Interleukin one beta (IL-1B) gene polymorphisms in Iraqi patients with susceptibility to develop hepatitis C infections

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ABSTRACT
Hepatitis C virus (HCV) infection is the main cause of chronic hepatitis, affecting an estimated 150 million people worldwide. Initial exposure to HCV is most often followed by chronic hepatitis, with only a minority of individuals spontaneously clearing the virus. This study aimed to find an associated relationship between HCV susceptibility and Polymorphic gene of IL-1B targeting a total of 45 individuals: 30 patients with chronic hepatitis C and 15 healthy control from different cities of Iraqi recruited the Hospital for diseases of the digestive system and liver in Baghdad between November 2022 to February 2023. Genomic DNA was extracted and specific promotor sequence was amplified by polymerase chain reaction (PCR) followed by sequencing then measuring serum concentrations of IL-1β by Enzyme-Linked Immunosorbent Assay (ELISA). The results revealed significant difference in serum levels of IL-1B ($P \leq 0.01$), from 1.10-+0.09 in Chronic HCV patients in comparison with its level in healthy controls 7.58± 0.28. Molecular results suggested that single nucleotide polymorphisms in the promoter of IL-1B is probably associated with susceptibility to HCV chronic infection according to this sample of Iraqi patients. In conclusion, the results suggested that variation in serum level and polymorphism in IL-1B is probably associated with susceptibility to hepatitis C virus chronic infection.

1. Introduction

The Hepatitis C virus (HCV) is an enveloped RNA virus of the Flaviviridae family, transmitted through contaminated blood (1). HCV targets primarily human liver cells, evades innate and adaptive immunity, and establishes chronic infections in 70% of cases. Ultimately, infection by HCV leads to hepatocellular carcinoma (HCC) in 20% of the cases (2), a unique feature for a flavivirus (3,4). Hepatitis C virus is the major cause of chronic liver diseases and the only cytoplasmic RNA virus known to be oncogenic in humans (5). According to the World Health Organization (WHO) estimation, 58 million people are chronically infected with Hepatitis C virus (HCV). Approximately 1.5 million persons are infected with this virus every year, and 290,000 persons die from hepatitis C complications such as cirrhosis and liver cancer (6). The Chronic hepatitis C virus (HCV) infection affects 58 million people globally. The frequency of HCV infection in the general Iranian population is less than 0.5%; however, a concentrated epidemic was reported among people who use drugs, particularly those with a history of drug injection (7). The common SNPs rs1143623, rs1143627, and rs16944 in the IL-1β gene with the development of liver cirrhosis and HCC in a large multicenter cohort of Caucasian patients with chronic HBV infection (8). Similar concept was suggested with other types of viral infections such as SARA-CoV-2 (9).

2-Materials and methods

2.1 Patients, controls, and sampling

The patient group included 30 chronic HCV carriers and 15 healthy group in the Hospital for diseases of the digestive system and liver in Baghdad between November 2022 to February 2023 for HCV as part of a pretherapeutic investigation. The patient group originated from different cities of Iraqi and included 36 males aged ≥ 50 years (mean = 66.66 years) and 24 females aged < 50 years (mean = 33.33 years). The control group was composed of 15 age-related and sex-matched healthy individuals who came to the Hospital for diseases of the digestive system and liver in Baghdad. The control group was composed of 10 males and 5 females aged ≥ 50 years (mean = 66.66 years) and aged < 50 years (mean = 33.33 years) demographic data are presented in Table 1.

Table1: Demographic data of HCV patients and controls.

Characteristics	Patients	Control
Male / Female	36 / 24	10 / 5
Mean Age (years) ≥ 50 / < 50	66.66 / 33.33 years	66.66 / 33.33 years

Written informed consent was obtained from all study participants prior to their enrolment in the study. The protocol was approved by the Ethics Committee of Hospital for diseases of the digestive system and liver.

Primer design:

Primer have been designed in this study based on the Bioinformatics tools using the international databases (NCBI) using primer3 plus to detect and sequence a 163bp of the promoter of IL-1B gene. The forward primer 5'- GAGGGTGTGGGTCTCTACCT -3' and the reverse primer was 5'-CTTATCTCCAGGGTTGCCCC -3'.

Blood sample collection and Laboratory diagnosis of hepatitis B

The Blood samples were collected from the patients and apparently healthy people. Two ml of blood was collected then kept in EDTA anticoagulant tubes in freezer (-20) to be a source for DNA extraction and 3 ml of blood was kept in Gel tube to procedure ELISA Tests after separate by centrifugation. Laboratory diagnosis of hepatitis after isolation of serum, it was tested by enzyme linked immunosorbent assay (ELISA) to detect IL-1B (BT LAB Human Interleukin 1B, IL-1B ELISA Kit; Korea). the diagnosis was confirmed further by PCR analysis to detect the IL-1B gene.

