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Research Article

Association of Codon 72 of *P53* Gene Polymorphism with Chronic Hepatitis C Virus Infection: A Case Control Study

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Abstract

Single nucleotide polymorphism in codon 72 of *p53* gene (Arg/Pro) changes *p53* protein structure and affects its activities. Hepatitis C virus (HCV) is believed to induce hepatocellular carcinoma and *P53* polymorphisms have been associated with human cancers. The aim of this study was to evaluate genetic variants of codon 72 of *p53* gene polymorphism in HCV patients and its relationship with HCV infection. The study was conducted on 67 HCV patients, who were referred to medical centers of Mashhad city, Iran, and 73 healthy people from the same region. Genotyping of codon 72 of *p53* gene was performed by PCR-RFLP method. The distributions of different alleles of *p53* polymorphisms did not differ significantly between groups. The respective proportions of Proline homozygotes, heterozygotes, and Arginine homozygotes were 37.31%, 35.82%, 26.86% in patients and 39.72%, 27.39%, and 32.87% in the control group respectively. However, we found no significant differenece for the allelic or genotype distribution between cases and controls. Our results indicated no strong evidence of association of the *p53* polymorphism with HCV infection; however, further investigation is needed in different ethnic groups to elucidate the role of this polymorphism in HCV infection.

***Keywords*:** Polymorphism, P*53* gene, HCV, Genetic epidemiology, Iran

Introduction

The effects of genetic and environmental factors have been definitively proved in cancers (Ziech et al., 2010a; Ziech et al., 2010b; Ziech et al., 2011). Genetic factors are not only effective in hereditary cancers such as bilateral retinoblastoma or xenoderma pigmentosum, but it seems that these factors play a significant role in common cancers and so far many related genes have been identified (Kraemer et al., 1987; Sepahi et al., 2014).

P53 mutations have extensively been studied in human tumours (Chen et al., 2010; Goh et al., 2011). *P53* has 11 exons and codes for a protein containing 393 amino acids (Marcel et al., 2011). *P53* has different functions such as DNA binding (Hagn et al., 2010) cell cycle control (Leontieva et al., 2010), DNA restoration (Sotiropoulou et al., 2010), differentiation (Molchadsky et al., 2010), genomic plasticity (Zhao and Xu, 2010), and programmed cell death (apoptosis) (Li et al., 2012). Therefore, the overall function of p53 is to maintain genomic integrity. Genetic polymorphisms are the natural differences of DNA nucleotide sequence which can cause variations in genes products or responses to different stimuli. Mutation of *p53* has a close relationship with cancers in patients who have Li-Fraumeni syndrome, which is caused by deactivation of preventive gene of *p53* tumour through mutation (Srivastava et al., 1990). Genetic polymorphisms of *p53* can be found in different locations of the gene and their relations with various malignancies have been underlined (Själander et al., 1995; Sjalander et al., 1996). In cervical cancers, *p53* Arg (CGC) at codon 72 has been associated with the disease in patients infected by HPV (Storey et al., 1998; Zehbe

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et al., 1999). However, many researchers have challenged this claim (Minaguchi et al., 1998).
Japanese researchers found that the frequency of *p53* Pro in patients who suffer from hepatocellular carcinoma is higher than non-cancerous cases (Xiong et al., 2009). Most of these patients were infected with hepatitis C virus (HCV).
We aimed to assess if polymorphism in this gene could increase the risk of HCV infection in our population. Accordingly, in the present study, *p53* Arg72Pro single-nucleotide polymorphism was compared in HCV infected patients and healthy individuals.

Materials and Methods

Study Population

Patients and control individuals in this study were from Mashhad city, Northeast of Iran. All samples were recruited from those who were referred to Ghaem and Imam Reza academic teaching hospitals during a 12-month period starting from September 2011 to September 2012.
The case group included 67 patients with HCV (59 men and 8 women). HCV-infected patients were individuals positive for anti-HCV IgG with ELISA kit (Delaware Biotech., USA) and HCV RNA with RT-PCR method as described previously (Afshari et al., 2014).
Control subjects (73 HCV negative individuals; 32 men and 41 women) were selected. All the procedures were carried out according to the principles of the institutional guidelines and the study was approved by the Ethics Committee of Mashhad University of Medical Sciences.
A written informed consent was obtained from all subjects prior to recruitment. ALT levels were determined using Pars Azmoon Kit (Pars Azmoon, Iran) according to the manufacturers' instructions.

Extraction of Genomic DNA

Genomic DNA was obtained from peripheral blood samples and collected in EDTA tubes. The DNA was extracted using DNA Extraction Kit (Genet Bio, Korea).

