Evaluation of T- Helper (Th-1) Cytokines during the Treatment Responses of chronic Hepatitis C Virus

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Summary:

Background:
It is widely assumed that cellular immune response and cytokine (especially proinflammatory cytokines) production play an important role in the course and treatment effectiveness of chronic hepatitis C infection.

Aim of the study: evaluate the level of Th-1 cytokines during treatment of chronic HCV infection and their dynamic changes in response to treatment.

Patients and methods: The study was performed on sixty patients with chronic HCV infection, the patients were recruited from medical city gastroenterology and hepatology teaching hospital for the period from February 2007 till February 2008 and 50 healthy control group. Diagnosis was made using third generation ELISA-based screening test, RIBA-based test and PCR-based test. On the other hand, Cytokines (serum level) were determined by ELISA method before, during (6, 12, 24 weeks) and after the treatment.

Results: This study showed that after 6 weeks from the initiation of the treatment by pegilated interferon-α in the combination with ribavirin, concentration of serum cytokines IL-1β, TNF- α and INF- γ have significantly changed and at the end of the treatment concentration of all cytokines have decreased and this change was statistically significant for all cytokines.

Conclusion: dynamic changes of some cytokines during INF- α and ribavirin treatment may be used as a predictor factor for evaluation of the therapy effectiveness.

Key wards: chronic hepatitis c; Pro-inflammatory cytokines.

Introduction:

Hepatitis C virus (HCV) is a ubiquitous virus infection. It has been estimated that about 3% of the world's population have HCV and there are about 4 million carriers in Europe alone [1]. Chronic hepatitis C has been a public health concern during the last decade in most developed countries [2]. It has been shown that the serum levels of cytokines are elevated in chronic hepatitis C patients [43]. It has also been demonstrated that T cells play a role in HCV clearance in HCV-infected patients helper T cells (Th) help in the functions of the immune system as the major regulator and also help to destruct antigen and to reinforce antibody production [3-5]. The reason for the hepatocellular injury in hepatitis C infection is still unknown [6]. Cytokines are proteins and glycoproteins, which modulate the activity of target cells through binding to specific receptors. Cytokines can be classified either on the basis of function, i.e. pro-inflammatory and anti-inflammatory cytokines or on the basis of whether they are produced principally by either Th-1 or Th-2 lymphocytes. Elimination of HC-virus significantly depends on the status of the balance between the two groups of cytokines (7-9).

Cytokines are increasingly recognized as the important factors in the pathophysiology of chronic hepatitis C. Markers of cytokines expression at the early stage of the disease may be used as the criteria for prediction of further immune response (10). Treatment of chronic hepatitis C is a prolonged process and very costly. In some cases the treatment is ineffective. Although the effectiveness of combination therapy with pegilated interferon-α and ribavirin Increased to almost 60-65%, there are still a lot of cases of non-responders and relapsers. Little is known about the production development and progression of cytokines in hepatitis C infections. This study was conducted to assess the serum levels of Th1 cytokines and also their association with inflammatory indicators in HCV-infected and normal individuals. Measuring values and correlating them with responses should bring very important information in assessing and monitoring patients with HCV during treatment.

Materials and Methods:-

We have studied 60 Patients with chronic hepatitis C, recruited from medical city gastroenterology and hepatology teaching hospital for the period from February 2007 till February 2008 compared to 50 healthy volunteers. Diagnosis was made using third generation ELISA-based screening test that uses antigen coated beads with an antibody coupled with

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an enzyme to produce florescent end product that is proportional to the amount of bound antibody. according to the Lab kits Anti-HCV antibody less than one unit considered as negative, 1-1.2 unit borderline, and higher than 1.2 considered as positive. Patients with positive results were retested using a more specific test, a RIBA-based test than allows for the detection of antibodies against specific HCV antigens. Further tested for the presence of HCV RNA by PCR-based test. Patients with chronic hepatitis B, haemochromatosis, Wilson's disease, α1- antitripsin deficiency, autoimmune hepatitis were excluded from our study. The following scheme of treatment was used: pegilated α-interferon-2b ("Peg-Intron", Essex Pharma) 1,5 μg/kg weekly s.c. for 48 weeks plus ribavirin twice a day orally ("Rebetol") 10 mg/kg .Cytokines (serum level) were determined by ELISA method (Marseille Cedex 9/ France) before, during (6, 24 weeks) and after the treatment.Collected data were analyzed by SPSS software. ANOVA was used to compare means of more than two independent groups. The level of significance in all cases was set at a two-tailed (p<0.05)

Results:
Thirty two male and eighteen female with age range between (20-52 years) with mean age of 31.6 ± 11.2 healthy control , and 45 male and 15 female with HCV infection +ve age was between (14 – 54 years) with mean age of $34..6 \pm 10.8$

