Iranian Journal of Virology 2016;10(2-3): 40-47

Original Article

Study on the Relationship of Demographic Characteristics of rs1053004 in STAT3 Gene in Patients with HCC following Chronic HBV Infection

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Abstract

Background and Aims: Hepatocellular carcinoma (HCC) is one of the most important common cancers in the world. The main etiology of this cancer in developing and third world countries is due to the infection with hepatitis B and C viruses. Hepatitis B and C viruses (HCV) would both cause liver cancer but the incidence of the disease in relation to the age and gender has not been determined. The present study was conducted to evaluate the relation between some demographic characteristics with rs1053004 polymorphism in STAT3 gene among patients with liver cancer following chronic hepatitis B infection and its comparison with healthy subjects.

Materials and Methods: In this study, 33 tissue samples of liver cancer from pationts with HBV infection, 50 blood samples from patients with chronic hepatitis B and 50 blood samples from healthy subjects, as the control group, were obtained to determine rs1053004 polymorphism in STAT3 gene (signal transducer factor and activator of transcription in the nucleus) using Real Time PCR method.

Results: In the present study 133 subjects were evaluated and from them, 50 (37.6%) were healthy and 50 of the participants (37.6%) had chronic hepatitis B and 33 (24.8%) had HCC. 69.9% of the participants (93 participants) were male and 30.1% (40 participants) were female. According to the results, the gender of the participants in the studied groups had no significant relation with their SNPrs1053004 polymorphism. But the relation between gender and liver cancer was statistically significant ($p < 0001$); indicating that the prevalence of liver cancer was higher among men than women. The average age of the healthy group was 35.86 years, of the chronic hepatitis B group was 40.4 years and of the HCC group was 53.78 years. Based on the results, the difference in age groups of chronic hepatitis B group and HCC pationts was statistically significant as compared with the control group ($p < 0.001$).

Conclusions: Results of the present study showed no significant relation between the presence of rs1053004 polymorphism in STAT3 gene (signal transducer factor and activator of transcription in the nucleus) and gender of the participants but the difference between the ages of the healthy group, chronic hepatitis B group and HCC group was statistically significant. In other words, age could be a predicting factor in developing HCC.

Keywords: Polymorphism, STAT3 Gene, Hepatocellular Carcinoma, Hepatitis B.

[DOI: 10.21859/isv.10.2.3.40]

Introduction

Hepatitis B virus is one of the most important human viral pathogens which causes a wide range of acute and chronic liver diseases (1). This disease is a viral infection in humans which, despite the production of its vaccine, has involved many people all around the world and its prevalence among adults is increasing. About 5 to 10% of the cases infected with hepatitis B, would lead to chronic hepatitis B due to the incomplete and inadequate respond of the immune system(2, 3) . Most of the patients with chronic hepatitis B are asymptomatic carriers and are not aware of their disease, since they have no obvious clinical symptom; therefore they would pass the virus to others unintentionally(4, 5).

Hepatocellular carcinoma has various risk factors, which differ from one country to another. In developing countries and third world countries, due to the high prevalence of hepatitis B, this virus is the most important cause of liver cancer(6, 7). Hepatocellular carcinoma is one of the most important common cancers in the world especially in Africa and East Asia. Also it is the third cause of death by cancer among men and the seventh among women and would lead to the death of one million people annually, globally. About 360 million people have the chronic from of hepatitis B in the world and in more than about 25% of the cases, the chronic infection would lead to hepatocellular carcinoma. Chronic infection with HBV, with or without cirrhosis of the liver, in most regions of the world, including Iran, is considered one of the important risk factors for liver cancer(4, 5). Liver cancer is the most death-related cancer. Liver cancer is usually related to different risk factors including alcohol consumption, expression of Aflatoxin B and chronic viral hepatitis infections such as B and C; about 80% of the reported cases of liver cancer in the world were developed following the infection with hepatitis B(6, 7) .

