

Immunological role of TLR-7 , IL-17A and IL-10 In a Sample of Iraqi Chronic Renal Failure Patients

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Abstract

Objectives: The present study designed to investigate the Immunological role of toll-like receptor-7,IL-17A and IL-10 in sera of 66 Chronic Renal Failure CRF patients at IBN-SINA center for Dialysis in Diyala governorate - Iraq.**Methods:** Sandwich Enzyme Linked Immuno-Sorbent Assay(ELISA) technique were used in this study to evaluate concentration of TLR-7 ,IL-17A and IL-10 between study groups.**Results:** The findings of the present study demonstrated that significantly increased level of TLR-7 in the CRF group (0.24±0.05 ng/ml) compared to controls (0.02 ±0.08 ng/ml), the findings revealed a significant association between CRF and IL-17A serum level in the studied groups,There was a significantly increased level of IL-17A in the CRF group (6.50±1.37 pg/ml) compared to controls (2.19±0.32 pg/ml) also decreased level of IL-10 in the CRF group (26.20 ±5.16 pg/ml) compared to controls (79.27±23.44 pg/ml).**Conclusion:** These findings concluded that TLR-7 , IL-17A and IL-10 might have a role in CRF pathogenesis.

Keywords: Chronic Renal Failure, TLR-7, IL-17A, IL-10, Pro-inflammatory cytokines.

Introduction

The kidneys are paired organs that lie on the posterior wall of the abdomen behind the peritoneum on either side of the vertebral column ^[1]. The kidneys are able to maintain Fluids, electrolytes, and regulate blood pressure ^[2]. Renal failure (RF) is the condition in which the kidney is unable to remove the final metabolites from the blood and regulate fluids, electrolytes, and acid balance in extracellular fluid ^[3].(CRF) occurs when the kidneys are destroyed or decreased functions for at least 3 months (Glomerular Filtration Rate (GFR) <60 ml / min / 1.73 m² ^[4]. The severity of CRF is directly proportional to diabetic disease and hypertension, and the most serious complication of chronic renal insufficiency is the risk of cardiovascular disease (CVD), as the blockage of the arteries, which has a negative effect on the kidney, leads to further deterioration of kidney function ^[5]. many pathogens in patients with CRF

include: Immune disorders such as glomerulonephritis , Metabolic Disorders such as diabetes, Renal vascular disorders such as atherosclerosis, Injuries Infections such as tuberculosis, Urinary Tract Obstruction such as Renal Calculi and Congenital and hereditary disorders such as polycystic disease and congenital nephropathy ^[6]. Toll-like receptors (TLRs) have recently emerged as a key component of the innate immune system that detect microbial infection and trigger antimicrobial host defense responses, TLRs activate multiple steps in the inflammatory reactions that help to eliminate the invading pathogens and coordinate systemic defenses In addition, TLRs control multiple dendritic cell functions and activate signals that are critically involved in the initiation of adaptive immune responses ^[7]. When triggering TLR, signals are generated transcription factors that stimulate expression of genes that encode cytokines, enzymes, antimicrobial, and phagocytes^[8]. Acute Renal Failure (ARF) caused by Injuries, drug interactions or toxicity is identified and generated by biological memory. This process is not simple and may take several weeks. Pro-inflammatory cytokines are generated with an increase in the circulatory flow , (TLR-7 and TLR-9) play a role in increasing the severity

