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Evaluation of Serum Complement Proteins and Biochemical Parameters in Patients with Chronic Kidney Disease-associated Pruritus

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Abstract

Chronic kidney disease-associated pruritus (CKD-aP) is a common, troubling, and in some cases a debilitating problem for patients with chronic kidney disease (CKD), end-stage renal disease (ESRD), as well as those maintained on hemodialysis (HD) or peritoneal dialysis (PD). This study aims to assess the serum complement 3 (C3), complement 4 (C4), and biochemical parameters levels in HD patients with CKD-aP.

The research was performed between 4th of October 2021 – 5th of March 2022 on 60 HD patients, who suffer from CKD-aP, and 30 healthy controls in Baqubah Teaching Hospital-Ibn Sina Dialysis Center. The findings of our investigation revealed that levels of C3 and C4 were significantly decreased in HD patients with CKD-aP (57.83 ± 2.91 mg/dl and 14.59 ± 0.88 mg/dl, respectively) as compared to healthy controls (110.76 ± 2.20 mg/dl and 32.02 ± 1.09 mg/dl, respectively) (P = < 0.001).

The results of this study also indicate that urea, creatinine, and phosphorus levels were significantly higher in HD patients with CKD-aP (120.95 ± 2.56 mg/dl, 5.27 ± 0.18 mg/dl,

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4.98 ± 0.15 mg/dl, respectively) when compared with healthy controls (22.16 ± 0.77 mg/dl, 0.46 ± 0.03 mg/dl, 3.21 ± 0.10 mg/dl, respectively) (P = < 0.001). In contrast, albumin was significantly decreased in HD patients with CKD-aP (36.98 ± 1.68 g/L) as compared to healthy controls (40.96 ± 0.67 g/L) (P = 0.03). Furthermore, high serum creatinine had a statistically significant correlation with the severity of pruritus (P= 0.029).

Keywords: Chronic kidney disease-associated pruritus, Uremic pruritus, Complement proteins.

التقييم المصلي لبروتينات المتمم والمؤشرات الكيموحيوية لدى المرضى الذين يعانون من الحكة المرتبطة بأمراض الكلى المزمنة (CKD-aP)

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الخلاصة

الحكة المرتبطة بأمراض الكلى المزمنة (CKD-aP) هي مشكلة شائعة، مزعجة، وفي بعض الأحيان تكون منهكة للأشخاص الذين يعانون من مرض الكلى المزمن (CKD) والفشل الكلوي في مراحله النهائية (ESRD) وكذلك أولئك الذين يخضعون لغسيل الكلى الدموي (Hemodialysis) أو البريتوني (Peritoneal Dialysis). آلية نشوء وتطور الحكة اليوريمية غير مفهومة تماما وقد تم اقتراح العديد من الفرضيات فيما يتعلق بتطور هذا المرض. تهدف هذه الدراسة لتقييم مستوى المتمم 3 (C3)، والمتمم 4 (C4)، بالإضافة إلى بعض المؤشرات الكيموحيوية بين المرضى الخاضعين للغسيل الكلوي والمصابين بالحكة اليوريمية. أجريت هذه البحث في الفترة ما بين 4 أكتوبر 2021 و 5 مارس 2022 على 60 شخصا من المرضى الخاضعين للغسيل الكلوي الدموي والذين يعانون من الحكة المرتبطة بأمراض الكلى المزمنة (CKD-aP) وعلى 30 من الأشخاص الأصحاء (السيطرة) في مركز ابن سينا للغسيل الكلوي في مستشفى بعقوبة التعليمي. تشير نتائج هذه الدراسة إلى أن مستويات الـ C3 و C4 في المصل كانت منخفضة بشكل ملحوظ لدى المرضى الخاضعين للغسيل الكلوي الذين يعانون من الحكة اليوريمية (CKD-aP) (57.83 ± 2.91، 14.59 ± 0.88

