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Research Article

Evaluation of Interleukin-31 Serum Levels in Patients with Chronic Kidney Disease on Hemodialysis with and without Uremic Pruritus

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Abstract:

Background: Interleukin-31 has been linked with developing and maintaining pruritus in various dermatological and non-dermatological diseases.

Objectives: To evaluate interleukin-31 serum levels in hemodialysis patients with and without uremic pruritus as a potential contributor.

Methods: This cross-sectional study involved ninety adult chronic kidney disease patients on hemodialysis. All of the enrolled patients were on a three-times/week hemodialysis regimen. Patients were divided into two groups of 45 patients each. Group 1 involved those with pruritus and Group 2 involved those free of pruritus; based on the itching severity scale (ISS). Serum levels of interleukin-31, intact parathormone, urea, creatinine, and calcium were assessed before the hemodialysis session. Serum interleukin-31 levels were also assessed after the hemodialysis session. The statistical analysis was performed using the Statistical Package for Social Science (SPSS) .The median and interquartile range (IQR) were used to present data on the continuous variables. Mann-Whitney test was used to compare the differences between medians of the two groups, and Wilikson test was used to compare the differences between medians of IL-31 before and after hemodialysis. Spearman's correlation was employed to assess the correlation among the studied variables. A P-value less than 0.05 was considered significant.

Results: In the pre-dialysis samples, serum levels of interleukin-31 in patients with uremic pruritus were not significantly different from those in patients without uremic pruritus [1361.55 (741.96) pg/mL and 1395.75(624.75) pg/mL, respectively; P=0.36]. However, patients with uremic pruritus had higher serum creatinine levels than patients without uremic pruritus [9.8(5.5) mg/dL and 8.15(3.18) mg/dL, respectively; P=0.02]. The two groups had no significant differences in intact parathormone, calcium, or urea serum levels. In both of the study groups, serum interleukin-31 levels in patients with and without uremic pruritus were significantly reduced by hemodialysis. In the post-dialysis samples, serum interleukin-31 levels in patients with uremic pruritus were not statistically different from those in patients without uremic pruritus [864 (164.58) pg/mL and 879.7(84.19) pg/mL, respectively; P=0.83].

Conclusions: The association of serum interleukin-31 levels with uremic pruritus in chronic kidney disease CKD patients on hemodialysis need further verification.

Keywords: Chronic kidney disease**;** Hemodialysis**;** Itching; InterlukinL-31; Uremic Pruritus.

Introduction:

Chronic kidney disease (CKD) is one of the leading public global health problems. The estimated worldwide prevalence of CKD is more than 10%, affecting about 843.6 million individuals in 2017; and patients with end-stage renal disease (ESRD) needing renal replacement therapy, including hemodialysis, peritoneal dialysis, and kidney transplantation, is estimated between 4.902 and 7.083 million(1). Numerous etiological factors have been associated with the development of CKD(2,3). Generally, ESRD patients on hemodialysis have a lower quality of life compared to the general population; this has been attributed to several factors including, physical limitation, psychological distress, sleep disturbances, comorbidities, and medication-related issues(4). Uremic pruritus (UP) also called CKD-associated pruritus is one of the most common complications of CKD. It is a prevalent, unpleasant, yet seldom fatal symptom. Over 70% of hemodialysis patients had pruritus, with 40% reporting at least moderate itching. The pruritus significantly lowers the quality of life for many people. The high proportion of pruritus that happens at night may cause sleep disruption. 60% of cases involved patients with significant itching and frequent sleep problems(5). The causes of UP are currently not fully obvious. Furthermore, The knowledge regarding patients receiving hemodialysis

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about UP is limited(6). Some pathogenetic factors such as histamine, calcium, magnesium, immunological dysregulation, neuropathy, and opioid imbalance have been proposed as potential contributors(7). Interleukin-31 (IL-31) is a cytokine that plays a crucial role in skin inflammation and has been implicated in pruritus. Several studies have proposed a connection between IL-31 and the pathogenesis of various dermatological and non-dermatological diseases; thus IL-31 has been linked with the development and maintenance of pruritus(8). Chronic pruritus with no apparent cause is one of the disorders where IL-31 has been linked to this type of pruritus(9). In addition, IL-31 has an obvious role in itching related to liver problems, especially those involving cholestasis(10). The possible mechanism for itching induced by IL-31 is that; humoral immunity reaction is induced by T-helper-2 cells, involving the secretions of some interleukins such as (IL-4, IL-5, IL-6, IL-10, IL-15, IL-16, and IL-31), then the sensory neurons in the skin were activated by IL-31, leading to itching(11). Finally, the pre-dialysis IL-31 levels were found to be elevated in CKD patients on hemodialysis compared to healthy individuals, and the levels in hemodialysis patients were associated with the development and intensity of UP(12,13). However, Haggag M et al. (2022) reported no association between serum IL-31 levels and UP in hemodialysis patients.

