Interleukin-6 Level in serm of Iraqi Patients on Maintenance Hemodialysis Therapy

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Summary

Back ground : *Chronic renal failure (CRF) is characterized by progressive destruction of renal mass with irreversible sclerosis and loss of nephrons over a period of at least months to many years which is treated either by dialysis which includes hemodialysis (HD) and peritoneal dialysis (PD) or renal transplantation.*

Objective : *To document the elevation of interleukin-6 (IL-6) in Iraqi HD patients, and to what degree that elevation influences secretion of C-reactive protein (CRP), in addition to its association with other acute phase proteins and kidney function tests .*

Method : *A total of 75 individuals were included in the present study, 50 individuals were treated with HD compared to 25 healthy volunteers.*

Result : *The level of serum IL-6 showed a significant increase in HD patients , and this elevation lead to a high increase in the secretion of CRP as well as the differences in levels of other serum acute phase proteins; ferritin revealed an increase in its level , while alb shows a decrease in its level . Kidney function tests were detected in sera of all the studied groups, which showed an increase in urea and Cr with a decrease in CrCI in patients group.*

Key words: *IL-6, CRP,Acute phase proteins, And Hemodialysis*

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 and 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).

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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 . This is common in hemodialysis (HD) patients and it is likely that elevation of interleukin-6 (IL-6) may play a central role for the close relationship between inflammation, malnutrition and cardiovascular disease in HD patients (4).

Subjects and Methods

1- Patients Study Group: -

During the period from June 2003 to August 2003, blood samples were collected from fifty patients (32 males & 18 females), age range 14-75 year with chronic renal failure undergoing hemodialysis (HD) at the center of renal diseases and transplantation in the Hospital of Surgicals Specialties (the Medical City in Baghdad). Clinically unstable patients and those with tumours, inflammatory diseases (such as diabetic ulcers, chronic active hepatitis or chronic pulmonary disease), or those treated with immunosuppresives, patients had chronic allograft nephropathy now on dialysis and does that *affect* 1L-6 level and any body had received blood or plasma or albumin for at least a week before sampling were excluded. No patient showed signs of inflammation or infection during the study period that potentially influencing IL-6. Body temperature was measured before each dialysis session, and was never elevated.

All patients had been subjected to HD one

to three times per week for 3-4 hours using cuprophane membrane and dialysate that consists of granules (sodium bicarbonate, sodium chloride, glucose, potassium chloride, magnesium chloride and calcium chloride) which dissolved in softened water. All dialysis parameters (such as blood flow, dialyzer membrane, and dialysis time) were kept constant during the study.

2- **Control Group: -**

Twenty five apparently healthy individuals (15males & 10 females) with no fever within last week, not receiving any medication, didn't have a history of any chronic or acute diseases were selected age range 19- 63 year.

Methods:

Collection of Blood Samples: -

Ten milliliters (mls) of venous blood were collected from each patient in hemodialysis unit before heparinization and healthy individuals after 12 hours fast. The blood samples were allowed to stand for 15 minutes (min) then centrifuged at 3000 rpm for 10 min.Serum was dispensed into two aliquots and immediately frozen at -20°c till used for the estimation of acute phase proteins,
and kidney function tests .

1 Serum Interleukin -6 (IL-6) Level:

Interleukin-6 was measured by enzyme immuno assay method using. a kit supplied by immunotech-France (Cat.No. 1120).

2 Serum C-Reactive Protein (CRP) Level:

C-reactive protein was measured by rapid test for the qualitative and semi quantitative determination of CRP in serum by agglutination of latex particles on slide using a kit supplied by linear chemicals-Spain (COD.3800-0146).

3 Serum Ferritin Level

Ferritin was measured by immunoradiometric assay method using a kit for
the quantitative determination of ferritin in human serum supplied by CIS bio international -France (B.p.32-F91192-GIF-SUR-YVETTECEDEX).

4 Serum Albumin (alb) urea , creatinine(Cr) :
were measured by colorimetric method using kits supplied by Randox laboratories ltd., united kingdom (Cat.No.AB 362).

5. Creatinine Clearance Level

Creatinine clearance was predicted from serum creatinine level according to Cockcroft DW and Gault MH formula (5) .
(Male) Creatinine clearance =1.23 (140 - age)
x weight (kg)/serum creatinine (i imol/1)
(Female) Creatinine clearance = 1.04
(140 - age) x weight (kg)/serum
creatinine(.tmol/1)

Statistical Methods:To compare the significance of the difference in the mean values of any two groups student t-test was applied .$p<0.05$ or $p<0.01$ was considered statistically significant.