Different factors are associated with occurrence of hepatocellular carcinoma form chronic hepatitis B infection. One of these factors that has recently attracted the attention of researchers is the increase in expression of STAT3 protein. STAT protein is an inflammatory mediator, a signal transducer in the entire cytoplasm and the activator of transcription in the nucleus. STAT protein has seven members including: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 (8, 9). From the family of STAT proteins, STAT3, due to its relation with hepatocellular carcinoma, has attracted more attention. Recently the polymorphisms of this protein in formation of cancer have been studied and some of these polymorphisms are SNPrs1053004 (T > C), rs4796793 (C > G), rs2293152 (C > G) and etc. This polymorphism itself is consisted of different alleles and genotypes including T alleles (thymine) and C alleles (cytosine) and these alleles have different forms of dominant and recessive (9-14). Some studies have shown that STAT3 protein has a potential role in inflammation, survival, extension and invasion of hepatocyte cells and development of liver cancer(9, 15). In another study, inhibition of STAT3 caused an average reduction in expression of hepatitis B virus and consequently development of hepatocellular carcinoma was prevented; this indicated the role of STAT3 in spreading cancer in liver cells(16, 17). Another study has shown that one of the most important factors for phosphorylation and consequently activation of STAT3 protein, is the X protein from the hepatitis B virus, IL11, IL6 (interleukins are a group of secretory protein that are produced by different cells including leukocytes and are responsible for mediation and regulation of all the aspects of innate and adaptive immunity), NFKB and acute phase proteins, which by activating this protein would develop metastasis in the cell(13, 18). In study that was conducted on rs1053004 polymorphism of

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[DOI: 10.21859/isv.10.2.3.40]

STAT3, a relation was revealed between this polymorphism and the increased risk of hepatocellular carcinoma; in this study the CC allele in comparison to the TT allele, and men compared to women, were significantly associated with higher risks of HCC. In this study one of the main activators of STAT3 was the X protein of hepatitis B virus. Also in this study the mentioned polymorphism was associated with chronic hepatitis B infection and low viral load. Therefore this polymorphism could be associated with immunity tolerance too (13). According to this study SNPrs1053004 could be a new genetic marker that its recognition might be helpful in the advancement of the disease in developing hepatocellular carcinoma among patients with chronic hepatitis B(13).

Considering that liver cancer is the third cause of one of the deadliest gastrointestinal cancers(19, 20), and also, since in Iran, cases of liver cancer are usually diagnosed at the intermediate or advanced stages of the cancer, it is necessary to find solutions for diagnosing the disease before these stages which will help to prevent its development and provide timely treatment (21). Also, in Iran, no studies have evaluated the relation of STAT3 and its polymorphism with liver cancer. So, the present study was conducted to evaluate the relation between some demographic characteristics and rs1053004 polymorphism in STAT3 gene among patients with liver cancer following chronic hepatitis B infection and its comparison to healthy subjects. If possible, the results of this study might be used as a basis for future studies.

Methods

In the present study, 33 tissue samples as liver biopsy from patients with hepatocellular carcinoma infected with hepatitis B virus and 50 blood samples from 50 patients with chronic active hepatitis and 50 from healthy subjects were obtained. liver biopsy specimen were used for the HCC group. Chronic active HBV infection was confirmed by ELISA test and PCR test for HBsAg after 6 months and hepatocellular carcinoma was confirmed by imaging and histological examinations(22). The inclusion criteria for the healthy subjects were having no indication of hepatitis B infection or any other infectious diseases, having no autoimmune diseases, and not using any immunosuppressive drugs. HIV and HCV positive patients, pregnant subjects, and those who had an infection or surgery in the previous two weeks of the study were excluded. The participants of the control group were selected in a way that they would match the patients group regarding their age and gender.

To determin HBV-DNA copy and rs1053004 polymorphism, DNA was extracted from peripheral blood samples for tissues. Three cross-sectional cuts were made out of each tissue sample. The cuts were deparaffinized using xylene, and at the next step, to remove the xylene, ethanol was used. After drying the samples at room temperature, they were put into buffer and proteinase K solutions. Then, from the prepared tissue samples and gathered blood samples, DNA was extracted. For extracting the genome DNA, Magcore DNA Extraction kit (RBC, Germany) was used through automatic extraction device, MagCore 36, at the virology department of the Besat specialized clinic.

