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The impact of IL-10 gene polymorphism 1082A/G (rs1800896) on increased IL-10 secretion in patients with chronic kidney disease in the Kurdistan Region of Iraq.

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Key words: chronic kidney disease; polymorphism; interleukin-10.

Abstract. Studies have indicated that interleukin-10 gene polymorphism at 1082A/G is associated with chronic kidney disease (CKD). The aim of this study was to determine Il-10 gene polymorphism in CKD patients and identify the risk factors and prevalence of the disease among Kurdish patients. It was also aimed at finding out the serum levels of IL-10 in different genotypes, AA, GA and GG. The study included 108 patients with CKD: 54 on hemodialysis (HD) and 54 renal-transplanted (RT) and 54 healthy subjects. The mean age for HD, RT and healthy subjects was respectively 46.1, 36.8 and 40.2. Half of the HD patients and healthy subjects (50%) were male and half were female, while 55.6% of the RT patients were male and 44.4% female. According to the allele frequency of both G and A, there was no significant difference between both groups of patients and the healthy subjects (P=0.42). The AG genotype was independently associated with increased risk of CKD undergoing HD and RT, while the GG genotype showed an increased risk for renal failure. The levels of serum IL-10 concentrations increased significantly in both groups of patients compared to the healthy subjects. Regarding the genotypes, the genotype AA had the highest concentration among the patients, while a high level was found in genotype GA in the healthy subjects. The lowest level of this cytokine was found in both genotypes GG and AA in the healthy subjects. The findings of the present study revealed that IL-10 gene polymorphism at 1082 A/G (rs1800896) and increased concentration of serum IL-10 were associated with increasing chronic kidney disease in the Iraqi Kurdish population.

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Impacto del polimorfismo 1082A/G (rs1800896) del gen de IL-10 sobre la secreción de IL-10 en pacientes con enfermedad renal crónica en la región Kurdistán de Irak.

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Palabras clave: enfermedad renal crónica; polimorfismo; interleucina-10.

Resumen. Varios estudios han indicado que el polimorfismo 1082A/G (rs1800896) del gen de IL-10 está asociado con la enfermedad renal crónica (ERC). El propósito de este estudio fue determinar el polimorfismo del gen de la IL-10 en pacientes con ERC e identificar los factores de riesgo y prevalencia de la enfermedad entre pacientes Kurdos. También se determinaron las concentraciones séricas de IL-10 en los diferentes genotipos AA, GA y GG presentes. El estudio incluyó 108 pacientes con ERC: 54 en hemodiálisis (HD) y 54 con trasplante renal (TR) y en 54 sujetos sanos. Las edades promedio para los HD, TR y sujetos sanos fueron 46,1, 36,8 y 40,2 años, respectivamente. La mitad de los pacientes en HD y de los sujetos sanos eran hombres y la otra mitad eran mujeres, mientras que el 55,6% de los pacientes con TR eran hombres y 44,4 % eran mujeres. Considerando la frecuencia de los alelos G y A, no hubo diferencias estadísticamente significativas entre ambos grupos de pacientes y los sujetos sanos (p= 0,42). El genotipo AG se encontró independientemente asociado con un riesgo aumentado de pacientes con ERC bajo HD y TR, mientras que el genotipo GG mostró un riesgo mayor de falla renal. Las concentraciones séricas de IL-10 se encontraron aumentadas significativamente en ambos grupos de pacientes, comparadas con las de los sujetos sanos. En consideración a los genotipos, el genotipo AA tenía las más altas concentraciones entre los pacientes, mientras que se encontró un mayor nivel en el genotipo GA en los sujetos sanos. Las concentraciones más bajas de esta citocina se encontraron en los genotipos GG y AA de los sujetos sanos. Los hallazgos del presente estudio revelaron que el polimorfismo 1082A/G (rs1800896) del gen de la IL-10 y las concentraciones séricas aumentadas de IL-10 estaban asociadas con aumentos de la enfermedad renal crónica en la población Kurda de Iraq.

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INTRODUCTION

Progressive kidney dysfunction is known as CKD. The HD, peritoneal dialysis and RT, which are renal replacement therapies, may extend survival in patients with end-stage-renal-disease (ESRD) and provide a good quality of life in most cases (1). In HD patients, the levels of several cytokines, such as IL-10, IL-2, IL-4, IL-5 and IFN-γ, increase (2). 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 cardiovascular disease (CVD) (3).

