conduct routine check-ups for the elderly in health care centers [23].Patients who are >55 year old are more likely to develop kidney failure compared to younger age groups. The age groups are associated with glomerular filtration rate(GFR). whereas, in elderly patients, the glomerular filtration rate is less compared to younger people, so the death rate for elderly patients with renal failure is more [24][25], in addition to the related diseases such as hypertension and diabetes mellitus that are more common in senior patients, postulate that RF disease was elevated gradually

with age of patients and that this may contribute to decline in GFR and increase renal injury [25] .The results were consistent with those of other studies conducted around the world. The prevalence of RF in the adult US population was much higher in those who were 55 years and older than in those who were 40 to 50 years old and 20 to 39 years old, respectively [26]. The GFR test determined how well a person's kidneys are working. The severity of kidney disease is inversely correlated with GFR and is linked to a decline in GFR regardless of the cause. A normal GFR (125 mL/min) is taken as evidence that the kidneys are healthy and functioning [27]. Many people's GFR begins to fall in their third decade of life at a rate of roughly 10 cc/min. As a result, in people 70 years of age or older, normal GFR may drop to 60 cc per minute or less; this implies deteriorating kidney function, though not usually progressive RFs [28]. The older age has become independent predictors of lower GFR, possibly representing age and muscle mass well-known associations. Older people tend to have lesser muscle mass, which results in decreased urine excretion of creatinine and lower plasma creatinine at any GFR [29]. In the current study ,the genotypic distribution of *TNF-alpha* -264bp are significantly different between patients and control ($p = 0.014$), the CC Genotype was more frequent in RF patients than' control, the allelic frequency 'of *TNF-alpha* C was also significantly different in cases than in controls for -264 bp ,where ($P = 0.029$) . To our knowledge, this is the first investigation into the relationship between RF, as assessed by levels of filtration and tubular damage indicators, and a functionally significant variation in the *TNF-alpha* gene. All genetic analyses revealed a connection between the TNF- gene and RF susceptibility, indicating that the mutation may increase the risk of RF in the population, albeit this connection needs to be confirmed in a larger sample size [30]. This study demonstrated an inverse correlation between effect of *TNF-alpha* gene on end-stage renal disease and decreased eGFR, believed that the progression of kidney failure into later stages occurs when the ectodomain of TNF-alpha is shed into the lumen as a result of renal cell damage. However,our findings will need to be confirmed in future functional studies and larger samples because RF is a complex disease.

6-Conclusion: The prevalence of RF patients in Thi-Qar province recorded a significant rise in the rate of hemodialysis yearly. Where incidence of renal failure can be distributed equally between males and females , while the disease is more severe in men, who also have a higher prevalence of the end-stage renal disease . Elderly people over 50 years old are more likely to be affected than young people, diabetic mellitus are the important risk factor that cause and advances renal failure disease. The TNF- α Gene Polymorphism is linked with markers of RF and distal organ dysfunction between patients with RF.

References:

[1] Obesity, Weight Loss, and Number Sex Age Range. "Clinical Research Protocol National Institute of Diabetes and Digestive and Kidney Diseases." (2017).

[2] Corsello, Andrea, et al. "Walter E. Dandy: his contributions to pituitary surgery in the context of the overall Johns Hopkins Hospital experience." *Pituitary* 20 (2017): 683-691.

[3] Sameen, Faisal Younus, et al. "Assessment Knowledge of the patients' with acute renal failure concerning dietary pattern in dialysis unite of AL-Zahraa Teaching Hospital at AL-Kut city." *Prof.(Dr) RK Sharma* 21.1 (2021): 249.

[4] Liao, Min-Tser, et al. "Insulin resistance in patients with chronic kidney disease." *Journal of Biomedicine and Biotechnology* 2012 (2012).

[5] Bonventre, Joseph V.; WEINBERG, Joel M. Recent advances in the pathophysiology of ischemic acute renal failure. *Journal of the American Society of Nephrology*, 2003, 14.8: 2199-2210.

[6] Akcay, Ali; Nguyen, Quocan; Edelstein, Charles L. Mediators of inflammation in acute kidney injury. *Mediators of inflammation*, 2009, 2009.

[7] Lee, D. W.; Faubel, S.; Edelstein, CL21888852. Cytokines in acute kidney injury (AKI). *Clinical nephrology*, 2011, 76.3: 165-173.