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مليغرام/ديسيلتر، على التوالي) مقارنة بالأصحاء (Control) (110.76 ± 2.20، 32.02 ± 1.09 مليغرام/ديسيلتر، على التوالي) (P تساوي 0.001). ايضا، أظهرت نتائجنا ان مستويات الـ يوريا، كرياتينين، والفسفور ازدادت بشكل ملحوظ في دم مرضى الغسيل الكلوي الذين يعانون من الحكة اليوريمية (120.95 ± 2.56، 5.27 ± 0.18، 4.98 ± 0.15 مليغرام/ديسيلتر، على التوالي) مقارنة بالأصحاء (Control) (22.16 ± 0.77، 0.46 ± 0.03، 3.21 ± 0.10 مليغرام/ديسيلتر، على التوالي)(P تساوي 0.001). في المقابل، انخفض مستوى الالبومين بشكل ملحوظ في المرضى (36.98 ± 1.68 غرام/لتر) مقارنة بالأصحاء (40.96 ± 0.67 غرام/لتر) (P تساوي 0.03). علاوة على ذلك، كان لارتفاع الكرياتينين في الدم علاقة ذات دلالة إحصائية مع شدة الحكة (P تساوي 0.029).

الكلمات المفتاحية: الحكة المرتبطة بأمراض الكلى المزمنة: الحكة اليوريمية: بروتينات المتمم

Introduction

Itch (also known as pruritus) is an irritating sensation that induces a desire to scratch. Chronic kidney disease-associated pruritus (CKD-aP), also known as "uremic pruritus (UP)", is an unpleasant symptom related to severe chronic kidney disease (CKD) and end-stage renal diseases (ESRD) [1]. The prevalence of UP has differed significantly among research studies. It has been reported that it can range between 20% and 90% among CKD and ESRD patients and has a significant clinical impact associated with poor sleep, depression, reduced quality of life, and increased mortality among these patients [2,3, and 4]. CKD-aP can develop without any associated diagnosable skin disorders or primary skin lesions, although, over time, secondary skin alterations such as excoriations may occur as a result of intense scratching. Symptoms can be either localized, affecting large symmetrical areas of the body, or generalized, affecting the whole body [5,6, and 7].

The pathogenesis of CKD-aP is complex and not fully understood, and many hypotheses have been proposed regarding its development. Multiple biomarkers have been reported to be associated with CKD-aP. Conventionally, it was assumed that efficiency of dialysis and metabolism biomarkers such as phosphorus, calcium, and parathyroid hormone are associated with an increased risk of UP [1,8]. In individuals with CKD, decreased renal function leads to

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insufficient metabolite excretion. This results in the buildup of cytotoxic metabolites, which cause a variety of adverse effects. When the levels of serum phosphorus exceed the usual range, it mixes with serum calcium to produce calcium phosphate, which is accumulated in the skin as well as other organs. The accumulated calcium-phosphate compound stimulates local nerve endings, resulting in pruritus [9]. According to Hu *et al*. [9] and Gobo-Oliveira *et al.* [10] hemodialysis (HD) patients with CKD-aP have higher levels of serum creatinine and urea than those without CKD-aP, emphasizing the necessity of limiting these uremic toxins in CKD-aP patients. The role of immune system and pro-inflammatory cytokine dysfunction in the pathogenesis of CKD-aP has been discussed previously, but it is still unclear. According to Melchior *et al*. [11] complement activation occurs during the interactions between blood and synthetic HD membranes, resulting in systemic inflammation in CKD patients. In hemodialysis patients, activation of the complement system may also induce an allergic response, hence many patients experience pruritus [12].

All of the abovementioned studies suggest that the immunological and uremic toxin hypotheses may be the main contributors to CKD-aP. Furthermore, with an increasing number of supporters and opponents of these hypotheses, the high incidence of CKD-aP, and the psychological and physical difficulties that CKD-aP patients face, this study aimed to investigate CKD-aP and its hypotheses by measuring the levels of some complement proteins and biochemical parameters, as well as their correlations with the itch severity in HD patients with CKD and ESRD at the Ibn Sina Dialysis Center in Diyala, Iraq.

