Determination of Interleukin-35 in Sera of Male Patients with Chronic Renal Failure on Hemodialysis

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Abstract

Background:- Renal failure may lead to increased inflammatory responses through a number of mechanisms which include decreased clearance of proinflammatory cytokines, decreased levels of antioxidant , that lead to increase of oxidative stress , nephrons are destroyed and kidney function is reduced .

Objective:- The aims of this study were to determination the differences in the levels of interleukin-35 (IL-35) in patients with chronic renal failure (CRF) on hemodialysis and compare with control group.

Method:- Sixty male individuals (age 40-60) were enrolled in this study which were divided into two groups as follows: Group (C) consisted of 30 healthy individuals as a control subject, (30) patients with (CRF) which were collected of their serum pre and post hemodialysis and control. IL-35, hs-CRP, TAC, MDA, MDA/TAC, urea, creatinine, uric acid, albumin levels were evaluated.

Results:- The results in this study revealed that mean of serum IL-35 levels were significantly increased differences in patients with (CRF) pre and post hemodialysis compared with control group and a significant positive correlation between IL-35 and (MDA, MDA/TAC) in patients with pre and post dialysis patients with CRF.

Conclusion:- Our findings suggest that a high IL-35 level most possibly reflect that the patients with (CRF) are associated and the positive correlation with oxidant-antioxidant status. Therefore, we suggest that possible antioxidant strategies that can be used therapeutically for better management of (CRF).

Keywords :IL-35, antioxidant, chronic renal failure.

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Introduction

The interleukin -12 (IL-12) cytokine family contain IL-12, IL-23, IL-27 and IL-35. Interleukin 35 (IL-35) is produced by regulatory, but not effector, T-cells and plays a role in immune suppression. It is a dimeric protein composed of IL-12α and IL-27β chains (p35 and EB13), which are encoded by two separate genes called IL12A and EBI3, respectively. IL-35 is not constitutively expressed in tissues, but the gene encoding IL-35 is transcribed by vascular endothelial cells, smooth muscle cells and monocytes after activation with proinflammatory stimuli. [1, 2]

Renal insufficiency may develop, when nephrons are destroyed as in chronic glomerulonephritis, infection of renal pelvis and nephrons or loss of a kidney or when kidney function is reduced by damage or blockage. This can cause retention of salt, water, uremia and inability to excrete urea, H^+, K^+. If the blood contains too much

creatinine or urea and urine contains protein, kidneys may not be functioning properly. [3]

Renal failure may lead to increased inflammatory responses through a number of mechanisms which include decreased clearance of proinflammatory cytokines, decreased levels of antioxidant. [4]

One of the most dramatic changes is increase in blood serum levels of an inflammatory marker known as C-reactive protein (CRP). [5] Interleukin-1 (IL-1),interleukin-6 (IL-6),and tumor necrosis factor (TNF) are the major cytokines that stimulate the liver to synthesis CRP and other positive acute-phase proteins. [6]

Reactive oxygen species (ROS) play a key role in the pathophysiological processes of renal diseases. The cellular damage is mediated by an alteration in the antioxidant status, which increases the concentration of ROS in the stationary state (oxidative stress). Oxidative stress mediates a wide range of renal impairments, from acute renal failure, rhabdomyolysis, obstructive nephropathy, hyperlipidemia, and glomerular damage to chronic renal failure and hemodialysis. [7]

The aims of this study were to determination of IL-35 levels in patients with (CRF) on hemodialysis and study the relationships between IL-35 and some biochemical parameters such as urea, creatinine, uric acid, albumin, high sensitive c-reactive protein (hsCRP) and antioxidant status.

Materials and Methods

Blood samples were collected from sixty male individuals (age 40-60) years; thirty male were patients with (CRF), which were enrolled pre and post hemodialysis in Medicine City Hospital; the other male individuals were normal healthy subjects. Other complications such as blood pressure, diabetic mellitus, smoker, or inflammation were excluded from patients and normal healthy subjects.

