Original Article

Association Between Hepatitis C Virus Infections and Toll-Like Receptor 3 Gene Polymorphism, Erbil City

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Abstract

Introduction: Globally, hepatitis C virus (HCV) has infected about 180 million people by 2019. The World Health Organization (WHO) reports that 71 million people worldwide are infected with chronic HCV. HCV is considered as a main public health problem globally, and recently it has been observed that the prevalence of this virus is declining but still populations with asymptomatic chronic HCV exists. The main objective of the present study was to determine the effects of TLR3 gene single nucleotide polymorphism (SNP) on the susceptibility of HCV infections in Erbil city of Iraq. **Materials and Methods:** For this purpose, samples were collected from many private hospitals, which included 50 samples from patients with HCV and 10 samples as controls from healthy people. The sample followed molecular techniques for determining toll-like receptor 3 (TLR3) SNP. Finally, the results were analyzed using Chromas-Pro software. **Results:** The results of the present study showed that both age and gender have a crucial role in HCV infection. About 66% of the HCV patients were males and 64% of patients were above 40 years. **Conclusion:** The results concluded that there was a significant relation between TLR 3 SNP (rs78726532 polymorphism) and the risk of HCV infection in population of Erbil city.

Keywords: Age, gender, PCR, polymorphism, sequence, virus

Introduction

For very first time in 1989, hepatitis C virus (HCV) has been discovered and diagnosed, since that time this virus is recognized as a main problem that causes death through chronic liver disease.[1,2] It has been reported in 2007 that HCV has infected around 130 million people,[3] whereas the rate of infection has been increased in 2019 to around 180 million people.[4] The most recent article on HCV reported that 71 million people suffer from chronic HCV worldwide.[5] A previous study in the northern part of Iraq, Dhouk city reported the prevalence of HCV by only 1/7900 (0.013%).[6] HCV is an enveloped virus which has a single RNA strand that infects liver cells (hepatocytes) and causes serious damages.[7] This virus belongs to the family Flaviviridae which can escape its host's immune mechanism.[8,9] It can be transmitted sexually and perinatally.[10,11] However, the main route for transmission of HCV is direct contact with blood, through the utilization of intravenous drugs, blood transfusions, and/or blood products.[12]

Over time, the prevalence of HCV is decreased but still considered as a main health problem due to the asymptomatic population which might develop clinical symptoms and cause death over coming decades.[11,13] Thus, determining the symptoms of HCV is important, which aids in the treatment and recovery processes.[14] A comprehensive study in USA revealed more deaths due to HCV than AIDS in 2000 and suggested intensive monitoring of liver function test if important during the HCV infection; also liver biopsy and education and awareness of HCV transmission decrease the rate of HCV infections.[13,15] Additionally, people with chronic liver infections suffer from fibrosis and liver cirrhosis, and 3–5% of people with liver cirrhosis will develop liver

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cancer in the future.[16,17] Hepatitis C-related diseases are usually treated with antivirals such as interferon-alpha and ribavirin, which are standardized for the care of chronic HCV patients.[18] However, such treatment is only effective in 50–60% of the patients. In addition to the development of drugs that stimulate cellular immunity in the host, significant progress has been made in the treatment and management of HCV through antiviruses that directly affect it.[19]

TLR is an abbreviation of toll-like receptors that categorized as a family of receptors that recognize motifs shared by different classes of microbial pathogens, and these receptors have a role in the recognition of microbial invasion.[20,21] TLR is expressed on numerous cell types, including professional APC such as MDC, PDC, and TLR3; also their ligation by microbial elements is thought to be critical in DC activation and maturation.[21-23] TLR-mediated activation of DC therefore provides an important pathway for the formation of an adaptive pathogen-specific T cell response.[24] TLR3 is encoded by a gene located in the 4q35.1 region and expressed intracellularly, and many studies have shown that genetic changes in the TLR3 gene are associated with high susceptibility or resistance to immune and infectious diseases, including AIDS, liver disease in people with HCV infection, herpes simplex virus infection (herpes virus) type 2, viral infection hepatitis C, type 1 diabetes, and autoimmune diseases.[25-27] The present study aimed to determine the effects of TLR3 gene single nucleotide polymorphism (SNP) on the susceptibility of HCV infections.

