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Study of Association between Interleukin 20 Polymorphism (Rs1518108) and Chronic Hepatitis C Infection

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Abstract

***Background*:** Chronic hepatitis C is a major concern for global health as it causes liver problems, cirrhosis and liver cancer. Immune factors have a determinant role in susceptibility to chronic infection or clearance of infection in body. As a defensive agent, cytokines are important factors of immune system, since they can activate immune response or inhibit virus replication directly. The aim of this study is the evaluation of interleukin 20 polymorphism (rs1518108) in hepatitis C patients.

***Materials and Methods*:** This survey was a case–control study. By using PCR-RFLP method, 105 patients and 135 controls were studied randomly. We used SPSS-16 software for statistical analysis.

***Results*:** A significant association was found between polymorphism (rs1518108) of interleukin 20 and hepatitis C patients (p=0.035) (OR=2.283). The incidence of hepatitics C in males was observed five times more than that one females (p=0.01) (OR=5.18). In addition, no significant association between polymorphism of genotypes and liver harms (chronic and cirrhosis) was found in this study (p=0.362).

***Conclusion*:** Our findings show that variants of interleukin 20 polymorphism (rs1518108) in the population of the study are important factors for being affected by hepatitis C. The incidence of heterozygote allele CT was more than of homozygote genotype TT.

Introduction

Chronic hepatitis C is caused by the hepatitis C virus (HCV) infection. This virus is a RNA virus and belongs to *Flaviviridae* family that can develop a wide range of clinical complications including hepatocellular carcinoma, liver failure and liver cirrhosis [1]. Hepatitis C is one of the main causes of mortality in the world and HCV has infected 170 million people in the world that 15% of its population is symptomatic [2, 3]. This viral infection leads to pro inflammatory cytokines production. The cellular immune responses in hepatitis C have an important role in liver damages and viral functions, although the exact mechanisms of such functionality are not well known yet [4, 5].

Cytokines play a crucial role in the body against infectious agents. They generally activate the immune system as a defense factor and also prevent viral replication in the body [6]. The variation of polymorphisms in cytokine genes could be an effective factor in the control of hepatitis C virus infection; therefore, in this study the association between interleukin 20 (IL 20) polymorphism and HCV infection was investigated. IL 20 is produced by different cells in liver including hepatocytes, sinusoidal endothelial cells, liver kupffer cells and lymphocytes. IL 20 belongs to the interleukin 10 (IL 10) family and has a chief regulatory part in HCV infection like IL10 [7-9].

The level of IL 20 production is associated with autoimmune diseases such as autoimmune hepatitis and it seems that its production level has an effect on the incidence of hepatocellular carcinoma and lesions related to immune response such as liver failure and liver cirrhosis [10, 11]. IL 20 coding sequence is a 195 kilo base region which is located on the long arm of chromosome 1 [12].

Genetic variations such as nucleotide polymorphisms in cytokine genes can raise or lower the severity of the inflammatory infections [13]. Changing the alleles can be considered as an effective factor to prevent diseases from progression to liver failure and cirrhosis. Several polymorphisms have been identified in the sequence of human IL 20 gene. In this study, IL 20 polymorphism (rs1518108) at position 3978 (in a region near to the gene)

was investigated. The aim of this study was to assess the association between nucleotide polymorphism at position 3978 of IL 20 gene and chronic HCV infection in patients and healthy control subjects who were referred to gastroenterology ward of Tehran Taleghani Hospital.

Materials and Methods

This research was conducted as a case-control study. Patients' and healthy controls' samples were collected from the gastroenterology ward of Tehran Taleghani Hospital between years 2010 and 2011, then according to the clinical criteria, ELISA tests were performed on samples. ELISA test positive results for patients' samples and negative results for the healthy control group samples were confirmed and the results were evaluated. The patient group consisted of 105 patients and the control group consisted of 135 healthy people. The demographic data and clinical history of patients were gathered based on a questionnaire completed by the assistance of a trained general practitioner. Peripheral blood samples were taken and informed consent was obtained from all participants as well.

