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Evaluation Level and Effect of IL-2 on Lipid Profile and Liver Function of Patients with Liver Fibrosis

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Abstract

Background: role of IL-2 in liver disorders not clear therefore present study conducted to determine level and effect of IL-2 on lipid profile and liver function of patients with liver fibrosis. **Method:** this study included sample collection from 44 people suffering from liver fibrosis and 44 healthy people as a control group. ELISA test used for quantitative determination of IL-2. FUJI DRI-CHEM SLIDE test used to liver enzymes and lipid profile. C-REACTIVE PROTEIN TEST kit is a rapid immunochromatographic device for the semi-quantitative detection of CRP in whole blood samples. **Results:** we institute a upper concentration of IL-2 in sick (242.56 ± 100.1 ng/ml) compared to controls (43.11 ± 21.3 ng/ml). we did not find a clear relationship or effect of IL-2 on liver function, as the increase or decrease of this immune indicator was not accompanied by an effect on the concentration of TSB, ALT, AST, CRP and PT (r= 0.169, 0.091, 0.032, 0.190 and 0.063 respectively), except for ALP and albumin, which was slightly affected by the change in the concentration of IL-2 (r= 0.341 and 0.314 respectively). The results showed a clear relationship between the concentration of IL-2 with the level of cholesterol, triglyceride and LDL, as the increase or decrease in IL-2 coincided with the increase or decrease of these lipids indicators in the blood of patients (r= 0.393, 0.316, 0.409 respectively), except HDL that it was not affected or interacted with IL-2 (r = 0.009). **Conclusion**: IL-2 significantly associated with liver fibrosis might be linked to its effects on lipid profile, ALP and albumin.

Keywords: IL-2, Liver, Fibrosis, Lipid, ELISA

Introduction

Enzymatic antioxidants comprised superoxide dismutase (SOD), catalase (CAT), Alanine amino transferase (ALT), aspartate amino transferase (AST), glutathione

peroxidase (GPx), alkaline phosphatase (ALP) linked by liver function [1]. Oxidative stress is the state that results from a disruption in the liver's antioxidative balance. Excessive reactive species originating from nitrogen and oxygen can nevertheless cause oxidative damage to organs and tissues[2]. Liver injury is triggered and worsened by oxidative stress, which has been identified as a joint pathogenic mechanism. Numerous risk factors, such as drugs, alcohol, irradiation, chronic viral hepatitis, and environmental pollutants, can cause oxidative stress in the liver, which can lead to severe liver fibrosis (LF)[3]. By causing irreversible changes to the contents of lipids, proteins, and DNA—and, more crucially, by altering pathways that regulate regular biological functions—oxidative stress causes hepatic damage [4]. These pathways control the transcription of genes, the expression of proteins, cell death, and the activation of hepatic stellate cells[5]. Research has shown that liver function and lipids metabolism are indirectly linked to each other, and that immune disorders have a direct relationship with these indicators, especially when liver disorders are in the stage of chronic immune inflammation [6,7].

The course of liver injury is determined by the interactions between immune effector cells, local fibroblasts, and tissue macrophages at sites of scar formation. Chronic inflammation and fibrosis are closely related [8]. It is now evident that both the innate and adaptive immune systems play a role in the regulation of fibrosis due to our growing understanding of the mechanisms regulating inflammation and fibrosis [9]. Hepatic KC are tissue macrophages that are derived from circulating monocytes and make up 15% of the total population of liver cells. The phenotype and function of macrophages play a crucial role in determining whether an injury resolves or leaves fibrotic scars [10]. Upon being stimulated by bacterial products, KCs release an abundance of fibrogenic and pro-inflammatory mediators. An example of an interleukin, or cytokine signaling molecule in the immune system, is interleukin-2 (IL-2). This protein, which has a molecular weight of 15–16 kDa, controls the immune-stimulating properties of leukocytes, or white blood cells [11]. The body naturally produces IL-2 in response to microbial infection and in order to distinguish between "self" and "non-self" substances. By attaching to IL-2 receptors, which are expressed by macrophages and lymphocytes, IL-2 mediates its effects. Activated CD4+ and CD8+ T lymphocytes are the main sources of IL-2 [12]. There are generally very few studies discussing the function of IL-2 in liver disorders. Thus, the purpose of this investigation was to ascertain IL-2's function in liver fibrosis as well as how it relates to lipid levels and liver function.

