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Interleukins and Chronic Hepatitis

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Abstract: This study was performed on 60 patients classified as follows: 20 patients with viral hepatitis, 20 patients with non viral hepatitis and 20 apparently healthy subjects as control group. Laboratory investigations included complete blood picture, liver function tests and hepatitis B surface antigen and hepatitis C antibody detection. Cases and control groups were investigated for quantitative measurement of serum level of interleukin 2, 4 and 6 using ELISA kits. As regards interleukin 2 (IL-2), there was a statistical highly significant decrease in viral hepatitis patients (10.0 ± 7.1) and statistically significant increase among cases of non viral hepatitis (311.7 ±124.2) in relation to control group. The serum level of (IL-4) was normal in viral hepatitis patients and a statistically highly significant increase (0.971 ± 0.5) in cases of non viral hepatitis (1.29 ± 0.42) than control group. The serum level of (IL - 6) was significantly increased among both groups of viral and non viral hepatitis with (24.5 ± 3.5; 20.6 ± 9.1) respectively. Red blood cells, platelet counts and hemoglobin concentration showed a significant decrease ($F < 0.001$), while, the erythrocyte sedimentation rate showed significant increase. Also, most of the liver function tests were significantly higher than with control group. In conclusion, changes may occur in the level of interleukins 2, 4, 6 in hepatitis. The level of interleukin may be variable in different chronic liver disease. Detection of interleukin levels 2, 4 and 6 are easy method and can be applied in the field of diagnosis and following up of such chronic liver diseases.

Key words: Interleukins · Chronic Hepatitis · ELISA · Control

INTRODUCTION

Hepatic fibrosis is an outcome of many chronic liver diseases, such as viral and autoimmune hepatitis, alcohol consumption and biliary obstruction. Prolonged liver injury results in hepatocyte damage, which triggers the activation of hepatic stellate cells (HSCs) and the recruitment of inflammatory cells into the liver [1, 2]. Liver fibrosis is characterized by an accumulation of extracellular matrix (ECM), resulting from its increased production and decreased degradation and leading to distorted reconstruction of the liver parenchyma that accompanies liver function impairment during most chronic liver diseases [3].

Infection with hepatitis B virus (HBV) is a problem of immense dimension with over 200 million people being infected worldwide; the virus is believed to be non-cytopathic [4]. The major features of immunological interest in HBV infection show that a proportion of those exposed to the virus become chronically infected and that once chronic infection is established, most will gradually eliminate the nucleocapsid antigen from the liver, while the envelope antigens continue to be expressed. During the elimination of nucleocapsid antigens, many patients develop permanent liver damage [5].

There are multiple factors and regulatory mechanisms operating to modulate the immune responses during HBV infection. In this respect, the cellular immune response (presented as T-cells) complemented by the non antigen-specific amplification systems (T-cell cytokines) is involved in the efficient elimination of HBV [6].

One striking clinical feature of hepatitis C virus (HCV) infection is that more than 50% of patients with acute hepatitis C will develop chronic infection [7,8]. Activation of type 2-like T-helper (Th2-like) cells is related to the development of chronicity. Also, peripheral blood $CD4^+$ T-cell proliferation and cytokine secretion occur in response to a panel of recombinant HCV antigens [3, 9].

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Immune-mediated mechanisms are believed to play an important pathogenetic role in chronic hepatitis C virus infection [10]. Interleukin 4 (IL-4) and IL-10 are secreted by T helper-2 type cells (Th2) which may down regulate cell-mediated immune effector mechanisms important in the host defense against intracellular pathogens [11, 12].

Factors determining the outcome of chronic liver diseases are not fully understood, but it is clear that both cellular and humoral immune responses of the host are important. Chronic liver diseases may interfere with endotoxin detoxification resulting in a high local concentration of biolocytokines production [13].

