https://doi.org/10.33472/AFJBS.6.10.2024.4184-4196

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Effect of *TNF-α* G/A gene polymorphism and it is gene expression on renal failure

Sarhang Hasan Azeez[1*] ,Mariwan Fathalla Abdalfatah [2], Suhaila Nafiq Darogha[3], Hama Hussein Hama Hussein[4]

[1, 3]Department of Biology, College of Education, Salahaddin University- Erbil, Erbil, Kurdistan Region, Iraq.
[2]Department of Medical Laboratory, Kurdistan Technical Institute, Sulaymaniyah, Iraq.
[4]Ministry of Education, General-Directorate Education in Raparin, Educational Department, SubDirectorate of Pshdar, Qaladiza Scientific High School, Kurdistan Region, Iraq.
***Corresponding author: Sarhang Hasan Azeez, Email: sarhnag.azeez@su.edu.krd**

Volume 6, Issue 10, 2024

Received: 19 March 2024

Accepted: 20 April 2024

Published: 10 May 2024

doi:10.33472/AFJBS.6.10.2024.4184-4196

Abstract

This study was carried out in Hawler Dialysis Center and Hala private clinic in Erbil city, from the period of 7th January to 24th November 2017. A total of 108 patients suffering from chronic kidney disease (CKD) (54 on hemodilaysis (HD) and 54 renal transplanted (RT)) and 54 healthy individuals as a control group. Some demographic distributions were collected, immunological and molecular study of cytokine gene polymorphism were determined in patients and control group. *TNF-α* $_{-308}$ gene polymorphism revealed that genotype GA was in 37% and 33.3% of HD and control group respectively, χ^2 =0.16, (RR: 1.18, CI: 0.5-2.8) (P>0.05). Results for AA genotype recorded 3-folded higher in HD patients than those of control group, χ^2 =1.03, (RR: 3.12, CI: 0.32-83.52). Allele frequencies of HD patients were 0.76 and 0.24 for HD patients, P>0.05. However, for RT patient's genotyping and allele frequency the results of this study showed different percentages. Therefore, 50% of RT patients were GG genotype, while higher percentage but not significant difference of control group had the same genotype, which was GG, 64.8%, χ^2 =2.42 (RR: 0.54, CI: 0.23-1.26), (P>0.05). Thus, 42.6% of RT patients had GA genotype and 33.3% of control had the same genotype, χ^2 =0.16 (RR: 1.18, CI: 0.5-2.8), P>0.05. Whereas, AA genotype was found 4-folded higher in RT patients than control group, χ^2 =1.88 (RR: 4.24, CI: 0.51-106.74), P>0.05. Regarding the allele frequencies, the results for G and A allele were 71%, 29% and 81%, 19% for RT patients and control group respectively. Genetic variations in TNF-α may interfere with the production of pro-inflammatory cytokine and may result in inflammatory diseases such as CKD. The GG genotype was protective against the renal failure by almost 70%. AA genotype was at risk to have the renal failure by 4 folded higher than the GG and GA genotype.

Kew Words: Gene polymorphism, *TNF-α* G/A, gene expression, Renal Failure

Introduction

As a global public health challenge, chronic kidney (CKD) disease has relatively unchanged magnitude and leads to mortality despite growing technological advancement and progress in renal replacement therapy (Schoolwerth et al., 2006). CKD refers to abnormality in the structure or function of the kidneys, prolonged for 3 months, and cause health complications (Wanner and Tonelli, 2014). Different risk factors for progression of CKD were including life style, demographic variables, family history, and pre-existing diseases were described in research (Gheewala et al., 2018).

There is a direct relationship between CKD prevalence and age, such that its prevalence is about 17% of those are over 60 years old (Zhang and Rothenbacher, 2008). The main causes of chronic kidney disease are diabetic nephropathy, hypertension and glomerulopathy. Diabetes has been referred to as the leading risk factor for CKD (Alicic et al., 2017).

Inflammation has been referred to as a significant factor since its activation is caused by the hemodynamic, biochemical, and metabolic derangements that are reported to be available diabetic kidney (Lim and Tesch, 2012). As a result, it seems that overproduction of proinflammatory cytokines has a significant role in kidney injury and inflammation and as a result of altered expression of immune cells has been correlated with renal injuries (Bonventre and Zuk, 2004). Study demonstrated an increased turnover of specific cytokines in ESRD (Vaziri, 2012). The rate of cytokine turnover differs individually and maybe related to genetic susceptibility (Felger and Lotrich, 2013).

