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Association of Interleukin-1 beta Gene Polymorphisms with Susceptibility to Hepatitis B Virus Infection in Chronic Hepatitis B Patients

[1]Ghasaq A. Kadhum ， [2]Mohammed A. A. Al-Bedhawi

[1,2] Institute of Genetic Engineering and Biotechnology for post graduate studies, University of Baghdad, Baghdad, Iraq.

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Abstract: A persistent infection with the hepatitis B virus (HBV) can cause significant morbidity and mortality. Although antiviral therapy, monitoring, and liver cancer surveillance are not considered therapeutic, they can lower morbidity and mortality. The Polymorphic gene IL1B analyzed in a total of 45 individuals: 30 patients with chronic hepatitis B from different cities of Iraqi recruited the Hospital for diseases of the digestive system and liver in Baghdad between November 2022 to February 2023 and 15 controls. Genomic DNA was obtained from both patients and control and polymerase chain reaction (PCR) was perform with specific primer designed to target the promoter region of IL-1β gene, followed by sequencing. Serum concentrations of IL-1β were measured using Enzyme-Linked Immunosorbent Assay (ELISA). The results showed serum levels of IL-1B which significantly decreased (P≤0.01), from 1.10-+0.09 in hepatitis specimen in comparison with the level in healthy controls 7.58± 0.28. The results also revealed that the age group > 30 years have a significant (P≤0.01) low level of IL-1B (0.83±0.06) in comparison with the age group ≤ 30 years (1.71±1.36). The molecular results suggested that IL-1B single nucleotide polymorphism (insertion/deletion) is probably associated with susceptibility to HBV chronic infection patients, the sequencing alignment data for 30 samples from patients with hepatitis B were compared to 15 control samples. The findings indicated frame shifts (deletion) in 124 and 125 positions in patients' comparison with control groups. The results showed a cystosine base pair (C) in the patient group, while the control group noticed a deletion. In conclusion, the results suggest that IL-1B is probably associated with susceptibility to hepatitis B virus chronic infection.

Keywords: Hepatitis B, interleukin-1β, polymorphisms, polymerase chain reaction.

Corresponding author: (Email: ga.abd90@gmail.com).

Introduction

Hepatitis B virus (HBV) chronically infects approximately 350 million individuals worldwide. HBV chronic infection is a common cause of liver cirrhosis and hepatocellular carcinoma (HCC)(1). HBV is a communicable disease that can be transmitted vertically from mothers to their neonates or horizontally by infected blood, blood products transfusion and body secretion (2-3). The pre-existing chronic liver diseases are inflammation, liver fibrosis, and cirrhosis caused by hepatitis virus infection, heavy alcohol consumption, autoimmunity, nonalcoholic steatohepatitis, and genetic diseases (4-7).

Interleukin-1 beta (IL-1β) promotes liver disease progression and hepatocarcinogenesis in chronic

hepatitis B (CHB). Single nucleotide polymorphisms (SNPs) within the promotor region of the IL-1β gene can affect the progression towards liver cirrhosis and hepatocellular carcinoma (HCC), to investigate the association of three common IL-1β SNPs with hepatitis B virus (HBV)-related HCC in Caucasian patients (8). The IL-1B genetic variants that have been involved in the immune response and the analyzed their role in the susceptibility to develop chronic hepatitis B in the Tunisia population. IL 1B is a potent proinflammatory cytokine that plays an important role in inflammation of the liver (9). In the promoter region within the IL-1β gene, three SNPs were described at position rs1143623, rs1143627 and rs16944. Previous studies reported that the IL-1β SNPs were linked to the development and pathogenesis of numerous chronic inflammatory diseases (10–12) as well as the progression towards chronic hepatitis B (13–15).

Materials and methods

Subjects

Subjects included 30 HBV patients and 15 healthy groups who attended the Hospital for Diseases of the Digestive System and Liver in Medical city in the period between November 2022 to February 2023 as a part of a pre-therapeutic investigation. The protocol was approved by the Ethics committee of the hospital and the committee of the institute of genetic engineering and biotechnology/university of Baghdad.

Sample collection

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.

DNA extraction

Genomic DNA was extracted from whole blood using Adprep Genomic DNA extraction kit (AddBio, Koria).

Primer design

Primers have been designed in this study based on the Bioinformatics tools using the international databases (NCBI) and number of tools that are available on website (online tools and software). Starting with reviewing the Gene of IL-1B followed by selecting the targeted sequence, which includes sequence of the promoter of IL-1B gene. The designing of the primers was carried out using primer3 plus (online at website (http://www.bioinformatics.ngi-bin/primer3plus.cgi/). Primers for IL-1B gene are illustrated in (Table 1).

