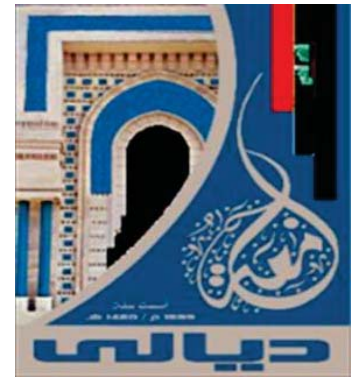




Republic of Iraq
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and Scientific Research
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College of Medicine



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

A Thesis

Submitted to the council of the College of Medicine - University of
Diyala in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Medical Microbiology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَى

(صدق الله العظيم)

سورة الضحى الآية (٥)

Dedication

To my father, who taught me the value of education and supported me to realize the person I am today.

To my mother, my source of encouragement and inspiration.

To my husband who supported me and gave me endless love.

To my soul... My children Tameem & Yazin.

To my beloved family who are always supporting, helping, and standing by me.

To everyone who helped and supported me in my study.

The researcher

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The researcher

Abstract

Uremic pruritus (UP) is a common problem in hemodialysis patients. It remains a clinical priority for patients with Chronic Kidney Disease (CKD). Despite the strong impact on the quality of life, its pathogenesis is not completely understood. Multiple factors are found associated with UP in several studies .

The study aims to assess the role of Interleukin-2 and Interleukin-31 in the pathogenesis of uremic pruritus among hemodialysis patients attending the Ibn Sina Dialysis center in Diyala, and correlation between Interleukin-2 and Interleukin-31 with severity of uremic pruritus .

Study is performed from 1 September 2020 to 20 January 2021 in Ibn Sina Dialysis center, Diyala province. Among 226 hemodialysis patients, the researcher excluded hemodialysis patients: under 20 age, hepatitis C and hepatitis B, 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 were 26 healthy persons. Itch intensity was scored by VAS as mild, moderate and severe. Blood samples were collected from each one and serum samples was directly stored at -20 °C to be analyzed later for Interleukin-2 and Interleukin-31. Serum IL-2 levels and serum IL-31 levels were determined by ELISA methodology. The various characteristics of pruritus was assessed using an interview. MS excel package and SPSS 20 software was used for analysis.

The result showed that the level of Interleukin-2 and Interleukin-31 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 patients without uremic pruritus p-value 0.006, but the results showed no 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 ± 10.32 pg/ml, and 25.49 ± 3.55 pg/ml, respectively) p-value 0.183. While the results 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, respectively. 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, respectively.

Eosinophils increased significantly in hemodialysis patients with uremic pruritus ($0.24 \pm 0.02 \times 10^9$ /L) when compared with control ($0.14 \pm 0.02 \times 10^9$) p-value 0.010, and patients without uremic pruritus ($0.09 \pm 0.00 \times 10^9$ /L) p-value 0.000, but the results showed no any significant differences between hemodialysis patients without uremic pruritus and control group p-value 0.137

Basophils and neutrophils cells showed a significant increase in hemodialysis patients with pruritus ($0.04 \pm 0.00 \times 10^9$ /L, and $4.29 \pm 0.17 \times 10^9$ /L respectively) p-value 0.000 and 0.001, and patients without pruritus ($0.04 \pm 0.00 \times 10^9$ /L, and $4.29 \pm 0.17 \times 10^9$ /L respectively) p-value 0.000 and 0.005 when compare with control ($0.02 \pm 0.00 \times 10^9$ /L, and $3.17 \pm 0.19 \times 10^9$ /L respectively), while it not showed any significant differences between hemodialysis patients with and without uremic pruritus p-value 0.051 and 0.376.

Lymphocytes increased significantly in hemodialysis patients with uremic pruritus ($1.77 \pm 0.06 \times 10^9$ /L) when compared with patients without uremic pruritus ($1.60 \pm 0.04 \times 10^9$) p-value 0.002, but the results

showed no any significant differences between hemodialysis patients with uremic pruritus and control group p-value 0.094 and patients without uremic pruritus and control group p-value 0.938.

Eosinophils were showed no any different significant among patients with mild, moderate, and severe itch ($0.28 \pm 0.07 \times 10^9 /L$, $0.20 \pm 0.02 \times 10^9 /L$, and $0.29 \pm 0.06 \times 10^9 /L$ respectively), p-value 0.341, 0.901, and 0.181. Also Basophiles showed no any different significant among patients with mild, moderate, and severe itch ($0.04 \pm 0.00 \times 10^9 /L$, $0.04 \pm 0.00 \times 10^9 /L$, and $0.04 \pm 0.02 \times 10^9 /L$ respectively), p-value 0.351, 0.639, and 0.634, Neutrophils were showed no any different significant among patients with mild, moderate, and severe itch ($4.36 \pm 0.48 \times 10^9 /L$, $4.78 \pm 0.35 \times 10^9 /L$, and $4.13 \pm 0.42 \times 10^9 /L$ respectively), p-value 0.530, 0.762, and 0.261, Lymphocytes showed no any significant differences among patients with mild, moderate, and severe itch ($1.68 \pm 0.12 \times 10^9 /L$, 1.79 ± 0.88 , 1.92 ± 0.15), p-value 0.551, 0.253 and 0.421.