Materials and Methods

Sample collection

Samples have been collected from 60 adults, which included 50 patients with HCV infection and 10 healthy controls. All blood samples were transferred to an advanced molecular laboratory, and DNA was collected from white blood cells by using a human genomic extraction kit. The ages of enrolled people in the present study were 33–52; all patients were registered in private hospitals which were located in Erbil city.

PCR run

In the present study, only one pair of primers has been designed (TLR3-F 5'-GCTGGAAAATCTCCAAGAGC-3' and TLR3-R 5'-AGAGACCAAGCCAGCTAACC-3') and gradient PCR determined the best amplification condition. PCR was performed with GoTaq Green PCR Master Mix (Promega), according to the manufacturer's instructions. An aliquot of 50 µL of reaction mixtures is composed of 25 µL Master Mix, 1 µL (ranging from 100 to 500 ng) DNA templates, and 1 µL for each forward and reverse specific primer (10 pmol), and the volume was completed by adding 22 µL of nuclease-free water. Electrophoresis was carried out using 1% agarose gel to analyze DNA according to its molecular weight/electromobility and electrophoresis was run on 90 V for 35–45 min. Finally, ethidium bromide was used for staining the gel, and bands were visualized by a UV lamp at 365 nm and photographed by Canon D100.

Sequencing

Amplicons or PCR products were submitted for sequencing to determine the genotype of rs7872653 TLR3 gene polymorphism, and the results were analyzed using ChromasPro software.

Statistics

GraphPad Version 8 has been used to find *P*-value via the χ^2 test, and the *P*-value less than 0.05 considered significant. Thus, for each table *P*-value is calculated and interpreted.

Results

Genomic DNA was extracted and amplicons were analyzed using electrophoresis, which was carried out using 1% agarose gel; the PCR product was 515 bp, as shown in Figure 1. The result of the present study showed that TLR3 polymorphism at position (187000464) chromosome four is present in 90% of the patients with HCV infection. However, this polymorphism presented in only 20% of people without HCV infection as shown in Table 1. Thus, significant relationship has been observed

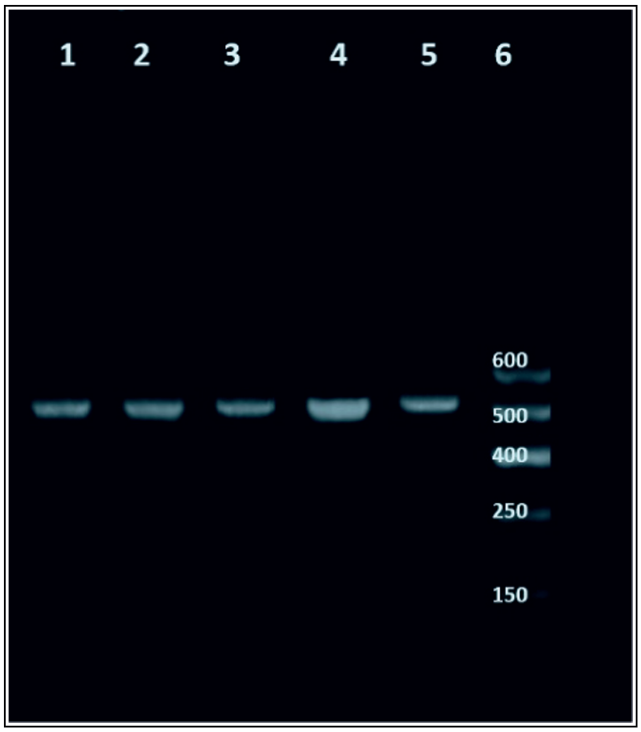

Figure 1: Amplification of TLR3 gene (set 1) which is examined through agarose gel electrophoresis (1%) under UV light. Lanes 1, 2, and 3: control group, lanes 4 and 5: HCV patients, and lane 6: ladder

($P < 0.001$) between HCV infections and rs78726532 polymorphism.