Material and Methods

1. Patients and healthy control

This study was performed between 4th October 2021 – 5th March 2022 on HD patients who suffer from CKD-aP and healthy controls in Baqubah Teaching Hospital-Ibn Sina Dialysis Center. It was conducted on 90 chronic HD patients with CKD-aP. Their ages ranged between

21 and 83 years. A secondary control group of 30 healthy adults was recruited from outpatient clinic visitors and colleagues. Their ages ranged between 25 and 55 years. The control groups were chosen based on two criteria: first, that they did not suffer from any chronic skin disorders (e.g. eczema, psoriasis, or others); and second, that they did not have any other chronic diseases (e.g. diabetes, malignancy, autoimmune disease, and others).

2. Exclusion criteria

Patient samples who tested positive for hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV), as well as patients with cancer, active autoimmune disorders, skin diseases (e.g. dermatitis and psoriasis), and patients under 20 years old, numbering around 30 patients, were excluded.

3. Hemodialysis characteristics

A questionnaire was used to collect data from each hemodialysis patient, which included age, gender, chronic disease, dialysis duration, number of dialysis sessions per week, number of hours per day, risk factor for CKD, risk factor for itching, distribution of itching, duration of itching, severity of itching, and type of medication used.

4. Pruritus severity assessment

The severity of itch or pruritus was evaluated and measured using a visual analogue scale (VAS). It is a scale of 1 to 10 (1-2 mild pruritus, 3-7 moderate pruritus, and 8-10 severe pruritus, while 0 no pruritus).

5. Blood collection and preparation

Approximately 4 mL of blood was drawn from CKD-aP patients during the first 10 minutes of hemodialysis, and the same volume of blood was obtained from healthy controls under completely aseptic conditions. The blood was then put into gel tubes and allowed to clot at

room temperature. The samples were centrifuged at 3000 rpm for 15 minutes, and the serums were separated into four Eppendorf tubes and stored at −20°C to be analyzed later for detection of C3 and C4. Biochemical parameter test results (urea, creatinine, phosphorus, and albumin) were obtained from the records of the Ibn-Sina laboratory. The informed consent was taken from all hemodialysis patients.

6. Determination of complement proteins (C3 and C4)

RID assay was performed on 60 hemodialysis patients with chronic kidney disease-associated pruritus (CKD-aP) and 30 healthy controls.

6.1. Assay Principle

The quantitation of serum complement components is usually performed by radial immunodiffusion (RID). RID assays are based on an antigen-antibody precipitation reaction. When a serum sample (antigen) is added to wells cut in an agarose gel containing a particular antibody, it forms an immune-complex that is visible as a ring around the well. The ring diameter is directly proportional to the complement protein concentration of the sample [13,14].

7. Statistical analysis

Statistical Package for Social Science (SPSS) version 26 software was used to analyze the data. The data were presented as mean ± standard error (M ± SE). Independent-samples T test was used to find a significant difference in the levels of study parameters between CKD-aP patients and healthy controls. Furthermore, the values of these parameters in correlation to itch severity were statistically analyzed using a one-way ANOVA test. Statistically significant differences were defined as those with a *P* value less than 0.05.

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Results

1. Demographic characteristics of the study groups

After the exclusion of 30 patients, a total of 90 individuals participated in this study, including 60 HD patients with CKD-aP and 30 healthy controls. The mean ages for HD patients and healthy controls were 48.03 ± 1.84 and 36.33 ± 2.32 years, respectively.