Furthermore, ESRD is associated with impaired cellular and humoral immunity

and persistent immune system activation (4,5). Large amounts of pro and anti-inflammatory cytokines produced by circulating monocytes and regulatory T cells (CD4+/CD25+) lead to limitation of inflammatory activation and subsequent pathogen elimination (6).

Because of endothelium function, atherosclerosis is accelerated directly by pro-inflammatory cytokines during early stages of CKD 3 and 4 (7-9). Constant inflammatory state, during which the patient suffers from its nutritional state, suppresses bone marrow stem cells leading to anemia, and shares essentially in erythropoietin therapy resistance, is correlated with poor outcomes of dialysis (10-13). Development of immune tolerance and pro-inflammatory cytokines reduced by the activity of IL-10 leads to a decrease in recipient T-cell activation and allogenic responses (14).

Individuals with adequate IL-10 production during ESRD have a better control over infection and uremia-associated state and experience less coronary artery disease (15-18). There are multiple polymorphisms in IL-10 gene promoter especially at positions _1082 (rs1800896), _819 (rs1800871), and _ 592 (rs1800872) which intensely affect the IL-10 levels (19-21).

The presence of essential biological impacts on transcriptional activity results in high-, intermediate-, and low-producing phenotypes of IL-10 gene promoter polymorphism. IL-10 high producers show better immune response and infection control and lower risk of cardiovascular death (22).

Cytokine production and expression are genetically determined; therefore, allelic variants of cytokine genes arising from nucleotide polymorphisms within regulatory region have been described in the last two decades (23).

MATERIALS AND METHODS

Patients and control group

The required samples were collected from January to November 2017. In so doing, 54 patients on maintenance hemodialysis at the Hawler Dialysis Centre and 54 patients on renal replacement therapy from private clinics at Hawler city were chosen for this study. Fifty-four healthy subjects were also selected as a control group, who were homogeneous with the patient groups regarding their age and gender. Clinical and laboratory data were obtained from the subjects, and a questionnaire was filled up for each patient and healthy individual in this study.

Blood samples

Peripheral blood samples (5 mL) were obtained from the groups and placed into two different tubes. About 2 mL of the blood samples were placed in heparinized tubes for DNA extraction from the whole blood or mononuclear cells, and the tubes were stored at -70°C until genomic extraction was obtained. The remaining 3 mL were placed in a gel tube to obtain the serum. The tubes were centrifuged at 10000 rpm for 10 minutes, and the serum was put into 1.5 ml Eppendorf tube and stored at -70°C until cytokine level estimation was carried out.

Determination of serum IL-10

Levels of serum IL-10 pg/mL were determined by enzyme linked immune sorbent assay (ELISA), and the Cloud Clone Corp. kit was used according to the manufacturer's protocol.

Cytokine genotyping

Using the manufacturer's instruction for QIAmp DNA mini kit (Qiagen, Hilden, Germany), peripheral blood mononuclear cells were used to obtain genomic DNA for both patients and control groups. For the IL-10 genotype at (-1082 G/A) (rs1800896), the amplification refractory mutational system method (ARMS-PCR) was utilized. The assays were performed in a 20 μL reaction volume containing 40 ng genomic DNA, 1.5 mM dNTPs, 25 mM $MgCl_2$, 1 μL of 10 pmol each primer and 0.4 units of Taq polymerase

(Fermentas, Maryland, USA) in 1X Reaction Buffer. The primer sequences were as follows: IL-10 generic primer, 5′-CAGTGCCAACTGAGAATTTGG-3′, IL-10 (G) Allele Primer 5′-CTACTAAGGCTTCTTTGGGAG- 3, and IL-10 (A) Allele Primer 5′-ACTACTAAGGCTTCTTTGGGAA-3. The PCR reaction was carried out in a thermal cycler (PX2) with the following cycling conditions: 95°C for 3 minutes, followed by 35 cycles at 95°C for 45 seconds, 58°C for 40 seconds, 72°C for 1 minute, and finally a 7-minute extension at 72°C. The amplicon size was 254bp. The amplified products were analyzed on 2% agarose gel.

Statistical Analysis

All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Normally distributed variables were expressed as mean ± SD as appropriate. The level of statistical significance was set at $P<0.05$. Intergroup comparisons were assessed for categorical variables and serum cytokine concentration using ANOVA. For IL-10 gene polymorphism 1082A/G (rs1800896), allele was counted by direct allele counting. The Hardy–Weinberg equilibrium was assessed with the chi square (X^2)-test. Descriptive data were presented as mean ± standard deviation (SD). Genotype and allele frequencies were compared between the groups using a (X^2)-test of independence with 2 x 2 contingency tables and z statistics. Statistical significance of the variables was established at the level $P<0.05$.