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of the injury and if this continues to progressively lead to the chronic stage of this disease [9]. (IL-17A) is a pro-inflammatory cytokine produced by natural killer cells (NK cell), neutrophils, macrophages, dendritic cells (DCs), mast cells plasma cells and Thelper17 (Th17) [10]. IL-17A has is pleiotropic cytokine which play a role in development of atherosclerosis, hypertension, diabetic nephropathy, ischemia-reperfusion injury, fibrosis, glomerulonephritis, nephrotic syndrome and acute renal rejection [11].(IL-10) is an important Homodimer anti-inflammatory agent known as the human Cytokine Synthesis Inhibitory Factor (CSIF), the length of each subunit 178 amino acid [12].(IL-10) is a pleopetric multifunctional cytokine that affects the immune response and inflammation, It also reduces the expression of T Helper(Th1) and Major Histocompatibility Complex Antigens (MHC II) and enhances the survival and proliferation of B cells and production of antibodies , also inhibits cell differentiation and(DCs) maturation [13]. Renal failure may increase inflammatory responses through a number of mechanisms, including immune response, which include increased concentration of pro-inflammatory cytokines and reduced levels of anti-inflammatory cytokines, as well as antioxidants and increased acute phase proteins [14]. Therefore, the aim of the present study is to assess the presence of innate immunity represented by TLR-7 serum level and cytokines (IL-17A and IL-10) in the pathogenesis of CRF patients.

Materials and Methods

Patients

Sixty-six Iraqi CRF patients (24 females and 46 males) were enrolled in this study with age mean ± standard deviation (SD)(48.1 ± 14.3years). They referred to Ibn-Sina center for dialysis, Baqubaa Teaching

Hospital. during the period beginning of October 2018 to end of March 2019 for treatment after diagnosis by the specialist physician in Ibn-Sina Center . In addition to patients, 22 apparently healthy individuals (11 females and 11 males) with no clinical symptoms or a family history for CRF were enrolled in the study as a control group with the age mean ± SD (37.1 ± 12.6 years). The study was permitted by the local ethics committee of the Health ministry, Baqubaa Teaching Hospital. In addition to the informed permission that was obtained from the study volunteers.

Methods

Five milliliters of venous blood was dropped from all the study volunteer groups. The withdrawn blood amount was separated in test tubes to collect serum for immunological examination, the blood left to clot at room temperature(25°C), then the tubes were centrifuged for 10 minutes, and the sera were separated in Eppendorf tubes. The sera of studied groups were tested to assess the level of TLR-7 (ABBEXA company, UK), IL-17A and IL-10 (PEPPROTECH Company/ UK), by using Sandwich ELISA technique.

Statistical Analysis

The data were analyzed by using the IBM SPSS computer program version 24 and expressed as mean ± SD, one-way ANOVA table, and Pearson’s two-tailed. The statistical significance level was set at (P < 0.05),(P≤ 0.01),(P≤ 0.001).

Finding

In the present study, Table 1 showed the level of TLR-7 in the sera of CRF patients and healthy control groups.

Table 1: Toll-like receptors7 level in the studied groups

TLR-7 Level ng/ml (mean±SD)	
CRF Patients	Healthy Control
0.248±0.057***	0.08±0.021***

*** significant differences (P ≤ 0.001).

When comparing (TLR7) with sex for CRF patients, we found that the level in males was higher than in females with significant difference between the two groups (P≤ 0.01), Table 2 showed the levels of TLR-7 in the sera of patients .

Table 2: Toll-like receptors7 level in CRF patients

TLR-7 Level ng/ml in CRF patients (mean±SD)	
Males (42)	Females (24)
0.528±0.264**	0.219± 0.351**

** significant differences (P ≤ 0.01).

The results of the present study showed a significant increase in the level of the IL-17A in people with CRF comparing with control group ,Table 3 showed the level of IL-17A in the sera of CRF patients and healthy control groups.alsoresults showed a significant decreased in levels of IL-10 for patients with CRF compared to healthy control group where we found an increase in the concentration of this cytokine ,Table 3 showed the level of IL-10 in the sera of CRF patients and healthy control groups.

Table 3: Interleukin-17 A and 10 level and in the studied groups

IL-17 A Level pg/ml (mean±SD)	
CRF Patients	Healthy Control
6.50±1.37*	2.19±0.32*
IL-10 Level pg/ml (mean±SD)	
26.20 ±5.16***	79.27 ±23.44***

* significant differences (P ≤ 0.05). *** significant differences (P ≤ 0.001).

When comparing IL-17A with sex, the level in males was decreased compared with females with no statistically significant difference (P>0.05), as shown in Table 4.