The study protocols and written informed consent were reviewed and approved by the ethics committee at the Research Center for Gastroenterology and Liver Diseases, Shaheed Beheshti University of Medical Sciences (Code 522). Genomic DNA was extracted from blood samples using standard phenol-chloroform method [14]. The subjected gene sequence was retrieved from GenBank and examined for primer design, and a primer pair was designed for the gene using Gene Runner software subsequently. Finally, sequences of the primers were subjected to BLAST online search engine (NCBI). Nucleotide sequences of primers used in this study are as follow: IL20 primer pairs Forward: 5'GCCCAGACAGGTGTATGAGC3' Reverse: 5'GAGTTATCAAAAGTTAAAGTCATTG 3′

The PCR-RFLP method was used to investigate the presence of variation at polymorphism site of IL20 gene. Polymerase chain reaction was performed using 100 ng of DNA in a total volume of 25 µl in the thermo cycler device (Ependorf). Thermocycling condition after optimization was as follow: 1) Primary denaturation phase: 10 min at 95ºC for 1 cycle, 2) Secondary denaturation phase: 45 sec at 95ºC, annealing phase, 45 sec at 45ºC and extension phase, 45 sec at 72ºC all of them for 35 cycle, 3) Final extension: 10 min at 72ºC for 1 cycle.

PCR performance accuracy was also controlled and confirmed by positive and negative controls. PCR products were then subjected to apal1 restriction enzyme digestion. This enzyme cuts the interleukin 20 at position 223 if the nucleotide changes at that position. RFLP products were run in 2% agarose gel and stained with ethidium bromide. Finally, 10% of whole samples were genotyped using sequencing technique (direct sequencing method) to confirm PCR-RFLP results. In this study, the statistical analysis was performed using SPSS-16 software. Logistic regression was performed to analyze the association between genotypes and a chi-square test was used to evaluate Hardy-Weinberg equilibrium. The risk of chronic hepatitis C was calculated with 95% confidence interval.

Results

In this study, general and clinical information of patients and healthy individuals such as, age gender and liver damages are shown in table 1. The mean age of both the patient and the healthy control groups are close. The prevalence of hepatitis C diseases was higher in men. The adjusted and unadjusted data for age and gender are reported in table 2. Statistical analysis showed that no association between genotypes of studied polymorphism and liver damage could be spotted.

Genotype frequencies of CC, TT, CT in patient group were 36.2, 15.2, 48.6 and in the healthy group were 52.6, 13.3, 34.1% respectively.

The results of this study highlighted a significant difference in genotype frequencies between patients and control group (p=0.053). The results of enzymatic digestion of Apal1 restriction site are as follow: length of CC genotype (homozygote C) undigested product was 386 bp, and CT genotype (heterozygote) digested products were 386, 223, 163 bp and TT genotype (homozygote T) digested product were 223 and 163 bp. The enzymatic digested products are illustrated in figure 1.

Table 1. General and clinical information of studied population

General information	Patients	Healthy controls	p-Value
Mean age (Years)	47.92	46.50	0.455
Female	20	73	0.001
Male	85	62	0.001
HCV	105	-	0.362

Table 2. Adjusted and unadjusted data for age and gender

Genotype	Patients N (%)	Healthy controls N (%)	OR[a] (CI 95%)	p-Value	OR[b] (CI 95%)	p-Value
CC	38 (36.2)	71 (52.6)	Reference 1	-	Reference 1	-
CT	51 (48.6)	46 (34.1)	2.072 (1.183-3.628)	0.011	2.283 (1.210-4.306)	0.011
TT	16 (15.2)	18 (13.3)	1.66 (0.761-3.624)	0.203	1.835 (0.789-4.267)	0.158
C	127 (60.5)	188 (69.6)	-	-	Reference1	-
T	83 (39.5)	82 (30.4)	1.498 (1.025-2.189)	0.035	-	-

a: adjusted for age and gender, b: unadjusted for age and gender

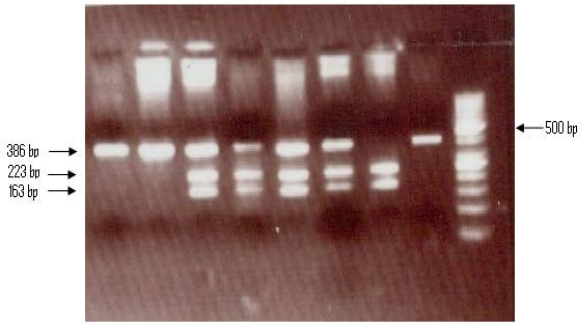

Figure 1. Fragments were made by ApaII enzyme. Marker 50 bp in well number 1, well 2: CC genotype, well 3: CT genotype and well 4: TT genotype

