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Immunological Role of IL-3, IL-22 and Some Physiological Markers in Iraqi Patients with Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) is a global health issue that is linked to early death and low quality of life. Management in its early phases may lead to better health results. Chronic inflammation related to advanced CKD, as indicated by higher levels of different pro-inflammatory cytokines or impacted levels of acute-phase proteins. This study was carried out in order to assess the roles of interleukins (IL-3 and IL-22), some kidney functions, complete blood count (CBC) and erythrocyte sedimentation rate (ESR) in CKD progression. Commercial enzyme linked immunosorbent assay (ELISA) kits were utilized to calculate interleukin (IL-3 and IL-22) levels in the serum of 60 patients with CKD (age range 20-87 years) and 30 age-matched healthy group. The levels of ESR, CBC, creatinine, urea, uric acid and albumin were also measured. The results showed a significant rise in IL-3 and IL-22 in CKD patients in comparison to healthy controls. CKD Patients were exposed to high levels of some CBC parameters and ESR and there was a significant difference in contrast to group of healthy control. There was a significant rise in creatinine and urea levels in CKD patients compared to healthy controls. The level of albumin was reduced in patients diagnosed with CKD and there was a significant difference between CKD patients and healthy controls. However, the level of uric acid increased in patients diagnosed with CKD but there was no significant difference between patients diagnosed with CKD and healthy group. There is a possible role of interleukins IL-3 and IL-22 in the usage of them as biomarkers for the progression of CKD.
Keywords: Interleukin-3, Interleukin -22, Creatinine, Urea, Albumin and CKD.

1. Introduction

Chronic kidney disease (CKD) is defined by kidney structure or function abnormalities that have been established for 3 months and have a negative impact on health. Albuminuria, urine sediment abnormalities or other defects owing to tubular problems, histological abnormalities, and imaging structural abnormalities are all indicators of kidney impairment [1]. Interleukins (ILs) are cytokine-related secretory immunomodulatory proteins that perform extensive immunological roles. The primary function of ILs in the immune system is to facilitate intercellular communication, which includes proliferation, cell migration, adhesion, and maturation. Interleukins play a role in both acute and chronic inflammation [2].

However, their complicated and vast range of functions and influences on other cells could result in depression and the progression of disease. This applies to diabetes, autoimmune diseases, neurological conditions, and kidney disorders [3]. Chronic inflammation is related to advanced CKD, which can be observed by higher levels of pro-inflammatory cytokines (IL-6, etc.) or altered levels of acute-phase proteins (albumin, etc.) [4].Interleukin-3 (IL-3), also known as multi-CSF, is a pleiotropic cytokine that promotes the survival, proliferation, and differentiation of multipotent hematopoietic stem cells. Interleukins-3 (IL-3) is mainly generated by activated T lymphocytes, although it is also secreted by NK cells, myeloid lineage cells, astrocytes, keratinocytes, and thymic epithelial cells [5]. Human IL-3R is made up of a component (IL3Rα) that binds Interleukins -3 specifically and a common signal transducing β subunit (βc) that is shared with the GM-CSF receptors and IL-5 [6]. Interleukin-22 (IL-22) is a cytokine that belongs to the Interleukin-10 (IL-10) family and is primarily generated by innate and adaptive T cells [7]. It has been demonstrated that interleukin-22 (IL-22) is constitutively expressed in a wide range of tissues, including the gut, pancreas, liver, lung, and kidney [8]. Interleukins-22 (IL-22) interacts with a class II cytokine receptor (IL-22R) consisting of IL22RA1 and IL-10RB2 subunits, activating signal transducer and transcription factor 3 (STAT3)-dependent downstream signaling pathways [9]. The erythrocyte sedimentation rate (ESR) is a standard hematological test that detects and tracks a rise in inflammatory activity in the human body as a result of one or more medical conditions, like infections or tumors [10]. Creatinine is created as a consequence of creatine and creatine phosphate [11]. It is filtered through the glomerulus, and small amounts of it are expelled through the proximal tubules during glomerular filtration [12]. Creatinine determination in biological fluids is becoming an increasingly significant clinical marker in evaluating renal impairment, thyroid dysfunction, and muscle injury [13]. Urea, or BUN, is a nitrogen-containing compound [14]. It is the main excretory outcome of protein metabolism [15]. Urea abundance has traditionally been used as an alternative indication of the function of the kidney, protein intake, and dialysis [16].Albumin is one of the tiniest plasma proteins known [17]. Every day, the liver produces 10 to 15 g of albumin, which is then discharged into the vascular space [18]. Albumin is an important prognostic and therapeutic marker in critical care [19]. Uric acid is the outcome of purine metabolism; it is generated from xanthine by the xanthine oxidase enzyme in many steps and eliminated in the urine [20]. Endogenous UA synthesis occurs mostly in the liver, muscle, intestines and vascular endothelium [21]. An excess of UA in the human body causes chronic hyperuricemia, this buildup has the potential to worsen hypertension, chronic renal disease, cardiovascular, and metabolic syndrome [22]. The most popular and simple laboratory test, the CBC, gives a plethora of details on a human being's health state. This test helps for the early detection of numerous health disorders that should be studied further through laboratory and clinical examination. White blood cells, red blood cells and platelets are the three types of CBC parameters [23]. The goal of this study was to establish whether IL-3 and IL-22 could be used as a potential biomarker for the progression of CKD.