In HD patients, the various cytokine levels, such as IL-2, IL-4, IL-5 and IFN-γ, were increased (Rios et al., 2017). Morbidity and mortality, which are independent risk factors in CKD patients, remain unchanged despite undoubted improvements in haemodialysation techniques, and are due to high levels of interleukins, the presence of metabolic acidosis, chronic inflammation, malnutrition, anemia, and CVD (Stancu et al., 2018).

Functional single nucleotide polymorphisms (SNPs) within the promoter region of cytokine genes were identified as influencing the activities of gene promoters and the levels of gene products (Bayley et al., 2004). It has been shown that such polymorphisms are associated with susceptibility to a variety of atherosclerotic diseases in CKD (Okada et al., 2012; Kubo et al., 2017). However, it has not been fully clarified whether cytokine polymorphisms are risk

factors for CKD itself. Study has not shown such associations, possibly because of the small sample size, and their findings are controversial (Okada *et al.*, 2012).

TNF-α have been reported to be accompanied with a number of infectious and autoimmune diseases and have an important role in ESRD pathogenesis (Imig and Ryan, 2013). Infiltration of immune cells such as neutrophils and macrophages can increase the development of TNF-α in the kidney (Parameswaran and Patial, 2010). Increased innate immune activation of neutrophils and macrophages and increased production of TNF-α were observed in infections such as cisplatin nephropathy, diabetic nephropathy, antiglomerular membrane nephropathy and obstructive nephropathy (Ortega and Fornoni, 2010, Tanaka et al., 2014). In such cases, triggers for the development of TNF-α include activation of TLR 4 in response to LPS, oxidative stress, deposition of antibodies and additional activation (O'Neill et al., 2009).

Materials and method:

One hundred and eight Iraqi Kurdish patients with chronic kidney disease were enlisted in the study. 54 patients were selected from the Hawler Hemodialysis Centre for HD, and the other 54 were RT patients from private clinics at Hawler city from 7th January 2017 to 24th November 2017 in order to be diagnosed and treated. The diagnosis was made in the hospital's medical consultant on the basis of a clinical review and laboratory test results. The mean age for HD and RT were respectively 46.1± 1.6 and 36.8± 2.8. Half of the HD patients (50%) were male and half were female, while 55.6% of the RT patients were male and 44.4% female. A questionnaire form was prepared and filled for each patients through direct interview with them. The questionnaire form is attached in appendices. In addition to patients, the study included 54 seemingly healthy Iraqi Kurdish subjects (27 males and 27 females) as a control group and their age range was 18-71 years (mean: 40.2 ± 1.9 years).

Ten ml of venous blood was drawn from each participant (patients and controls) using a syringe, and dispersed in two aliquots. The first aliquot (7 ml) was applied to a plain tube and centrifuged for 15 minutes (min) at 3000 round per minute (rpm). In Eppendorf tube, the isolated serum was distributed in 6 aliquots, which were frozen at -20℃ before cytokine serum concentrations were measured. For the genotyping of cytokine gene polymorphisms, the second aliquot (3 ml) was transferred to the Ethylene Diamine Tetra Acetic Acid (EDTA) tube and frozen at -20℃ before deoxyribonucleic acid (DNA) extraction.

Cytokine gene polymorphism was done by using amplification refractory mutation system polymerase chain reaction ARMS-PCR. Certain primers and protocols were used to detect all polymorphisms at promoter positions of cytokine (TNF-α). Details of these polymorphisms are given in table (1).

Table 1. studied polymorphism in cytokine. The location of the chromosome, the names of dbSNP, the locations of active polymorphisms in relation to the starting site of the gene and the substitutions of nucleotides.

Cytokines	Chromosome	db SNP	Position	SNP
TNF-α	6p21.3	rs1800629	-308	G/A

The used primers and protocols with the final product size were given in Table 2. The protocols of ARMS-PCR of the studied cytokine for gene polymorphism were as shown in the Table 3.

Table 2. Cytokines and their primers.

Cytokine	Primer Name	Primer Sequence
TNF-α	TNF-α -308 Common primer	5'-TCC TCC CTG CTC CGA TTC CG-3'
	TNFα A Allele primer	5'-CAA TAA GTT TTG AGG GGC ATG A-3'
	TNFα G Allele primer	5'-CAA TAA GTT TTG AGG GGC ATG G-3'

Table 3. studied gene polymorphisms ARMS-PCR protocol and product size.