One of the most important advances related to the regulation of specific immune responses to infectious agents is the identification of two subpopulations of T helper (Th) lymphocytes based on cytokine secretion profiles [14].Th1 lymphocytes predominantly produce interleukin (IL) -2, interferon gamma and tumor necrosis factor, while Th2 lymphocytes are predominant producers of IL-4, IL-5, IL-6 and IL-10. Th1-like cytokines, inflammatory cytokines, contribute to cellular immune attack, cytotoxic T lymphocyte response and viral clearance. Th1-like cytokines have also been shown to occur in the HBV clearance without killing HBV-infected hepatocytes by inhibition of HBV gene expression and replication. In contrast, Th2-like cytokines, anti-inflammatory cytokines, lead to T cell tolerance and viral persistence and play an important role in regulation of humoral immune response. Thus an imbalance of Th1- and Th2-like cytokines production may be related to active or inactive disease state in chronic HBV infection [15, 16].

Interleukin-2, a product of helper T cells, is essentially involved in the regulation of cell-mediated immunity. Two monocyte-derived factors, interleukin-1 and prostaglandin E2 influence interleukin-2 synthesis with opposite actions. To analyze immunoregulatory function in HBsAg-positive chronic active hepatitis, T cell subsets in peripheral blood and the levels of interleukin-2, interleukin-1 and prostaglandin E2 in supernatants from lectin- or lipopolysaccharide-activated peripheral mononuclear cell cultures were determined. However, in those groups with reduced interleukin-2 activity, an increased number of T8 cells were observed. It is suggested that the low levels of interleukin-2 found in the replicative phase of chronic active hepatitis B and in delta super-infection reflect a disturbed immunoregulation that may contribute to persistent viral replication in these two conditions [17]. The level and activity of cytokines may be abnormal in many liver diseases. However, little information exists on the role of cytokines in initiation of liver diseases [18].

Our study was an attempt to determine the possible occurrence of abnormal level of interleukins 2, 4 and 6 among patients with chronic liver diseases. Furthermore, to detect the correlation of these interleukins and different types of chronic liver disease and to clarify if these serum tests could be used in the field of diagnosis of chronic liver disease.

MATERIALS AND METHODS

Subjects: The present study was performed on 60 (20 with viral hepatitis, 20 with non viral hepatitis and 20 apparently healthy) patients admitted to the Internal Medicine Departments, King Abdulaziz University Hospital. The patients' age ranged from 18 to 75 years and they were 21 males and 19 females. The examined subjects were classified into three groups. First group: 20 patients with viral hepatitis B and hepatitis C diagnosed by presence of hepatitis B surface markers and antibodies against hepatitis, respectively .Second group: 20 patients with non viral chronic hepatitis. Third group: 20 apparently healthy subjects as control group.

The Patients and Control Group Were Subjected to the Following:

- Complete history taking: age, sex, residence, history of liver and gastrointestinal diseases.
- Complete Clinical examinations of liver, spleen and investigation of gastrointestinal tract.
- Laboratory investigations: urine, stool analysis, complete blood picture, ESR and complete liver function tests (SGOT, SGPT, bilirubin and alkaline phosphatase, prothrombin time, total protein and albumin).
- Abdominal ultrasonography for liver, spleen, portal vein and presence of ascites.
- Sigmoidoscopy and rectal snip with microscopic examination.
- Hepatitis B surface marker and core antibody: All sera were tested for hepatitis B markers using the enzyme immuno-assay (EIA kits, Behring-Germany)

which included hepatitis surface antigen, Hbs Ag), immunoglobulin M to hepatitis B core (anti-HBC IgM), hepatitis Be antigen (HBe Ag) and antibody (anti-HBe) manufactures instruction were strictly followed and readings were obtained by the multiscan EIA plate reader (flow laboratories-Scotland).

- Hepatitis C antibody: Antibodies to hepatitis C virus (Anti-HCV were tested using second generation commercially available kits (Abbott-USA) that was recommended by Mattsson *et al.* [19]. The results were obtained by Abbott Quantium II EIA tube reader.
- Determination of IL-2, 4 and 6: Quantitative measurements of serum interleukin 2, 4 and 6 by using enzyme linked immunosorbent assay Medgenix ELISA kits from Biosource Europea, SA (Belgium).

Statistical Analysis: Statistical analysis was performed by an analysis of variance using standard deviation SD and student (t) test.