Table (1): Sequences of Primers and the expected size of PCR product.

Gene	primer	sequences	PCR product size
ILB1	Forward	5'- GAGGGTGTGGGTCTCTACCT -3'	163bp
	reverse	5'- CTTATCTCCAGGGTTGCCCC -3'	

Single plex PCR

The components required for the reaction mixture of single plex PCR, were mixed as follow: Four µl DNA were mixed with 20 µl PCR master mix. Then 1 µl of each forward and reverse primers then the volume was completed up to 40 µl with nucleases free water. The reaction conditions were optimized first, then fixed as follows: initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at

95 °C for 30 s, annealing at 58 °C for 20 s and extension at 72 °C for 20 s. Both the extracted genomic DNA and PCR products were profiled by electrophoresis using 1% and 2% agarose-gel respectively.

Profiling of PCR products

Seven microliter of the PCR products were analyzed by electrophoresis in 2% agarose gel which was stained with ethidium bromide and run for 1hour in 1X TAE buffer. After that, it was visualized under UV light using ultraviolet transilluminator. A DNA ladder of (100-1000 bp) was used and the gel was photographed by a digital camera.

Sequencing of PCR product

PCR product direct sequencing for all samples (30 samples of hepatitis B and 15 samples of control) to confirm the positive results of PCR in this study and to reveal the types of SNPs in the targeted sequence. Frozen PCR products with primers were sent for sequence analysis to Macro gene Company in Korea.

Statistical analysis.

The data was presented as means ± standard deviation (SD). The program that has been used for statistical analysis was SPSS 26.0 (SPSS Inc., Chicago, USA). Statistics were judged significant at pvalue < 0.05. One-way ANOVA was used to statistically analyze the differences between the mean values of the control participants and hepatitis B patients.

Results and Discussion

Distribution of IL-1β serum levels according to age group

The results showed significant differences in serum levels of IL-1β, of hepatitis B patients compared with age matched healthy controls. The level of IL-1β protein significantly increased (P≤0.01), from (1.10-+0.09) in hepatitis specimen in comparison with its level in healthy controls (7.58± 0.28) as shown in (table 2). The interleukin-1 family (IL-1 family) plays an important role in the pathogenesis of HBV infection. IL-1 family includes: IL-1A, IL-1B, IL-18, IL-33, IL-36, IL-37, and IL-38 (16). The present study was agreed with Li *et al.* (17) that was showed the older age was a risk factor for HBsAg infection among children younger than 15 years. Among adults 15-59 years old, the risk factors were male gender. In the similar study, the proportion of individuals aged under 45 declined from 10.6 to 1.6%, while people aged over 55 increased from 70.3 to 91.0%, Persistent or chronic infection was more common in younger adults and males (18). The elevation of the serum IL-1β level was demonstrated in subjects with liver fibrosis. IL-1β was upregulated in Huh7 cells transfected with HBV (Wu etal.,2018) (19). HBV transmission could be reduced in more developed areas because people had more opportunities to receive health education or medical service. It was previously reported that the combination of these two factors played a more important part in HBV transmission than either individually Xu *et al*. (20). The age was an important factor with hepatitis B, the results showed that the age group > 30 years have a significant (P≤0.01) low level of IL-1B (0.83±0.06) in comparison with the age group ≤ 30 years (Table 3). The Interleukin-1 beta (IL-1β) promotes liver disease progression and hepatocarcinogenesis in chronic hepatitis B (CHB). Single nucleotide polymorphisms (SNPs) within the promotor region of the IL-1β gene can affect the progression towards liver cirrhosis and hepatocellular carcinoma (HCC) (21).

Table (2): Distribution of IL-1B level according to 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			

Table (3): Distribution of IL-1B level according to age group.

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≤0.01) Highly Significant			

Detection of *IL-1B* gene by singleplex PCR

The Agarose gel electrophoresis of 10µl of the PCR products that represent an approximately 160bp segment of IL-1B DNA. The PCR was conducted using specific primers. the presence of the Amplified amplicon of 15 control (healthy people) DNA sample in figure (1) and 30 patients with Hepatitis B DNA sample the presence of the targeted amplicons of in figure (2) and figure (3).