Discussion

In this study, the association between genotype CT of interleukin 20 gene and hepatitis C was investigated in patients and healthy populations; it was determined that the risk of hepatitis C in individuals with genotype CT was approximately two fold higher than individuals with genotype CC; likewise, the prevalence of hepatitis C in men was estimated 5 times higher than women. Therefore, individual's genetic background is one of the most effective factors in disease progression toward chronic infection; especially in viral infections such as hepatitis C and changes in cytokines production levels have deep impact on infection's severity and chronicity [15-19].

Factors such as disease progression, rate and response to treatment were in various manners affected by genetic background; and as a result, elicit various responses in different people. IL 20 has also an important immunomodulatory role as well as IL 10, inhibits T cell proinflammatory cytokine genes transcription and macrophage growth and activation. Recent studies on hepatitis C patients suggest that decline in IL 20 production may slow progression to liver cirrhosis [20]. Kingo et al. who studied IL 20 rs1518108 polymorphism on 76 samples from Asian patients with vitiligo and 236 Asian healthy subjects, could find a significant association between the polymorphism and vitiligo. The T minor allele frequency of IL 20 rs1518108 polymorphism was 36.8 and 46.4% in the patient and the healthy control groups respectively [21]. In the present study, the frequency of T Minor allele in the patient and the healthy control groups was 39.5 and 30.4% respectively which represents higher percentage of T minor allele patients with hepatitis C. This reflects the high prevalence of IL 20 rs1518108 polymorphism in the studied patients. A meaningful difference in genotypes prevalence was also observed in the present study.

Thus, the present report demonstrates the increased risk of hepatitis C for carriers of rs1518108 polymorphism. Tracks et al., studied rs1518108 in 227 healthy controls and 153 MDD (Major Depressive Disorders) patients. They couldn't find any significant association [16-23]. Truelove et al., have investigated IL 20 gene polymorphisms and their association with hepatitis B virus infection in African-American and American-European populations and they have observed meaningful difference in both populations but among IL 20 polymorphisms, rs1518108 showed a clear association with chronic disease in African-American population [13]. Oleksyk et al., have reported a deeper association between rs1518108 polymorphism and hepatitis C in African-American population [24]. However, these differences in various studies may be a result of difference in IL 20 expression level. In a survey on American-European population, the minor T allele frequencies were reported the same, in both patient and control groups (49%). Nevertheless in a study on African-American population, T allele was more frequent in healthy controls than patients (64% in controls vs. 48% in patients) [13, 25].

In the present study, heterozygote CT genotype was more than TT homozygote in rs1518108 polymorphism. Similar to this study, Truelove et al., have reached to the same results on hepatitis B patients which heterozygote CT genotype was reported higher than homozygote TT in rs1518108 polymorphism [13, 16]. Another key aspect in studying patients with chronic hepatitis infection is frequency of cirrhotic patients among them.

Hamada et al., studied the association between IL 10 level variations and liver cirrhosis; they concluded that high production of IL 10 can reduce disease progression to cirrhosis phase [17] but there hasn't been any report of association between IL 20 and cirrhosis process yet. No significant difference was found between patients with chronic disease and people with cirrhosis in the present study. Further surveys on treatment response at different stages of the disease and IL 20 polymorphisms are suggested.

