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Evaluation the interleukin-6 Serum Level on Hemodialysis Patient of Al-Diwanyah Province

A Research
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BY

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بسم الله الرحمن الرحيم

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صدق الله العظيم

سورة البقرة: الآية 32

Dedication

To who was present with me at all times in my heart and mind to prophet of peace

"Mohammed"

Peace and prayer be on him and his purified family.

To ours great family who encouraged us

To all our friends

Hayat Maeen, Anwar Sabri , Ryam Amar

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*First of all, praise is to "**Allah**", who enabled us to overcome all the difficulties that were associated with this work till I brought it to the present state. Peace and prayer be on the most honourable Mohammed and his purified family.*

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Abstract

The end-stage renal failure (ESRD) patients have significant immune dysregulation compared with the general population and subsequently, have a higher susceptibility to infection and a high incidence of malignancy Our study was designed to determine whether a patients undergoing hemodialysis session leads to an acute substantial alteration in the plasma levels of inflammation IL-6 or not. Where this study designed to evaluate the concentration of IL-6 serum level in 28 of hemodialysis Iraqi patients who were referred the Department of Dialysis at AL-Diwaniyah Teaching Hospital for period from November 2017-february 2018, in addition to 20 sample for health group. by using the ELISA technique (Sandwich ELISA formate).The result showed low concentration of IL-6 serum level in the patients (28.037pg/ml) significantly at ($P < 0.000$), when compared with those of healthy (96.1pg/ml)

Conclusion: the hemodialysis patients display decrease in the concentration of IL-6 serum level and this condition referred to susceptibility to infection by various disease due to the anti-inflammatory state.

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Introduction:

Chronic renal failure (CRF) is a progressive loss of function of more and more nephrons that gradually decreases overall kidney function (1), develops over many months or years, and is irreversible, leading eventually to end-stage renal failure (ESRF) which requires either long-term renal replacement treatment (dialysis) or a successful renal transplant to survive (2). In general, CRF can occur because of disorders of the blood vessels, glomeruli, tubules and lower urinary tract (1).

There are important metabolic features such as retention of waste products of metabolism and biochemical changes especially in urea, creatinine(Cr) and creatinine clearance (CrCl) , in addition to , changes in acute phase proteins {interleukin-6 (IL-6) , C-reactive protein (CRP), ferritin and albumin (alb)} and lipid profile in plasma of ESRF patient. Severe loss of kidney function, either acutely or chronically, is a threat to life or requires removal of toxic waste products and restoration of body fluid volume and composition toward normal. This can be accomplished by dialysis with an artificial kidney (3).

Hemodialysis and inflammation:

Inflammation is a physiological response to infections, trauma, or toxic injury, and in the form of acute phase response, it may lead to malnutrition and atherosclerosis (4). ESRD is associated with an inflammatory state characterized by elevated circulating levels of proinflammatory cytokines such as interleukin-6 (IL-6), which has been recognized as a predictor of mortality in both incident and prevalent dialysis patients [5,6]. Although the causes of elevated IL-6 in ESRD patients are not fully understood.

Recent evidence points to chronic inflammation as a major contributor to morbidity and mortality in end-stage renal disease (ESRD)(7). It has been proposed that a chronic inflammatory state could account for the high risk of ischemic heart disease in patients with ESRD(8).

About 35-65% of ESRD patients receiving hemodialysis (HD) show signs of inflammation, whereas the prevalence in predialysis patients may be somewhat lower(7).

A significant part of immune alterations in the course of ESRD could probably be attributed to the presence of protein energy wasting (PEW) (9). This severe, yet common, complication of ESRD has been shown to correlate with increased morbidity and mortality in this patient population (10), and has been found to be related to lymphocytopenia and to impaired T lymphocyte function (11, 12)

IL-6 and the hemodialysis:

Interleukin-6 (IL-6) was discovered in 1986 as a B cell stimulatory factor initiating IgG production (13). Later, it was demonstrated to be a multifunctional cytokine that regulates numerous biological processes including the organ development, acute-phase responses, inflammation, and immune responses (14).