RESULTS

Table (1) shows non-significant differences between patients and controls according to age, sex and residence.

IL-2 was significantly decreased among patients with viral hepatitis (10.0 ±7.1) and P< 0.001. On the other hand, IL-2 was significantly increased among patients with non viral hepatitis (311.7 ±124.2) and P < 0.001. Results also revealed that 1L-4 was normal in viral hepatitis patients (0.97 ± 0.5) and P>0.05, while it was highly significantly increased in patients with non viral hepatitis (1.29 ± 0.42) (P<0.001). Also, on recording the serum IL-6amongthe different groups, It was significant highly increased in viral and non viral groups (24.5 ±3.5 and 20.6 ±9.1), respectively (Table 3). Also P-value was highly significant in both groups (P <0.001).

Table 4 shows comparison between interleukins level in the different investigated groups. It revealed that there were high significant difference between examined groups and IL-2 levels (P <0.001). On the other hand that IL-2 was significantly increased among patients with non viral hepatitis (311.7 ±124.2) (P < 0.001).

Table 1: Demographic characteristics of the examined patients.

Clinical data	Patients (40)	Control (20)	t-test	p-value	
MeanAge	32.6 ±10.1(15-60)	30.2 ± 8.2(18-46)	0.93	0.64	NS
MeanAge	25 (62.5%)15 (37.5%)	12 (60%)8 (40%)	0.04	0.08	NS
Sex MalesFemale	28 (70%)12 (30%)	11(7.55)9 (7.45)	11.32	0.25	NS

NS = Non significant

Table 2: Different types of virus among patient with viral hepatitis.

	Patient with viral hepatitis (20)	
Type of virus	No.	%
Hepatitis B virusHepatitis C virusCombined hepatitis B& C virus	5	25
	12	60
	3	15

Table 3: Serum interleukin 2, 4 and 6 level among the examined groups

Type of interleukin	ControlN = 20 (X±SD) Range	Group IN = 20 (X±SD) Range	Group IIN = 20 (X±SD) Range	F	P-value
IL-2t- test P- value	80.2 ± 45.8(12 -150)	10.0 ± 7.1(0 -20)6.77< 0.001	311.7 ±124.2 (100 - 490)7.8<0.001	85.04	<0.001
IL-4t- test P- value	0.816 ±0.23(0.37 -1.2)	0.971 ± 0.5(0.35 -1.9)1.26>0.05	1.29 ± 0.42(0.26 -1.3)4.44<0.001	7.35	<0.001
IL-6t- test P- value	1.8± 0.13(0.5 - 4)	24.5± 3.5(16.5 - 32)14.43< 0.001	20.6± 9.1(11.1 - 30.4)9.135<0.001	92.9	<.001

IL-2 = Interleukin 2 IL-4 = Interleukin 4 IL-6 = Interleukin 6

Table 4: Determination of interleukins Level among the examined groups.

Types of interleukins	Group I (X ± SD)	Group II(X± SD)	t- test	P- value
IL-2	10.0± 7.1(0-20)	311.7±124.2(100-490)	10.8	<0.001H.S.
IL-4	0.97 ± 0.5(0.35-1.9)	1.291 ± 0.42(0.75-1.85)	2.18	<0.05Sig.
IL- 6	24.5 ± 3.5(16.5 - 0.32)	20.6 ± 9.1(14.4 - 0.5)	1.65.	>0.05Non Sig.

H.S= High significant Sig. = Significant Non Sig. = Non significant

Table 5: Comparison between laboratory blood parameters of the examined groups.

Blood parameter	Group 1	Group II	Group III	F	P- value
RBCsHbPlateletESR	4.8 ± 1.3	3.3 ± 12.1	3.0 ± 0.3	9.88	<0.001
	15 ±0.97	9 ± 0.8	9.2 ± 0.9	291.5	<0.001
	302.5 ± 126.7	157 ± 16.8	172 ± 13.5	325.0	<0.001
	4.1 ± 1.2	27.9 ± 8.6	27.9 ± 24.2	85.0	<0.001

Table 6: Comparison of Liver Function Tests among the examined groups.