2. The level of complement proteins

The results revealed that the serum levels of complement 3 (C3) and complement 4 (C4) in hemodialysis (HD) patients with CKD-aP were (57.83 ± 2.91 mg/dl and 14.59 ± 0.88 mg/dl, respectively) as compared to healthy controls (110.76 ± 2.20 pg/ml and 32.02 ± 1.09 mg/dl, respectively). According to these findings, serum levels of C3 and C4 were significantly decreased in HD patients with CKD-aP as compared to healthy controls (P = < 0.001), as shown in **Figure 1**.

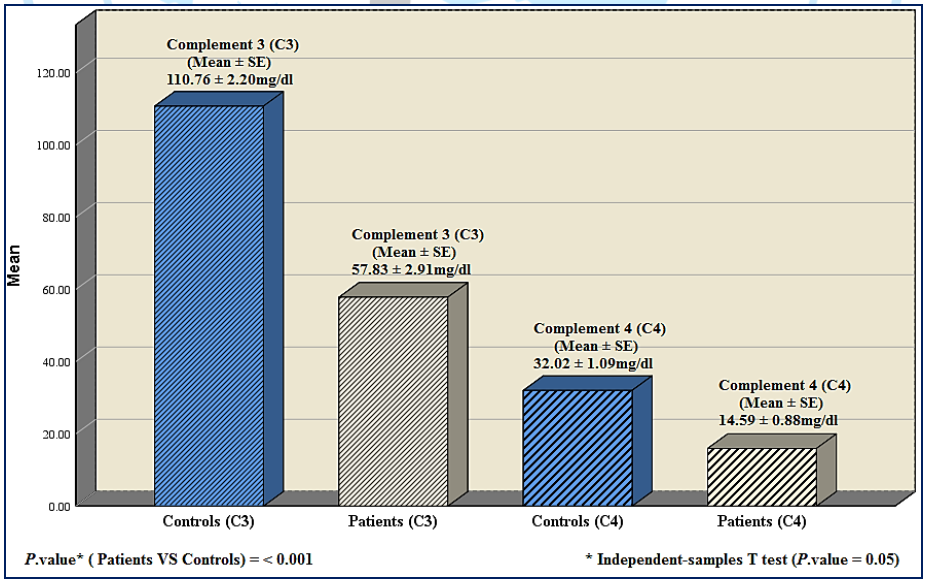

Figure 1: The serum levels of C3 and C4 in HD patients with CKD-aP and healthy controls

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3. The level of biochemical parameters

The results showed that the serum levels of urea, creatinine, phosphorus, and albumin in hemodialysis patients with CKD-aP were (120.95 ± 2.56, 5.27 ± 0.18 mg/dl, 4.98 ± 0.15 mg/dl, and 36.98 ± 1.68 g/L, respectively) when compared with healthy controls (22.16 ± 0.77 mg/dl, 0.46 ± 0.03 mg/dl, 3.21 ± 0.10 mg/dl, and 40.96 ± 0.67 g/L, respectively). From these results, it is clear that serum levels of urea, creatinine, and phosphorus were significantly higher in HD patients as compared to healthy controls (P = < 0.001), whereas albumin was significantly decreased in HD patients compared to healthy controls (P = 0.03) as shown in **Table 1.**

Table 1: The level of biochemical parameters in patients with CKD-aP and healthy controls

GROUPS	N (90)	UREA (MG/DL) (*MEAN ± SE*)*	CREATININE (MG/DL) (*MEAN ± SE*)*	PHOSPHORUS (MG/DL) (*MEAN ± SE*)*	ALBUMIN (G/L)(*MEAN ± SE*)*
Patients with CKD-aP	60	120.95 ± 2.56	5.27 ± 0.18	4.98 ± 0.15	36.98 ± 1.68
Controls	30	22.16 ± 0.77	0.46 ± 0.03	3.21 ± 0.10	40.96 ± 0.67
P-Value*		0.001	0.001	0.001	0.03
P value <0.05	* *Values are expressed as mean ± standard error (SE)*			* *Independent-samples T test*	

4. Correlation between itch severity and biochemical parameters

The findings of our investigation demonstrated that the levels of creatinine among severe, moderate, and mild itch patient groups differed significantly (P = 0. 029). The difference in urea, phosphorus, and albumin levels, on the other hand, were statistically insignificant among these patient groups (P = 0.546, 0.594, and 0.631, respectively) as shown in **Table 2**. According to these findings, serum levels of creatinine increase significantly as the severity of itch increases in patients with CKD-aP.