Results:

The specific role of interleukin-6 (IL-6) in this context is not clear, and little attention has been paid to study the measurement of IL-6, therefore this study was undertaken to show the role of IL-6 and, its sequelae in hemodialysis (HD) patients.

1. Acute Phase Proteins

The mean' (±SD) of serum interleukin-6 (IL-6), ferritin and albumin (alb) concentrations and the sensitivity limit of C-reactive protein (CRP) in serum of hemodialysis (HD) patients and healthy individuals are illustrated in table (1).

Table (1): Mean (±SD) of serum acute phase proteins concentrations in HD patients and healthy individuals.

Parameters	n	Healthy individuals	n	HD patients
IL-6(pg/ml)	25	7.5±2.4	50	39±19.0**
CRP(mg/l)	25	<6	15	<6
			35	≥6
Ferritin(µg/l)	25	127±35.0	50	1990±1200**
Albumin(g/l)	25	39±2.5	50	37±4.4*

** P<0.0

* P<0.05

Table (1.1) Mean (±SD) of serum IL-6 according to seroreactivity for CRP

Seroreactivity for CRP	n	IL-6
Negative	15	30±5.0
Positive	35	43±17.0*

Negative: less than 6 mg/l

Positive: equal or more than 6 mg/l

* P<0.01

2-Kidney function tests:

Mean (±SD) of urea, creatinine (Cr) and creatinine clearance (CrCI) in serum of HD patients and healthy controls are illustrated in table (2).

Table (2): Mean (±SD) of serum kidney function tests Concentrations in HD patients and healthy individuals.

Parameters	n	Healthy individuals	n	HD patients
Urea(mmol/l)	25	6.5±0.66	50	23±7.1**
Cr(µmol/l)	25	85±17.68	50	890±224.96**
CrCl(ml/min)	25	97.72±23	50	7.83±2.8**

** P <0.01

Discussion:

Normal CRP levels do not exclude the presence of minor degrees of acute, localized inflammation or some chronic diseases. This may be related to a single measurement which is only useful to indicate inflammation within about 3 days of an. acute event, in addition to, low level of IL-6 in this group (6).

The increase in serum IL-6 of HD patients obtained in the present study may be due to the stimulation of it's production by hemodialysis procedure (i.e . bioincompatible membrane and the use of non-sterile dialysate) (7), since uremic patients with peritoneal dialysis or without dialysis showed no such high level (8), moreover the characteristics of the dialyzer membrane may affect the production of IL-6 during HD session , a significant increase in circulating IL-6 was observed at the end of HD in patients using cellulose membrane but not polysulfone membrane (9). On the other hand reduced renal function is related to IL-6 elevation in ESRD patients , in addition to, a number of factors prevalent in uremic patients,
such as hypertension, adiposity, insulin resistance, fluid overload, oxidative stress infections 'and chronic heart failure which may all contribute to this elevation (10).

Interleukin-6 (IL-6) is one of the most potent proinflammatory cytokines that initiates the acute phase response by hepatocytes and induces the synthesis of CRP (11), however this increase in serum CRP level has been linked to multiple factors, including effects of hemodialysis procedure, biocompatibility of the dialysis membranes as well as multiple hospitalization because of infections (12), (13).

The high blood CRP a marker of inflammation is demonstrated as a powerful predictor for the mortality in HD patients (14,15,16, 17,18,19).

Also others reported that levels of plasma CRP in the absence of active disease are low but can rise up to 1000- fold in patients with an inflammatory reaction and they found that the serum concentration of CRP reflects the activity of cytokine- mediated acute phase processes and is roughly proportional to the extent of tissue injury (14,20).

Hemodialysis patients showed a higher synthesis of ferritin than healthy individuals that may be related to the acute phase response to infection and injury that increases secretion of ferritin (21).

A reduction in the rate of albumin synthesis which may be caused by metabolic acidosis, impaired protein intake and inflammation shows a significant decrease in albumin level in HD patients (22). These results are supported by findings of other workers who suggested that proinflammatory cytokines (TNF-a and interleukins) induce an acute phase response in the liver, resulting in an increase in the degradation of albumin (23).

The increase in urea and creatinine level in serum of HD patients is due to the decrease in the number of functioning nephrons, which would reduce the GFR, which causes major decreases in renal excretion of water and solutes (1). It is well known that the increase in urea level is proportionally more than creatinine level because of its reabsorption by the tubular cells, particularly at low urine flow rate. This lead to a relatively higher serum urea level than creatinine which is not so reabsorbed (2),as a result this lead to loss of the ability of the kidney to clearance which can explain the decline of creatinine clearance level.

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