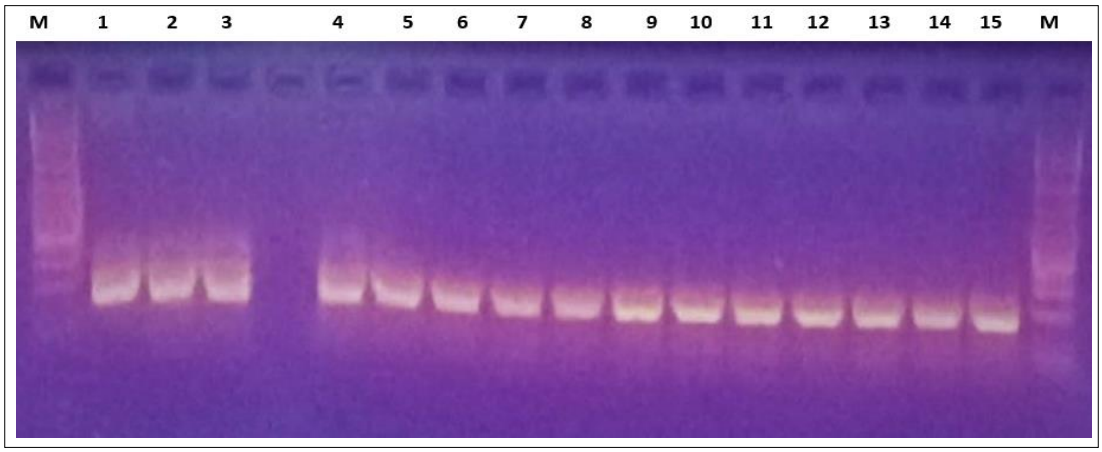

Figure (1): Gel electrophoresis of PCR products at 7 volt/cm for 1 hr. Lane M : 100bp ladder, Lanes 1-15: PCR product of ILB1 gene (160bp) of control group.

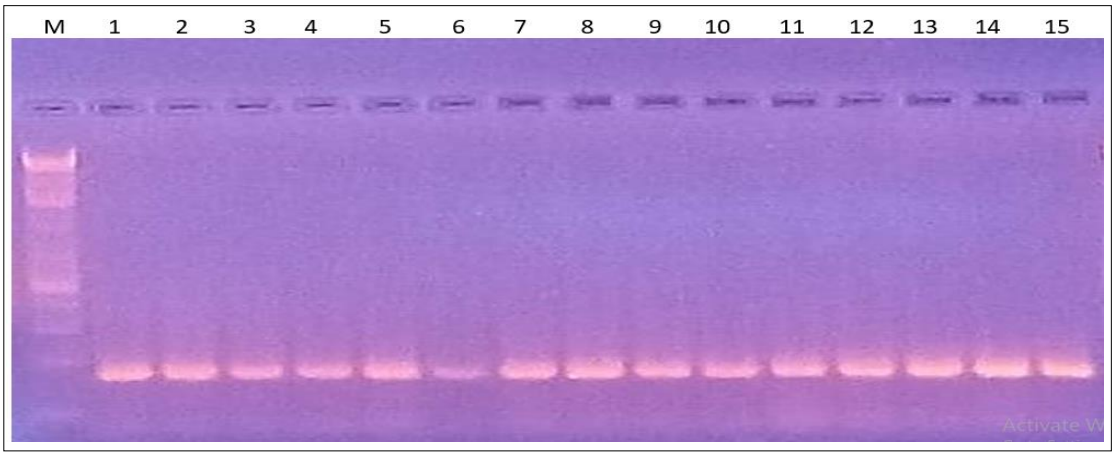

Figure (2): Gel electrophoresis of PCR products at 7 volt/cm for 1 hr. Lane M: 100bp ladder, Lanes 1-15: PCR product of ILB1 gene (160bp) of patient group.