The result showed that no significant difference in mean age of patients with HCV and healthy control group since they represent 34.2 ± 10.8 and 31.6 ± 11.2 respectively. There were 45 males and 15 females with chronic HCV infection with M:F ratio 3:1, compared to 32 male and 18 female as a healthy control group with M:F ratio 1.7:1 (table-1)

Table -1: Age and gender distribution of study groups

Groups		Healthy control N = 50 (%)	Chronic HCV N = 60 (%)
Age (years) Range Mean± SD		(20-52) 31.6 ± 11.2	(14-54) 34.26 ±10.8
Gender	Male	32 (64)	45 (75)
	Female	18 (34)	15 (25)

 P = 0.153 non significant for age between patients & control. P= 0.53   non significant for gender between patients   & control. P=0.0001 significant between male and female.
Regarding serum levels of cytokines, We have studied changes of Th1 derived cytokines: TNF-α, IL-1β and additionally proinflammatory cytokines

INF-γ concentrations in the blood sera. As it is shown in the table-2, a significant elevation of above cytokines shows among HCV patients than healthy control group with P-values (0.05, 0.001 and 0.05) respectively. In addition their changes were statistically significant as early as after 6
weeks of treatment. This status did not change significantly after completion of the treatment.

Table -2: Level of serum cytokines before, during

Cytokine (pg/ml) Level	Before treatment	After 6 weeks of treatment	After 12 weeks of treatment	After the treatment	Healthy control (N=50)
IL-1 β	4.9±1.5	9.5±1.3	3.8±0.9	3.0 ±0.8	1.8±0.6
TNF- α	12.7±2.3	22.2±2.5	9.0±1.9	9.5 ±1.2	5.3±2.7
IFN-γ	115.7±20.5	57.5±10.5	48.3±12.5	40.7±10.5	30.5±8.5

after the treatment.

Discussion:
Approximately 80-90% of patients acutely infected with hepatitis C virus develop persistent infection (6). It has been thought that cytotoxic T lymphocyte responses early in infection may be important for viral clearance (3). Several cytokines and chemokines induced by viral infection play directly or indirectly roles in antiviral defense. These include TNF-α , IFN-γ, IL-1 β (11). In the context of an inflammatory response against the virus, different cytokine responses of the host may be responsible for the variable liver damage (6). In our study initial levels of this cytokines and changes after 6 weeks during the treatment (especially IFN-γ) should be predictors for sustained response in combination therapy with interferon-α and ribavirin.It has been reported that, IL-1 modulate the immune system and exert direct antiviral activity by cytopathic and non cytopathic mechanism (12).
Also Torre et al., (13) and Geneva, (14) reported that, increased IL-1 production may be important for viral clearance and its level probably related to hepatitis activity and thus have some role in hepatocytic injury. On the other hand, TNF-α is the principal mediator of the acute inflammatory response to infectious pathogens they also triggers a partially overlapping set of antiviral defense mechanisms and serum level of TNF-α reflects the progression of inflammation [3].Like other studies (15,16,17), results here showed that a high levels of IL-1 and TNF- α were frequently observed in chronic HCV infected patients than healthy control group. Actually, this finding is attributed to the production of IL-α by activated monocytes and macrophages (18).This study also documented a

significant increase of IL-1B in patients with chronic HCV after 6 weeks treatment. This due to the biological activities of IFN-α, that induce rises in spontaneous production of IL-1 by peripheral blood mononuclear cells (PBMCs) (19).This suggestion could be supported by the finding reported by Daniels et al., (20), who demonstrated substantial rises in spontaneous invitro production of TNF-α and IL-1 β by PBMCs from patients responded to IFN-α.Interferon-γ named IL-18 (inducing factor) is a cytokine synthesized by Kupffer cells and macrophages. A lot of studies provide significant evidence indicating that IL-18 plays a prominent role in liver injury. It is structurally related to IL-1β. Our study shows a significant up-regulation of INF-γ in chronic HCV infection, suggesting a role of this cytokine in the chronic cellular immune response toward hepatocytes in the course of this disease. It is important to note that INF-α promotes anti-inflammatory effects via two cytokine families prominently involved in the liver pathology, namely TNF-α and IL-1. We show that in HCV patients Interferon-α reduces INF- γ concentration. We propose that this anti-inflammatory mechanism contributes to the treatment efficacy with Interferon-α. A persistent Th1 response may cause a gradual accumulation of liver injury induced by cytotxic T-lymphocytes and macrophages. These macrophages should then express a range of cytokines such TNF-α, IL-1β, INF- γ and some chemokines, which are responsible to the continuation of a Th1 response. This study shows an association between IL-18 and TNF-α, IL-1β production. The notable upregulation of INF- γ correlated to a significance increase of TNF-α and IL-1β. Taking together, measuring values and correlating them with responses should bring very important information in assessing and monitoring patients with HCV during treatment.

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