Patients and control groups were determined the following parameters:

- Serum interleukin-35 (IL-35) and (hsCRP) levels were measured by enzyme-linked immune sorbet assay (ELISA) method. [8, 9]
- Malondialdehyde (MDA) level was determined in serum as a thiobarbituric reactive species according to the method of Buege and Aust [10] .
- Total antioxidant capacity (TAC) was measured in serum using Randox Total Antioxidant Capacity (Cat No. Nx 2331) according to the method of Miller. [11]
- Urea, creatinine, uric acid, albumin concentration were measured using enzymatic colorimetric method. [12, 13, 14, 15]

Results

- Mean±SD of IL-35, hsCRP, MDA, TAC, MDA/TAC, urea, creatinin, uric acid and albumin for CRF pre and post dialysis patients with CRF were revealed in table (1). Mean of serum IL-35 , hsCRP, MDA, TAC, MDA/TAC, urea, creatinin, uric acid, albumin levels in pre dialysis patients with CRF were significantly differences higher than in the control group $P<0.05$. Mean of serum hsCRP, MDA, TAC, MDA/TAC, urea, creatinin, uric acid, albumin levels in post dialysis patients with CRF were significantly differences lower than in the control group $P<0.05$. Mean of serum hsCRP, MDA, TAC, MDA/TAC, urea, creatinin, uric acid levels in pre dialysis patients with CRF were significantly differences higher than in the post dialysis patients $P<0.05$, and albumin insignificantly difference higher than in the post dialysis patients.
- Table (2) showed the correlation between IL-35 and hsCRP, MDA, TAC, MDA/TAC, urea, creatinin, uric acid, albumin for the pre and post dialysis patients with CRF. The results in table (2) can be classify into:-
- 1-Correlation of IL-35 with special test:-

- The results in table (2) revealed significant positive correlation between IL-35 and hsCRP in patients with pre and post dialysis patients, significant positive correlation between IL-35 and (MDA, MDA/TAC) in patients with pre and post dialysis patients with CRF, significant positive correlation between IL-35 and TAC in patients with pre dialysis patients and significant negative correlation between IL-35 and TCA in patients with post dialysis patients with CRF.

- 2-Correlation of IL-35 with general criteria test:-
- The results in table (2) revealed no significant positive correlation between IL-35 and urea, creatinin, uric acid in patients with pre and post dialysis patients with CRF and no significant negative correlation between IL-35 and albumin in pre and post dialysis patients with CRF.

Table (1): Mean±SD and p-value of IL-35, hsCRP, MDA, TAC, MDA/TAC, urea, creatinin, uric acid, albumin for the control, pre and post dialysis patients with CRF.

Groups / parameter	Mean±SD			P-Value		
	Control	Pre	Post	C-Pre	C-post	Pre-Post
IL-35(pg/mL)	25.2±5.4	٤3.4±8.3	34.2±10.7	P<0.05	P<0.05	P<0.05
hsCRP(g/mL)	2.25±0.42	24.8±15.2	19.4±11.5	P<0.05	P<0.05	P<0.05
MDA(µmol/L)	0.73±0.2	2.81±0.51	1.63±0.35	P<0.05	P<0.05	P<0.05
TAC(mmol/L)	0.8±0.12	0.33±0.04	0.51±0.07	P<0.05	P<0.05	P<0.05
MDA/TAC	1.2±0.32	10.7±2.0	5.87±1.72	P<0.05	P<0.05	P<0.05
Urea(mmol/L)	5.1±1.2	32.7±2.3	22.1±5.7	P<0.05	P<0.05	P<0.05
Creatinin(µmol/L)	67.3±12.2	822±153.2	574±83.7	P<0.05	P<0.05	P<0.05
Uric acid(mg/dL)	٣.3±0.2	5.6±2.2	4.1±1.3	P<0.05	P<0.05	P<0.05
Albumin(g/L)	41.02±9.3	33.5±8.8	33.1±10.2	P<0.05	P<0.05	P>0.05

Table (2): Correlation between IL-35 and other parameters for the patient groups.

groups / IL-3٥(pg/ml)	Pre		post	
	r	p-value	r	p-value
hsCRP(g/L)	0.55	S	0.61	S
MDA(µmol/L)	0.42	S	0.83	S
TAC(mmol/L)	0.41	S	-0.44	S
MDA/TAC	0.42	S	0.64	S
Urea(mmol/L)	0.3	NS	0.1	NS
Creatinin(µmol/L)	0.33	NS	0.25	NS
Uric acid(mg/dL)	0.05	NS	0.24	NS
Albumin(g/L)	-0.08	NS	-0.16	NS

Discussion

This is the first study to document, as far to knowledge, which depict the determination of IL-35 levels in CRF patients on hemodialysis.