The study aimed to evaluate interleukin-31 serum levels in hemodialysis patients with and without UP.

Materials and Methods:

This cross-sectional study was conducted at the hemodialysis unit of Al-Fallujah Teaching Hospital in Fallujah City, Iraq, from July to November 2023. A total of 90 adult patients with CKD of both sexes [51 males, and 39 females].and were on a hemodialysis regimen three times weekly. The participants were divided into two groups: the first group consisted of 45 patients with UP, and the second group consisted of 45 patients without UP. The grouping of patients was based on the Itch Intensity Scale (ISS), where a score of 0 indicated no pruritus, 1 indicated mild pruritus, 2 indicated moderate pruritus (stressful but not interfering with regular activities or sleep), and 3 indicated severe pruritus (impacting regular activities or sleep) (14).Patients with the following conditions were excluded from the study: skin rash, primary skin disorders, systemic causes of pruritus (including polycythemia vera, chronic liver disease, thyroid and parathyroid diseases, malignancy, and neuropsychiatric disorders), or communication problems, as well as those on antipruritic therapy. Pre- and post-dialysis session blood samples (5 -ml) were collected from each participant. The pre-dialysis serum samples were used to measure the levels of urea, creatinine, calcium, phosphorus, intact parathormone, and IL-31; and the post-dialysis serum samples were used to measure the serum levels of IL-31 only.

All participants were informed about the aim and the expected benefits of the study; verbal consent was obtained from participants before being enrolled in the study.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS), version 26 software. Shapiro-Wilk test was used to check the uniformity of data distribution. The median and interquartile range (IQR) were used to present data on the continuous variables. Mann-Whitney test was used to compare the differences between medians of the two groups, and Wilikson test was used to compare the differences between medians of IL-31 before and after hemodialysis. Categorical variables were expressed as numbers (percent) and the difference was checked by the Chi-square test. Spearman's correlation was employed to assess the correlation among the studied variables. A *P*-value less than 0.05 was considered significant.

Results

Characteristics of patients: The age range for CKD patients without UP, who were recruited in this study, was 18 to 82 years, with a median (IQR) of [56 (24)]. On the other hand, the age range for CKD patients with UP was 18 to 79 years, with a median (IQR) of [53 (24)]. Table (1) shows that there is no age difference between CKD patients with UP and those without (P = 0.580). Of the 45 patients with CKD who did not have uremic pruritus, 27 (60%) were men and 18 (40%) were women; However, of the 45 patients who did have uremic pruritus, 24 (53.3%) were men and 21 (46.7%) were women. Table (1) shows that there is no gender difference (P = 0.523) across the research groups. Regarding the intensity of pruritus, among the 45 patients with CKD who had uremic pruritus, 5 (11.1%) had mild pruritus, 19 (42.2%) had moderate pruritus, and 21 (46.7%) had severe pruritus (P < 0.001; Table 1).

Table 1. Characteristics of patients

Variable	CKD without UP (n=45) No. (%)	CKD with UP (n=45) No. (%)	*P*-value
Age (Years)	[56 (24)]	[53 (24)]	0.580
Gender			
Male	27(60)	24 (53.3)	0.523
Female	18 (40)	21(46.7)	
Severity of pruritus			
Mild	-----	5(11.1)	0.001
Moderate	-----	19(42.2)	
Severe	------	21(46.7)	

Where; n=number; CKD=chronic kidney disease; UP=uremic pruritus

Some Biochemical characteristics: The current study found that there was a statistically negligible difference in the two groups' serum urea, serum Calcium, serum phosphorus, and serum intact

parathormone levels before dialysis. The median (IQR) of these tests is greater in CKD with UP than in those without UP. There were statistically significant differences in the serum Cr levels. (P = 0.02). Table (2).