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Table 2: The level of biochemical parameters in severe, moderate, and mild itch patient groups.

PATIENTS GROUPS		N (60)	UREA (MG/DL) (*MEAN ± SE*)*	CREATININE (MG/DL) (*MEAN ± SE*)	PHOSPHORUS (MG/DL) (*MEAN ± SE*)	ALBUMIN (G/L) (*MEAN ± SE*)*
1. Severe Pruritus		24	124.25 ± 4.56	5.85 ± 0.31	4.83 ± 0.26	38.16 ± 2.99
2. Moderate Pruritus		23	117.82 ± 3.76	4.96 ± 0.26	5.18 ± 0.25	37.47 ± 2.72
3. Mild Pruritus		13	120.38 ± 5.18	4.75 ± 0. 32	4.90 ± 0.25	33.92 ± 2.71
P-Value*	Severe vs Moderate		0.276	0. 029	0. 325	0. 858
	Severe vs Mild		0.577	0. 022	0. 859	0. 353
	Moderate vs Mild		0.714	0.649	0.514	0.439
	Difference Between Groups		0.546	0.029	0.594	0.631
P value <0.05 * *Values are expressed as mean ± standard error*				* *One-way ANOVA*		

Discussion

Complement activation probably plays an essential role in the pathogenesis of chronic kidney disease-associated pruritus (CKD-aP). The results of our study showed that the serum C3 and C4 levels were significantly decreased in HD patients with CKD-aP as compared to healthy control **(Figure 1)**. These findings were consistent with a study conducted in Spain by Rodríguez-Sanz *et al*. [15], which found that serum C3 and C4 levels were decreased in polysulfone-allergic HD patients as compared to non-allergic HD patients (control). Furthermore, these results were partially in agreement with the findings of Al-dulaimy *et al*.[16] and Albayati, [17], who demonstrated that C3 and C4 were typically decreased in the general population of HD patients with CKD as compared to the control group.

On the other hand, our findings relatively disagreed with the findings of Elia and Mustafa, [18], who observed that there was a significant increase in C3 levels for patients with renal failure compared to the control group, while C4 levels for renal failure patients showed no significant change compared to the control group. C3 is an acute-phase protein and a protein crucial to all complement activation pathways [19]. Hence, the high serum C3 level indicates

a low-grade of systemic inflammation. In contrast, the low C3 level may indicate chronic inflammation and excessive complement activation [20]. Moreover, chronic renal disease and long-term dialysis may have a direct correlation with chronic inflammation. As a result, the discrepancies across studies may be explained by the stages of renal disease and the length of dialysis.

Our results of lower serum complement proteins (C3, C4) in hemodialysis patients who suffer from uremic pruritus suggest a high rate of complement activation and proteolytic degradation, resulting in the release of chemotactic molecules known as anaphylatoxins. Anaphylatoxins (C3a and C5a) can induce activation and degranulation of mast cells. The latter releases mediators like tryptase, causing systemic symptoms such as itching in HD patients. In a study conducted in Germany, Dugas-Breit *et al*.[21] found that the mast cell tryptase levels were elevated in CKD-aP patients, and the severity of pruritus in these patients was correlated significantly with tryptase levels. Moreover, C5a may induce the release of histamine from human skin mast cells and peripheral blood basophils via C5a receptors without antigens and IgE interaction. Notably, C5aRs are expressed on the surfaces of skin mast cells but not of other kinds of human mast cells [22]. This may explain the itch-related complement activation in hemodialysis patients.

