Biochemical studies of interleukin-2, 4, 6 and 8 in patients with chronic liver and kidney diseases

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Abstract: Background: *Interleukins are a group of cytokines (secreted proteins/signaling molecules) that were first seen to be expressed by white blood cells (leukocytes). In chronic hepatitis C, intra-hepatic expression of both IL-8 and IL-2 increased with fibrosis and inflammatory activity. Positive correlations were found between IL-8 and other cytokines and between cytokines themselves. These findings suggest that these interacting cytokines play an active role in the pathogenesis of CHC, and maybe involved in the up regulation or induction of one and other, and interleukin-6 (IL-6), the major cytokine inducers of the acute phase response, are markedly raised in acute alcoholic hepatitis and correlate closely with clinical and laboratory indicators of disease severity.*

Methods: *We measured (IL-2, IL-4, IL-6 and IL-8) serum levels in 60 patients classified into three different groups twenty chronic renal failure patients, twenty liver disease patients and twenty patients of chronic renal failure combined with liver diseases in comparison to twenty healthy controls. Serum (IL-2, IL-4, IL-6 and IL-8) were determined using ELISA technique.*

Results: *IL-2 and IL-6 were significantly highest in HCV and combined groups with no significant difference between them, followed by renal group compared to IL-4 and IL-8 were significantly highest in combined group, followed by renal group, followed by HCV group and lowest in control group.*

Conclusions: *IL-2 and IL-6 are elevated in patients with chronic HCV disease. IL-4 and IL-8 are elevated in chronic renal failure.*

Keywords: *Chronic HCV; Chronic renal failure; Interleukins*

I.   Introduction

IL-2 is necessary for the growth, proliferation, and differentiation of T cells to become 'effector' T cells. IL-2 is normally produced by T cells during an immune response. Antigen binding to the T cell receptor (TCR) stimulates the secretion of IL-2, and the expression of IL-2 receptors IL-2R. The IL-2/IL-2R interaction then stimulates the growth, differentiation and survival of antigen-specific CD4+ T cells and CD8+ T cells [1]. As such, IL-2 is necessary for the development of T cell immunologic memory, which depends upon the expansion of the number and function of antigen-selected T cell clones.IL-2 is also necessary during T cell development in the thymus for the maturation of a subset of T cells that are termed regulatory T cells (T-regs) [2].

Interleukin-4, is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4. The cell that initially produces IL-4, thus inducing Th0 differentiation, has not been identified, but recent studies suggest that basophils may be the effector cell [3]. It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces B-cell class switching to IgE, and up-regulates MHC class II production. IL-4 decreases the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell IL-12. Overproduction of IL-4 is associated with allergies [4]. Tissue macrophages play an important role in chronic inflammation and wound repair. The presence of IL-4 in extravascular tissues promotes alternative activation of macrophages into M2 cells and inhibits classical activation of macrophages into M1 cells. An increase in repair macrophages (M2) is coupled with secretion of IL-10 that results in a diminution of pathological inflammation. Release of arginase, proline and polyaminases by the activated M2 cell is tied with wound repair and fibrosis [5].

Interleukin-6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL-6 gene. IL-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage

leading to inflammation. IL-6 also plays a role in fighting infection, as IL-6 has been shown in mice to be required for resistance against bacterium Streptococcus pneumonia **[6].** IL-6 is an important mediator of fever and of the acute phase response. It is capable of crossing the blood-brain barrier and changing the body's temperature set point. In muscle and fatty tissue, IL-6 stimulates energy mobilization that leads to increased body temperature. IL-6 can be secreted by macrophages in response to specific microbial molecules, referred to as pathogen-associated molecular patterns (PAMPs). These PAMPs binding to an important group of detection molecules of the innate immune system, called pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). These are present on the cell surface and intracellular compartments and induce intracellular signaling cascades that give rise to inflammatory cytokine production **[7].** IL-6 is also essential for hybridoma growth and is found in many supplemental cloning media such as briclone. Inhibitors of IL-6 (including estrogen) are used to treat postmenopausal osteoporosis. IL-6 is also produced by adipocytes and is thought to be a reason why obese individuals have higher endogeneous levels of CRP. Intranasally administered IL-6 has been shown to improve sleep-associated consolidation of emotional memories **[8].** IL-6 is responsible for stimulating acute phase protein synthesis, as well as the production of neutrophils in the bone marrow. It supports the growth of B cells and is antagonistic to regulatory T cells.