Table 2. Some biochemical characteristics of patients before hemodialysis

Variable	CKD without UP (N=45) No. (%)	CKD with UP (N=45) No. (%)	*P*-value
Urea (mg/dL)	[127 (50.15)]	[127 (74.75)]	0.75
Creatinine (mg/dL)	[8.15 (3.18)]	[9.8(5.5)]	0.02*
Phosphorus (mmol/L)	[5.3 (1.65)]	[5.8 (3.3)]	0.18
Calcium (mg/dL)	[9 (0.8)]	[9.5 (1.4)]	0.08
Intact Parathormone (Pg/ml)	[15.85 (9.8)]	[17.78 (17.8)]	0.386

Where n=number; CKD=chronic kidney disease; UP=uremic pruritus
**P<0.05 was a significant difference*

Serum Interleukin-31 Level of Patients: In the pre-dialysis session, the current study revealed a statistically non-significant difference between the groups' measured serum IL-31 levels. The median (IQR) of this test is 1395.75(624.75) pg/mL for those without the UP group, while the median (IQR) is 1361.55 (741.96) pg/mL for those with the UP group. (*P=0.36*; Figure 1). In the post-dialysis, the current study revealed a statistically non-significant difference between the groups' measured serum IL-31 levels. The median (IQR) of this test is 879.7(84.19) pg/mL for those without the UP group, while the median (IQR) is 864 (164.58) pg/mL for those with the UP group. (*P*=0.831; Figure 1).

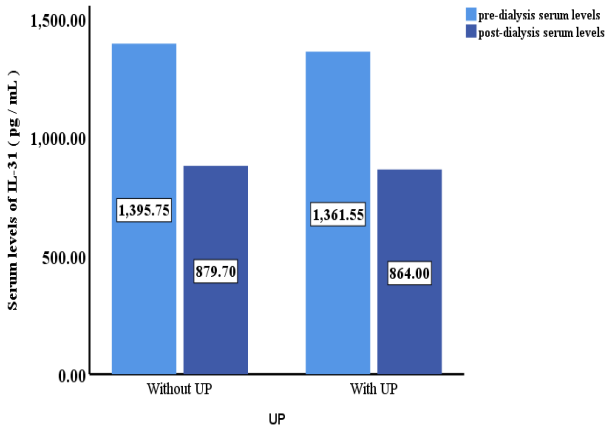

Figure 1: pre- and post- hemodialysis serum interleukin-31 levels of participants

Comparison between the levels of IL-31 before hemodialysis and after hemodialysis: The current study revealed a statistically significant difference between the groups' serum L-31 before hemodialysis and those tests after hemodialysis. The median (IQR) of these tests is higher in the pre-dialysis group than the post-dialysis [1435 (601.61) pg/mL and 873.45 (109.54) pg/mL, respectively; P <0.001].

Correlation studies between IL-31 and variables of all patients in the pre-dialysis session: Table (3) shows the correlations of serum IL-31 levels with the studied variables of the patients before the dialysis session. Regarding the IL-31 level, it showed no significant correlation with age, gender, urea, creatinine, calcium phosphorus, or intact parathormone (P>0.05; Table 3).

Table 3. Correlation of IL-31 with the studied variables of all patients before hemodialysis
**P<0.05 was significant.*

Parameters	Interleukin-31	
	spearman's correlation	*P*-value
Age(year)	0.4	0.71
Gender	-0.16	0.13
Urea (mg/dL)	0.051	0.636
Creatinine (mg/dL)	-0.202	0.057
Phosphorus (mmol/L)	-0.099	0.352
Calcium (mg/dL)	0.154	0.148
Intact parathormone (pg/mL)	-0.41	0.485

Discussion:

A variety of variables have been studied as possible causes of UP in dialysis patients, with varying degrees of outcome.(15,16). Multiple mechanisms have been hypothesized to explain the incomplete understanding of the exact pathophysiology of UP. (17,18).The present study aimed to measure serum levels of IL-31, during pre- and post-dialysis sessions, and to investigate the possible contribution of IL-31 in the pathogenesis of itch in patients on chronic hemodialysis. The impact of age and sex on the development of UP in CKD patients on chronic dialysis was inconsistent in the literature. According to one study, male sex and older age were risk factors for UP.(19). Conversely, another study found that among dialysis patients, UP is linked to younger age and female sex.(20). Other studies predicted that below this age range were susceptible to UP, due to many causes although these age ranges were not included in this study.(21–23).yet, clinical studies observed no association between sex and UP development among CKD patients (24,25).