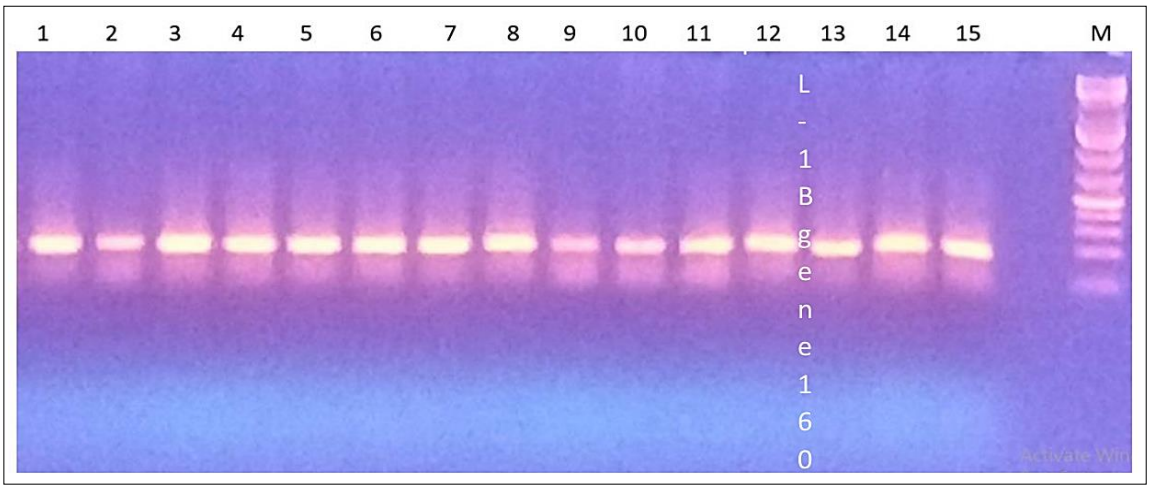

Figure (3): Gel electrophoresis of PCR products at 7 volt/cm for 1 hr. Lane M: 100bp ladder, Lanes 1 15: PCR product of ILB1 gene (160bp) of patient group.

Distribution of IL-1β gene polymorphism with hepatitis B infection.

The table (3) and table (4) were showed that SNP rs33775 G/A 86.6 of G and 13.3 of A, the SNP rs1402741993 C/T 86.6 of C and 13.3 of T, the SNP rs779164486 T/C 90% of T and 10% of C, the SNP rs748357841 T/CG 90% of T ,6.67 of C and 3.33% of G, the SNP rs1402741993 C/TG 86% of C, 6.67% of T and 6.76 of G and the SNP rs5748357841 T/C 93.33% of T and 6.67 of C in control groups ,while the patients of hepatitis B group the SNP rs774164486 T/CG 86.67% of T, 10% of C and 3.33% of G , the SNP rs1682114917 C/G 96.67% of C and 3.33% of G, the SNP rs779164486 T/C 93.33 of T and 6.67% of C.

Table (3) Data analysis of number of SNPs located at the promoter of IL-1β of hepatitis B (control).

	T/GC	G/A rs33775	A/CG	C/gap	gap	gap	C/T rs1402741993	T/T	T/C rs779164486	T/CG rs748357841	C/TG rs1402741993	T/C rs779164486	T/C rs748357841	G/G
Control 15 samples	12T 2G 1Y(C+T)	13G 2A	13A 1C 1G	14C 1gap	15 gap	15 gap	13C 2T	15T	13T 1C 1Y (C+)	13T 1C 1K (G+T)	13C 1T 1G	14T 1C	14T 1C	15G
	88.33%T 13.33%G 3.33%C	86.67%G 13.33%A	86.67%A 6.67%C 6.67%G	93.33%C 6.67%gap	100%gap	100%gap	86.67%C 13.33%T	100%T	90%T 10%C	90%T 6.67%C 3.33%G	86%C 6.67%T 6.67%G	93.33%T 6.67%C	93.33%T 6.67%C	100%G

Table (4): Data analysis of number of SNPs located at the promoter of IL-1B of hepatitis B (patients).

HBV 30 samples	T/CG rs774164486	G/T	A/G	C/G rs1682114917	gap/TC	gap /TC	gap /TG	C/AGgap	T/AG	T/AG	T/AC	C/A	T/AC	T/C rs779164486	G/T
	26T 3C 1G	29G 1T	28A 1G 1R (G+A)	29C 1G	27 gap 2T 1C	27 gap 1T 2C	28gap 1T 1G	24C 2A 1G 3 gap	27T 2A 1G	27T 2A 1G	27T 2A 1C	28C 2A	27T 2A 1Y (C+T)	28T 2C	29G 1T
	86.67%T 10%C 3.33%G	96.67%G 3.33%T	95%A 5%G	96.67%C 3.33%G	90%gap 6.67%T 3.33%C	90%gap 3.33%T 6.67%C	93.33%gap 3.333%T 3.33%G	80%C 6.67%A 3.33%G 10%gap	90%T 6.67%A 3.33%G	90%T 6.67%A 3.33%G	90%T 6.67%A 3.33%C	93.33%C 6.67%A	91.67%T 6.67%A 1.67%C	93.33%T 6.67%C	96.67%G 3.33%T