2.4 The estimation of IL-1B serum levels

Serum concentrations of IL-1β was measured by the Enzyme-Linked Immunosorbent Assay (ELISA) sandwich immunoassay. Dosages were made from plasma of subjects in the Hepatitis B patient group and controls. That use a (BT LAB Human Interleukin 1B, IL-1B ELISA Kit; Korea) to detect of IL-1B .

2.5 Detection of IL-IB gene SNP

Genomic DNA was extracted from EDTA blood using Genomic DNA extraction kit (Addbio, Korea). the SNPs of IL-1B gene promoter was detected by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel. The thermo-cycling conditions were optimized, as follow : initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 30 s, and then annealing at 58 °C for 20 s and extension at 72 °C for 20 s.

2.6 Direct sequencing

For detecting and analysing of potential SNPs causing susceptibility for hepatitis C infection, PCR products of all samples were sent for direct Sanger sequencing. (Macro gene, Korea).

3- Result and discussion

3.1 The age factor in relationship between serum levels of IL-1β in chronic Hepatitis C patients and healthy controls.

There was a significant differences in serum levels of IL-1β, of hepatitis C patients compared with age matched healthy controls. The results showed that the level of IL-1β significantly decreased (P≤0.01), from (1.10-+0.09) in chronic hepatitis C specimen in comparison with the level in healthy controls 7.58±0.28 as shown in table (2).

Table 2: IL-1B level for chronic HC patients and control

Groups	Sample number	IL-1B level	P-value
Patients	30	1.10±0.09	0.0001
control	15	7.58±0.28	
(P≤0.01) Highly Significant			

The results revealed age as an important factor with chronic hepatitis C, the group of age more than 30 years was decreased the level of IL-1B (0.83±0.06), while the group of age lees than 30 years record a significant (P≤0.01) decrease in the level of 1L-1B (1.71±1.36).

Table 4-4: Age the risk factor in chronic Hepatitis C patients (serum levels of IL-1β).

Age groups	Sample number	IL-1B level	P-value
≥ 30 y	21	0.83±0.06	0.0001
<30 y	9	1.71±1.36	
(P≤0.05) Significant			

Recently, a significant drop in the level of some interleukins were recorded in chronic HC patients (Lafta *et al*., 2023). Other studies revealed a combination of response inclouded the effect of polymorphism of the genes of cytokines IL-1β, IL-6 and TNFα on the pathogenesis of chronic HBV and HCV infection, response to treatment, and development of complications (Khayrulla *etal*., 2021).

3.1 Genomic DNA extraction.

The results of DNA extraction showed that blood samples yielded adequate DNA concentration for PCR amplification Figure (1–A).

3.2 PCR of IL-1B promotor for hepatitis C patients

The PCR was conducted using specific primers. the presence of the Amplified amplicons were profiled using agarose gel electrophoresis of 10µl of the PCR products that represent an approximately 160bp segment of ILB1 gene, which revealed single specific bands of the 15 sample of control as shown in figure (1-B).and the patients with hepatitis C with thier 30 samples as shown in figure (1-C and D).