2. Materials and Methods

2.1 Study design

This research comprised sixty participants with chronic kidney disease and was conducted at the Kidney Diseases and Transplant Center in Medical City, Baghdad, Iraq. The study extended between September 2022 and February 2023. The ages of CKD patients range from 20 to 87 years, and the ages of 30 healthy patients who participated in this study ranged from 20 to 70 years.

2.2 Collection of blood samples

Venous specimens of blood have been gathered from CKD diagnosed patients in the sitting posture using a set of five-milliliter syringes that were disposable. A total of 3 milliliters of blood are gradually squeezed into disposable tubes of serum that contain separating gel, and the additional two milliliters are placed in EDTA tubes (ethylene diamine tetraacetic acid). After allowing the blood that is found in the gel tubes to coagulate for 15 minutes at room temperature, the serum is stored at (-20°) C until later use. The serum was then used for measuring (IL-3 and IL-22) and also to measure some kidney function. The blood in the EDTA tubes was stored at -20°C until needed. The blood that was collected in the EDTA tubes was utilized for measuring ESR and CBC tests.

2.3 Quantitative measurements of parameters study

Interleukins-3 (IL-3) and Interleukins-22 (IL-22) were quantified in human serum samples using an (ELISA) kit (SunLong Biotech, China), according to the manufacturer's guidelines. All samples of serum and reagents were thawed and brought to room temperature before use. The wells of a 600-ml wash buffer were then filled with 50 µl of standards (S1, S2, S3, S4, S5, and S6), with a single well left empty to serve as a blank control. Following the addition of 40 µl of sample dilution buffer and 10 µl of serum samples, to wash the wells, the solution in each well was eliminated, and the washing solution was poured into each well five times. After adding fifty µl of Horseradish peroxidase reagent (HRPconjugate) to all wells, stirring them, and sealing them, the plate was incubated at 37 degrees Celsius for thirty minutes before being discarded and rinsed five times as previously. Then, in the dark, 50 µl of each chromogen solution was gently mixed into all wells. The reaction ended by adding fifty µl of stop solution to all wells, which turned the wells yellow. As a result, the reaction was complete.

2.4 Statistical analysis

For the statistical analysis, (SPSS) the statistical program for the social sciences (version 23), was utilized. The result was presented as the mean ± SE. For the statistical differences across groups, a T test was used, and ($p \leq 0.05$) and ($p \leq 0.01$) were considered significant values.

3. Results

Based on the statistical analysis, IL-3 was significantly ($P \leq 0.01$) higher in CKD-suffering patients (457.28 ±10.45) than in the healthy control group (154.66 ±9.24) as shown in **Table 1.** The results of Table 1 indicated that IL-22 level was significantly ($p \leq 0.01$) higher in CKD suffering patients (341.89 ±18.70) than in the control group (98.80 ±2.90).

Table 1. Comparison between CKD patients and control group in IL-3 and IL-22.

Groups	IL-3 (Pq/ml)	IL-22 (Pq/ml)
Patients	457.28 ±10.45	341.89 ±18.70
Control	154.66 ±9.24	98.80 ±2.90
T-test	30.878 **	48.394 **
P-value	0.0001	0.0001

Data are presented as mean ± standard error (SE). **P<0.01.