Interleukin-6 (IL-6) is a pleiotropic cytokine that not only regulates the immune and inflammatory response but also affects hematopoiesis, metabolism, and organ development.

The ESRD patients have significant immune dysregulation compared with the general population and subsequently, have a higher susceptibility to infection and a high incidence of malignancy and cardiovascular disease, and a poor response to vaccination [15,16,17].

The aim of the study: Our study was designed to determine whether a patients undergoing hemodialysis session leads to an acute substantial alteration in the plasma levels of inflammation IL-6 or not.

Sample ,Materials and Methods

Sample:

The study was carried out on 32 hemodialysis Iraqi patients who were referred the Department of Dialysis at AL-Diwaniyah Teaching Hospital for period from November 2017-february 2018. The selected samples were classified into two groups: the first group included (20) healthy; the second group included 26 hemodialysis patients (9 female and 17 male).

Material:

Instrument & Equipments:

	Equipments	***Manufacturing***
1.	Centrifuge –universal 16 A	Hittch Germany
2.	ELISA system	Bio-test, Germany
3.	Fine and adjustable micropipettes	Gilson, France
4.	Freezer	Ishtar (Iraq)
5.	Oven	Olympus, Japan
6.	PH-meter	Thermo Electron , USA
7.	Refrigerator	Arcelik, Turkey
8.	Sensitive balance	Sartorius (U.S.A)

2. Plastic and Glass Wares

	Plastic and Glass Ware	***Manufacturing***
1.	Automatic pipette	Birhit, Finland
2.	Disposable syringes	Meheco, China
3.	Eppendorf Tubes (0.25, 0.5,1 and 1.5 ml)	Star Lab,UK
4.	Eppendorf tube	EAPIF, Germany
5.	Gloves	Hungary
6.	Pasteur’s pipettes	Volac (England)
7.	Pipette Tips (10 µl , 20 µl , 100 µl , 200 µl and 1 ml)	Star Lab,UK

3. Imunological Kit :

Use Interleukin-6 ELISA kit from Peprotech Company (USA)

It is a Human IL-6 ELISA development kit contains Human IL-6 in a sandwich ELISA format in the range of 24-1500pg/ml.Using the ELISA protocol described below.

ELISA Solutions Required:

- Block Buffer - 1% BSA in PBS with 0.05% NaN3.
- **Wash Buffer:** 0.005% Tween-20, 0.1% BSA in PBS.

Solutions Preparation:

- **Phosphate Buffer Saline (PBS):** One tablet of PBS was dissolved in 200 ml distilled water and the pH was adjusted to 7.2, then the solution was autoclaved (121ºC, 15 pounds per square inch for 20 minutes) and stored in the refrigerator (4ºC) until use
- Reagent Diluent1 - 0.1% Bovine Serum Albumine (BSA),5 0.05% Tween 20 in Tris-buffered Saline (20 mM Trizma base, 150 mM NaCl) pH 7.2 - 7.4, 0.2 µm filtered.
- Stop Solution - 2 N H2SO4
- Substrate Solution - 1:1 mixture of Color Reagent A (H2O2) and Color Reagent B (Tetramethylbenzidine)
- Tween 20

Methods:

Collection of Samples

The blood samples were collected from hemodialysis patients according to the blood aspirated technique, which is mentioned by (Baron *et al.,* 1995). The blood sample collected(1-5ml) was transferred to a plain tube, and then it was let to clot at room temperature (20-25 °C) for 15 minutes,

centrifuged at 3,000 RPM for 10 minutes to separate the serum, then they were stored at -20ºC until assayed of the serum level.

RECOMMENDED MATERIALS

PLATE PREPARATION

1 - Dilute capture antibody with PBS to a concentration of 100µg/ml. Immediately, add 100µ to each ELISA plate well.Seal the plate and incubate overnight at room temperature .

