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Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3

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Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3

Zaigham Abbas, Tariq Moatter, Akber Hussainy, Wasim Jafri

Methods: Patients with chronic hepatitis C genotype 3 were analyzed for single nucleotide polymorphisms of interleukin (IL)-10, IL-1 beta, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) by polymerase chain reaction using sequence-specific oligonucleotide primers. Liver biopsies were assessed by modified histological activity index (HAI) scoring system using a scale of 0–18 for grading the necro-inflammatory activity and 0–6 for staging the fibrosis. HCV RNA levels were determined by bDNA assay. The patients were treated with interferon-alpha and ribavirin for 6 mo. Sustained virological response was assessed 6 mo after the completion of the treatment.

Results: Out of the 40 patients analyzed, 26 were males. Mean age was 40.5 ±12.5 years (range 18–65 years). The frequencies of different dimorphic polymorphisms based on single nucleotide substitution were as follows: IL-10-1082 G/A 85%, A/A 12.5%, G/G 2.5%; IL-10-819 A/C 87.5%, C/C 10%, A/A 2.5%; IL-10-592 C/A 72.5%, C/C 27.5%; IL-1 C 90%, U 10%; IFN-874 T/A 50%, T/T 27.5%, A/A 22.5%; TNF-308 A/G 95%, G/G 5%; TGF-10 T/C 52.5%, C/C 35%; T/T 12.5%.

For IL-10 -819 genotypes, mean scores of fibrosis were A/A = 6.0, A/C = 2.2, and C/C = 1.0 (P = 0.020) though the inflammatory activity was not much different. No significant differences in HAI were noted among polymorphisms of other cytokines. Moreover, ALT and HCV RNA levels were not significantly different among different cytokine polymorphisms. There was a significant correlation of HAI and HCV RNA levels with the duration of disease. TGFβ-10 genotype CC patients had a better end of treatment response than those with other genotypes (P = 0.020). Sustained virological response to the treatment was not influenced by the cytokine polymorphism. No effect of other factors like viral load, degree of fibrosis, gender, steatosis, was observed on sustained virological response in this population infected with genotype 3.

Conclusion: There is no significant correlation between cytokine polymorphisms and HAI except for the polymorphisms of anti-inflammatory cytokine IL-10, which may influence hepatic inflammatory activity and fibrosis in patients with chronic hepatitis C genotype 3. Sustained virological response in this genotype does not seem to be influenced by cytokine gene polymorphisms.

Key words: Interleukin; Interferon gamma; Tumor necrosis factor alpha; Transforming growth factor; Cytokines; Gene polymorphism; Hepatitis C; Alanine aminotransferase; Liver biopsy

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Introduction: HCV infection is a leading cause of chronic liver disease worldwide. The infection leads to viral persistence and chronic disease in a very high proportion of cases. Pathogenesis of liver injury is not fully understood. There is a complex relationship between HCV and its host. Liver lesions could be the result of immune...
responses or pathogenic action of the virus. Cytotoxic T cells and cytokines produced by both CD4+ (T helper) and cytotoxic T cells may be responsible for much of the damage that occurs in the livers of infected patients.[3]

Two distinct patterns of cytokine production may occur.[2] Type 1 responses are characterized by production of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), which are prime and maintain antigen-specific cellular immunity[3,4] and are important in defense against viruses. Type 2 responses are characterized by IL-4, IL-5, and IL-10, which promote humoral immune responses. An imbalance in helper T-cell type 1 (Th1) and type 2 (Th2) cytokines is suggested to play an important role in the pathogenesis of chronic hepatitis C. The progressive liver injury seen in chronic HCV infection is associated with the upregulation of intrahepatic Th1-like cytokines. Intrahepatic IFN-γ and IL-2 mRNA expression is upregulated in chronic hepatitis C, while the expression of IL-10, a Th2-like cytokine, is downregulated.[5]

Intrahepatic CD4+ T cells play a pathogenetic role in the hepatic injury of HCV infection.[6] Vigorous HCV-specific CD4+ Th1 response, particularly against the nonstructural proteins of the virus, may be associated with viral clearance and protection from disease progression.[7] Patients without viremia after HCV infection frequently have strong Th lymphocyte responses of the Th1 type to multiple HCV antigens many years after the onset of infection, whereas antibody responses are less marked. These results suggest that control of HCV replication may depend on effective Th lymphocyte activation.[8-9]. There is also an enhanced Th2 response during chronic HCV infection, which may partly be responsible for the persistence of HCV infection.

In addition to the altered intrahepatic cytokine expression, there might be a significant correlation between circulating cytokines and degree of inflammation in the liver. One study has shown such a correlation between baseline TNF levels and histologic grading score of hepatitis.[10]. The maximal capacity of cytokine production varies between individuals and may correlate with polymorphism in cytokine gene promoters. The objectives of our study were to analyze the role of allelic or genotype variations of IL-10, IL-1β, IFN-γ, TNF-α and TGF-β and its association with hepatocellular injury as suggested by liver biopsy and ALT and treatment outcome. We selected genotype 3 for this study which is the main genotype in our country.

