Detection of Interleukin-2 and Interleukin-31 among Patients with Uremic Pruritus

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Abstract

Uremic pruritus is a form of chronic itching that affects patients with advanced end-stage renal disease. It is one of the most common symptoms in patients with end-stage renal disease, with approximately 60–90 percent of patients on hemodialysis (HD) experiencing it. The study aims to assess the role of interleukin-31 and Interleukin-2 in the pathogenesis of uremic pruritus among hemodialysis patients attending the Ebn Sina Dialysis Center in Diyala, and Correlation between Interleukin 31 and Interleukin-2 with severity of uremic pruritus. A study was performed from 1 Sep 2020 to 20 Jan 2021 in Ebn Sina Dialysis center, Diyala Governorate. the study was conducted on 150 patients with CKD divided to two groups, 75 patients with uremic pruritus and 75 patients without uremic pruritus, the control groups was 26 healthy persons. Itch intensity was scored by VAS as mild, moderate and severe. Serum IL-31 levels and serum IL-2 levels were determined by the enzyme-linked immunosorbent assay methodology. The various characteristics pf pruritus was assessed using an interview questionnaire. MS excel package and SPSS 20 software was used for analysis. Among 226 HD patient excluded under 20 age and hepatitis C and Hepatitis B HD patients, 33.8% had uremic pruritus. The patients with pruritus had higher serum IL-31 levels and IL-2 than those without uremic pruritus and control groups, it was statically significant. As well as the IL-31 and IL-2 showed no significant differences among patients with mild, moderate, and severe itch. IL-31 and IL-2 play important role in the pathogenesis of uremic pruritus. Further studies to confirm the role of cytokines in the pathogenesis of UP.

Keywords*: uremic pruritus, Itching, Interleukin-31, Chronic Kidney disease, hemodialysis, Diyala.*

Introduction

Uremic pruritus, also known as "Chronic Kidney Disease associated Pruritus" (CKDaP), is a form of chronic itching that occurs in patients with advanced or end-stage renal disease [(1)]. Symptoms can be localized, affecting large symmetrical regions of the body, or they can be generalized, affecting the whole body. It may occur daily or near-daily [(2,3)]. Many factors may cause uremic pruritus, including xerosis, elevated serum calcium, phosphate, calcium–phosphate product, hyperparathyroidism, and insufficient dialysis. Researchers recently confirmed that the immune hypothesis may be a major cause of uremic pruritus [(4)]. multisystem dysfunction that is comorbid with renal failure. T-helper (Th)-1 cytokine and interleukin (IL)-2 are pro inflammatory mediators that may play a role in pruritus. Previous research has linked interleukin (IL)-31 to the development of UP [(5)]. Multiple health-related quality-of-life outcomes, such as sleep quality, mood, and social function, are linked to uremic pruritus severity [(6,7)]. Certain risk factors for uremic pruritus in dialysis patients have been discovered by researchers. Males and those with certain comorbidities (e.g., congestive heart failure, hepatitis C infection, neurological disorders, and higher serum calcium/phosphorus levels) were found to be more frequently affected [(6)]. Despite modern day dialytic management, 60% of dialysis patients experience itching and approximately 30–45% experience moderate or severe/extreme pruritus [(6)].

Material & Methods

Study protocol

This study was conducted on HD patient to evaluate the levels of Interleukin-31 in-patient with uremic pruritus. It was performed from the period between September 2020 to March 2021 in in Ebn Sina Dialysis center/ Diyala. The number of patient in Ebn Sina Dialysis center in this period was 226 patients we excluded the age under 20 and hepatitis c and hepatitis so the study conducted on 150 patients (75 with UP and 75 without UP). Questionnaire was prepared, distributed, and collected through each patient and control group. Patients answer on the questions of questionnaire in Dialysis Center. For each case of Uremic Pruritus, a questionnaire was applied to obtain demographic information regarding age, sex, duration of Dialysis, number of dialysis session per week, number of hour of dialysis session per day, severity of uremic pruritus.

Ethical considerations

Diyala Medical College and Health Directorate of Diyala Iraq, approved this study. The patients and control endorsements to the questionnaire in the study and blood sample.

Blood sample

All patients were examined by a dermatologist referred from Ebn Cina Dialysis Center. Blood sample was taken from each participant in the study 3 ml of venous blood was withdrawn under complete aseptic condition from the cubital vein, put in gel tube and allowed to clot at room temperature. the samples were centrifuged at 3000 rpm for 15 min and the obtained serum divides in to two Eppendorf tube then kept frozen at −20°C to be analyzed by ELISA technique later for Interleukin-31 and Interleukin-2.