Materials and methods

Study strategy and samples gathering: The current training is a case - control study that was conducted during the period from 3/9/2022 to 10/10/2023. Samples were collected from Al-Diwaniyah Teaching Hospital, outpatient clinics, Medical City in Baghdad and Gastrointestinal Unit at Al-Hakim Hospital/Najaf Al-Ashraf. The number of cases was 44 people suffering from liver fibrosis and 44 healthy people as

a control group. Moreover, consent was also obtained from the participants before collecting the questionnaire or taking blood samples. Six ml of blood stood taken as of completely contributors for conducting the required tests.

Human Interleukin 2 (IL-2) evaluation: In this study, ELISA was utilized in accordance with business guidelines (BT-LAB/USA) to quantitatively determine the amount of IL-2 in serum samples from both patient and healthy control subjects.

Liver function tests: FUJI DRI-CHEM SLIDE (China) are used to determine total Albumin, glutamic oxalacetic transaminase/aspartate aminotransferase activity (AST-P III), total serum bilirubin (TSB), alanine aminotransferase activity (ALT-P III) and alkaline phosphatase activity (ALP-P III).

C-reactive protein (CRP) test: C-REACTIVE PROTEIN EXAMINATION kit (USA) stays a rapid immunochromatographic device for the semi-quantitative detection of CRP in whole blood samples.

Lipids test: FUJI DRI-CHEM SLIDE (FUJIFILM /Takeo /Japan) are used to determine total Cholesterol, high-density lipoprotein (HDL) triglyceride, and low-density lipoprotein (LDL) concentration.

Ethics approval and consent to participate: The study received ethical agreement as of the Continuing Education Unit of Hospitals. After the researcher explained the aim, objectives, and methods of the training, the patients provided their written well-versed consensus during they contributed in the training.

Statistical analysis: The current study included information examination using the Statistical Package for the Social Sciences, version 22. We also used Excel 2010. Significant differences smaller than 0.05 were considered statistically association [13].

Results

The current study is a case- control Study, included 44 patients distress as of LF, whose ages ranged between 29-78 years, through an average age of 61.16 ± 11.16 years, and the majority of them were male, at a rate of 68%, as in Table (1). On the other hand, the study included 44 healthy people as a control group, whose ages ranged from 25 to 76 years, through an average age of 55.6 ± 11.14 years. Statistically, we did not invention significant alterations when comparing sick with healthy controls in positions of age or sex ($P > 0.05$).

Table (1): Demographic properties of sick and controls groups

Properties	Cases	control	*P* value
Age range	29 - 78	25 - 76	
Mean ± SD	61.16 ± 11.16	55.6 ± 11.14	0.226

SE	1.68	1.67	
Gender	**N (%)**	**N (%)**	***P* value**
Males	30 (68%)	26 (59%)	0.077
Females	14 (32%)	18 (41%)	0.081
***P* value**	0.0015*	0.041*	
Total number	**44**	**44**	

SD: Standard deviation, SE: standard error, * significant association (*P* <0.05

To evaluate liver function, we measured TSB, ALP, ALT, AST, albumin, CRP and PT concentration. The results of Table (2) showed an increase in the concentration of TSB, ALP, ALT, AST, CRP in patients (15.3 ± 2.50 mg/dl, 370 ± 108.59 U/L, 42.36 ± 5.97 U/L, 47.76 ± 2.96 U/L, 15.45 ± 2mg/L respectively) compared to controls (0.488 ± 0.09 mg/dl, 122.56 ± 10.96 U/L, 26.36 ±4.95 U/L, 11.92 ± 2.16 U/L, 6.76 ± 0.828 mg/L respectively) and on the contrary, the concentration of albumin increased in healthy people (3.90 ± 0.44 g/dl) compared with patients (1.78 ± 0.42 g/dl) (P<0.05).