Genotype Analysis

The genotypes of *p53* Arg72Pro polymorphism were determined using PCR-based restriction fragment length polymorphism (RFLP) method.
The forward primer used was 5'-ATCTACAGTCCCCCTTGCCG-3', whereas the reverse primer was 5'-GCAACTGACCGTGCAAGTCA-3' (Okada et al., 2001). Each PCR reaction mixture (30 µl) contained 0.4 mM of each primer, 1.5 mM $MgCl_2$, 0.4 mM dNTP, 0.4 U of *taq* DNA polymerase (Genet Bio, Korea), and 40 ng of genomic DNA in 10X reaction buffer. PCR conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s.
The final extension was at 72°C for 7 min. After confirmation of an amplified fragment of the expected size (296 bp) on an agarose gel, 10 µl of PCR product was digested with 5 units of restriction enzyme *BstUI* (Fermentase, Germany) at 60°C for at least 3 h.
DNA fragments were checked through electrophoresis on a 3% agarose gel and stained with green viewer (Pars Tous, Iran).
The Arg allele is cleaved by *BstUI* and yields two small fragments (169 and 127 bp). The Pro allele was not cleaved by *BstUI* , resulted in a single 296-bp band.
The heterozygotes give three bands on gel electrophoresis (296, 169 and 127 bp) (Figure 1).
Although the HCV genotype frequencies in the patients of our study were determined previously, we randomly selected 30% of patients and genotyped them using genotype specific primers (Afshari et al., 2014).

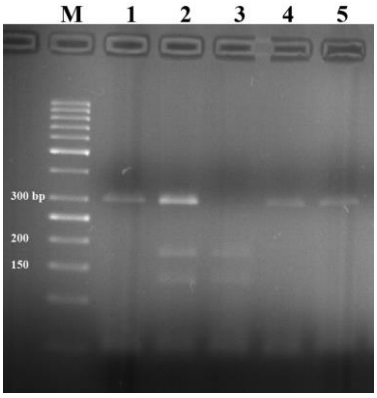

Figure 1. PCR products were digested by *BstUI* restriction enzyme. The Arg allele *BstUI* digestion showed 169 and 127 bp fragments (lane 3). The Pro allele was not cleaved by *BstUI*, resulted in a single 296-bp band (lanes 1, 4, 5). The heterozygotes showed three bands (296, 169 and 127 bp) on gel electrophoresis (lane 2); Lane M: 50 bp DNA size marker.

Statistical Analysis

Difference in gender, addiction, alcohol consumption, transfusion and tattoo operation between HCV patients and controls were evaluated using the chi-square test. The association between the *p53* polymorphism and HCV was determined using the logistic regression method to assess odds ratio (OR) and 95% confidence intervals (95% CI).

Difference between values were considered significant when a two-tailed *P* was <0.05. Statistical analysis was performed with SPSS 21.0 software (SPSS Inc., Chicago, IL).

Results

Descriptive Characteristics of Cases And Controls

The distribution of demographic data for all patients and controls is shown in Table 1.

Male patients were slightly more than females while females in the control group had a higher proportion (11.94% vs. 56.16%).

The mean age was 43.52 and 36.22 years in cases and controls, respectively. Predictably, HCV patients were significantly more likely to be addicts.

Table 1. Demographic characteristics of the study population

Characteristics	Patients	Controls	P-Value
Male: Female	59:8	32:41	<0.05
Age (years; mean ±S.D.)	43.52 ±10.52	36.22 ±12.49	<0.05
Addiction N	32	0	<0.05
Alcohol N	28	2	<0.05
Transfusion N	26	7	<0.05
Tatoo N	24	0	<0.05
ALT (U l-1, mean±S.D.)	41.15 ±41.36	25.97 ±21.63	<0.05
Increased ALT* N (%)	23	14	<0.05

*(>41 U. L-1 for men and >31 U.L-1 for women)

Distribution of P53 Codon 72 Genotypes Among Patients And Controls

As shown in Table 2, the frequencies of the Arg/Arg, Arg/Pro, and Pro/Pro genotypes among cases were, 26.86, 35.82 and 37.31%, respectively. In controls frequencies of Arg/Arg, Arg/Pro, and Pro/Pro were calculated as 32.87, 27.39 and 39.72%. We then analysed the distribution of Arg72Pro genotypes in patients compared to control group which showed no significant difference.

There was also no significant difference in allele frequencies. In addition, in a recessive model analysis of this position (Pro-Pro vs. Pro-Arg+Arg-Arg), Pro-Pro genotype was more common in controls (*P*=0.77; OR=0.90, 95% CI: 0.46 -1.79).

Genotype Distribution of HCV

The dominant genotypes of the virus in our population were 1a and 3a, and these results were in line with previous results that reported 1a and 3a as the most common genotypes in HCV patients (Afshari et al., 2014; Vossughinia et al., 2012).