Detection of Human interleukin 31 (IL-31)

For in vitro diagnostic used Abbexa Ltd, Cambridge, UK. Human Interleukin 31 (IL-31) ELISA Kit (Cat No: abx251292). For quantitative detection of IL-31 in Human serum.

Detection of human Interleukin-2 (IL-2)

For in vitro diagnostic used Abbexa Ltd, Cambridge, UK. Human Interleukin-2 (IL-2) ELISA Kit (Cat No: abx152027). For quantitative detection of IL-2 in Human serum.

Statistical Analysis

Data procession software package is used SPSS 20 for windows. Data are expressed as mean ± standard error (M ± SE). Differences between means of two major groups are analyzed by using t-test and the significance is tested at two- tail Pvalue. However, differences among subgroups are analyzed by using one-way analysis of variance (ANOVA), then if there are significant differences, they are analyzed by least significant difference (LSD) test. The Pvalue of differences <0.05 is considered significant. Pearson correlation (R) accounted to explain type and strength of relationship between variables. A level of significance of $\alpha=0.05$ was applied to test. Using SPSS version [21] statistical peak age of social science version.

Results

Characterization of Pruritus Patients under hemodialysis session

Table 1 shows the general characteristics of uremic pruritus patients under HD session, include (duration of hemodialysis, age, sex, type of itch, and severity), also the most important risk factors for kidney failure.

Table (1) Characterization of uremic pruritus patients under hemodialysis treatments

Characters Pruritus Patients under hemodialysis treatments N = 75			
Age (year)	**Range (20-75)**		
Duration of Hemodialysis	More than 6 months	N= 42	
	Less than 6 months	N= 33	
Sex	Men	N= 45	(60 %)
	Women	N= 30	(40 %)
Type of itch	Localize	N= 49	(65.3 %)
	Generalize	N= 26	(34.6 %)
Severity of itch	Mild	N= 13	(17.3 %)
	Moderate	N= 42	(56 %)
	Sever	N= 20	(26.6 %)

The Results among patients under hemodialysis with uremic pruritus and without uremic pruritus.

The results were comparing among three groups uremic pruritus patients without uremic pruritus who taking regular hemodialysis sessions and control.

Cytokine levels in Patients with uremic pruritus and without uremic pruritus.

The results of the statistical analysis showed no any significant differences in the level interleukin-2 and interleukin-31 in hemodialysis patients without uremic pruritus (179.39 ± 21.19 pg/ml, and 39.01 ± 4.74 pg/ml respectively) when compared with control (73.55 ± 10.32pg/ml, and 25.49 ± 3.55 pg/ml respectively) p-value 0.183, but increase significantly in hemodialysis patients with uremic pruritus (339.07 ± 57.55 pg/ml, and 55.87 ± 5.36 pg/ml respectively) when compared with control p-value 0.001 and without uremic pruritus patients p-value 0.006 (table 2).

Table (2) Cytokine levels among patients with uremic pruritus, without uremic pruritus and healthy individuals.

Groups	**Interleukin 2 (pg/ml) (Mean ± SE)**	**Interleukin 31 (pg/ml) (Mean ± SE)**
Patients with Pruritus (N=75)	339.07 ± 57.55	55.87 ± 5.36
Patients Without Pruritus (N=75)	179.39 ± 21.19	39.01 ± 4.74
Control (N=26)	73.55 ± 10.32	25.49 ± 3.55
P-Value*	P vs W. P 0.006 C vs P 0.001 C vs W. P 0.183	P vs W. P 0.013 C vs P 0.001 C vs W. P 0.151
P value <0.05, SE stander error,		

*ANOVA test

C control. U.P. uremic pruritus, and W.U..P without uremic pruritus.

The results according to severity of itch mild, moderate, and sever

The results were comparing among groups dividing the patients with pruritus according to severity of itch mild, moderate, and sever itch.

Cytokine levels among mild, moderate, and severe itch groups

The results of the statistical analysis showed that there were no significant differences in the level of interleukin-2 among patients with mild, moderate, and severe itch (247 ± 56.21 pg/ml, 324 ± 77.43 pg/ml, and 429.62 ± 138.47 pg/ml), p-value 0.633, 0.312, and 0.441. as well as the interleukin-31 showed no significant differences among patients with mild, moderate, and severe itch (50.27 ± 9.30 pg/ml, 64.10 ± 8.28 pg/ml, and 42.22 ± 7.47 pg/ml respectively), p-value 0.348, 0.626, and 0. 085 (Table 3).

