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Th1 Cytokine Profiles in Hepatitis C Virus Infected Patients and Their Contribution to Inflammatory Responses.

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Abstract:

Background: Th1 cytokines are required for host antiviral immune responses. However little is known about the production and progression of cytokines in hepatitis C virus (HCV) infected patients. The aim of this study was to assess the serum levels of Th1 cytokines and also their association with inflammatory indicators in HCV-infected and normal individuals.

Methods: Fifty four HCV-infected patients along with thirty one healthy controls were selected using the sequential sampling method. Serum levels of interleukine-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor-alfa (TNF-α) was determined in all the precipitants by enzyme-linked immunosorbent assay (ELISA). Moreover serum levels of alanine aminotransferase (ALT), aspartat aminotransferase (AST), alkaline phosphatase (ALP), C-reactive protein (CRP) and rheumatoid factor (RF) were also determined in both patient and control groups.

Results: The results showed that serum levels of IFN-γ, TNF-α and IL-2 were higher in HCV-infected patients than controls group but the difference was significant only for TNF-α ($p<0.05$). A positive correlation was found between the serum level of TNF-α and IL-2 in patient group ($p<0.05$).

Conclusion: TNF-α is the main mediator of the acute inflammatory responses to microbial infections and in our study serum level of TNF-α in HCV-infected patients was higher than healthy subjects. Positive correlation of serum TNF-α and IL-2 levels in HCV-infected patients may contribute to the role of innate immunity in stimulating the adaptive immune responses, thus suggests role of TNF-α in antibody production.

Key Words: Cytokine, Hepatitis C, Inflammation.

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]. The reason for the hepatocellular injury in hepatitis C infection is still unknown [3]. It has been shown that the serum levels of cytokines are elevated in chronic hepatitis C patients [4]. 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 [4-6]. Th1 favors the promotion of cellular immunity whereas Th2 favors the promotion of humoral immunity [7]. Cytokines produced by both T helper and cytotoxic T cells may be responsible for much of the damage that occurs in the livers of HCV-infected patients [3]. Th1 cytokines, such as interleukine-2 (IL-2) and interferon-gamma (IFN-γ), and tumor necrosis factor-alfa (TNF-α) are required for host antiviral immune responses [4,8,9]. Different types of microbes induce different innate immune responses, which then stimulate the types of adaptive immunity[9]. 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.

Materials and Methods:

Subjects of this study were the people referred to Iran Red Crescent Society clinic in Baku, Azerbaijan Republic. Blood samples were collected from each subject and tested, twice, for anti-HCV antibody by ELISA (Monobind, USA). Anti-HCV antibody less than one unit considered as negative, 1-1.2 unit borderline, and higher than 1.2 considered as positive. Accordingly, Thus fifty four serologically confirmed HCV-infected patients and thirty one healthy controls were enrolled using sequential sampling method. Mean age of patients and healthy controls were 42.40 ± 12.75 and 43.55±12.97 respectively. Serum levels of IFN-γ, TNF-α and IL-2 cytokines was checked in all participants, using ELISA (Euro clone, Italy). Serum levels of ALT, AST, ALP, CRP and RF were also determined (using STATFAX 1902, USA) for the patient group. Serum

ALT less than 38 unit, AST less than 42 unit, ALK less than 180 unit per litre, and ESR less than 10 mm/hr were considered as normal. Collected data were analyzed by SPSS software. ANOVA was used to compare means of more than two independent groups. T test was used to test the differences between the patients and control groups. Associations between the quantitative data were studied by correlation analysis. The level of significance in all cases was set at a two-tailed ($p<0.05$).

Results:

There was no significant difference in age and sex between control and patient groups (Table 1). A significant difference was found in levels of all inflammatory parameters, apart from CRP, between two groups.

Serum levels of IFN-γ, TNF-α and IL-2 were higher in HCV-infected patients than controls but the difference was significant only for TNF-α ($p<0.05$, Table1). The serum levels of studied cytokines were higher in male compared with female but the differences were not significant ($P>0.05$). Highest serum levels of IFN-γ and TNF-α was found in patients higher than 60 years old while the highest level of IL-2 was detected in patients lower than 31-years old. However, these differences were not significant ($p>0.05$). A positive correlation was found between the serum level of TNF-α and IL-2 in HCV-infected patients ($p<0.05$, $r = +0.65$, Table 1). The results showed no significant difference in serum levels of cytokines between the healthy controls and those patients with increased inflammatory parameters (Table 2).