The mechanism of complement activation during hemodialysis may involve binding of ficolin-2 to synthetic hemodialysis membranes, which leads to lectin pathway (LP) activation. On the other hand, the binding of C3b or properdin to synthetic HD membranes may induce alternative pathway (AP) activation. The end results of complement activation involve membrane attack complex (MAC), opsonins (C3b, iC3b), and anaphylatoxins (C3a, C5a) formation [12]. Activation of this system promotes recruitment of leukocytes, resulting in an oxidative burst and the release of pro-inflammatory cytokines (e.g. IL-6, IL-1, and TNF-α) and chemokines [23, 24]. Additionally, in HD patients with CKD-aP, overactivation of the

complement system may lead to a high rate of C3 and C4 consumption, which might explain our findings.

The results of this study also indicate that serum levels of creatinine and urea were significantly higher in HD patients with CKD-aP as compared to healthy controls (P = < 0.001) (**Table 1**). These findings were consistent with several recent studies, including Albayati, [17], Ghassan *et al*. [25], Jasim, [26], Ko *et al*. [27], and Alghazal *et al*. [28], who observed an increase in serum creatinine and urea levels in HD patients as compared to healthy control group. However, no significant differences were detected between HD patients with and without pruritus in any of these studies.

The kidneys are the primary organs in charge of eliminating blood urea and creatinine. In CKD, decreased blood flow to the glomerulus and reduced glomerular filtration rate (GFR) lead to a lower distal tubular flow rate and increase the reabsorption of urea and decrease its excretion, which may explain the increased serum urea concentration [29]. Moreover, the elevation of serum creatinine levels may be attributed to the decrease in creatinine clearance due to the decrease in the GFR [30].

The results of this study also indicate that serum levels of phosphorus were significantly greater in HD patients with CKD-aP as compared to healthy control group (P= < 0.001) (**Table 1**). These findings were consistent with a cross-sectional study conducted on 382 hemodialysis and peritoneal dialysis patients in China, which reported that serum phosphorus levels were significantly higher in HD patients with uremic pruritus (UP) as compared to those without UP [9]. Similar results were reported by Gatmiri *et al*. [31] and Schricker *et al*.[32], who found that the frequency of pruritus and its severity were significantly higher in patients with a higher serum phosphorus level. On the other hand, a cross-sectional study conducted on 175 hemodialysis patients in Taiwan found no significant differences in the serum phosphorus levels between HD patients with and without pruritus [27].

During CKD, the kidneys become unable to remove extra phosphorus from the body. When the levels of serum phosphorus exceed the usual range, it mixes with serum calcium to produce calcium phosphate, which is accumulated in the skin as well as other organs. The accumulated calcium-phosphate compound stimulates local nerve endings, resulting in pruritus [9].

Moreover, the findings of our investigation revealed that the serum levels of albumin were significantly decreased in HD patients with CKD-aP as compared to healthy controls (P= < 0.003) (**Table 1**). These results were in agreement with the findings of Schricker *et al*.[32], Chen *et al*.[33], and Kimmel *et al*.[34], who found that blood albumin levels were lower in the general population of HD patients with pruritus as compared to those who did not have pruritus. On the other hand, Ko *et al*. [27] and Alghazal *et al*. [28] did not find any differences in the serum albumin levels between HD patients with and without pruritus. The discrepancies among these studies may be explained by the stages of renal disease, duration of dialysis, type of synthetic dialysis membrane, chronic comorbidities, and the complex pathogenesis of CKD-aP.

Hypoalbuminemia is common in patients with CKD and ESRD. It is caused by a combination of the reduced synthesis and the increased degradation and renal clearance of albumin [35]. Furthermore, hemodialysis procedures, especially the dialyzer membranes, expose patients to foreign bodies, which trigger an inflammatory response that is a known cause of low albumin levels. Hemodialysis also induces protein adsorption on the synthetic membranes and tubes, resulting in a decrease in albumin levels [36,37]. In conclusion, the altered albumin homeostasis in ESRD patients is mainly caused by a systemic inflammation, which is a known cause of uremic pruritus.