The severity of pruritus in CKD patients can be mild, moderate, or severe(26).In an Indian study carried out on 120 eligible participants, (55.83%) of them had UP; the majority (73.1%) had mild pruritus, while moderate and severe pruritus was reported in (19.4%) and (7.5%) respectively(27). Another Chinese study

on 148 CKD patients receiving hemodialysis showed that (40.54%) of patients had UP; half of the patients with UP had moderate pruritus; while mild and severe pruritus was reported in (36.7%), and (13.3%) of the patients; respectively(26). Furthermore, a Pakistani clinical trial on 173 male patients on hemodialysis observed that (49.1%) of the patients had UP; (55.3%) of patients with UP had mild pruritus; while moderate and severe pruritus was reported in (34.1%), and (10.6%) of the patients respectively(28). In the present study, a higher percentage (46.2%) of patients with UP had moderate pruritus, followed by severe (42.2%), and mild (11.1%) pruritus. The difference in serum levels of urea, creatinine, phosphorus, calcium, and intact parathormone among CKD patients with and without UP was the subject of several studies, with very variable findings. Hu et al. (2019) reported higher levels of all of these analytes in patients with UP as compared to those without UP(29); Makhlough et al.(2014*)* reported elevated serum levels of blood urea nitrogen and intact parathormone only(30); while Tajbakhsh et al. (2013) reported abnormal serum calcium levels only(25). In the present study, only the serum creatinine levels of the pre-dialysis session samples were significantly higher in patients with UP. Elevation of serum creatinine levels has multiple reasons such as polycystic kidney disease(31). Several studies have proposed a connection between IL-31 and the pathogenesis of various dermatological and non-dermatological diseases; thus IL-31 has been linked with the development and maintenance of pruritus(8,32). The effect of IL-31 is mediated by binding to a heterodimeric receptor consisting of IL-31 receptor A (IL31RA) and Oncostatin M receptor (OSMR)(33). Activation of the IL-31 receptor triggers the activation of various signaling pathways, such as the JAK, STAT, or PI-3 kinase pathways. This subsequently affects a variety of different cell types, including epithelial cells, keratinocytes, peripheral sensory neurons, and the dorsal horn of the spinal cord(34).IL-31 inhibits the normal differentiation of keratinocytes, which are the predominant cells in the outermost layer of the skin. This inhibition of keratinocyte differentiation leads to epidermal thickening and an increase in trans-epidermal water loss(35). Furthermore, it has been observed that the overexpression or increased levels of IL-31 are associated with increased sensory neuronal outgrowth. This suggests a potential role for IL-31 in enhancing nerve fiber density and potentially contributing to the perception of pruritus(36). The role of IL-31 in UP has gained a lot of attention; most of the studies in this regard have reported an association between serum IL-31 levels and the development as well as intensity of UP(12,13). However, Haggag et al. (2022) reported no association between serum IL-31 levels and UP in hemodialysis patients(37). The finding of the latter occurs by the finding of this study; where there was no association between serum IL-31 levels and UP in both pre-and post-dialysis session samples. It is worth mentioning that Ardinata et al. (2021) showed that 6 weeks of acupuncture was associated with a reduction of pruritus dimensions in CKD patients on hemodialysis without altering serum IL-31 levels(38). Therefore, the role of IL-32 in UP needs further clarification. Secondary hyperparathyroidism is an important feature of CKD–mineral and bone disorder and plays an important role in the development of bone disease and vascular calcification. The mechanism responsible for secondary hyperparathyroidism may be that: low levels of vitamin D and hypocalcemia resulted in stimulation of the parathyroid glands to secrete parathormone as a compensatory mechanism for maintenance of hemostasis (39). There were significant differences regarding intact parathormone levels in one clinical trial conducted on CKD patients on hemodialysis(40). The current study showed no significant difference between patients with and without UP regarding serum intact parathormone levels. The disparity of findings regarding prevalence, the severity of UP, and biochemical findings in CKD patients with and without UP among different studies may be attributed to the differences in the inclusion and exclusion criteria, the number of participants, the categorizing criteria of the severity of pruritus, duration since the onset of hemodialysis, and the type of dialysate and the semipermeable membranes used in hemodialysis. One limitation of the present study is the relatively low number of patients in the two study groups which might mask the association of IL-31 with UP. Another limitation of the study is the cross-sectional nature of the study which does not permit testing of the causal relationship between serum IL-31 levels and UP. Furthermore, the effect of diet on the biochemical findings, and the residual renal function difference among participants were not considered because of the lack of cooperation of patients. Another limitation of the current study is the relatively small sample size of patients that were enrolled from a single center may impact the statistical power. hence, a larger-scale, multicenter study is recommended to establish the present study's findings.