MATERIALS AND METHODS

Out of the 40 patients analyzed, 26 were males. Mean age was 40.5±12.5 years (range 18-65 years). The participants did not receive interferon therapy and had neither coinfection with human immunodeficiency virus and hepatitis B virus nor other associated forms of chronic liver disease.

Quantitative serum HCV RNA was determined by DNA assay (Bayer, USA) according to the manufacturer's instructions. The minimum quantification limit of the assay was 3 000 HCV RNA copies/mL serum. HCV genotyping was performed using PCR and reverse hybridization assay (Innogenetics, Belgium). All patients included in this study were of HCV genotype 3. For cytokine gene polymorphism, DNA was extracted by proteinase K digestion from peripheral mononuclear cells, followed by phenol chloroform extraction and ethanol precipitation. After amplification polymorphisms in IL-10 (592, 819, 1082), IL-1β (511), IFNγ (874), TNFα (308) and TGF (codon25) were examined as described previously.[11,12]. The number in parenthesis indicates the location of the polymorphism on the DNA sequence. Briefly, single nucleotide polymorphisms (SNPs) were determined using sequence specific oligonucleotide primers. Each PCR reaction consisted of 1× PCR buffer, 0.2 mmol dNTPs. Concentration of MgCl2 varied with the type of SNP examined, 50 ng of each polymorphism specific primer, 1 U of Taq polymerase in a final volume of 10 µL. To monitor PCR inhibition, growth hormone gene was simultaneously amplified as an internal control. Thermal cycling was performed in a Perkin-Elmer 9700 thermal cycler. Following PCR amplification, amplicons were stained with ethidium bromide and visualized on a UV transilluminator. The size of product generated in each PCR assay was ascertained and scored as positive/negative for the presence/absence of a particular polymorphism. The PCR product obtained with IL-1β-511 specific primers was digested with the restriction enzyme AvaII. The digested product was then visualized for the presence of restriction fragments.

Liver biopsy specimens were analyzed by a single pathologist, who was unaware of the patient's identity, treatment regimen, response, or timing of the biopsy relative to the treatment. Liver biopsies were assessed by modified histological activity index (HAI) scoring system[13] using a scale of 0-18 for grading and 0-6 for staging. Degree of steatosis was scored at a scale of 0-3. Presence or absence of lymph follicles was also documented.

Duration of disease was determined by calculating time interval from the exposure to a possible risk factor. Patients were treated with standard doses of interferon alpha (3 mega units subcutaneous, thrice a week) and ribavirin (800-1 200 mg/d) for 6 mo and followed up for another 6 mo. HCV RNA was repeated 6 mo after the treatment to document sustained response.

Statistical analysis

Statistical analysis was performed by two-tailed tests. P values were calculated by one-way analysis of variance (ANOVA), Pearson’s χ² and Spearman’s rho correlation tests. P<0.05 was considered statistically significant.

RESULTS

The frequencies of different dimorphic polymorphisms based on single nucleotide substitution were as follows:
Table 1 Statistical significance of effects of cytokine gene polymorphisms on different parameters

| Cytokine gene polymorphism | ALT (upper limit normal) | HCV RNA level | Grade of inflammation (0-18) | Stage of fibrosis (0-6) | Steatosis (0-3) | Lymph follicles | End of treatment response | Sustained response |
|---------------------------|--------------------------|---------------|----------------------------|------------------------|----------------|-------------|------------------------|------------------|
| IL 10-1082 (GA, AA, GC)   | 0.636                    | 0.241         | 0.029                     | 0.079                  | 0.267         | 0.848       | 0.078                  | 0.331            |
| IL 10-819 (AC, CC, AA)    | 0.794                    | 0.781         | 0.412                     | 0.020                  | 0.57          | 0.295       | 0.063                  | 0.331            |
| IL 10-392 (CA, CC)        | 0.582                    | 0.198         | 0.243                     | 0.281                  | 0.351         | 0.626       | 0.416                  | 0.283            |
| IFN-γ (CA, GC)            | 0.389                    | 0.848         | 0.919                     | 0.921                  | 0.955         | 0.382       | 0.21                   | 0.933            |
| TNF-α (AA, TT, TA)        | 0.952                    | 0.436         | 0.777                     | 0.307                  | 0.32          | 0.85        | 0.674                  | 0.451            |
| IL-1β (AG, GG)            | 0.734                    | 0.72          | 0.386                     | 0.959                  | 0.232         | 0.643       | 0.026                  | 0.206            |
| IL-10 (A/C)               | 0.144                    | 0.591         | 0.964                     | 0.826                  | 0.326         | 0.836       | 1.00                   | 1.00             |

P values were calculated by ANOVA and Pearson’s χ² test. Statistically significant values. IL: interleukin; IFN-γ: interferon gamma; TNF-α: tumor necrosis factor alpha; TGF-β: transforming growth factor beta.
whereas the mean IL4 and IL6 levels are lower in patients infected with HCV[23]. Another study shows the higher levels of serum IL-1β, IL-4 and IL-6 (0.221, 0.104 and 1.393 pg/mL) in all HCV patients than in healthy adults (0.188, 0.025 and 0.600 pg/mL) [23].