Cytokines	Positions	PCR protocol	Product size
TNF-α	-308	An primary 4-min denaturation at 95°C , followed by 35 30-s cycles at 95°C, 60°C, and 74°C. The final extension step was at 74°C for 6 min.	104 bp

Statistical analysis:

The cytokine serum rate was analyzed statistically using the statistical system for social sciences (SPSS) version 19 computer program. Their data were given as a mean ± standard deviation (S.D), and the mean difference was evaluated by analysis of variance (ANOVA), followed by Duncan test.

Cytokine genotypes were presented as percentage and allele frequencies, and the exact probability (P) of two-tailed Fisher was evaluated by considerable variation between their discrepancies in CKD patients and controls. Furthermore, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) calculated also to describe the relation between a genotype and the disease. The RR value can differ between less than one (negative association) and more than one (positive association). The EF was determined if the relationship was positive, while the PF was given if it was negative (Ad'hiah, 1990). Using WINPEPI computer programs for epidemiologists, these figures are determined. At http:/www.brixtonhealth.com, the new version of the WINPEPI kit is freely available.

Results and Discussion

Patients and controls were presented in regard to sex and age groups. For sex, HD patients were distributed as 50% males and 50% females, while such frequencies among RT were 55.6 and 44.4%, respectively. In controls, males and females represented equal distribution which were 50%. Distribution of patients into two age groups (< 40 years and ≥ 40 years) showed discrepancies between patients with HD and RT. The age group < 40 years was less frequent in HD patients than in RT patients (42.6 vs. 59.25%), while an opposite picture was drawn in the age group ≥ 40 years (57.4 vs. 40.75 %). About the mean age of the patients, the results were 46.1, 36.8 and 40.2 years for HD, RT and control respectively. This study showed no variations in BMI between the groups, whereas, both groups of patients were at normal BMI, which were 24.83 and 24.17 for both HD and RT patients. The results revealed that 27.8% and 20.4% of the patients respectively in HD and RT groups smoked. However, about 26% of the HD patients and 13% of the RT patients had diabetes. Moreover, hypertension was observed in about 50% and 16.7% of the HD and RT patients, respectively. It was also seen that 20.4% and 24.1% of the HD and RT patients had family history, respectively, (Table 4.1)

Table 4.The demographic distribution of the studied groups.

Groups	Hemodialysis	Renal	Control

Characters	N=54	Transplanted N=54	N=54
Age (Mean± SD)	46.1±1.6	36.8±2.8	40.2±1.9
Sex:			
Male	27/54(50%)	30/54(55.6%)	27/54(50%)
Female	27/54(50%)	24/54(44.4%)	27/54(50%)
BMI (Mean± SD)	24.83±0.5	24.17±1.0	23.62±0.45
Smokers n (%)	15/54(27.8%)	11/54(20.4%)	-------
Diabetes n (%)	14/54(26%)	7/54(13%)	-------
Hypertension n (%)	27/54(50%)	9/54(16.7%)	-------
Family History n (%)	11/54(20.4%)	13/54(24.1%)	-------

Age has been referred to as a significant effective modifier in renal disease. Evidence has shown that there have been multiple prognostic effects with respect to age in kidney disease. The significance of age difference in ESRD reflects various phenomena (Prakash and O'Hare, 2009). Similar to our results, lower ESRD incidence among older patients was likely due to the greater competing risks for death as a result of various coexisting age-related comorbidities (Obi et al., 2010). Other explanation could be that older patients were survivors for a longer period and therefore have slowly progressive or non-progressive disease than younger patients. In older patients, comorbidities that are related to age can predict global outcomes such as mortality, although obese patients can predict different renal outcomes (Rosansky et al., 2017).

According to the current study, results cited by neighbouring country, the average age of patients was smaller than that indicated in the United States and the Kingdom of Saudi Arabia, which was found to be 60 years and 55 years, respectively and slightly lower than that found in Iran (51,6 years) (Al-Zahrani et al., 2019).

TNF-α level was also significantly increased in HD patients compared to controls (80.57 ±5.71 vs. 38.74±3.38 pg/ml) but RT showed no significant difference p>0.05.

Table 5. Serum level of TNF-α in total HD and RT patients and controls.