The strength points of the present study include controlling for clinical parameters and the measurement of the serum levels of the IL-31 pre- and post-hemodialysis session that enables the examination of the accumulation of this cytokine, as well as, the efficiency and adequacy of the hemodialysis procedure.

Conclusions

The association of serum IL-31 levels and UP in CKD patients on hemodialysis need further verification.

Authors' declaration:
We hereby confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the University of Baghdad's College of Pharmacy's Ethics Committee gave its approval (RECAUBCP2572623 on 25/7/2023). Before the participation agreement was recorded, each participant was given information about the study's goals and anticipated advantages.
Conflicts of Interest: none
Funding: None

Authors' contributions:
Study conception & design: (Mustafa Sh. Abdulqahar& Ali. A. Kasem). Literature search: (Mustafa Sh. Abdulqahar). Data acquisition: (Mustafa Sh. Abdulqahar). Data analysis & interpretation: (Mustafa Sh. Abdulqahar& Ali. A. Kasem). Manuscript preparation: (Mustafa Sh. Abdulqahar& Ali. A. Kasem). Manuscript editing & review: (Ali. A. Kasem).

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تقييم مستويات مصل إنترلوكين 31 لدى المرضى الذين يعانون من مرض الكلى المزمن على الديلزة الدموية مع أو بدون حكة يوريمية

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الخلفية: تم ربط الإنترلوكين -31 بتطور الحكة والحفاظ عليها في العديد من الأمراض الجلدية وغير الجلدية.
الأهداف: تقييم مستويات المصل انترلوكين-31 في مرضى غسيل الكلى الذين يعانون من أو بدون حكة يوريمية.
طرق البحث: شملت هذه الدراسة المقطعية تسعين مريضا بالغا مصابا بمرض الكلى المزمن يخضعون لغسيل الكلى. كان جميع المرضى المسجلين يخضعون لنظام غسيل الكلى ثلاث مرات أسبوعيا. تم تقسيم المرضى إلى مجموعتين كل منهما 45 مريضا. المجموعة 1 شملت أولئك الذين يعانون من الحكة والمجموعة 2 شملت أولئك الذين لا يعانون من الحكة. بناء على مقياس شدة الحكة. تم تقييم مستويات الإنترلوكين-31، الباراثورمون، واليوريا، والكرياتينين، والكالسيوم قبل جلسة غسيل الكلى. تم أيضا تقييم مستويات الإنترلوكين-31بعد جلسة غسيل الكلى.
النتائج: مصل ما قبل غسيل الكلى غير مختلف بين المرضى غير المصابين بحكة والمصابين بها. [1395.75(624.75) بيكوغرام/مل و1361.55 (741.96) بيكوغرام/مل، على التوالي؛ (قيمة الاحتمال = 0.36) من ناحية أخرى، كان لدى المرضى الذين يعانون من الحكة اليوريمية مستوى كرياتينين في الدم أعلى بكثير [9.8(5.5) ملغم/ديسيلتر] من المرضى الذين لا يعانون منها [8.15(3.18) ملغم/ديسيلتر] (قيمة الاحتمال = 0.02). لم يكن هناك اختلاف كبير في مستويات مصل الباراثورمون أو الكالسيوم أو اليوريا بين المرضى الذين يعانون من حكة أو بدون
كان متوسط قياس مصل الانترلوكين-31في المرضى الذين يعانون من الحكة والمرضى الذين لا يعانون منها مختلفا بشكل كبير، عند 1435 (601.61) بيكوغرام/مل و873.45 (109.54) بيكوغرام/مل، على التوالي، قبل وبعد جلسات غسيل الكلى.).
كان لدى المرضى الذين لا يعانون من الحكة مستوى انترلوكين-31أكبر بشكل غير ملحوظ في عينة مصل ما بعد غسيل الكلى [879.7(84.19) بيكوغرام/مل] من المرضى الذين يعانون من حكة [864 (164.58) بيكوغرام/مل] (الاحتمال = 0831).
الاستنتاج: مستويات المصل انترلوكين 31 لدى مرضى مرض الكلى في المرحلة النهائية الذين يعانون من الحكة اليوريمية ليس لها أي دور في الحكة.
الكلمات المفتاحية: اعتلال الكلية المزمن , ديلزة دموية, ، حكة ,الإنترلوكين 31, الحكة اليوريمية

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