Different liver enzyme	Control	Group I	Group II	F (test)	P- value
SGOT	16.0 ± 4.7	97.8 ± 17.4	146.2 ± 6.8	2110.95	<.0.001
SGPT	9.4 ± 3.6	80.4 ±20.9	9.7 ± 4.1	214.29	<0.001
Akaline phosphate	8.7 ± 2.3	18 ± 1.9	25.1 ± 8.6	48.98	<0.001
Bilirubin	4.2 ± 0.3	2.6 ± 0.1	3.1 ± 0.6	98.05	<0.001
Albumin	0.5 ± 0.2	3.1 ± 0.2	3.3 ± 0.3	861.1	<0.001

Table (5) shows different laboratory blood parameters among different investigated patients. It revealed variable difference of blood picture among investigated groups. Our findings detected highly significant decrease of RBC and platelets count and Hb concentration(P <0.001). While, highly significant increase of ESR was observed among patients with chronic liver diseases (P<0.001) (Table 5).

Table (6) shows comparison of mean ± SD of different liver function testes among investigated groups. It shows highly significant increase of SGOT, alkaline phosphate and albumin and statistically significant decrease in bilirubin level (P <0.001) among viral and non viral hepatitis patients.

DISCUSSION

Viral hepatitis is a major public health problem throughout the world affecting several hundred millions people. Viral hepatitis is a cause of considerable morbidity and mortality in the human population both from acute infection and the chronic squeals which include, with at least two types of infection, chronic active hepatitis, cirrhosis and primary liver cancer [20, 21]. Liver cirrhosis may be caused by viral hepatitis which includes infection with hepatitis B and C or non viral causes as alcohol overuse, direct hepatotoxicity, idiosyncratic hepatotoxicity, cholestatic reactions and metabolic and autoimmune disorders as acute exacerbations of sub clinical liver disease, such as autoimmune hepatitis and Wilson's disease [3]. The level and activity of cytokines may be abnormal in many liver diseases; however, little information exists on the role of cytokines in the initiation of liver diseases. In our study we detected the level of IL2, IL4 and IL-6 among 20 patients with viral hepatitis and 20 patients with non viral and comparing them to 20 apparently healthy subject.

The level of IL-2 was significantly decreased among patients with viral hepatitis (10.0 ±7.1, P, 0.001), while was significantly increased among patients with chronic non viral hepatitis (311.7 ± 124.2, P <0.001). This in agreement with Ohno *et al.* [22] and Bat and Kong [23] explained the decrease in the level of IL-2 by being one of the mechanisms used by the virus to escape the immune response by defective generation of cytotoxic T-cells which are directed against the viral antigen expressed on the surface of hepatocytes. They also found no correlation between the functional T- cell defect and the severity of liver damage, degree of viral replication and duration of the disease. Also, Vingerhoets *et al* [24] detected a lower level of IL-2 among patient with chronic viral infection. Our results are not in agreement with Fransesco *et al* [25] and Sabahatti *et al* [17] who found high level of Il-2. Sabahatti *et al* [17] and Mizukosh *et al* [26] who showed that the mean IL-10 level was higher in asymptomatic carrier (AC) with replicative HBV infection than in the replicative chronic hepatitis B, nonreplicative chronic hepatitis B and controls (p<0.05) . In contrast, the mean concentration of IL-2 was found to be significantly elevated in chronic hepatitis patients with replicative HBV infection compared to the replicative and nonreplicative HBsAg carriers and controls (p<0.05). They also reported that the interleukin-2 levels were significantly higher in the patients with hepatitis C than in control subjects (p<0.0001). A progressive and significant increase has occurred in soluble interleukin-2 receptor levels with increasing severity of liver injury (p<0.001) [17, 26].

On studying the variation of serum IL-2 and IL-4 levels among the study groups, IL-2 was significantly higher (311.71 ±124.2) (P<0 .001) in non viral hepatitis than viral group. Furthermore, IL-4 was highly significantly increased (1.29±0.42) than the control group (0.816±0.23). The P-value was highly statistically significant (P <0.001) in both groups. Markus*et al* [11] also found an elevated

level of IL-4 in the examined patients and they concluded that elevated Th2 cytokine levels may represent a systemic response and not a result of increased local production within the liver [11].