[8] White, Laura E.; Hassoun, Heitham T. Inflammatory mechanisms of organ crosstalk during ischemic acute kidney injury. *International journal of nephrology*, 2012, 2012..

[9] O'malley, W. Edward; Achinstein, Betty; Shear, Murray J. Action of bacterial polysaccharide on tumors. II. Damage of sarcoma 37 by serum of mice treated with Serratia marcescens polysaccharide, and induced tolerance. *Journal of the National Cancer Institute*, 1962, 29.6: 1169-1175.

[10] Hayashi, Kentaro, et al. A systems biology approach to suppress TNF-induced proinflammatory gene expressions. *Cell Communication and Signaling*, 2013, 11: 1-15.

[11] Zhang, Bei-Bei, et al. Association between TNF α gene polymorphisms and the risk of duodenal ulcer: a meta-analysis. *Plos one*, 2013, 8.2: e57167.

[12] Locksley, Richard M.; Killeen, Nigel; Lenardo, Michael J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*, 2001, 104.4: 487-501.

[13] Elahi, Maqsood M., et al. Tumor necrosis factor alpha− 308 gene locus promoter polymorphism: an analysis of association with health and disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 2009, 1792.3: 163-172.

[14] Barbara, Jeffrey AJ; Van ostade, Xaveer; LOPEZ, Angel F. Tumour necrosis factor-alpha (TNF-α): The good, the bad and potentially very effective. *Immunology and cell biology*, 1996, 74.5: 434-443.

[15] Tsukamoto, Kazuhiro, et al. A highly polymorphic CA repeat marker at the human tumor necrosis factor alpha (TNFAα) locus. *Journal of human genetics*, 1998, 43.4: 278-279.

[16] Kadiroglu, Ali Kemal, et al. The evaluation of effects of demographic features, biochemical parameters, and cytokines on clinical outcomes in patients with acute renal failure. *Renal failure*, 2007, 29.4: 503-508.

[17] Jaber, Bertrand L., et al. Polymorphism of immunomodulatory cytokine genes: implications in acute renal failure. *Blood purification*, 2004, 22.1: 101-111.

[18] Jaber, Bertrand L., et al. Cytokine gene promoter polymorphisms and mortality in acute renal failure. *Cytokine*, 2004, 25.5: 212-219.

[19] Levey, Andrew S., et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Annals of internal medicine*, 1999, 130.6: 461-470.

[20] O'hare, Ann M., et al. Age affects outcomes in chronic kidney disease. *Journal of the American Society of Nephrology*, 2007, 18.10: 2758-2765.

[21] Ribero, Rita de Cássia Helú Mendonça, et al. Characterization and etiology of the chronic renal failure in a countryside nephrology unit of São Paulo State. *Acta Paulista de Enfermagem*, 2008, 21: 207-211.

[22] MohammedJumaah, Israa A. A study of some biochemical parameters in blood serum of patients with chronic renal failure. *Journal of Basrah Researches (Scinces)*, 2013, 39.4.

[23] O'HARE, Ann M., et al. Mortality risk stratification in chronic kidney disease: one size for all ages?. *Journal of the American Society of Nephrology*, 2006, 17.3: 846-853.

[24] Lindeman, Robert D.; TOBIN, Jordan; SHOCK, Nathan W. Longitudinal studies on the rate of decline in renal function with age. *Journal of the American Geriatrics Society*, 1985, 33.4: 278-285.

[25] Hemmelgarn, B. R., et al. Progression of kidney dysfunction in the community-dwelling elderly. *Kidney international*, 2006, 69.12: 2155-2161.

[26] Phoon, Richard KS. Chronic kidney disease in the elderly: assessment and management. *Australian family physician*, 2012, 41.12: 940-944.

[27] Higgins, Chris. Urea and creatinine concentration, the urea: creatinine ratio. *Acute Care Test Hand*, 2016, 1-8.

[28] Carroll, E.,. The Stages of Chronic Kidney Disease and the Estimated Glomerular Filtration Rate. *The Journal of Lancaster General Hospital Fall*, (2006), 1(2), 64–69.

[29] Levey, Andrew S., et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Annals of internal medicine*, 1999, 130.6: 461-470.

[30] Chen, Guanzhong, et al. Ethnicity-stratified analysis of the association between TNF-α genetic polymorphisms and acute kidney injury: a systematic review and meta-analysis. *BioMed Research International*, 2020, 2020.