Table (2):Compared liver function checks between cases and control groups

Liver function tests	Cases	Control	T test	95% CI	*P* value
	Mean ± SD	Mean ± SD			
TSB (mg/dl)	15.3 ± 2.50	0.488 ± 0.09	39.27	14.07 to 15.56	0.031*
ALP (U/L)	370 ± 108.59	122.56 ± 10.96	15.15	215.5 to 280.5	0.022*
ALT (U/L)	42.36 ± 5.97	26.36 ± 4.95	14.03	14.07 to 18.72	0.037*
AST (U/L)	47.76 ± 2.96	11.92 ± 2.16	64.88	34.74 to 36.94	0.019*
Albumin (g/dl)	1.78 ± 0.42	3.90 ± 0.44	23.12	-2.30 to -1.94	0.0219*
CRP (mg/L)	15.45 ± 2	6.76 ± 0.828	26.63	8.041 to 9.34	0.043*
Total number	**44**	**44**			

The results of the current study showed higher concentration of cholesterol, triglycerides and LDL in patients (316.12 ± 96, 256.08 ± 41 and 147.48 ± 11.69 ml/dl respectively) compared to healthy people (136.24 ± 10.5, 126.48 ± 6.96 and117.72 ± 3.59 ml/dl respectively) (P< 0.05) as in Table (3), and on the contrary, a lower rate of HDL concentration in patients (53.8 ± 4.89 ml/dl) compared with controls (59.28 ± 6.31 ml/dl) but without significant association (P = 0.227).

Table (3): Comparison case – control groups according to lipids profile

Lipid profile (ml/dl)	Cases	Control	T test	95% CI	*P* value
	Mean ± SD	Mean ± SD			
Cholesterol	316.12 ± 96	136.24 ± 10.5	12.355	150.94 to 208.82	0.049*

Triglycerides	256.08 ± 41	126.48 ± 6.96	20.67	117.14 to 142.06	0.029*
HDL	53.8 ± 4.89	59.28 ± 6.31	4.554	-7.872 to -3.088	0.227
LDL	147.48 ± 11.69	117.72 ± 3.59	16.143	26.095 to 33.424	0.047*
Total number	**44**	**44**			

Immunologically, we found a higher concentration of interleukin in patients (242.56 ± 100.1 ng/ml) compared to controls (43.11 ± 21.3 ng/ml) with *P* value equal to 0.0013 as shown in Table (4). Moreover, serum concentration of IL-2 increased significantly in females (393.95 ng/ml) while decreased in males (171.47 ng/ml) as seen in Figure (1). A binary test with a variable cut-off point can be visually represented and its optimal performance can be identified using the ROC curve. Using the values produced by a ROC curve, the ideal cut-off for IL-2 was determined to be >250 ng/ml by optimizing both sensitivity and specificity (Figure 2).

In Table (5) we did not find a clear relationship or effect of IL-2 on liver function, as the increase or decrease of this immune indicator was not accompanied by an effect on the concentration of TSB, ALT, AST, CRP (r= 0.169, 0.091, 0.032, 0.190 respectively), except for ALP and albumin, which was slightly affected by the change in the concentration of IL-2 (r= 0.341 and 0.314 respectively). The results in Table (6) showed a clear relationship between the concentration of IL-2 with the level of cholesterol, triglyceride and LDL, as the increase or decrease in IL-2 coincided with the increase or decrease of these lipids indicators in the blood of patients (r= 0.393, 0.316, 0.409 respectively), except HDL that it was not affected or interacted with IL-2 (r = 0.009).