Cytokine	Cytokine Serum Mean Level ± S.D. (pg/ml)

	Patients		Controls (No. = 54)
	HD (No. = 54)	RT (No. = 54)	
TNF-α	80.57 ±5.71^{A}	43.28±1.15^{B}	38.74±3.38^{B}

Numerous researches on the formation and release of IL-1β, IL-2, IL-6, IL-8, IL-10 and TNF-α proinflammatory cytokines in patients with chronic renal failure on maintenance HD provide conflicting data. Study found that some of HD patients have shown high serum levels of proinflammatory cytokines before and during HD, other studies have shown that cellular activation and cytokine synthesis are only temporary and serum levels are rather moderate (Rysz et al., 2006, Iyer and Cheng, 2012). Regarding levels of serum TNF-α, it was observed that there was a relationship between elevated levels of TNF-α and CKD, because higher TNF-α levels were observed in patients with HD in the present study than the control group. This finding is in agreement with the study conducted by (Navarro-Gonzalez and Mora-Fernandez, 2008). Increased levels of serum TNF-α and their soluble receptors in patients with differing grades of renal failure have been related to the worsening of renal function (Colombo et al., 2012). Decreased concentration of TNF-α in RT patients was carry a significant reason. The RT patients received immunosuppressant drugs and this had most likely to related to decrease in immune response and low TNF-α production in their serum (Krishna and Nadler, 2016).

In a recent study, Alwahaibi *et al.* found that the serum TNF-α level in dialysis and chronic patients with renal failure was not higher than in control group (Alwahaibi et al., 2016). In addition, the increase in serum TNF-α found during the HD session was also demonstrated by Borazan et al., (2004), who also measured serum TNF-α concentration before and after dialysis and reported similar findings.

About the TNF-α serum level from males of HD the increase was significant, while in RT patients there was slight increase. Same results were found in females of the mentioned cytokine.

Table 6. Serum level of TNF-α in total HD and RT patients and controls dispensed by sex.

Cytokine	Sex	No.	Cytokine Serum Mean Level ± S.D. (pg/ml)		
			Patients		Controls
			HD	RT	
TNF-α	Males	84	72.81 ± 26.5^{A}	45.35 ± 25.12^{B}	42.17 ± 5.88^{B}

	Females	78	87.47 ± 37.87^{A}	44.39 ± 8.48^{B}	32.87 ± 4.08^{B}
P ≤			**N.S.**	**N.S.**	**N.S.**

The genetic polymorphism of the *TNF-α* gene was studied at location (TNF-α-$_{308}$), which was identified in HD and RT patients and controls with three genotypes (GG, GA and AA). No significant departure from H-W equilibrium were recorded in both group of patients and controls. For *TNF-α*-$_{308}$, there was no significant variation between genotypes and alleles. HD and RT patients and controls (Tables7, 8, 9, 10) and (Figure 1).

Table 7. Observed numbers and percentage frequencies and H-W equilibrium of TNF-α-308 genotypes and alleles in HD patients and controls.

Groups			*TNF-α* -308 Genotype or Allele					H-W P ≤
			GG	GA	AA	*G*	*A*	
HD (N=54)	Observed	No.	31	20	3	82	26	Not significance
		%	57.4	37	5.6	76	24	
	Expected	No.	31.1	19.7	3.1	Not Estimated		
		%	57.6	36.5	5.8			
Controls (N=54)	Observed	No.	35	18	1	88	20	Not significance
		%	64.8	33.3	1.9	81.5	18.5	
	Expected	No.	35.9	16.3	1.9	Not Estimated		
		%	66.4	30.1	3.5			

Table 8. Statistical evaluations of associations between TNF-α-308 genotypes or alleles and HD patients.

TNF-α-308 Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
GG	0.73	0.17	Not significance	0.34-1.58
GA	1.18	0.05	Not significance	0.54-2.57
AA	3.12	0.03	Not significance	0.32-30.31
G	0.72	0.23	Not significance	0.37-1.38
A	1.4	0.06	Not significance	0.73-2.68

Table 9. Observed numbers and percentage frequencies and H-W equilibrium of TNF-α-308 genotypes and alleles in RT patients and controls.

Groups			*TNF-α* $_{-308}$ Genotype or Allele					H-W P ≤
			GG	GA	AA	*G*	*A*	
RT (N=54)	Observed	No.	27	23	4	77	31	Not significance
		%	50	42.6	7.4	71.3	28.7	
	Expected	No.	27.4	22.1	4.4	Not Estimated		
		%	50.8	40.9	8.2			
Controls (N=54)	Observed	No.	35	18	1	88	20	Not significance
		%	64.8	33.3	1.8	81.5	18.5	
	Expected	No.	35.9	16.3	1.9	Not Estimated		
		%	66.4	30.1	3.5			

Table 10. Statistical evaluations of associations between TNF-α-308 genotypes or alleles and RT patients.