Table 1: Demographic features and level of biochemical factors of patients and control groups.

Variable	**Control**	**Patient**
Age	43.55±12.97	42.40±12.75
Sex(F/M)	16/15	19/35
ALT	25.58±9.23	41.90±13.62
AST	32.41±7.77	39.86±7.29
ALP	169.22±36.22	214.04±52.08
ESR	9.40±5.08	14.60±10.14
CRP (Nl/Increased)	7/8	16/29
RF (Nl/Increased)	15/0	35/10
IFN-γ	0.49±1.114	3.045±13.383
TNF-α	0.393±2.117	2.892±8.245
IL-2	0	0.517±2.184

Table 2: Cytokine levels in normal and elevated serum inflammation parameters.

Variable groups	IFN-γ	TNF-α	IL-2
normal ALT	1.84±4.37	3.00±8.01	0.05±0.24
elevated	0.60±1.67	3.16±9.16	1.12±3.23
normal AST	1.29±3.67	3.17±9.27	0.58±2.47
elevated	1.31±2.46	1.31±1.94	0.12±0.23
normal ALP	1.51±3.51	4.56±11.81	1.32±4.01
elevated	1.17±3.42	2.51±6.95	0.23±0.79
normal ESR	0.11±0.88	0.07±0.04	0.075±0.024
elevated	0.10±0.66	0.06±0.03	0.074±0.023
normal CRP	0.89±2.78	3.25±10.21	1.01±3/61
elevated	0.90±2.86	3.55±8.21	0.33±0.94
normal RF	0.72±2.31	3.21±9.02	0.71±2.46
elevated	1.48±4.10	4.15±8.97	0.22±0.70

Discussion:

Approximately 80-90% of patients acutely infected with hepatitis C virus develop persistent infection, about one half of them have elevated transaminase indicative of ongoing liver inflammation [3]. Results of our study showed elevated serum levels of transaminases (ALT and AST) and other inflammatiory indicators such as ALP, ESR and RF in hepatitis C patients.

Factors involved in the progression to liver disease in HCV-infected patients are not well characterized. It is thought that cytotoxic T lymphocyte responses early in infection may be important for viral clearance [4]. Immune-mediated mechanisms are believed to play an important phatogenetic role in HCV infection [10]. Several cytokines and chemokines induced by viral infection play directly or indirectly roles in antiviral defense. These include TNF-α , IFN-γ, IL-2 [11]. Most of acute and chronic liver diseases are characterized by inflammatory processes with enhanced expression of various pro- and anti-inflammatory cytokines in the liver [12]. In the context of an inflammatory response against the virus, different cytokine responses of the host may be responsible for the variable liver damage [3]. In our study serum cytokine TNF-α level in patients infected with HCV was higher than healthy controls. Many other studies have showed increased circulating immunoregulatory cytokines in patients with HCV liver disease [3,13,14]. Alvarado and colleagues showed TNF-α level elevation in patients with chronic

HCV in their study [15]. Many bacteria and viruses stimulate Th1 responses which activate the effecter mechanisms that are best to eliminating these microbes [9]. TNF-α is the principal mediator of the acute inflammatory response to infectious pathogens and is responsible for many of the systemic complication of sever infections [16]. TNF-α also triggers a partially overlapping set of antiviral defense mechanisms and serum level of TNF-α reflects the progression of inflammation [4]. Serum levels of IFN-γ and IL-2 in HCV-infected patients were similar to healthy controls in our study. The level of expression of type 1 cytokines, such as IL-2 and IFN-γ, is correlated with the degree of histologic injury.

There was no any association betwee n cytokines

expression and alterations

in inflammatory parameters in this study. This is consistent with Kusumoto, et al. study where they found no corelationship between cytokines expression and alterations in ALT levels [17].

Taken together positive correlation of serum TNF-α and IL-2 levels in HCV-infected patients may contribute to the role of innate immunity in stimulating the adaptive immune responses, thus suggests role of TNF-α in antibody production.

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