Based on the statistical analysis, the WBC level was significantly ($p \leq 0.01$) higher in CKD-diagnosed patients (9.19 ±0.50) than in the healthy control group (6.20 ±0.38), as illustrated in Table 2. Lymphocyte level was significantly ($p \leq 0.01$) higher in CKD-suffering patients (2.42

±0.16) than in the healthy control group (26.84 ±2.72), as shown in Table 2. ESR level was significantly ($p \leq 0.05$) higher in CKD patients (54.26 ±6.26) than in the healthy control group (8.05 ±1.10), as indicated in Table 2. Monocyte levels were significantly ($p \leq 0.01$) higher in CKD-suffering patients (0.645 ±0.06) than in the healthy control group (6.84 ±0.31), as shown in Table 2. The RBC level was significantly ($p \leq 0.01$) higher in CKD-suffering patients (4.11 ±0.19) than in the healthy control group (5.37 ±0.15), as indicated in Table 3. The results of Table 2 indicated that the IL-22 level was significantly ($p \leq 0.01$) higher in CKD-suffering patients (4.11 ±0.19) than in the control group (5.37 ±0.15). HGB level was significantly ($p \leq 0.01$) higher in CKD-suffering patients (10.63 ±0.44) than in the healthy control group (13.81 ±0.34), as illustrated in **Table 2.**

Table 2. Comparison between CKD patients and control group in CBC and ESR.

Groups	WBC	Lymphocyte	ESR	Monocyte	HGB	RBC
Patients	9.19 ±0.50	2.42 ±0.16	54.26 ±6.26	0.645 ±0.06	10.63 ±0.44	4.11 ±0.19
Control	6.20 ±0.38	26.84 ±2.72	8.05 ±1.10	6.84 ±0.31	13.81 ±0.34	5.37±0.1
T-test	1.391 **	4.562 **	15.194 **	0.544 **	1.250 **	0.552 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Data are presented as mean ± standard error (SE). **P≤0.01.

Based on the statistical analysis, creatinine was significantly ($p \leq 0.05$) higher in CKD patients (3.72 ±0.30) than in the healthy control group (0.706 ±0.06), as indicated in **Table 3.** Urea was significantly ($p \leq 0.05$) higher in CKD patients (110.55 ±8.28) than in the healthy control group (30.70 ±1.47), as shown in Table 3. However, the results of Table 3 indicated that the uric acid levels in the CKD patients and healthy control group did not differ significantly (5.87 ±0.52 and 4.67 ±0.27), respectively. Albumin level was significantly ($p \leq 0.01$) lower in CKD patients (3.61 ±0.11) than in the control group (4.57 ±0.06), as illustrated in **Table 3.**

Table 3. Comparison between CKD patients and control group in Kidney functions.

Groups	**Creatinine**	**Urea**	**Uric acid**	**Albumin**
Patients	3.72 ±0.30	110.55 ±8.28	5.87 ±0.52	3.61 ±0.11
Control	0.706 ±0.06	30.70 ±1.47	4.67 ±0.27	4.57 ±0.06
T-test	1.103 **	29.554 **	1.358 NS	0.337 **
P-value	0.0001	0.0001	0.0804	0.0001

Data are presented as mean ± standard error (SE). **P≤0.01; NS: Non-Significant.

4. Discussion

Chronic kidney disease (CKD) is a chronic disorder that causes significant morbidity and increases healthcare expenses. The result of this study showed a rise in the level of IL-3 in CKD-diagnosed patients compared to the control group that does not suffer from CKD. According to the acknowledgment of the author, this was the first time to study the effect of IL-3 on CKD patients in this research. The level of IL-22 was also elevated in patients with CKD in comparison to the healthy controls in this study. The results of IL-22 are in line with a study that found the expression of IL-22 to differ throughout the progression of multiple acute and chronic kidney disease models [24]. In this study, the level of erythrocyte sedimentation rate was elevated in CKD-suffering patients compared to the group of healthy controls, and this result agrees with the finding of a study that found the basal erythrocyte sedimentation rate level was greater in uremic patients without indications of inflammation compared to controls [25]. Erythrocyte sedimentation rate