TNF-α $_{-308}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
GG	0.54	0.29	Not significance	0.25-1.17
GA	1.48	0.13	Not significance	0.68-3.22
AA	4.24	0.05	Not significance	0.47-1.11
G	0.56	0.35	Not significance	0.3-1.07
A	1.77	0.12	Not significance	0.94-3.35

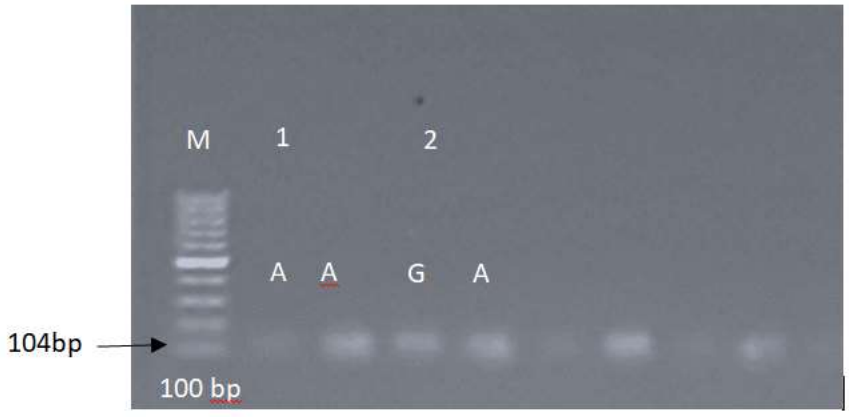

Figure 1. TNF-α product of ARMS-PCR on agarose gel (2%) amplicon size (104bp): M: 100bp size DNA ladder.

Though the studies investigating SNP (-308 GA) as a predisposing risk factor for nephropathy/ESRD was conflicting due to inter-individual differences and phenotypic heterogeneity (Lee et al., 2005, McKnight et al., 2007). The results of the present study was in line with the results of the study conducted by (Manchanda et al., 2006). Similar result, the homozygous rare AA genotype of TNF-α -308 SNP was correlated with raised TNF-α production that in turn was associated with a 3-folds increased risk for HD (OR=3.12) (Essadik et al., 2015). The data from this study affirms this risk in a relatively larger sample size with better study power. TNF-α GA genotype in HD patients was linked with an increased expression of TNF-α levels, carriers of AA genotype may experience an exacerbated inflammatory response. This association relates to either early initiation of symptoms or quick progression towards ESRD.

In accordance with the results of this study, the -308 G/A SNP has been reported as a potent predisposing CKD risk factor (Wang et al., 2005). The results of the study conducted by (Manchanda et al., 2006) demonstrated that genotypes that are reported to be associated with an increase in secretion of TNF-α are correlated with an increase in ESRD risk.

The results of SNP TNF- α -308 was in agreement with a study done on Asian Indians (Prasad et al., 2007). Vazquez-Huerta et al. were also observed that there was an associative relationship between CKD and any haplotype of these SNPs, indicating the fact that it is unlikely that susceptibility to CKD is significantly affected by the -308 G/A *TNF-α* gene SNP, while the contribution of other variants in the TNF-α gene promoter region have an undeniable contribution susceptibility to CKD (Vázquez-Huerta et al., 2014). The frequency of the – 308/A allele varies in geographical areas. It was reported to exist in about 30% of the white Caucasians in the United Kingdom and 5% of South African population (Elahi et al., 2009). Compared to the control group, the patients with HD and RT were found to have higher levels of the AA genotype for −308 region of the *TNF-α* gene. As shown by the results of a study conducted by Lee et al., AA genotype produced high levels of TNF-α, was significantly correlated with immunoglobulin A nephropathy, and was referred to as a significant cause of ESRD (Lee et al., 2001). Rao *et al.* revealed that the AA genotype of TNF-α −308 was significantly associated in patients with reflux nephropathy (Rao et al., 2007).