In our study, increased levels of IL-2 and IL-4 in non viral hepatitis in comparison with the normal control could represent the higher levels found during the early course of chronic infection and the levels of these important interleukins may get lower with chronicity which mean immunologic down regulation and persistence of such chronic infection. The level of IL-2 and IL-4 production is crucial for the maintenance of the granulomatous response. The gradual decline in IL-2 and IL-4 production with the chronic stage of the disease is a contributory factor to the diminished granulomatous responsiveness. IL-4 may promote the granulomatous inflammation by several pathways; In vitro, it was demonstrated that IL-4 acts as a co-stimulant on hemopiotic progenitor cells of the bone marrow to produce number of granulocyte and macrophage colonies which release different cytokines as IL-2 and IL-4 [27, 28].

On studying the correlation of IL-4 and viral hepatitis, non significant increase of IL-4 was found in comparison to control ($P>0.05$). Our results are in agreement with Dharancy *et al* [15] who found elevated level of IL-4 among patients with viral hepatitis. This difference may be due to variable immune response of the body to the antigen whether Schistosoma or virus B hepatitis [15]. Our results are in agreement with others who found non-significant change of IL-4 with viral hepatitis and they reported that different cytokine profiles of T-cells within the liver in chronic viral hepatitis may have different behavior of the local immune responses that may have pathogenetic implication.

On detecting the level of IL-6 and chronic liver disease, highly significant elevations of serum IL-6 in non viral hepatitis (20.6 ± 9.1, $P<0.001$).This is in agreement with Oyanagi *et al* [29] who confirmed the high level of IL-6 in non viral hepatitis and explained that by the fact that IL-6 is mainly metabolized at the hepatic level, so in the presence of hepatic insufficiency, the elevation of endogenous cytokines is alwayspresent. Another explanation is that chronic endotoxin anemia commonly occurs in patients with liver diseases and this might activate spontaneous IL-6 production [29].Also, our results detected a significant elevation of 1L-6 serum level in chronic viral hepatitis patients (24.5 ± 3.5) and $P<0.001$. Our results are in accordance with others as they attributed this increase to the activation of endothelial, Kupffer and infiltrating mononuclear cells and stimulate liver tissue to produce IL-6, contributing to the inflammatory and immune responses in chronic hepatitis.

Also, Osna *et al* [21] reported that the increase-plasma IL-6 in patients with chronic viral hepatitis may be due to various causes which effectively induced the production of IL-6.

On detecting different liver function tests in correlation with chronic liver disease, our study revealed impaired liver functions as evidenced by decrease serum total bilirubin and increase serum enzymes levels of SGOT, SGPT and alkaline phosphate ($P<0.001$). Our results are in agreement with Morsi *et al* [6] who observed the same association of chronic liver diseases and disturbances in liver function tests [6].

In the present study, there was a significant lower RBC count, hemoglobin concentration in all patient's groups than in control group. Our results are in agreement with Seeger and Mason [30] who stated the decrease of RBC count and HB in patients with chronic liver disease is due to reduction of RBCs life and inadequate erythropoietin [30]. Also, our results detected a significant decrease in platelet number/ this in accordance with Morsi *et al* [6] who reported that there is always thrombocytopenia in patients with chronic liver disease and hepatitis [6]. Our study showed significant elevation of the erythrocyte sedimentation rate (ESR) which is common in all patients with chronic liver disease. These results are in agreement with Ohno *et al* [22] who found increase of ESR in patients with chronic liver diseases which was attributed to disturbance in plasma protein [22].

CONCLUSION

Serum levels of cytokines including IL-2, IL-4 and IL-6 were variably different in patients with chronic liver diseases. Significant increase and decrease in the concentration of cytokines represent characteristic features of chronic liver diseases and changes occurring in the level of interleukin in chronic liver disease are considered as immunological mechanisms offered by the body. So we can relay on these interleukins for diagnosis and following up the disease.

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