According to the sequencing, the following genotype/allele distribution in chromosome 4 for TLR3 SNP has been determined in percentage, as shown in Table 2. The highest incidence among both control and HCV groups was AA genotype by 56% and 50%, respectively.

The result of the same study also revealed that HCV infection disproportionately affects men more than women, as shown in Table 3. In this investigation, 66% of the patients were males out of the 50 infected HCV patients whereas 34% were females. However, the relationship between gender and HCV was not significant ($P = 0.155$).

It has been observed that people with older age can be affected more than those with younger age. As reported in this study, 64% of the patients with HCV were above 40 years, whereas only 36% of the patients with HCV were below 40 years, as shown in Table 4. The relationship between age and HCV infection was not significant ($P = 0.225$).

Discussion

Infections caused by HCV can be very dangerous and can sometimes lead to hepatocellular carcinoma.[28] The finding of different susceptibilities to HCV infection in individuals who are homozygous for the variant allele of the TLR3 exon 4 SNP and different susceptibilities to viral infections concerning polymorphisms in PRRs or in molecules mediating type I IFN responsiveness is in line with our findings.[29] Another study concluded that TLR3 SNP of -705 was associated with chronic HCV infection.[30] The present study suggested that environmental factors have a great role in both innate and adaptive immune systems that affect the prevalence of HCV infections. Al-Anazi *et al*.[7] reported a significant association between rs78726532 polymorphism and the risk of hepatitis C when compared with the healthy population. Other studies have suggested that polymorphisms in the TLR3 gene's promoter sequence may influence the expression of this gene. In contrast, it has been observed that the HCV can affect the transcription of the TLR3 gene through the p53 factor.[31]

According to the present study, men are more likely to get infected with HCV than women, and this finding is in agreement with the previous finding which observed the same result as mentioned in environmental and lifestyle factors including smoking as causative factors.[32,33] Differently, another study in Pakistan reported more HCV infections among females than among males.[34]

The results of the present study showed that HCV infection is more common among those above 49 years. This result is not in agreement with another study in regions like Australasia, which reported that the estimated peak prevalence at age 20–24 years may reflect the high incidence of HCV.[35] Another study in Iraq reported the highest incidence of HCV among the age group of 26–45 years.[36]

Table 1: Percentage of rs78726532 polymorphism in some healthy individuals and HCV patients

Groups	rs78726532 polymorphism	Percentage	Normal genotype	Percentage
HCV patients	45	90%	5	10%
Healthy individuals	2	20%	8	80%

Conclusions

1. HCV affects approximately 180 million people worldwide.
2. Hepatitis C is an enveloped virus with single-stranded RNA that infects liver cells.
3. Age and gender are strongly associated with the incidence of HCV: 66% of HCV patients were males and 64% of the patients were above 40 years.
4. There is a significant relation between TLR3 SNP (rs78726532 polymorphism) and the risk of HCV infection.

Ethics

The corresponding author hereby confirms that ethics were considered for this research and that the article is original, and its contents are unpublished. The co-author has read and approved the manuscript for submission.

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Nil.

Conflicts of interest

The authors have no conflicts of interest to declare.

Table 2: Genotypic distribution for TLR3 gene polymorphisms when patient group was compared with the control group

Gene	SNP	Genotype/allele distribution	Controls, $n = 50$	Control percentage	Patients, $n = 10$	Patients percentage
TLR3	rs78726532	GG	12	24	3	30
		AG	10	20	2	20
		AA	28	56	5	50

Table 3: Association of gender and HCV infection

Gender	HCV patient	Percentage
Male	33	66
Female	17	34

Table 4: Role of age in HCV infection

Ages	HCV patient	Percentage
Below 40 years old	18	36
Above 40 years old	32	64

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