Similarly to our findings, on the other hand, the -308 G / A SNP has previously been correlated with North India as a powerful predisposing risk factor for CKD and Chinese

populations (Wang et al., 2005, Prasad et al., 2007). However, it seems that TNF-α levels do not suit the polymorphisms of the − 308 TNF promoter. Although multifactoral regulatory process might control circulating TNF-α level, concentration of local TNF-α might be more significant and be more controlled by particular polymorphisms (Elahi et al., 2009).

Associations between CKD and TNF-α (-308G/A) polymorphism have been reported to be varying, which can be attributed to variations in genotyping methods, sample size, and/or ethnicity, as frequencies of TNF-α (-308) alleles and genotypes can also be different in various ethnic healthy populations worldwide (Paskulin et al., 2011). Genetic variations in TNF-α and IFN-γ that interfere with the development of proinflammatory cytokine and may result in inflammatory diseases such as CKD. As the study found, genetic polymorphisms of these cytokines may play a crucial role in the outcome of patients with serious CKD (Spriewald et al., 2005).

Conclusion:

Patients mean age were 46.1 and 36.8 years for HD and RT respectively. Male and female were at risk of the HD and RT in CKD almost equally. Diabetes and hypertension were found more common in HD patients than those whom had RT. In HD patients the concentration of studied cytokines (TNF-α) were increased significantly when compared to controls, while for RT patients the results revealed different variations regarding the cytokine. Genetic variations in TNF-α may interfere with the production of pro-inflammatory cytokine and may result in inflammatory diseases such as CKD. The GG genotype was protective against the renal failure by almost 70%. AA genotype was at risk to have the renal failure by 4 folded higher than the GG and GA genotype.

References:

AD'HIAH, A. H. (1990). Immunogenetic studies in selected human diseases. University of Newcastle upon Tyne.

ALICIC, R. Z., ROONEY, M. T. and TUTTLE, K. R. (2017). Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Amer Soci Nephrol*, 12, 2032-2045.

ALWAHAIBI, N. Y., ALISSAEI, H. K., ALSHIHI, S. A., ALABRI, N., ALBALUSHI, S. S. and ALBALOOSHI, M. (2016). Serum levels of TNF-α, IL-6 and IL-10 in haemodialysis and renal transplant patients and in healthy subjects. *Port J Nephrol Hyper*, 30, 194-198.

AL-ZAHRANI, J. M., ALDIAB, A., ALDOSSARI, K. K., AL-GHAMDI, S., BATAIS, M. A., JAVAD, S. EL-METWALLY, A. (2019). Prevalence of Prediabetes, Diabetes and Its Predictors among Females in Alkharj, Saudi Arabia: A Cross-Sectional Study. Annals of global health, 85(1), 109.

BAYLEY, J., OTTENHOFF, T. and VERWEIJ, C. (2004). Is there a future for TNF promoter polymorphisms? *Genes Immun*, 5, 315.

BONVENTRE, J. V. and ZUK, A. (2004). Ischemic acute renal failure: an inflammatory disease? *Kid Inter*, 66, 480-485.

BORAZAN, A., USTÜN, H., USTUNDAG, Y., AYDEMIR, S., BAYRAKTAROGLU, T., SERT, M. and YILMAZ, A. (2004). The effects of peritoneal dialysis and hemodialysis on serum tumor necrosis factor-alpha, interleukin-6, interleukin-10 and C-reactive-protein levels. *Medi Inflam*, 13, 201-204.

COLOMBO, P. C., GANDA, A., LIN, J., ONAT, D., HARXHI, A., IYASERE, J. E., URIEL, N. and COTTER, G. (2012). Inflammatory activation: cardiac, renal, and cardio-renal interactions in patients with the cardiorenal syndrome. *Heart Fail Rev*, 17, 177-190.

ELAHI, MM., ASOTRA, K., MATATA, BM. and MASTANA, SS. (2009). Tumor necrosis factor alpha− 308 gene locus promoter polymorphism: an analysis of association with health and disease. Biochimica et Biophysica Acta (BBA)-*Mol Bas Dis*, 1792(3), 163-172

ESSADIK, A., JOUHADI, H., RHOUDA, T., NADIFIYINE, S., KETTANI, A. and MAACHI, F. (2015). Polymorphisms of Tumor Necrosis Factor Alpha in Moroccan Patients with Gastric Pathology: New Single-Nucleotide Polymorphisms in TNF-a -193 (G/A). *Med Inflam*, 2015, 5.

FELGER, J. C. and LOTRICH, F. E. (2013). Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience*, 246, 199-229.