The sequencing alignment data for patients of hepatitis B 30 samples compare with control samples (15). The result was show frame shifts (gaps) in patients with hepatitis B in 124 and 125 positions in the Iraqi population compared with control samples, they notice there is Cytosine base pair (C) while in control group they notice gap as shown in the figure (4) and Figure (5). The Interleukin-1 beta (IL-1β) promotes liver disease progression and hepatocarcinogenesis in chronic hepatitis B (CHB). Single nucleotide polymorphisms (SNPs) within the promotor region of the IL-1β gene can affect the progression towards liver cirrhosis and hepatocellular carcinoma (HCC) (21). Data analysis of number of SNPs located at the promoter of IL-1B of hepatitis B (patients). The present study was show that regarding to the rs774164486 T/CG as a genomic variant at a single base position in the promoter region of the IL-1B DNA, which showed 86.67% of T, 10% of C and 3.33% of G, however there was no recorded study referred to this SNP regarding susceptibility to hepatitis infection. the regarding to the rs1682114917 as a genomic variant at a single base position in the promoter region of the IL-1B DNA, which showed C/G 96.67% of C and 3.33% of G, however there was no recorded study referred to this SNP regarding susceptibility to hepatitis infection. the regarding to the rs779164486 T/C as a genomic variant at a single base position in the promoter region of the IL-1B DNA, which showed 93.33 of T and 6.67% of C, however there was no recorded study referred to this SNP regarding susceptibility to hepatitis infection. The clinical researches also revealed the elevation of IL-1β in serum and liver tissues of HBV infection patients (22). The occurrence of any mutation or polymorphism in IL genes may result in substantial changes in their biology and function and may be associated with a wide range of diseases and disorders (23).

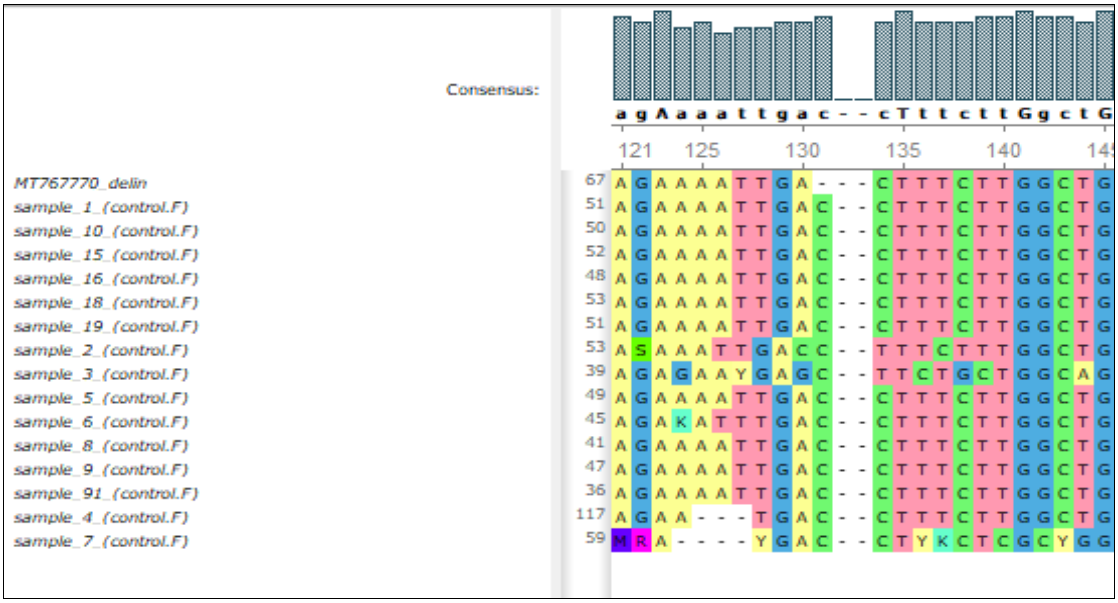

Figure (4): Alignment of sequenced PCR product. The first horizontal line represents the reference sequence (MT767770 delin). While the followed sequences represent the sequences of healthy control.

Figure (5): Alignment of sequenced PCR product. The first horizontal line represents the reference sequence (MT767770 delin). While the followed sequences represent the sequences of healthy control.

Conclusion

The findings imply that IL-1B is most likely linked to a person's vulnerability to a chronic hepatitis B virus infection, with chronic hepatitis B in comparison with healthy controls.

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