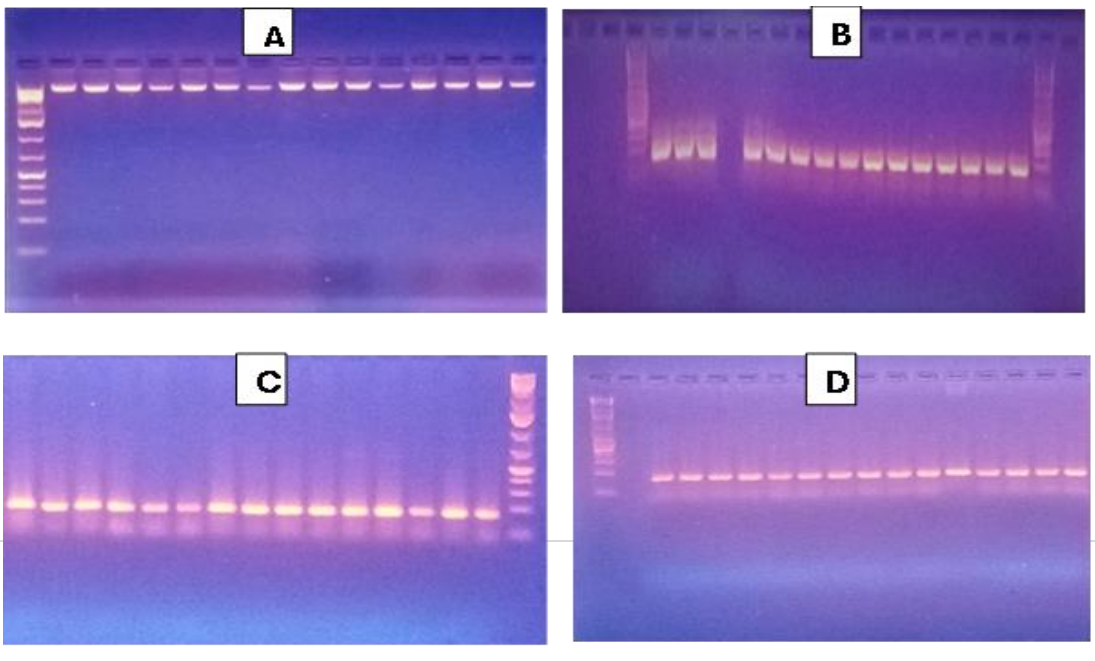

Figure (1) (A) 1 % Agarose gel electrophoresis of 10µl of Sample of chromosomal DNA bands visualized under UVlight.PCR products that represent an approximatly 160bp segment of ILB1 DNA. The PCR was conducted using specific primers. **(B)** picture reveals the presence of the targeted amplicons of 15 control (healthy people) DNA sample. **(C and D)** reveals the presence of the targeted amplicons of 30 HCV patients. The sequences were align to reveal the variances in selected SNPs for patients of hepatitis C (30) samples compare with control samples (15). The result showed SNPs around the frame shifts (gaps) for the SNP (rs1558784303) in patients with hepatitis C as showed in figures (2) and figure (3). The most of the samples of this study, noticing there is Cytosine base changing within and in comparison with control group.

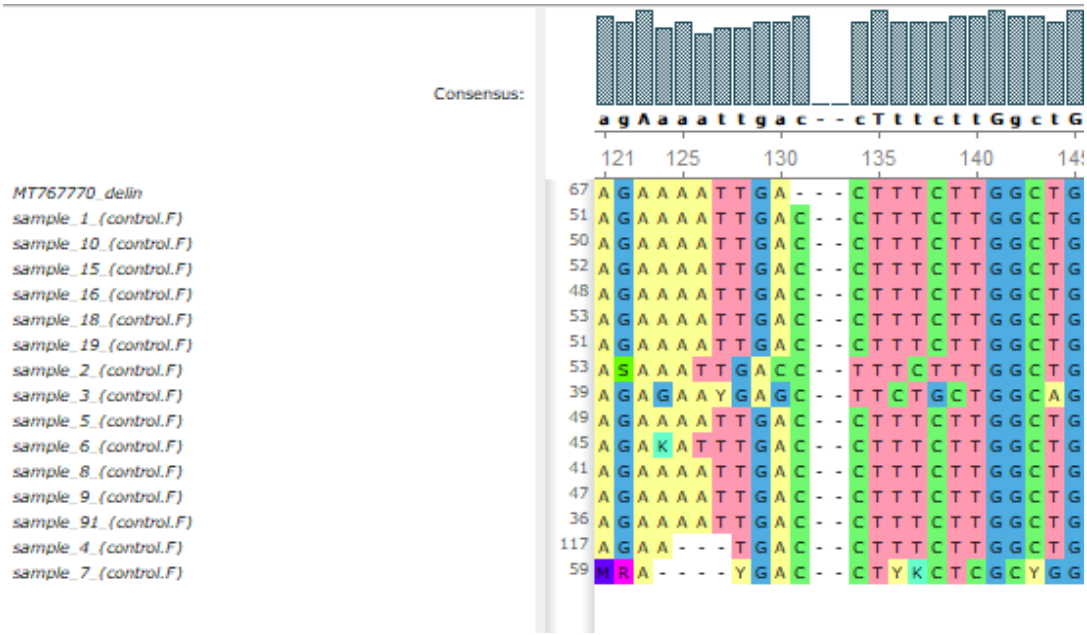

Figure (2): The sequencing Data were undergoing a bioinformatic analysis to show the variance differences of SNPs amongst the groups of healthy control.

Figure (3): The sequencing Data were analysis to show the variance differences of SNPs amongst the group's patients of chronic hepatitis C.

The results showed that regarding to the SNP rs774164486 T/C/gap as a genomic variant in the promoter region of the IL-1B DNA, which showed 91.67% of T, 5% of C and 3.33% of gap, however there was no recorded study referred to this SNP regarding susceptibility to hepatitis infection . Thus regarding to the rs748357841 T/C as a genomic variant at a single base position in the promoter region of the IL-1B DNA, which showed 96.67% of T and 3.33% of C, however There is no recognized study that specifically investigates the correlation between this (SNP) and susceptibility to hepatitis infection. However, several SNPs at the promotor target might associated with Necroinflammatory activity of HCV(11).

Conclusion

Furthermore, rs1402741993 C/T as a single base position in the promoter region of the IL-1B DNA, which showed 96.67 of C and 3.33% of T, has no noted study mentioned to this SNP as regarding susceptibility to hepatitis infection. finally, the SNP rs748357841 T/C as a genomic single base position in the promoter region of the IL-1B DNA, also showed 95% of T and 5% of C, Although, there was no recorded study referred to this SNP regarding susceptibility to hepatitis infection. Other researches refer to the association of hepatitis C virus genotype 4 which might required more investigations (12).

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