2 - Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well. After the last ash invert plate to remove residual buffer and blot on paper towel.

3 - Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.

4 - Aspirate and wash plate 4 times.

ELIZA PROTOCOL

Standard/Sample: Dilute standard from 1.5ng/ml to zero in diluent immediately add 100µl of standard or sample to each well in duplicate. Incubate at room temperature for at least 2 hours.

Detection: Aspirate and wash plate 4 times. Dilute detection antibody in diluents to a concentration of 0.50µg/ml. Add 100µl per well. Incubate at room temperature for hours.

Avidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 5.5µl of Avidin HRP-Conjugate. 1:2000 in diluents for total volume of 11ml. Add 100µl per well. Incubate 30 minutes at room temperature.

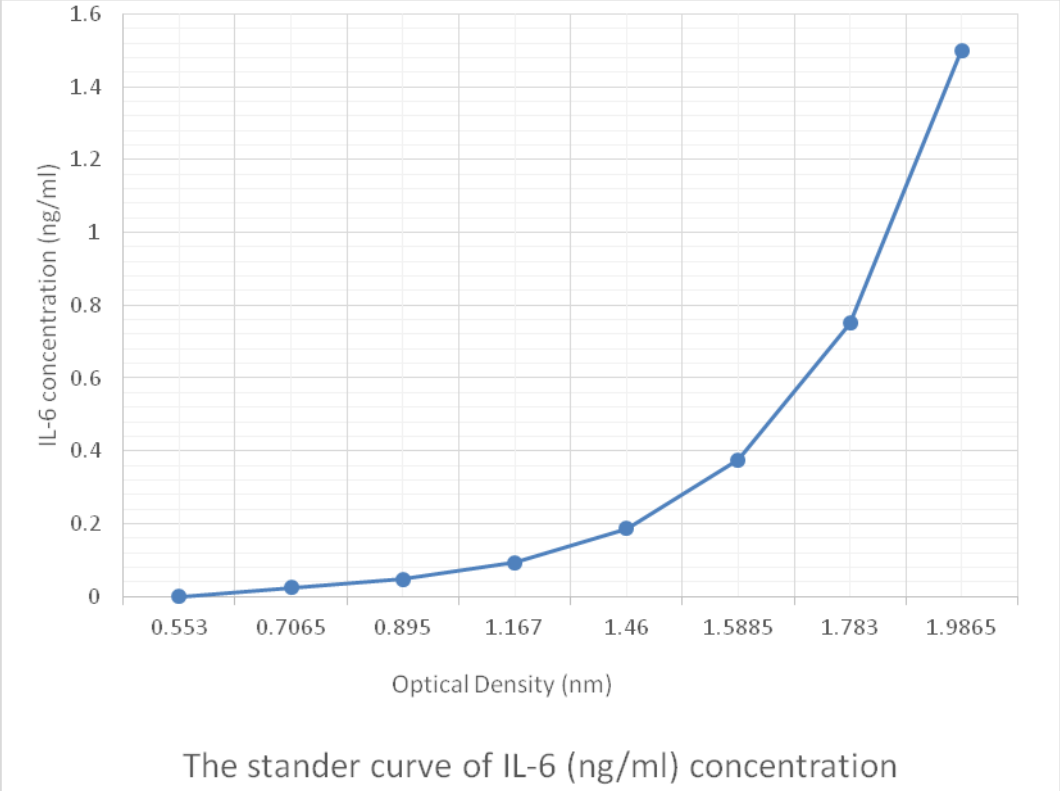
ABTS Substrate solution:

(ABTS Substrate should be at ambient temperature prior to use)

Aspirate and wash plate 4 times. Ad 100µl of substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 495 nm with wavelength correction set at 650nm.

Data Calculation:

Average the duplicate readings for each standard, control, and sample was subtracted from the average of zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-6 concentrations versus the O.D. and the best fit line can be determined by regression analysis.