Table (4): Evaluation mean of IL-2 concentration of patients and controls

IL-2 (ng/ml)	**Cases**	**Control**	***P* value**
Range	2.54 - 798.9	19.12 – 108.74	
Mean ± SD	242.56 ± 100.1	43.11 ± 21.3	0.0013*
SE	15.1	3.12	
Total number	**44**	**44**	

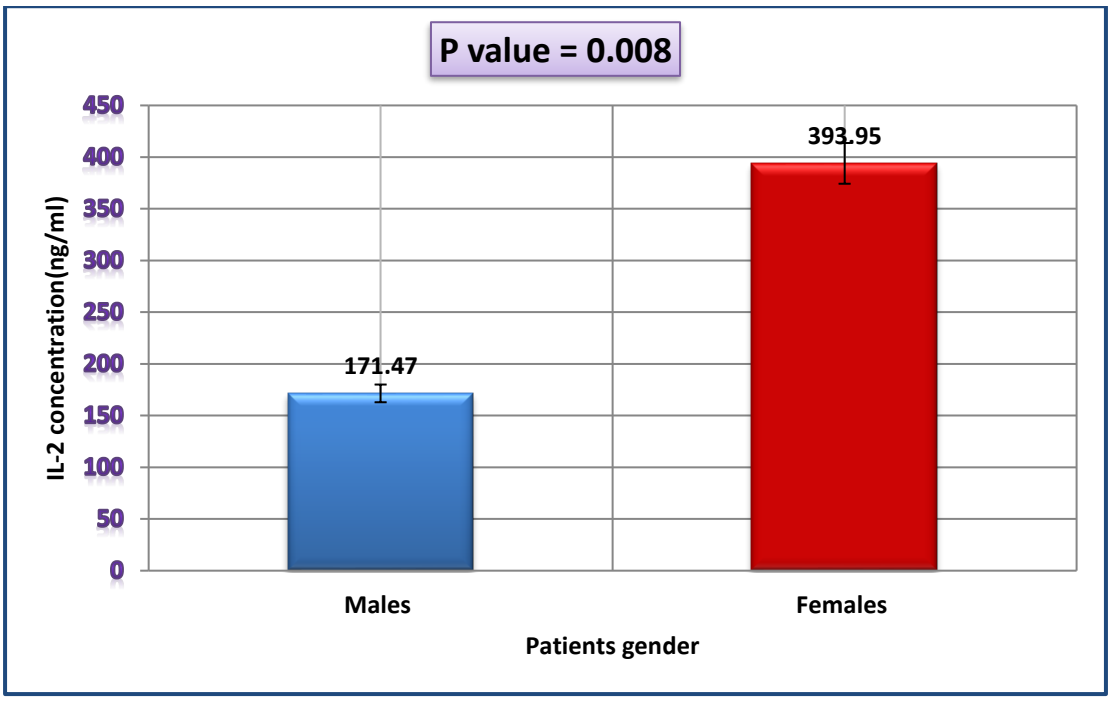

Figure (1): Evaluation serum concentration of IL-2 according to patients' gender (*P* value= 0.008)