Table (3) Cytokine levels among mild, moderate, and severe itch groups

Groups	Interleukin 2 (pg/ml) (Mean ± SE)	Interleukin 31 (pg/ml) (Mean ± SE)
Patients with mild itch (N=13)	247 ± 56.21	50.27 ± 9.30
Patients with moderate itch (N=42)	324 ± 77.43	64.10 ± 8.28
Patients with severe (N=20)	429.62 ± 138.47	42.22 ± 7.47
P-Value*	MI vs MO. 0.633 MI vs S 0.312 MO vs S 0.441	MI vs MO. 0.348 MI vs S 0.626 MO vs S 0.085
P value <0.05, MI mild, MO moderate, and S severe		

*ANOVA

Discussion

Uremic pruritus refers to the common and distressing symptom of persistent itch experienced by many patients with chronic kidney disease (CKD). Pruritus is the most common skin manifestation of kidney disease, affecting both patients with non-dialysis CKD and those with kidney failure on dialysis [8]. In our study cytokines levels (interleukin-2, interleukin-31) showed increase significant differences in hemodialysis patients with uremic pruritus when compared with control and without uremic pruritus patients, but the study showed no any significant differences the level of IL 2 and IL 31 in hemodialysis patients without uremic pruritus. ESRD patients can considered an immune compromised patient as they have increased the risk of infection [9], As renal function declines, uremic toxins and cytokines increase, causing significant oxidative stress and the release of inflammatory cytokines. The retention of uremic toxins, as well as interactions between blood and dialyzers during hemodialysis, are

thought to affect both the innate and adaptive immune systems, as well as altered cytokine balance [10]. Gibbes et al found that IL-31 was elevated in variety of skin conditions associated with itching [11]. Some studies explain the Overexpression of IL-31 associated with promotion of sensory neuronal outgrowth like a study of Feld et al [12], and stimulation like Furue et al [13] study, provide increase sensitivity to minimal itch inducing stimuli which can result in sustained pruritus. Expression of IL-31 in skin is also associated with a profound repression of the filaggrin protein, which is a critical in the differentiation of keratinocytes and the skin barrier maintenance [14]. While in Egypt Haggag et al found no significant elevation was detected in the IL-31 level in patients with UP as compared with patients without pruritus [15]. In Taiwan Ko et al demonstrated a significant increase in IL-31 in hemodialysis patients with UP compared with those without UP [10]. Many studies give a suggestion to the reason of why IL-2 elevated, Smith et al showed the release of IL-6, IL-1β and TNF-α make the lymphocytes to synthesis IL-2 [16]. In a study to Vink et al suggested that activation of central pruritus center in CNS and/ or activation of central or peripheral opoid μ- receptors, increase serum level of IL-2, which may result in the role of IL-2 in UP among HD patients [17]. A study to Kimmel et al which they showed that contact of blood with dialyzer membrane result in degranulation of leukocytes and release inflammatory mediators [18]. previous study in Iran by Fallahzadeh et al showed increase in interleukin 2 in the pruritus group was higher than in the non-pruritic group [19]. In Egypt Banha, Shafei and Nour found increase in serum IL-2 in pruritus patients when compared with hemodialysis patients without pruritus and control [20]. In present study the result showed that there were no significant differences in the level of interleukin-2 among patients with mild, moderate, and severe itch. As well as the interleukin-31 showed no significant differences among patients with mild, moderate, and severe itch. IL-31 was found to be elevated in variety of skin conditions associated with itch, [11], but it did not have a correlation with itch severity score or death during the follow up period, it can be explained by the complexity of pruritus pathogenic mechanisms which involves many mediators and mechanisms. In contrast, in a study to Ko et al they demonstrated a positive exposure-response relationship between IL-31 levels and VAS scores of pruritus severity [10]. The result that the serum levels of IL-31 correlated with pruritus intensity indicates that aberrant immune reactions may be important in the pathogenesis of uremic pruritus. Pranata et al found that there is a significant positive correlation was obtained between serum level of IL-2 and severity of uremic pruritus. These differences between the hemodialysis and pruritus groups were not statistically significant [20].

Conclusion

UP is a serious problem in dialysis patients, with complex pathogenesis involving various mediators. IL-31 and IL-2 was found to be elevated in HD patients with UP. The elevated IL-31 and IL-2 levels were not correlated with severity of itch.

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