The stander curve of IL-6 (ng/ml) concentration

Statistical Analysis:

The statistical analysis system- SAS (2010) was used to study the effect of difference factors on study parameters. Least significant difference –LSD test was used to significant compare between means of cytokine (IL-6) serum level.

Result and Discussion:

Our result showed low concentration of IL-6 serum level IL-6 levels in the patients (28.037pg/ml) were significantly lower at (P < 0.000),when compared with those of healthy (96.1pg/ml)and this result disagreed with other result, where some study demonstrate that the serum concentrations of IL-6 remained unchanged over the course of measurement, and so referred to unchanged serum IL-6(18),during HD concurrent with increased clearance or membrane adsorption of these cytokines(19).Other study reported the hemodialysis procedure caused a significant reduction of IL-6 levels, where the ESRD group had a significant increase in plasma IL-6 concentration before hemodialysis and reduce in IL-6 after hemodialysis procedure (20).

Table (1): **Explain the Serum IL-6 (pg\ml) Level for hemodialysis patients in comparative with healthy**

Group	**No.**	**Mean ± SE(pg\ml)**
		IL-6
Healthy	**20**	**96.1± 13.9**
patients	**27**	**28.037 ± 6.11**

P-Value	**--**	**0.0001**
LSD Value	**--**	**45 ***
*** (P<0.001)**		

The previous study explained the Diminished TLR4 expression has been associated with reduced synthesis of TNF-α,IL-1β, IL-6, and IL-8 in response to LPS challenge (21).

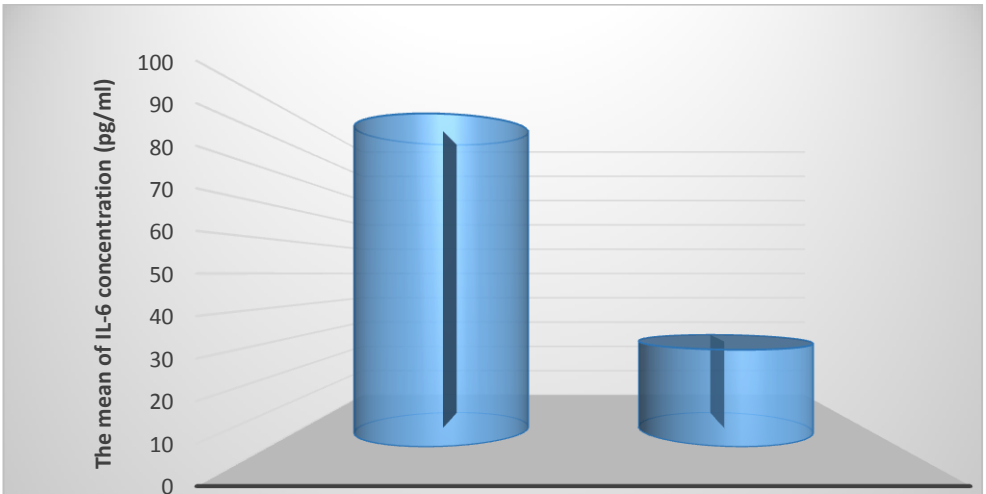


Figure (2) serum IL-6 level in hemodialysis and healthy groups

Similar results have been obtained in HD patients, and it has been suggested that, apart from uremia, endotoxins contained in the dialysate, by continuous stimulation, might eventually lead to a decrease in TLR4 expression (22), On

the other hand, clearance or membrane adsorption of cytokines during hemodialysis may alter circulating cytokine levels(18,23)

According to this result we can conclusion the uremia and hemodialysis is associated with immunosuppression due to the reducing the IL-6 serum level as inflammatory mediator

Conclusion and Recommendations

Conclusion:

The hemodialysis patients display decrease in the concentration of IL-6 serum level and this condition and this explain the high susceptibility to infection by various disease due to the anti-inflammatory state.

Recommendations:

Care about the patients' hygiene and maintenance them in clean environment after the dialysis route

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