GHEEWALA, P. A., PETERSON, G. M., ZAIDI, S. T. R., JOSE, M. D. and CASTELINO, R. L. (2018). Public knowledge of chronic kidney disease evaluated using a validated questionnaire: a cross-sectional study. *BMC Pub Health*, 18, 371.

IMIG, J. D. and RYAN, M. J. (2013). Immune and inflammatory role in renal disease. *Compr Physiol*, 3, 957-976.

IYER, S.S. and CHENG, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Crit Rev Immunol*, 32(1).

KRISHNA, M., and NADLER, S. G. (2016). Immunogenicity to Biotherapeutics - The Role of Anti-drug Immune Complexes. *Front Immunol*, *7*, 21.

KUBO, Y., IMAIZUMI, T., ANDO, M., NAKATOCHI, M., YASUDA, Y., HONDA, H., KUWATSUKA, Y., KATO, S., KIKUCHI, K. and KONDO, T. (2017). Association between kidney function and genetic polymorphisms in atherosclerotic and chronic kidney diseases: A cross-sectional study in Japanese male workers. *PloS One*, 12, e0185476.

LEE, E. Y., YANG, D. H., HWANG, K. Y. and HONG, S. Y. (2001). Is tumor necrosis factor genotype (TNFA2/TNFA2) a genetic prognostic factor of an unfavorable outcome in IgA nephropathy? *J Kor Med Sci*, 16, 751.

LEE, S. H., LEE, T. W., IHM, C. G., KIM, M. J., WOO, J. T. and CHUNG, J. H. (2005). Genetics of diabetic nephropathy in type 2 DM: candidate gene analysis for the pathogenic role of inflammation. *Nephrology*, 10, S32-S36.

LIM, A. K. and TESCH, G. H. (2012). Inflammation in diabetic nephropathy. *Medi Inflam*, 2012.

MANCHANDA, P. K., KUMAR, A., KAUL, A. and MITTAL, R. D. (2006). Correlation between a gene polymorphism of tumor necrosis factor-α (G/A) and end-stage renal disease: A pilot study from north India. *Clin Chim Acta*, 370, 152-157.

MCKNIGHT, A. J., SAVAGE, D. A., PATTERSON, C. C., SADLIER, D. and MAXWELL, A. P. (2007). Resequencing of genes for transforming growth factor β1 (TGFB1) type 1 and 2 receptors (TGFBR1, TGFBR2), and association analysis of variants with diabetic nephropathy. *BMC Med Genet*, 8, 5.

NAVARRO-GONZALEZ, J. F. and MORA-FERNANDEZ, C. (2008). The role of inflammatory cytokines in diabetic nephropathy. *J Amer Soc Nephrol*, 19, 433-442.

OBI, Y., KIMURA, T., NAGASAWA, Y., YAMAMOTO, R., YASUDA, K., SASAKI, K., KITAMURA, H., IMAI, E., RAKUGI, H. and ISAKA, Y. (2010). Impact of age and overt proteinuria on outcomes of stage 3 to 5 chronic kidney disease in a referred cohort. *Clin J Amer Soc Nephrol*, 5, 1558-1565.

OKADA, R., WAKAI, K., NAITO, M., MORITA, E., KAWAI, S., HAMAJIMA, N., HARA, M., TAKASHIMA, N., SUZUKI, S. and TAKEZAKI, T. (2012). Pro-/anti-inflammatory cytokine gene polymorphisms and chronic kidney disease: a cross-sectional study. *BMC nephrology*, 13, 2.

O'NEILL, L. A., BRYANT, C. E. and DOYLE, S. L. (2009). Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer. *Pharmacol Rev*, 61, 177-197.

ORTEGA, L. M. and FORNONI, A. (2010). Role of cytokines in the pathogenesis of acute and chronic kidney disease, glomerulonephritis, and end-stage kidney disease. *Inter J Interf, Cytok Med Res*, 2, 49-62.

PARAMESWARAN, N. and PATIAL, S. (2010). Tumor necrosis factor-α signaling in macrophages. Critical Reviews™ in Eukaryotic Gene Expression, 20.

PASKULIN, D. D. Á., FALLAVENA, P. R., PALUDO, F. J., BORGES, T. J., PICANÇO, J. B., DIAS, F. S. and ALHO, C. S. (2011). TNF-308G> a promoter polymorphism (rs1800629) and outcome from critical illness. *Braz J Infect Dis*, 15, 231-238.