Discussion and Conclusion

In addition to epigenetic factors and life style, some genetic factors contribute to cancers through different mechanisms.

P53 tumour suppressor gene is one of the most significant factors which can cause structural changes related to different kind of cancers (Hollstein et al., 1991). These changes could be observed in stem cells (genetic polymorphism) and somatic cells (mutation). Somatic mutation of p53 Arg72Pro in cancer, such as hepatocellular carcinoma, has been studied widely (Kawajiri et al., 1993; Papadakis et al., 2000; Själander et al., 1995). However, the probable relationship between p53 Arg72Pro gene polymorphism and HCV infection has not been studied in Iran. Several viral cancerous proteins react with p53 and can modulate its biological functions (Dobner et al., 1996; Friborg et al., 1999; Ko and Prives, 1996; Wang et al., 1994). It has been reported that in cervical cancer caused by human papillomavirus (HPV), an exon 4 polymorphism, which encodes for arginine in codon 72 (p53 Arg), confers more sensitivity to degradation by HPV E6 (Storey et al., 1998; Zehbe et al., 1999). Similar mechanism may be responsible for higher incidence of HCC in HCV infections. It has also been reported that NS3 protein of HCV has an interaction with p53. It has been found that natural set of NS3 and p53 aggregate in nucleus but mutant NS3 and p53 aggregate in cytoplasm (Ishido and Hotta, 1998; Muramatsu et al., 1997). The polymorphism of codon72 in p53 may affect NS3 aggregation and function. This process plays a role in virus proliferation, and may cause cell malignant transformation and deformation (Sakamuro et al., 1995). It is assumed that the core protein (central) of HCV is also included in malignant cell transformation (Moriya et al., 1998). It has been found that this protein reacts with p21 (Wang et al., 2000), which is a cell-cycle regulator and are induced by p53 (El-Deiry et al., 1993). P53 polymorphisms may also affect p21 induction and alter HCV proliferation and malignant hepatocytes transformation. It is worth noting that in cervical cancer caused by human papillomavirus, p53Arg is considered as the risky allele and this is inconsistent with present findings. This may be due to different reactions of p53 polymorphic protein with products of papillomavirus and HCV.

Table 2. Genotype distribution and allele frequencies of p53 codon 72 polymorphism in HCV patients and controls.

Codon 72 of *P53* gene	Controls N (%)	Patients N (%)	P value	OR	95% C.I.
Genotype					
Pro-Pro	29 (39.72)	25 (37.31)	Ref		
Pro-Arg	20 (27.39)	24 (35.82)	0.47	0.74	(0.33-1.66)
Arg-Arg	24 (32.87)	18 (26.86)	0.67	1.19	(0.53-2.69)
Pro-Pro	29 (39.72)	25 (37.31)	0.77	0.90	(0.46-1.79)
Arg-Arg/Pro-Arg	44 (60.29)	42 (62.68)			
Arg-Arg	24 (32.87)	18 (26.86)	0.43	0.75	(0.36-1.55)
Pro-Pro/Pro-Arg	49 (67.11)	49 (73.13)			
Allele Frequency					
Prolin	78 (53.42)	74 (55.22)	0.76	1.08	(0.67-1.72)
Arginin	68 (46.57)	60 (44.77)			

Since the dominant genotypes of HCV in Khorasan Razavi province and city of Mashhad were 1a and 3a (Afshari et al., 2014; Okada et al., 2001), in the current study only 20 samples were randomly genotyped for HCV types and the same results were achieved with a predominant 1a and 3a HCV genotypes. This may explain why the negative correlation between polymorphic p53 with HCV genotype was only observed in types 1a and 3a virus. It could be partly due to the fact that fewer numbers of cases were infected with other HCV genotypes. Consequently, structural differences of descriptive areas of HCV virus genotype may be included in p53 reaction. In women, other genetic or life style factors may influence HCV infection. In fact, as mentioned before, prevalence of HCV infection is significantly lower in men compared to women in this region (Shakeri et al., 2013). Leveri et al. did find any association between codon 72 genotypes and risk of cirrhosis and hepatocarcinoma in HCV patients (Leveri et al., 2004).In another study, it was shown that the Pro allele of the p53 Arg72Pro SNP has an increased risk for HCC in HBs Ag-negative subjects (Zhu et al., 2005). Okada et al. illustrated that at least in males, homozygosity for Pro in codon 72 of the p53 gene is one of the risk factors for infection with HCV genotype 1b (Okada et al., 2001). In addition, Anzola et al. showed that there is no significant correlation between codon 72 of p53 gene polymorphism and hepatocellular carcinoma in HCV patients (Anzola et al., 2003).

As our study has been done for the first time in Iran, further studies with larger sample size are required to explore this association.

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