Table 4: IL-17A level in CRF patients

IL-17A Level pg/ml in CRF patients (mean ± SD)	
Males (42)	Females (24)
5.75 ± 9.43NS	7.81± 13.79NS

NS No significant differences (P > 0.05).

When compared with the sex of the infected, IL-10 was found to be higher in males than in females and no significant difference between the two groups (P >0.05) As shown in Table 5 .

Table 5: IL-10 level in CRF patients

IL-10 Level pg/ml in CRF patients (mean ± SD)	
Males (42)	Females (24)
27.56 ± 49.87NS	23.91±23.03NS

NS No Significant differences (P>0.05)

The Relationship between TLR-7,IL-17A, IL-10 and Age in CRF patients showed in Table 6.

Table 6: showing levels of (TLR-7, IL-17A and IL-10) in age group of CRF patients

Age Group (year)	NO.	TLR-7ng/ml (mean±SD)	IL-17A pg/ml (mean±SD)	IL-10 pg/ml (mean±SD)
20-29	8	0.216±0.415	3.93±2.80	16.92±17.49
30-39	14	0.554±0.305	11.48±17.47	19.52±15.13
40-49	12	0.399±0.307	4.88±7.67	42.62±92.56
50-59	15	0.502±0.184	6.25±10.92	26.89±33.30
Over 60	17	0.477±0.232	4.98±8.93	29.30±50.37
Total	66			
P Value		0.185NS	0.316NS	0.715NS

NS No Significant differences (P>0.05)

Discussion

The results of TLR-7 level in the studied groups showed that there was a significantly increased level ($P \leq 0.001$) in CRF group compared to healthy control group (0.248 ± 0.057 ng/ml vs. 0.08 ± 0.021 ng/ml, respectively). This finding matched the findings of other studies that referred to high levels of TLR7 in sera of patients with CRF compared to healthy control is clearly indicative of presence infections due to the vulnerability of the immune system as they have increased chances of Bacterial infection are identified by cells of the immune system that are derived by the secretion of TLR-7,9,4,2 receptors that stimulate the phagocytosis and secretion of inflammatory cytokines [15]. Elevated levels of TLR-7 in sera may indicate the immune system’s response to inflammation and kidneys damage in patients with CRF who are constantly dialysis.

The level of TLR-7 may be associated with age, This finding matched the findings of other studies [16]. showing a high level of this receptor in the younger and middle age groups and generally decrease in aging. This high level may be associated with other diseases such as rheumatoid arithritis (RA), hepatitis C [17], immunodeficiencies, atherosclerosis and asthma [18]. The reason for decreasing level of this receptor may be attributed to the weakness of immunological mechanisms as a result of aging or may be affected by patterns and methods of life and smoking in addition to the duration of the incidence of kidney failure, where it is noted in

young groups that the incidence of kidney failure and the number of dialysis that was conducted less than age groups over 50 years.

The results of the present study showed a significant increase in the level of the IL-17A in people with CRF comparing with control group , The results were agreed with other study [19] as one of the pro-inflammatory cytokines, the reason for its rising is due to the higher frequency of the allele in the group of patients than the control group ,that the genetic differences of IL-17A may thus have an effect in The development of renal failure and its increased secretion by T cells due to inflammation[20,21].

These findings were agreed with previous studies indicated increased IL-17A concentration with age, showing that IL-17A was involved in the inflammatory process as a causative agent for aging diseases, and recent studies have enhanced the association of Th17 with age, These cells may contribute to significant changes in immune function [22].

The results of our study were agreed with [23] showed a significant decreased in levels of IL-10 for patients with CRF who are constantly undergoing dialysis compared to healthy control group. The absence of significant difference in level of IL-17A and IL-10 in CRF patients for both sexes may be due to immune defense mechanisms may be somewhat similar regardless of sex as the interaction within the body activates the

immune cells responsible for the immune response in the sera of patients, and the sex is not affected by the level of cytokines in the body [24,25].

Conclusion

TLR-7, IL-17A and IL-10 might have a role in CRF pathogenesis.

Conflict of Interest: Non

Source of Findings: Non

Ethical Clearance: Non

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