RESULTS

In the present study, CKD affected both young and old subjects. Both genders were equally influenced by this disease, too. The mean age in the patient groups with HD (n=54) and RT (n=54) were 46.1 and 36.8 years, respectively. With regard to the subjects' gender, 50% of the HD patients were males and 50% female, while 55.6% of the RT patients were males and the rest 44.4% were females. In this regard, there was no significant differences between the patient groups and the control group ($p>0.05$). The results also 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 a family history of these conditions, respectively (Table I).

TABLE I
THE DEMOGRAPHIC DISTRIBUTION OF THE STUDIED GROUPS

Groups / Characters	Hemodialysis N=54	Renal Transplanted N=54	Control 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	15/54(27.8%)	11/54(20.4%)	-------
Diabetes	14/54(26%)	7/54(13%)	-------
Hypertension	25/54(50%)	9/54(16.7%)	-------
Family History	11/54(20.4%)	13/54(24.1%)	-------

Detection of IL-10 gene polymorphism 1082A/G (rs1800896)

Genotypic frequencies of alleles showed different band patterns of amplified fragments for all of the HD, RT and healthy subjects (Table II). For IL-10 gene polymorphism1080, the results were as follows: GG: 30 (55.6%), 20* (37%) and 15* (27.8%) respectively for healthy subjects, HD and RT patients, while the distributions of GA were 20 (37%), 31* (57.4%) and 30 (55.6%) for healthy subjects, HD and RT patients, respectively. However, the distribution for AA was as follows: healthy subjects 4 (7.4%), hemodialysis 3 (5.6%), and renal transplanted patients 9 (16.6%). The results of the present study showed a significant difference between healthy subjects and both patient groups in terms of GG distribution ($P<0.05$). Also, healthy subjects and HD patients were significantly different regarding GA distribution ($P<0.05$). The Chi-square (X^2) result for the HD patients was 4.067 with a p-value of 0.047, which was consistent with HWE. However, the X^2 results for the RT patients and healthy subjects were 0.843 and 0.06, respectively ($p>0.05$), which was not consistent with HWE. Furthermore, the statistical analysis of genotypes and allele numbers was confirmed by direct allele counting and Chi-square test.

The results of the current study showed that the levels of serum IL-10 concentrations significantly increased in both groups of patients compared to the healthy subjects ($P<0.0001$). Regarding different genotypes and the concentration of serum IL-10, data analysis showed a significant increase in the patients compared to the healthy subjects ($p<0.0001$) for genotype GG, $p<0.001$ for genotype GA, and $p<0.01$ for genotype AA). Genotype GG had the highest concentration among the patients, while the highest level was found in genotype GA in the healthy subjects. The lowest level of this cytokine was found in genotype AA in the healthy subjects (Table III and Figs. 1 and 2).

TABLE II
IL-10 GENOTYPE AND ALLELE FREQUENCY OF STUDIED GROUPS

IL-10 1082 G/A (rs1800896)	Hemodialysis	Renal Transplanted	Healthy Subjects	p
No. of genotypes	N=54	N=54	N=54	
GG	20* (37%)	15* (27.8%)	30 (55.6%)	p<0.05
GA	31* (57.4%)	30 (55.6%)	20 (37%)	p<0.05
AA	3 (5.6%)	9 (16.6%)	4 (7.4%)	p>0.05
Allele frequency				
G	0.66	0.56	0.74	
A	0.34	0.44	0.26	
X^2	4.067	0.843	0.060	

* Significant difference.

TABLE III
CONCENTRATION OF SERUM IL-10 pg/mL (Mean±SD) IN STUDIED GROUPS REGARDING THEIR GENOTYPES

Cytokine		Hemodialysis (n=54)	Renal Transplanted (n=54)	Healthy Subjects (n=54)	p
IL-10 (pg/mL)		26.29[a] ± 2.422	24.41[b] ± 1.559	15.00 ± 0.918	p<0.0001
IL-10 (pg/mL)	GG	29.79[a] ± 1.45	25.85 ± 2.32	14.63 ± 1.392	p<0.0001
	GA	26.28[a] ± 3.173	23.72 ± 2.037	15.00 ± 0.918	p<0.001
	AA	25.43[a] ± 1.929	22.10 ± 3.306	14.38 ± 0.963	p<0.01

[a]: represents the significant difference between Hemodialysis and control group.
[b]: represents the significant difference between Renal transplanted and control group.