Interleukin-8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies**.** In humans, the interleukin-8 protein is encoded by the IL-8 gene **[9].** There are many receptors on the surface membrane capable of binding IL-8; the most frequently studied types are the G protein-coupled serpentine receptors CXCR1 and CXCR2. Expression and affinity for IL-8 differs between the two receptors (CXCR1 > CXCR2). Toll-like receptors are the receptors of the innate immune system. These receptors recognize antigen patterns (like LPS in gram negative bacteria). Through a chain of biochemical reactions, IL-8 is secreted and is an important mediator of the immune reaction in the innate immune system response. IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL-8 also induces phagocytosis once they have arrived. IL-8 is also known to be a potent promoter of angiogenesis. In target cells, IL-8 induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular Ca^{2+}, exocytosis (e.g. histamine release), and the respiratory burst **[10].**

Aim of the work

Biochemical utility of interleukins (IL-2), (IL-4), (IL-6) and (IL-8) as a marker in chronic renal diseases alone, liver diseases alone and also in chronic renal diseases combined with liver diseases. In addition some biochemical parameters, such as urea, creatinine, AST, ALT, Uric acid, albumin and glucose level, will be analyzed.

II.   Materials & methods

Group (1) included twenty healthy normal subjects (11 males & 9 females), they were studied as control group. Sixty patients who approved (signed informed written consent) to share in the study through Three groups:,
Group (2) consisted of twenty chronic renal failure patients included (11 males & 9 females) who were on periodic heamodialysis three times per week,
Group (3) consisted of twenty chronic HCV patients (10 males & 10 females) and
Group (4) included twenty chronic renal failures combined with chronic HCV disease patients (combined group), (10 males & 10 females).

Blood samples collection

Blood sample were collected from all subjects drawn after an overnight fast of at least 8 hours and for subjects which undergo heamodialysis the samples were drawn before the heamodialysis using a 10 ml disposable syringe. About 8 ml of blood were added to vacutainer plane tubes, the blood was left for about 10 minutes to allow blood to clot then, serum samples were obtained by centrifugation at (2000 rpm for 10 minutes). Serum samples were divided into two aliquots one for biochemical analysis and the other which stored at ≤ -20°C for cytokines.

Routine laboratory investigations (Liver function tests as (ALT, AST, and serum albumin), Kidney function tests as (urea, creatinine and uric acid), Fasting Blood Sugar) were determined using standard kits. Cytokines Interleukin-2, Interleukin-4, Interleukin-6 and Interleukin-8 were determined using ELISA kit **[11, 12, 13 and 14].** This assay employs the quantitative sandwich enzyme immunoassay technique. Polyclonal antibody specific for interleukins have been pre-coated onto a microplates. Standards and samples were pipette into the wells and any interleukins present were pounded by the immobilized antibody. After washing away any unbounded substances, an enzyme-linked polyclonal antibody specific for interleukins were added to wells.

Following a wash to remove any unbounded antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of interleukins bound in the initial step. The color development was stopped and the intensity of the color was measured.

Statistical methods

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 18.0. Descriptive statistics were done for the quantitative parametric data as mean ±SD (standard deviation) and minimum& maximum of the range, while they were done for qualitative data as number and percentage. Inferential analyses were done for quantitative variables using independent t-test in cases of two independent groups, ANOVA test with post hoc LSD for more than two independent groups. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions, while correlations were done using Pearson Correlation. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

III.   Results

The four studying groups were matched for age and sex. Chronic Renal group and combined group were homogenous for Duration of renal disease.

IL-2 and 6 were significantly highest in chronic HCV and combined groups with no significant difference between them, followed by renal group and lowest in control group. IL-4 and 8 were significantly highest in combined group, followed by renal group, followed by HCV group and lowest in control group. ALT and AST were significantly highest in HCV and combined groups and lowest in control and renal groups with no significant difference between them. Albumin was significantly highest in control groups with no significant difference between other groups. Urea, creatinine and uric acid were significantly highest in renal and combined groups with no significant difference between them, followed by HCV and control groups. Fasting was significantly highest in HCV and combined groups than control and renal groups (Table 1). In combined group there was a significant positive correlation between Interleukin-2 and creatinine in combined group (Table 2).

Table (1): Control subjects and patients.