PRAKASH, S. and O'HARE, A. M. (2009). Interaction of aging and chronic kidney disease. *Sem Nephrol*. *Elsevier*, 497-503.

PRASAD, P., TIWARI, A. K., KUMAR, K. P., AMMINI, A., GUPTA, A., GUPTA, R. and THELMA, B. (2007). Association of TGFβ1, TNFα, CCR2 and CCR5 gene polymorphisms in type-2 diabetes and renal insufficiency among Asian Indians. *BMC Med Genet*, 8, 20.

RAO, M., WONG, C., KANETSKY, P., GIRNDT, M., STENVINKEL, P., REILLY, M. and RAJ, D. (2007). Cytokine gene polymorphism and progression of renal and cardiovascular diseases. *Kid Inter*, 72, 549-556.

RIOS, D. R. A., PINHEIRO, M. B., DE OLIVEIRA JUNIOR, W. V., BRAGA GOMES, K., CARVALHO, A. T., MARTINS-FILHO, O. A., SIMÕES E SILVA, A. C. and DUSSE, L. M. S. A. (2017). Cytokine signature in end-stage renal disease patients on hemodialysis. *Dis Mark*, 2017.

ROSANSKY, S. J., SCHELL, J., SHEGA, J., SCHERER, J., JACOBS, L., COUCHOUD, C., CREWS, D. and MCNABNEY, M. (2017). Treatment decisions for older adults with advanced chronic kidney disease. *BMC nephrology*, 18, 200.

RYSZ, J., BANACH, M., CIALKOWSKA-RYSZ, A., STOLAREK, R., BARYLSKI, M., DROZDZ, J. and OKONSKI, P. (2006). Blood serum levels of IL-2, IL-6, IL-8, TNF-alpha and IL-1beta in patients on maintenance hemodialysis. *Cell Mol Immunol*, 3, 151-154.

SCHOOLWERTH, A., WNGELGAU, M., HOSTETTER, T., RUFO, K., CHIANCHIANO, D., MCCLELLAN, W., WARNOC, D. and VINICOR, F. (2006). Chronic idney disease: a public health problem that needs a public health action plan [Electronic Version]. *Preventing Chronic Disease: Public health research, practice and policy.*

SPRIEWALD, BM., WITZKE, O., WASSMUTH, R., WENZEL, RR., ARNOLD, ML., PHILIPP, T. and KALDEN, JR. (2005). Distinct tumour necrosis factor alpha, interferon gamma, interleukin 10, and cytotoxic t cell antigen 4 gene polymorphisms in disease occurrence and end stage renal disease in wegener's granulomatosis. *Ann Rheum Dis* 64(3), 457-461.

STANCU, S., MIRCESCU, G., MOCANU, A., CAPUSA, C. and STEFAN, G. (2018). Metabolic Acidosis of Chronic Kidney Disease and Cardiovascular Disorders. *Maedica*, 13, 267.

TANAKA, T., NARAZAKI, M. and KISHIMOTO, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spr Harb Pers Biol*, 6, a016295.

VAZIRI, N. D. (2012). CKD impairs barrier function and alters microbial flora of the intestine: a major link to inflammation and uremic toxicity. *Curr Opin Nephrol Hyper.* 21, 587.

VÁZQUEZ-HUERTA, DI., ALVAREZ-RODRÍGUEZ, BA., TOPETE-REYES, JF., MUÑOZ-VALLE, JF., PARRA-MICHEL, R., FUENTES-RAMÍREZ, F. (2014). Tumor necrosis factor alpha -238 G/A and -308 G/A polymorphisms and soluble TNF- α levels in chronic kidney disease: Correlation with clinical variables. *Int J Clin Exp Med*, 7(8), 2111-2119.

WANG, Y., NG, M. C., SO, W.-Y., MA, R., KO, G. T., TONG, P. C. and CHAN, J. C. (2005). Association between tumour necrosis factor-α G-308A polymorphism and risk of nephropathy in obese Chinese type 2 diabetic patients. *Nephrol Dial Transpl*, 20, 2733-2738.

WANNER, C. and TONELLI, M. (2014). KDIGO Clinical Practice Guideline for Lipid Management in CKD: summary of recommendation statements and clinical approach to the patient. *Kid inter*, 85, 1303-1309.

ZHANG, Q.L. and ROTHENBACHER, D. (2008). Prevalence of chronic kidney disease in population-based studies: systematic review. *BMC Pub Health*, 8, 117.