After extracting the genome, HBV DNA load determination was performed for all the samples to evaluate the severity; it was performed using HBV DNA Load Geneproof (Germany) kit, through Lightcycler96 (Rosche, Germany) device. Then using Real Time PCR technique, the number of HBV-DNA copies in the prepared samples was evaluated. (23) viral DNA load of the blood samples was evaluated using Inter Lab service (IVD,CE) kits, the level of viral load was determined and, in comparison with the standard number of HBV-DNA copies, the number of patients' HBV-DNA copies was determined. To evaluate the alleles of rs1053004 gene two types of probes have been designed, each for one allele which were marked with a specific color. So, in the present study, Real-Time PCR method on the basis of Taqman Probe was used (12, 23). Due to the proximity of this polymorphism's genomic position to other polymorphisms, using less expensive methods such as HRM

[DOI: 10.21859/isv.10.2.3.40]

was not possible. After performing Real-Time PCR, each allele was evaluated in a specific channel. Reactions were performed in a total volume of 25 µl. Briefly, 1x of magnesium-less PCR buffer, 0.2 mM of dNPTs combination, 1.5 mM of MgCL2, 0.2 unit of Taq DNA polymerase and 1x of primers and probes mixture were used for determining the genotypes of SNP(24) .

The gathered data were analyzed using SPSS version 18, by T-test and Mann-Whitney tests. To determine the relation between quantitative variables of gene expression and the serum levels of liver enzymes and also, in case of normal distribution of the data and the number HBV-DNA copies, Pearson and Spearman correlation coefficients were used. For all the statistical tests, the significant level was set at a p value less than 0.05.

Results

Frequency distribution of the studied participants in each group of the present study is shown in table 1.

As it is shown in the table, 50 of the participants (37.6%) were healthy, 50 (37.6%) had chronic hepatitis B and 33 (24.8%) had HCC.

Frequency distribution of the studied participants in the present study based on the frequency of their polymorphisms is shown in table 2. As it can be observed, 46.6% of the alleles were of TT type, 37% were of CC type and 30.8% were of TC type.

The results in table 3 indicates the relation between re1053004 polymorphism and the gender of the participants in each studied group. According to the results, no significant relation existed between the rs1053004 polymorphism and the gender of the studied participants.

Table 1: Frequency distribution of the studied participants in each group

Group	Frequency	Percent
Healthy	50	37.6
Chronic hepatitis B	50	37.6
HCC	33	24.8

Table 2: Frequency distribution of the studied participants based on frequency of their polymorphisms

Group	Frequency	Percent
TT	62	46.6
CC	30	22.6
TC	41	30.8

Table 3: Determining the relation between SNPrs1053004 polymorphism with the gender of the participants in each group

Polymorphism Group		Polymorphism			Test's statistics	P value
		TT	CC	TC		
Healthy	Male	18	2	12	2	0.133
	Female	14	2	2		
Chronic hepatitis B	Male	14	6	13	2	0.638
	Female	6	2	9		
HCC	Male	8	15	5	2	0.575
	Female	2	3	0		

[DOI: 10.21859/isv.10.2.3.40]

Table 4: Determining the relation between liver cancer and the gender of the studied participants

Gender	Number	Percent	ANOVA test	
			Test's statistics	P value
Male	28	84.8	29.164	0.000***
Female	5	15.2		

*** Statistically significant difference compared to other groups ($p < 0.001$)

Table 5: Determining the relation between SNPrs1053004 polymorphism with the age of the participants in each group

Polymorphism Group		Polymorphism			Test's statistics	P value
		TT	CC	TC		
Healthy	**10 to 30 years**	12	1	4	4	0.666
	31 to 50 years	11	2	8		
	51 to 70 years	9	1	2		
Chronic hepatitis B	**10 to 30 years**	6	2	4	4	0.836
	31 to 50 years	8	3	12		
	51 to 70 years	6	3	6		
HCC	**10 to 30 years**	4	2	1	2	0.019*
	31 to 50 years	6	16	4		
	51 to 70 years	10	18	5		

* Statistically significant difference compared to other groups ($p < 0.05$)

The relation between liver cancer and the gender of the studied participants is shown in table 4. According to the results, the gender of the participants had a significant relation with the risk of liver cancer ($p < 0.001$); meaning that the prevalence of liver cancer was higher among menthan women.