Sustained response to interferon and ribavirin, defined as undetectable HCV RNA at 6 mo after discontinuation of therapy, is achievable in 30-60% of treated patients [23,24]. Predictors of response include viral factors such as viral genotypes, viral load and early disappearance of HCV RNA after initiation of therapy, while the host factors include gender, age and degree of fibrosis [25-27]. In recent years, increasing attention has been drawn to the role of host variation in cytokine levels in inflammatory and immune responses. Polymorphisms in genes encoding immunoregulatory proteins, proinflammatory cytokines, and fibrogenic factors may affect the production of these factors and influence disease progression in patients with chronic liver disease due to alcohol, primary biliary cirrhosis, or hepatitis C [28].

There might be an association of cytokine gene polymorphism and susceptibility to hepatitis C infection. Hohler et al[29] have reported such an association with the polymorphism at the TNFα promoter. The TNF promoter variants TNF2 (-238A) and TNF3 (-308A) confer a 3.2-fold and 5.1-fold risk of cirrhosis respectively (P = 0.03 for both). Reciprocal effects have been observed with several TNF alleles and haplotypes defined by the -238 G/A and -308 G/A dimorphic sequences, thus polymorphisms in the TNF alpha promoter appear to be associated with the variability in the histological severity of chronic hepatitis C infection [30]. In our study done on hepatitis C genotype 3 patients, no such effect of TNFα -308 variability was observed.

Interleukin (IL)-10 is a cytokine that downregulates the proinflammatory response and has a modulatory effect on hepatic fibrogenesis and is a potent anti-inflammatory Th2 cytokine that downregulates the expression of major histocompatibility complex (MHC) class I and class II molecules, as well as the production of Th1 cytokines [31-36]. IL-10 levels differ widely between individuals, possibly because of polymorphisms in the promoter region of the IL-10 gene [37,38]. Specifically, three SNPs in the promoter (at positions -1082, -819, and -592 relative to the transcription start site) form three SNP combinations (ATA, ACC, GCC), which are associated with differential IL-10 expression [38-40]. It is reasonable to assume that hepatitis C patients who produce high levels of IL-10 have less hepatocellular injury and less ability to control infection and patients with low secretion of IL-10 have a better ability to eliminate the hepatitis infection. Perhaps low IL-10 production can skew the immune system into the Th1 type of response, facilitating the clearance of viral load. -1082A allele is associated with reduced IL-10 production in vitro [39]. In our study, individuals who were homozygous for IL-10 AA at position -1082 had a lower HAI.

It has been shown that hepatitis C patients, genotyped as high IL-10 producers, have a poor response to IFN-2 therapy [40]. Such polymorphisms may also predict the sustained viral response to antiviral therapy [41]. These patients may benefit from additional treatment strategies designed to enhance T-helper type 1 (Th1) response. In one study, the interleukin-10 -1082 G/G genotype was identified more frequently in patients than in controls (P = 0.048). The patients exhibiting transforming growth factor-beta 1+29 (codon 10) C/C genotype variables were less likely to respond to treatment than patients with the T/T or T/C genotypes [42]. Liver transplant recipients, who are genotyped as having a low production profile of IL-10, are more prone to rejection and less likely to have hepatitis C recurrence [43]. The IL-1beta-31 genotype T/T or the IL-1beta-511/-31 haplotype C/T is associated with the presence of HCC in Japanese patients with chronic HCV infection [44].

However, not all the studies favor such effect of polymorphisms. Three members of the interleukin-1 gene family (IL-1A, IL-1B and IL-1RN), three polymorphic sites in the interleukin-10 gene promoter (-1082, -819, -592) and two in the TNF-α promoter (-308, -238) were studied in two independent DNA banks, each with appropriate controls. Standard PCR-based genotyping techniques were used. No significant difference in the distribution of any of the polymorphisms has been found in either study set [45].

We, in this study, determined SNP at position -1082, -819 and -592 in case of IL-10, -874 for IFN-γ, -308 for TNF-α, -10 for TGF-β and IL-1 C/ U and analyzed the frequency of their distribution and correlation with the ALT and HCV RNA levels, HAI and response to treatment. In our series, we selected genotype 3 patients because this is the main genotype in our region and not enough data are available on the influence of host cytokine gene polymorphisms of this viral genotype. Another reason of selecting a single genotype was to make the group uniform as different genotypes have different response rates to antiviral therapy and influence of cytokines may also be different. We could not find any significant difference in the cytokine genotype profile while analyzing different variables except for some influence of polymorphisms of IL-10 on liver histology. These polymorphisms did not modulate the response to interferon plus ribavirin therapy. This may be due to the small sample size or the fact that viral genotype 3 is easy to treat genotype in any case.

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