A significant association between genotypes of healthy controls and hepatitis C at rs1518108 polymorphism of IL 20 was observed that represents higher risk of chronic hepatitis C infection for rs1518108 polymorphism carriers of IL 20. According to the results obtained in this study, allele T frequency of this polymorphism in the patient group was relatively higher than healthy control group which represents higher frequency of the rs1518108 IL 20 polymorphism in the studied population. No significant difference in the genotype of this polymorphism at the studied site has been found between chronic patients and people with cirrhosis.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5(9): 558-67.
2. Barrett L, Gallant M, Howley C, et al. Enhanced IL-10 production in response to hepatitis C virus proteins by peripheral blood mononuclear cells from human immunodeficiency virus-monoinfected individuals. BMC Immunol 2008; 9: 28.
3. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and independent mechanisms. Immunity 2006; 25(3): 455-71.
4. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998; 338(13): 853-60.
5. Lai CL, Ratziu V, Yuen MF and Poynard T. Viral hepatitis B. Lancet 2003; 362(9401): 2089-94.
6. Bommireddy R, Doetschman T. TGF beta1 and Treg cells: Alliance for tolerance. Trends Mol Med 2007; 13(11): 492-501.
7. Pestka S, Krause CD, Sarkar D, et al. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol 2004; 22: 929-79.
8. Blumberg H, Conklin D, Xu WF, et al. Interleukin 20: Discovery, receptor identification, and role in epidermal function. Cell 2001; 104(1): 9-19.
9. Gallagher G, Dickensheets H, Eskdale J, et al. Cloning, expression and initial characterization of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10). Genes Immun 2000; 1(7): 442-50.
10. Shin HD, Winkler C, Stephens JC, et al. Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL 10. Proc Natl Acad Sci U S A 2000; 97(26): 14467-72.
11. Vicari AP, Trinchieri G. Interleukin-10 in viral diseases and cancer: Exiting the labyrinth? Immunol Rev 2004; 202: 223-236.
12. Commins S, Steinke JW, Borish L. The extended IL-10 super family: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 and IL-29. J Allergy Clin Immunol 2008; 121(5): 1108-11.
13. Truelove AL, Oleksyk TK, Shrestha S, et al. Evaluation of IL 10, IL 19, and IL20 gene polymorphisms and chronic hepatitis B infection outcome. Int J Immunogenet 2008; 35(3); 255-264.
14. Sambrook J, Russell DW. Molecular cloning: A laboratory manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press; 2001: 31-38.
15. Xu W. Interleukin-20. Int Immunopharmacol 2004; 4(5): 627-633.
16. Koks S, Kingo K, Ratsep R, et al. Combined haplotype analysis of the interleukin-19 and -20 genes: Relationship to plaque-type psoriasis. Genes Immun 2004; 5(8): 662-667.
17. Hamada H, Yatsuhashi H, Yano K, et al. Interleukin-10 promoter polymorphisms and liver fibrosis progression in patients with chronic hepatitis C in Japan. J Hepatol 2003; 39(3): 457-458.
18. Parrish-Novak J, Xu W, Brender T, et al. Interleukins 19, 20, and 24 signal through two distinct receptor complexes. Differences in receptor-ligand interactions mediate unique biological functions. J Biol Chem 2002; 277(49): 47517-47523.
19. Romani S, Azimzadeh P, Mohebbi SR, et al. Investigation of transforming growth factor-β1 gene polymorphisms among Iranian patients with chronic hepatitis C. Hepat Mon 2011; 11(11): 901-906.
20. Tsai SL, Liaw YF, Chen MH, et al. Detection of type 2-like T-helper cells in hepatitis C virus infection: Implications for hepatitis C virus chronicity. Hepatology 1997; 25(2): 449-458.
21. Kingo K, Reimann E, Karelson M, et al. Association analysis of genes of the IL 19 cluster and their receptors in vitiligo patients. Dermatology 2010; 221(3): 261-266.
22. Traks T, Koido K, Eller T, et al. Polymorphisms in the interleukin-10 gene cluster are possibly involved in the increased risk for major depressive disorder. BMC Med Genet 2008; 9: 111.
23. Koks S, Kingo K, Vabrit K, et al. Possible relations between the polymorphisms of the cytokines IL-19, IL-20 and IL-24 and plaque-type psoriasis. Genes Immun 2005; 6(5): 407-415.
24. Oleksyk TK, Thio CL, Truelove AL, et al. Single nucleotide polymorphisms and haplotypes in the IL 10 region associated with HCV clearance. Genes Immun 2005; 6(4): 347-357.
25. Duggal P, Winkler CA, An P, et al. The effect of RANTES chemokine genetic variants on early HIV-1 plasma RNA among African American injection drug users. J Acquir Immune Defic Syndr 2005; 38(5): 584-589.

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