Table 5 has evaluated the relation between SNPrs1054003 and the age of the participants in different studied groups. Based on their age, participants were divided into three groups of 30 to 50 years old, 31 to 50 years old and 51 to 70 years old. According to the results, in the healthy and the chronic hepatitis B groups, the

[DOI: 10.21859/isv.10.2.3.40]

age of the participants had no significant relation with their SNPrs1053004 polymorphism but the relation between age and SNPrs1053004 in patients with liver cancer was statistically significant ($p < 0.05$); meaning that liver cancer is more prevalent among older patients who the allele of their SNPrs1053004 polymorphism is of CC type.

Discussion

The present study was conducted to evaluate the relationship between some demographic characteristics with rs1053004 polymorphism in STAT3 gene among patients with liver cancer following chronic hepatitis B infection and its comparison to healthy subjects. Regarding the nature and the goals, the present study was a case-control research. In the present study 133 participants were evaluated and from them, 50 (37.6%) were healthy subjects. 50 participants (37.6%) had chronic hepatitis B and 33 (24.8%) had HCC. From all the participants, 69.9% (93) were male and 30.1 (40) were female. According to the results the gender of the participants in the studied had no significant relation with SNPrs1053004 polymorphism but the relation between liver cancer and the gender of the participants was statistically significant. So, the prevalence of liver cancer among men was higher than women. In a study that was conducted by Nguyen and Hou results showed that HCC was reported more among men than women, which is similar to the results of the present study; this difference between men and women might be due to the environmental risk factors such as smoking and alcohol consumption. Also, gender differences in the risk of HCC might be caused by hormonal differences; meaning that estrogen has a protective against the spread of HCC by suppressing the effects and actions of STAT3, while androgens are able to improve some cancers such as prostate and HCC (25, 26). In a study that was conducted by Xie et al in 2013 on rs1053004 polymorphism of STAT3, a relation between this polymorphism and higher risk of hepatocellular carcinoma was shown. In this study CC allele in comparison to TT allele, and being male against female were significantly associated with increased risks of HCC. In this study one of the main activators of STAT3 was the X protein of hepatitis B virus. Also in this study the mentioned polymorphism was associated with chronic hepatitis B infection and low viral load. Therefore, this polymorphism might be related to immunity tolerance(13).

In the present study, the mean age of the participants in the healthy group was 35.86 years, in the chronic hepatitis B group was 40.04 years and in the HCC group was 53.78 years. According ot the results, there was a significant difference between the mean age of the participants in the healthy group, chronic hepatitis B and the HCC group. In other words, the variable of age could have a predictive role in development of HCC. Lamontagne in a study stated that development of a HBV-positive chronic infection is related to the age of the patients, in a way that younger patients are at more risks for developing this infection (27). Results of the present study are in line with the results of Lamontagne study. However, one part of the present study resulted that, in the healthy group and chronic hepatitis b group, the age of the participants had no significant relation with rs1053004 polymorphism but this relation was statistically significant in the liver cancer group ($p < 0.05$). So, liver cancer was more prevalent among older patients who the allele of their rs1053004 polymorphism was of CC type.

Acknowledgements

The present study was financially supported by the Kerman University of Medical Sciences.The authers would like to thank the medical pathology staff of the Shiraz University of medical Sciences for providing the tissue samples. The authors would like to thank all the patients and colleagues, who directly and indirectly, helped us in performing this study and writing this article.

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[DOI: 10.21859/isv.10.2.3.40]

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[DOI: 10.21859/isv.10.2.3.40]

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[DOI: 10.21859/isv.10.2.3.40]