The importance of regulatory T cells is playing a central role in preventing autoimmunity. These regulatory responses use a variety of mechanisms to mediate suppression, including soluble factors. The recently identified IL-35 has been shown to have potent suppressive function in vitro and in vivo. Furthermore, not only does IL-35 have the ability to directly suppress effector T cell responses, it is also able to expand regulatory responses by propagating infectious tolerance and generating a potent population of IL-35-expressing inducible Tregs. [2]

IL-23, a heterodimer composed of IL-12p40 and IL-23p19 subunits, and IL-17A, a cytokine produced by the IL-23-maintained Th17 subset, may play a pivotal role in the pathogenesis of coexisting lupus nephritis and anti-glomerular basement membrane disease. However, further studies are necessary to elucidate the exact molecular role and signaling transduction pathway of IL-23 and IL-17 during the disease process. [16]

Infiltrating T cells can induce tissue injury, either directly by cytotoxic functions and cytokine secretion or indirectly by activating macrophages. [17, 18] Infiltrating effector T cells of the Th1 type are supposed to initiate and perpetuate renal tissue damage in crescentic and proliferative forms of glomerulonephritis, which eventually leads to progressive loss of renal function ,deficient in the Th2 cytokines have more pronounced Th1 responses and develop more severe glomerulonephritis. [19, 20]

Pro-inflammatory cytokines (TNF-α and interleukins) induce an acute phase response in the liver, resulting in an increase in the degradation of albumin, increase in CRP levels in hemodialysis patients indicates inflammation. It is revealed that CRP is a powerful factor in predicting the complications and mortality in hemodialysis patients. [21]

Studies in mice show the absence of either IL-35 chain from regulatory Tregs reduces the cells' ability to suppress inflammation; this has been observed during cell culture experiments and using an experimental model for inflammatory bowel disease. [22] To produce its suppressive effects, IL-35 has selective activities on different T-cell subsets; it induces proliferation of Treg cell populations but reduces activity of Th17 cell populations. [23]

Cytokine activation is associated with reduced renal function or other pro-inflammatory conditions in dialysis patients such as the frequent contact with dialyzer membranes, vascular grafts, catheters or dialysate, each may constitute a pro-inflammatory factor. Increased release or activation of inflammatory cytokines, since albumin is a negative acute phase protein, may suppress appetite, cause muscle proteolysis and hypoalbuminemia. [24]

Serum albumin level in maintenance hemodialysis patients are dependent on the nutrition and inflammation, additionally plasma volume expansion can dilute the plasma pool. [25]

The increase in urea, creatinine and uric acid levels in serum of hemodialysis patients are due to the decrease in the number of functioning nephrons, which reduce the glomerular filtration rate and causes major decreases in renal excretion of water and solutes. [26]

Hemodialysis removes toxins from the blood by a closed loop process where the blood of the patients is continuously being withdrawn, dialyzed and returned to the patients [27].

Formation of ROS is part of the unspecific defense system of an organism against bacteria and other microbes. However, ROS may also affect cells of the host organism, in particular at sites of inflammation. The latter plays a role in a variety of renal diseases, such as glomerulonephritis, acute or progressive renal failure, or tubulointerstitial nephritis [28], oxygen radicals may contribute to hypertrophy of tubular cells. [29]

The imbalance between oxidant and antioxidant systems and the depletion of the protective antioxidant system and production of ROS in patients with CRF are leading to renal diseases, so the recruitment of T cells into the kidney is generated. Infiltrating T cells can induce tissue injury, generation of iTr35 cells, IL-35 expression and their conversion into iTr35 regulatory T cells, suggestion that these cells may play a role in promoting renal failure. The patients with renal failure, the renal functions are decreased. Serum levels of urea, creatinine , uric acid, IL-35, hsCRP, MDA, TAC, MDA/TAC are increased due to the reduce the glomerular filtration rate and causes major decreases in renal excretion of water and solutes, and the levels of these parameters post hemodialysis are lower than pre hemodialysis because of the plasma volume expansion can dilute the plasma pool. Other finding of the present study that IL-35 levels in patients group most possibly reflects the inflammatory component, we suggest that possible antioxidant strategies that can be used therapeutically for better management of patients with (CRF).

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