	Control group	Chronic Renal group	Chronic HCV group	Combined group	P
	Mean±SD				
N	20	20	20	20	
Age (years)	38.9±4.3	38.5±4.3	38.7±4.4	38.6±4.6	*>0.050
Sex (Male) n (%)	11 (55.0%)	11 (55.0%)	10 (50.0%)	10 (50.0%)	^>0.050
Duration of renal disease (days)		3.2±0.6		3.0±0.7	#>0.050
Duration of liver disease (days)			3.8±0.8	4.2±0.8	#>0.050
IL-2 (pg/ml)	9.4±0.6a	27.4±4.7b	102.3±6.3c	104.2±5.2c	*<0.001
IL-4 (pg/ml)	13.4±2.6a	100.5±6.9b	37.3±7.1c	121.9±14.6d	*<0.001
IL-6 (pg/ml)	20.3±3.9a	38.8±6.9b	98.6±7.0c	103.1±4.7c	*<0.001
IL-8 (pg/ml)	25.7±4.3a	103.4±8.7b	43.4±8.7c	130.3±8.6d	*<0.001
ALT (U/L)	18.7±4.4a	13.2±4.1b	252.9±14.1c	256.5±6.1c	*<0.001
AST (U/L)	19.4±4.3a	18.2±5.6a	80.0±8.9b	57.6±6.4b	*<0.001
Albumin (g/dl)	4.2±0.5a	2.9±0.3b	3.0±0.3b	2.9±0.3b	*<0.001
Urea (mg%)	39.5±6.8a	105.5±9.0b	43.9±2.8a	108.0±9.4b	*<0.001
Creatinine (mg%)	0.5±0.3a	7.3±0.9b	0.7±0.3a	7.3±1.3b	*<0.001
Uric Acid (mg/dl)	3.6±0.4a	6.2±0.5b	4.0±0.7c	6.5±0.6b	*<0.001
Fasting glucose (mg/dl)	95.4±10.4a	100.6±6.9a	114.3±9.3b	111.7±9.2b	*<0.001

IL: Interleukin, ALT: Alanine transaminase, AST: Aspartate transaminase, *ANOVA, #Independent t-test, ^Chi square test
Homogenous groups labeled as a, b, c and d upon post hoc LSD test

Table (2): Correlation between Interleukins and laboratory findings

	IL-2		IL-4		IL-6		IL-8	
	r	p	r	p	r	p	r	p
Chronic Renal group								
Urea	0.311	0.181	0.292	0.212	0.364	0.114	-0.031	0.898
Creatinine	0.186	0.433	0.152	0.523	-0.147	0.536	0.171	0.470
UA	0.048	0.840	0.016	0.947	-0.313	0.180	0.199	0.400
Chronic HCV group								
ALT	0.039	0.870	-0.096	0.688	0.009	0.969	-0.198	0.402
AST	-0.113	0.636	0.046	0.846	-0.135	0.569	0.046	0.848
Albumin	-0.059	0.806	0.003	0.99	-0.285	0.224	0.102	0.669
Combined group								
ALT	-0.036	0.882	-0.312	0.180	-0.323	0.165	0.200	0.397
AST	-0.170	0.474	-0.438	0.053	-0.160	0.501	-0.293	0.210
Albumin	-0.248	0.291	-0.014	0.952	0.193	0.415	-0.146	0.538
Urea	-0.238	0.311	-0.171	0.470	-0.025	0.916	0.061	0.797
Creatinine	0.502	**0.024**	-0.033	0.891	0.109	0.647	-0.064	0.787
UA	0.078	0.744	-0.312	0.180	-0.107	0.654	0.037	0.877

IV. Discussion

IL-2 is necessary for the growth, proliferation, and differentiation of T cells to become 'effector' T cells. IL-2 is normally produced by T cells during an immune response. Antigen binding to the T cell receptor (TCR) stimulates the secretion of IL-2, and the expression of IL-2 receptors IL-2R. The IL-2/IL-2R interaction then stimulates the growth, differentiation and survival of antigen-specific CD4+ T cells and CD8+ T cells [15].

Interleukin-6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL-6 gene. IL-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation [16].

The present study showed that the level of serum interleukin-2 and interleukin-6 were significantly highest in HCV and combined groups with no significant difference between them, followed by renal group and lowest in control group. The high increased level in case of HCV due to circulating inflammatory cells, especially from the monocyte lineage. These indicate that sIL-2R might be a potential marker for immune cell activation in CLD, especially for proinflammatory and profibrogenic non-classical CD14+CD16+ monocytes. This explanation mentioned by [17].

Elevated plasma IL-6 levels in ESRD patients may be related to (i) the loss of kidney function, (ii) uraemia per se (and its sequelae, such as fluid overload, oxidative stress and susceptibility to infections) and (iii) dialysis-related factors. Even before the initiation of dialysis therapy, patients with decreased renal function already demonstrated signs of inflammation and the deterioration of renal function has been associated with a significant increase in serum cytokine levels. This explanation mentioned by [18].

The present study was in agreement with [19] they pointed to an increased serum interleukin-2 in infection with hepatitis C virus and had demonstrated that hepatocytes represent the principal source of the cytokine in HCV in vivo infection. Mitochondrial localization of IL-2 suggested a direct involvement of the cytokine in disturbed function of the organelles.