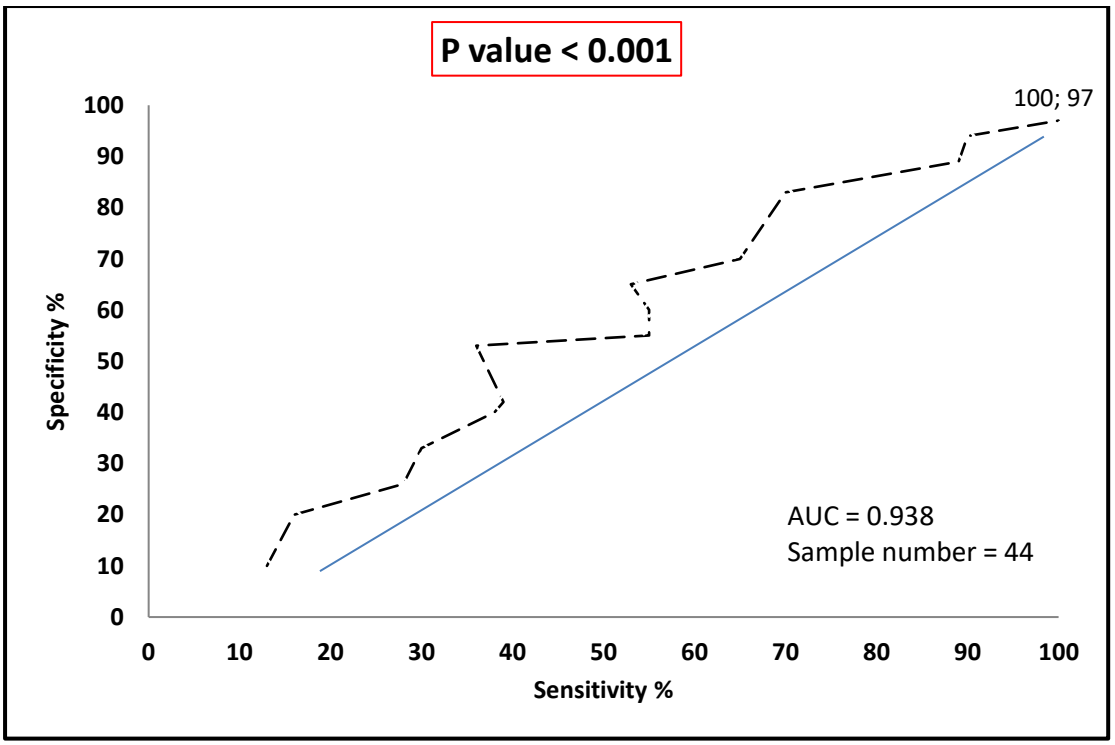

Figure (2): Receiver operating characteristic (ROC) curve of IL-2. The zone below the ROC (AUC) curve was 0.938 with sensitivity and specificity equal to 97% and 100% respectively at cut-off > 0.250 ng/ml.

Table (5): Pearson correlation (r) among IL-2 and liver function tests of patients

Biochemical markers	IL-2 concentration range (ng/ml)	Pearson correlation (r)	*P* value
TSB	2.54 - 798.9	0.169	< 0.0001
ALP	2.54 - 798.9	0.341	< 0.0001
ALT	2.54 - 798.9	0.091	< 0.0001
AST	2.54 - 798.9	0.032	< 0.0001
Albumin	2.54 - 798.9	0.113	< 0.0001
CRP	2.54 - 798.9	0.190	< 0.0001

Table (6): Pearson correlation (r) among IL-2 and lipids of patients

Lipid profile (ml/dl)	IL-2 concentration range	Pearson correlation (r)	*P* value
Cholesterol	2.54 - 798.9	0.393	< 0.0001
Triglycerides	2.54 - 798.9	0.316	< 0.0001
HDL	2.54 - 798.9	0.009	< 0.0001
LDL	2.54 - 798.9	0.409	< 0.0001