(ESR) elevation is caused by factors related to renal failure rather than dialysis [26].WBC levels were elevated in CKD patients in contrast to the group of healthy people, and this agrees with a study that stated white cell count was greater in the (pre-dialysis) chronic kidney disease-suffering patients in comparison to the healthy controls. Because of their associations with several other atherosclerotic risk factors, such as smoking, diabetes, or hypertension, WBC counts are predictive of future CKD risk [27, 28]. However, the levels of lymphocytes and monocytes were lower in CKD-suffering patients in contrast to healthy controls, and this result disagrees with a study that found people with clinically confirmed CKD have larger absolute counts of neutrophils and monocytes in their venous blood than those without CKD [29].Chronic kidney disease (CKD) is thought to be caused by immune system abnormalities, specifically systemic inflammation and immunological deficiency. Atherosclerosis, cardiovascular disease, and anemia are all caused by systemic inflammation. Immune deficits include poor phagocytic activity of granulocytes and monocytes/macrophages, decreased antibody generation by B lymphocytes, impaired T-cell-mediated immunity, and defective antigen presentation by monocytes/macrophages. These deficiencies can result in a poor response to vaccination and an elevated frequency of microbial diseases and tumors. Many studies have found a decrease in peripheral blood B cells in end-stage renal disease (ESRD) patients. The malfunctioning immune system has a significant clinical influence on ESRD patients' morbidity and mortality [30]. Also, there was a decrease in the levels of RBC and HGB in chronic kidney disease-suffering patients compared with the group of healthy controls, and these results correspond to studies that found that in the study population, there was a low RBC count, which worsened as renal function deteriorated, and the other study found a statistically significant link between anemia severity and renal failure stage, implying that severe anemia is more likely to be related to kidney failure at Stage V [31].Chronic renal anemia is caused by a variety of factors, the most common of which is reduced renal synthesis of erythropoietin (EPO), the hormone responsible for enhancing red blood cell production. Erythropoietin deficiency has recently been related to a decrease in hypoxia-inducible factor (HIF), a transcription factor that regulates erythropoietin gene expression. Other factors include uremia (which causes RBC deformity and hemolysis), folate and vitamin B12 insufficiency, iron deficiency, and bleeding from defective platelets [32]. There was a rise in creatinine levels in this study in CKD-diagnosed patients in contrast to healthy controls, and this result agrees with a study that found creatinine levels were greater in participants with CKD [33]. In the early stages of CKD, hormonal changes caused by the loss of renal parenchyma cause metabolic and nutritional derangements, including protein catabolism and a loss of lean body mass. Abnormalities in glucose metabolism, insulin resistance, dyslipidemia, and hypoalbuminemia are examples of such complicated changes, and these metabolic and nutritional derangements relate to a drop in creatinine in patients followed in a CKD clinic [34]. The level of urea also increased in CKD patients in comparison to the healthy group, and this result corresponds to a study that demonstrated higher BUN levels were associated with worse renal outcomes in individuals with moderate to severe CKD, regardless of eGFR [35]. This higher level indicated that there was a little obstruction in excreting urea in kidney disease patients, as well as an impairment of renal function, either due to a decrease in GFR or an obstruction that interfered with urinary excretion [36]. Uric acid levels didn’t differ significantly between CKD patients and the control group in this study, and this finding corresponds to a study that indicated there was no statistically significant link between uric acid levels and incident CKD, while several studies have linked increased levels of uric acid to the progression of renal disease or vice versa [37]. The level of albumin was decreased in CKD-diagnosed patients compared to

healthy people, and this agrees with a study that stated that in a sample of well-functioning, community-dwelling elders, lower blood albumin levels are substantially and independently linked with eGFR decline, fast eGFR decline, and incidence of CKD [38]. The effects of inflammation on the vascular endothelium and lipoprotein structure suggest that the influence of inflammation on albumin is principally responsible for the morbidity and mortality associated with hypoalbuminemia [39]. Hypoalbuminemia is common in chronic kidney disease-stricken patients and is caused by a complicated interaction of various variables, including malnutrition and inflammation [40].

5. Conclusion

This study indicated that levels of IL-3 and IL-22 in CKD suffering patients might be used as prognostic risk factors that could be utilized in the diagnosis of CKD disease, and this also study indicated statistically significant differences between CKD diagnosed patients and healthy controls in levels of creatinine and urea, but non-significant results were found in the level of uric acid in CKD-diagnosed patients.

Conflict of Interest

There is no conflict of interest.

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There is no funding for the article.

Ethical Clearance

This study was approved by the ethical committee of the Department of Biology, College of Science, University of Bagdad, Baghdad, Iraq, with authorization reference number CSEC/0922/0110 on September 29, 2022. This study was guided by the Declaration of Helsinki, the code of ethical principles for medical studies using human subjects.

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