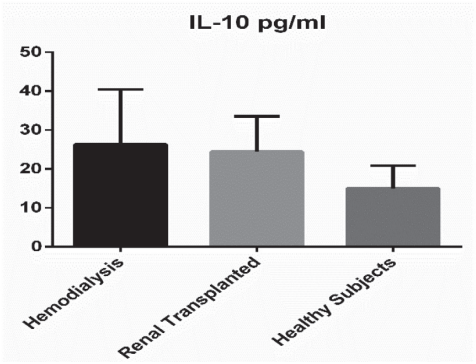

Fig. 1. Concentration of IL-10 pg/mL in the patients and healthy subjects.

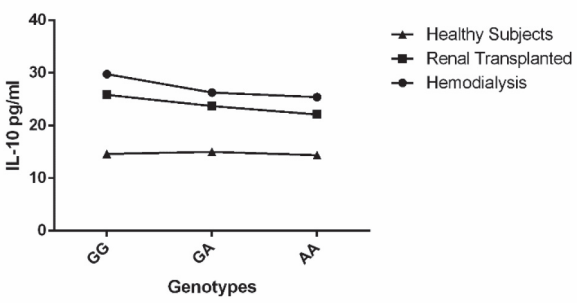

Fig. 2. Level of IL-10 according to the genotypes of studied groups.

As indicated in Table III above, intergroup comparisons indicated that the patient groups had a significantly higher level of the studied cytokine (i.e. IL-10) than the control group. However, intragroup comparison between the levels of different genotypes in each group revealed that the levels of the studied genotypes were not significantly different in any of the studied groups. This finding is clearly understandable by comparing the data presented in Table II above and Fig. 2 below.

DISCUSION

The results of the present study are in agreement with those of the study carried out by Zhang *et al* (24) who reported that IL-6 and IL-10 levels increased in HD patients. Another study showed a high level of IL-10 in CKD (HD) in comparison with control individuals, and a higher IL-10 expression in long-term HD patients compared with short-term HD ones (25).

Research has revealed that the serum level of IL-10 increases as a result of uraemic monocytes and that kidney clearance drops in ESRD (26). IL-10 genotype is considered as a risk factor for both ESRD and CVD (6, 27,28). High level of IL-10 was found in G allele at position -1082 (rs1800896) promoter region (29).

Although regular HD causes decreased levels of mortality in ESRD patients, it is considered a condition associated with inflammation (30). In CKD the variation in concentrations of both pro-inflammatory and anti-inflammatory cytokines were several times higher than healthy subjects, this

is due to both decreased renal clearance and increased production of cytokines (31,32).

The cytokine secretion varied between different individuals in this study due to different levels of their response to the stimuli. This alteration is determined by different promoter regions of polymorphism, which may lead to a person's susceptibility to a variety of chronic inflammation induced by cytokines.

Similar to the present study, there was a strong association between nucleotide polymorphism of IL-10 and functions of kidneys during CKD. Despite the patients' age, gender, and body mass index, the association remained stable, and polymorphism is reported as a risk factor for CKD outcomes (33). It is suggested that IL-10 production is determined genetically and can be controlled at the transcriptional level (34).

Research has indicated that the –1082 G allele is associated with higher IL-10 production, while the A allele with lower IL-10 production. These results confirm the findings of the present study (35). There is a higher IL-10 production in G/G genotype compared to other genotypes, which has been confirmed by previous studies. The susceptibility and/or severity of the disease by alteration in levels of both pro- and anti-inflammatory cytokines is genetically determined by lower IL-10 production (34).

In genotype combinations, there might be a strong regulatory increase in IL-10 production on the status of patients during CKD. Indeed, the results of the current study demonstrated a gradation of risk in the relationship between outcomes and genotype combinations that would be expected to predispose to a pro-inflammatory state with a consistent linear trend demonstrable on univariate analysis (36).

The results of the present study indicated that IL-10 serum level was genetically determined. They also showed differences between various genotypes in IL-10 gene polymorphism at the 1082 region (rs1800896) in CKD patients including HD and RT. The prevalence of the CKD was in the age group near 40 years. According to the results of this study, both genders were equally susceptible to CKD. It was also concluded that hypertension and diabetes were complications or risk factors for the development of the disease.

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