Discussion

The soluble interleukin-2 receptor (sIL-2R, sTAC, sCD25) has been identified as a valuable therapeutic tool for a number of diseases since its discovery in 1985 [14]. Seidler and associates. (2012) postulated that in individuals with chronic liver diseases, IL-2 may serve as a marker of both the severity of the disease and the activation of inflammatory cells. Remarkably, concentrations were linked to the stage of liver cirrhosis, significantly elevated in chronic liver diseases regardless of the underlying etiology, and correlated with other known biomarkers of liver function and hepatic fibrosis [15]. Basho and associates. , (2021) demonstrated that IL-2-driven Tfh cell impairment is a previously underappreciated aspect of fibrosis-associated immune dysfunction [16]. It's interesting to note that decompensated individuals with residual Tfh exhibit a "phenotypic imprint" that suggests increased interleukin 2 (IL-2) signaling, a characteristic that is closely linked to the impairment of TFH function and differentiation [17]. Elevated expression of the co-stimulatory molecule OX40 and the α-chain of the high affinity IL-2 receptor, CD25, are signs of this IL-2 imprint on Tfh. Likewise, serum concentrations of soluble-CD25, a surrogate marker linked to IL-2-induced immune activation, are higher in patients with decompensated cirrhosis, making CD4+ T cells in these patients more susceptible to IL-2 signaling [18].

Given that chronic inflammation is thought to be the primary cause of disease progression, sIL-2R may be a useful marker in chronic liver diseases (CLD) due to its

strong correlation with inflammatory processes [19, 20]. Studies looking at sIL-2R in CLD have found that hepatic disorders are associated with higher levels of sIL-2R. Nonetheless, a significant limitation of these investigations is their concentration on specific etiologies, primarily liver diseases associated with viruses or primary biliary cirrhosis [21, 22]. Therefore, it was unclear to what degree sIL-2R might represent activation of different leukocyte subpopulations in CLD or whether it would be generally helpful to monitor inflammatory activities in advancing CLD [23].

In present study, IL-2 increased HCV patients and correlated with liver function tests by weak positive linear relationship (except ALP evaluated in patients with high IL-2 level). The effects of IL-2 on the immune system are diverse. IL-2 levels in HBV patients were found to be higher than in healthy controls in a study by Mourtzikoua and colleagues, and this difference was statistically significant ($p<0.05$) [24]. Although IL-2 levels in HCV patients were not statistically significant, they were different from those in healthy controls, specifically 3.263 pg/mL compared to 1.668 pg/mL. Patients with HBV and HCV had significantly different IL-2 levels ($P < 0.05$) [24]. In HBV patients, a strong positive correlation was found between IL-2 levels and AST, ALT, and GGT, indicating that the degree of necroinflammation is correlated with IL-2 expression [25, 26]. For patients with HCV, Mourtzikoua et al. found a negative correlation ($r=-0.656$) between IL-2 and ALT and ($r=-0.642$) between IL-2 and AST [24]. Furthermore, ALT levels and IL-2 expression were found to be negatively correlated in chronic HCV patients with consistently normal serum transaminase levels, according to earlier research. The disease's progression is either nonexistent or extremely sluggish in these patients. [24,27].

When linking the level of lipids with the concentration of IL-2, we found a clear positive linear relationship between these indicators. This may be due to the fact that fibrosis of the liver and the accompanying injury to body tissues and its consequences represented by a change in liver enzymes, which play a role in lipids metabolism that are directly affected or indirectly effect on the immune system, including IL-2 [28]. Kao *et al*., showed that In patients who are morbidly obese, IL2RA is substantially correlated with NASH and may be a valuable biomarker for the diagnosis of NASH[29]. In the patients with liver fibrosis, excessive fatty acid is commonly secreted in peripheral adipose tissues, and the amount of fatty acid absorbed by the liver can be also increased correspondingly, leading to the deposit of fatty acid in liver tissues. As a result, TG synthesis speeds up, and the steatosis in patients is exacerbated when TG is increased to a certain degree in liver cells that associated with certain indirect immune changes as increase proinflammatory cytokines as IL-2 [30-32].

Conclusion

Present study detected a higher concentration of IL-2 in liver fibrotic patients (242.56 ± 100.1 ng/ml) especially in females compared to health individual in control group (43.11 ± 21.3 ng/ml). We found disturbances in liver enzymes and an increase in harmful lipids in patients with liver fibrosis. Moreover, IL-2 significantly

associated with liver fibrosis may be related to its effects on lipid profile, ALP and albumin concentration.

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