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List of abbreviations

Abbreviations	meaning
AD	Atopic dermatitis
AKI	Acute Kidney Injury
ASCVD	Atherosclerotic Cardio Vascular Disease
CGRP	Calcitonin gene-related peptide
CKD	Chronic Kidney Disease
CKD-ap	Chronic Kidney Disease associated pruritus
CNS	Central Nervous System
CRP	C- reactive protein
DOPPS	Dialysis outcomes and practice patterns study
DRG	Dorsal Root Ganglia
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
GPCRs	G protein-coupled Receptors
Hb1c	hemoglobin A1c
HD	Hemodialysis
H1R	Histamine receptors 1
hs-CRP	High sensitivity- C reactive protein
IFN	Interferon
IL-2	Interleukin-2
IL-31	Interleukin-31
IL-31RA	Interleukin-31 Receptor A

NaVs	Voltage-gated sodium channels
NF- κ B	Nuclear factor kappa B
OSMR	Oncostatin M Receptor
PKD	Protein kinase c
PTH	Parathyroid hormone
SP	Substance P
Th-1	T helper-1
Th-2	T helper-2
TLRs	Toll-like receptors
TNF	Tumor Necrosis Factor
TRPA1	Transient receptor potential vankyrin1
TRPV1	Transient receptor potential vanilloid1
UP	Uremic Pruritus
UP	Uremic Pruritus
VAS	Visual Analogue Score

1.1 Introduction

Uremic pruritus (UP), 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 (Mettang and Kremer, 2015).

In patients with CKD or kidney failure, uremic pruritus refers to a non-dermatomal itch pattern with no predominant skin lesion. 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 (Reszke and Szepietowski, 2018; Swarna *et al.*, 2019; Ragazzo *et al.*, 2020).

Despite modern daily dialytic management, 60% of dialysis patients experience itching and approximately 30–45% experience moderate or severe/extreme pruritus (Pisoni *et al.*, 2006; Ramakrishnan *et al.*, 2014). Many factors may cause uremic pruritus, including xerosis, elevated serum calcium, phosphate, calcium–phosphate product, hyperparathyroidism, and insufficient dialysis. Researchers recently confirmed that the immunohypothesis may be a major cause of uremic pruritus (Mettang *et al.*, 2002).

Multisystem dysfunction that is comorbid with renal failure. Some inflammatory cytokines such as Interleukin-2 are pro inflammatory mediators that may play a role in pruritus (Kimmel *et al.*, 2006). Previous research has linked interleukin-31 to the development of UP (Ko *et al.*, 2014; Gangemi *et al.*, 2017).

The symptoms of UP range from a generalized itch to a localized itch that affects the back, ears, and arms (Mettang Kremer, 2015). Multiple health-related quality-of-life outcomes, such as sleep quality, mood, and social function, are linked to uremic pruritus severity (Pisoni *et al.*, 2006; Mathur *et al.*, 2010).

Patients with UP have difficulty coping with it, and developing associated depression (Bíró *et al.*, 2005). UP is becoming an increasingly important problem among dialysis patients. Its effects on patients' quality of life, sleep, emotional state, and social relations (Narita *et al.*, 2006).

In dialysis patients, uremic pruritus typically appears after three months. 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, depression, and higher serum calcium/phosphorus levels) were found to be more frequently affected (Pisoni *et al.*, 2006). CKD-aP may occur even before pre-dialysis (Khanna *et al.*, 2010).

The association of chronic renal failure and pruritus has been recognized for more than a century (Chargin and Keil, 1932). Furthermore, patients with uremic pruritus have increased mortality rates when compared with hemodialysis (HD) patients without itching (Narita *et al.*, 2006).

According to several studies and trials, it appears that UP is a systemic inflammatory disease rather than a local dermatologic disorder (Kuypers, 2009) (Kimmel *et al.*, 2006). Derangement of T helper (Th) cells balance with Th1 predominance appears to be a major contributor to this systemic inflammation (Kimmel *et al.*, 2006). Fallahzadeh *et al.*, studied the correlation between uremic pruritus and the serum levels of IL-2, which were significantly higher in HD patients with itch versus those without it. Additionally, no correlation was detected between the level of IL-2 and UP severity (Fallahzadeh *et al.*, 2011).

Ko *et al.*, found that Interleukin-31 may play an important role in the pathophysiology of uremic pruritus (Ko *et al.*, 2014).

Aims of the study:

The study aims to:

- 1- Evaluating of Interleukin-2 and interleukin-31 among hemodialysis patients with and without uremic pruritus attending the Ibn Sina Dialysis center in Diyala.
- 2- Investigating socio-demographic distribution of uremic pruritus in these patients.
- 3- Investigating correlation between Interleukin-2 and Interleukin-31 with severity of disease.
- 4- Evaluating the frequency and severity of pruritus in maintenance hemodialysis patients, among biological marker which include: Eosinophils, Basophils, Neutrophils and Lymphocytes.