Finally, according to the intensity of the itch, our data revealed that high serum creatinine had a statistically significant correlation with the severity of pruritus (**Table 2)**. These findings

were consistent with Hu *et al*.[9] and Gobo-Oliveira *et al*.[10], who reported that the serum creatinine levels were significantly increased in HD patients with severe pruritus. Similar findings were reported by Narita *et al*.[38] and Shafei and Nour, [39], who found that the group with severe pruritus had significantly higher levels of serum creatinine as compared to those with moderate and mild pruritus. In contrast, Ko *et al*., [27] and Alghazal *et al*., [28] did not find any significant differences in the serum creatinine levels among HD patients with and without pruritus.

A recent study conducted in north India by Pratyusha *et al*., [40] reported that high creatinine levels were significantly associated with skin xerosis in hemodialysis patients. Persistent xerosis compromises the skin barrier and induces uremic toxin accumulation in the skin. This, along with an increase in urea secretion in sweat, may elicit pruritus in CKD patients [1]. This may explain why patients with severe pruritus have high levels of serum creatinine.

Another explanation of the elevation of creatinine in these patient groups may be associated with factors outside of kidney function, which include consuming large amounts of protein, the muscle bulk of patients, and taking certain medicines (e.g. trimethoprim and cimetidine), as well as some health conditions such as diabetes, high blood pressure, and heart disease [41,42, and 43].

The results of this study also indicate that serum levels of urea among severe, moderate, and mild itch patient groups differed insignificantly (P = 0.546) (**Table 2)**. These findings were consistent with the results of Ko *et al*., [27] and Alghazal *et al*., [28], which did not find any significant differences in the serum urea levels among HD patients with severe, moderate, and mild itch. Similar findings were reported in a recent study conducted in Iraq by Jasim, [26]. In contrast, Hu *et al*., [9] and Gobo-Oliveira *et al.*, [10] reported that the serum urea levels were significantly increased in HD patients with severe pruritus.

In addition, our results showed that there were no significant differences in the serum phosphorus levels among patients with mild, moderate, and severe pruritus (**Table 2)**. These findings were in agreement with different studies, including Gobo-Oliveira *et al.* [10], Ko *et al*. [27], Alghazal *et al*. [28], Chen *et al*. [33], and Kimmel *et al*. [34]. In contrast, Hu *et al*. [9], Gatmiri *et al*. [31], and Schricker *et al*. [32] reported that the serum phosphorus levels increase as the severity of pruritus increases in HD patients.

Furthermore, our results showed that there were no significant differences in the serum albumin levels among patients with mild, moderate, and severe pruritus (**Table 2)**. These findings were consistent with those of Gobo-Oliveira *et al.* [10], Ko *et al*. [27], Alghazal *et al*. [28], Chen *et al*. [33], and Narita *et al*. [38], who found that there were no statistically significant differences in the serum albumin levels between mild, moderate, and severe itch patient groups. In contrast, Schricker *et al*. [32] and Kimmel *et al*. [34] found that blood albumin levels were lower in the general population of HD patients with severe pruritus.

One possible explanation for the discrepancy among these different studies is chronic diseases, duration of dialysis, type of dialysis membrane used during HD, and age of patients under study, as well as cigarette smoking, since all of these may enhance the status of inflammation. In addition, individual genetic polymorphisms and the complex pathogenesis of uremic pruritus could also cause the discrepancies.

To conclude, the serum C3, C4, and albumin levels decreased significantly in HD patients with CKD-aP. In contrast, urea, creatinine, and phosphorus were significantly increased. Furthermore, high serum creatinine had a statistically significant correlation with the severity of pruritus.

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