Also the present study was in agreement with [20] they found that the plasma levels of sIL-2Ra were predictive of long-term renal disease progression in a large cohort of patients with biopsy-proven IgAN. Further studies were warranted to evaluate if sIL-2Ra levels feasibly contributed in the monitoring of effects of treatment, aimed to prevent the progression of interstitial fibrosis and progressive glomerulosclerosis in IgAN.

[21] analyzed the serum IL-2 in patients with renal failure and reported that sIL2-Ra levels were significantly higher in patients than in controls (P < 0.001). sIL-2Ra levels in the upper third tertile predicted a severe renal outcome, even after adjustment for the main clinical risk factors: time average albuminuria and GFR at baseline (Relative risk 5.35, P < 0.001). sIL-2Ra levels also correlated significantly to the yearly GFR slope ($\beta = -0.24$, P = 0.01).

Our experimental IL-6 results are in agreement with the study of [22] who reported that The experiments indicate an involvement of IL-6 in the pathogenesis of liver diseases and suggest a protective role of IL-6/gp130-dependent pathways in nonparenchymal liver cells during fibrosis progression in chronic liver diseases.

IL-4 is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4. The cell that initially produces IL-4, thus inducing Th0 differentiation, has not been identified, but recent studies suggest that basophils may be the effector cell [23].

Interleukin-8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies**.** In humans, the interleukin-8 protein is encoded by the IL-8 gene **[24].**

The present study showed that the serum level of interleukin-4 and interleukin-8 were significantly highest in combined group, followed by renal group, and followed by HCV group and lowest in control group. Because during a hemodialysis session, cytokines were released mainly by monocytes activated by endotoxin-type compounds in dialyzer fluid, complement factors and direct contact with dialyzer membrane. So they highly increased in case of heamodialysis patients. This explanation mentioned by **[25]**.

In case of HCV patients IL-8 are elevated this elevation, due to the elevation of Th2 cytokine levels may represent a systemic response and not a result of increased local production within the liver **[26].**

Our experimental IL-4 results are in agreement with the study of **[27]** who reported that higher levels of circulating proinflammatory cytokines (IL-2, IL-4, IL-5, IL-12, T-cell number and function) were associated with mortality, while immune parameters reflecting improved T-cell function were associated with survival in ESRD patients treated with HD, independent of other medical risk factors. These factors may serve as markers for outcome. The mechanism underlying the relationship of immune function and survival, and the effect of interventions to normalize immune function in HD patients should be studied.

On the other hand in case of liver diseases **[28]** found that one striking clinical feature of hepatitis C virus (HCV) infection was that more than 50% of patients with acute hepatitis C will develop chronic infection. They examined the activation of type 2-like T-helper (Th2-like) cells relating to the development of chronicity. Peripheral blood CD4+ T-cell proliferation and cytokine secreation in response to a panel of recombinant HCV antigens including core (C22), envelope 1 (E1), E2, nonstructural (NS) protein 4 (C100), fusion protein of NS3 and NS4 (C200), and NS5 were assayed in patients with acute hepatitis C.IL-4 and IL-10 (Th2 responses) were detectable, and C22-spicific Th2-like T-cell clones could be generated from patients with chronicity. So that activation of Th2 responses in acute hepatitis C patients may play a role in the development of chronicity.

Our experimental IL-8 results are in agreement with the study of **[29]** he found that serum IL-8 levels were .higher in patients with HCV compared with control subjects, and in patients with HCC associated with HCV infection compared with control subjects. They found also that serum level of IL-8 is significantly higher in patients of chronic hepatitis C with HCC than patients of chronic hepatitis C without HCC.

From Other hand in case in chronic renal failure patients **[30]** Found that the serum concentration of IL-8 was significantly higher in the HD patients before and after dialysis than in the controls.

The patients with chronic renal failure commonly present with abnormalities of immune function related with impaired kidney function and the accumulation of uremic toxins in addition to bioincompatibility of dialyzer membranes.

V.   Conclusion

IL-2 and IL-6 are elevated in patients with chronic HCV disease. IL-4 and IL-8 are elevated in chronic renal failure.

Abbreviations

IL= Interleukine; CLD= Chronic liver diseases; TCR= Tcell receptor; T-regs= Regulatory T cells; PAMPs= Pathogen-associated molecular patterns; PRRs= Pattern recognition receptors; TLRs= Toll-like receptors; SD= Standard deviation; HCV= Hepatitis C virus; GFR= Glomerular filtration rate; HD= Hemodialysis; sIL-2R= soluble interleukin-2 receptor.

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