RESEARCH ARTICLE

Genetic polymorphism detection of interleukin-10 (-1082 A/G) gene in hepatitis B patients

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Received: August 3, 2023; **accepted:** September 5, 2023.

Infection with the hepatitis B virus (HBV) varies greatly among the infected people. The genetic variation of some inflammatory immune proteins is highly important to determine the infection severity and the individual's resistance to the disease. Interleukin-10 (IL-10) is an important cytokine in the control of both infectious diseases and inflammatory processes in human beings. This study aimed to investigate the role of interleukin-10 gene polymorphism (-1082 A/G) in patients with HBV infection and to use it as an indicator to explore the body's resistance to the infection. A total of 90 blood samples from HBV infected patients with the age range from 15 to 60 years old were collected between January and March 2022. Additional 30 blood samples were collected from health people as the control group in this study. The DNAs were isolated from the blood samples of all participants, and then the genotypes of each sample were determined by using PCR amplification with specific primers. The results showed that the highest frequency of HBV infection was in the age group of 30-44 years old with 50% samples in this age stratum. The samples from males were the most infected (62.2%) compared to females (37.8%). The genotype AG showed the highest frequency of 42.2% in HBV group compared to that of 20% in healthy control group. The genotype AA demonstrated a frequency of 37.8% in HBV infected samples compared to that of 66.7% in healthy control group. In addition, genotype GG was presented at 20% in HBV infected patients compared to that of 13.3% in healthy control group. The results confirmed a strong correlation between vulnerability to HBV infection and IL-10 polymorphism (-1082).

Keywords: Hepatitis B virus; genetic polymorphism; IL-10; A allele; G allele.

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Introduction

Hepatitis B virus (HBV) belongs to the Hepadnaviridae family and has double-stranded DNA with a small envelop, which causes hepatocytes infection. The virus replicates in the nucleus and persists latently [1]. The HBV virion has a spherical lipid-based structure as a viral envelope containing small, medium and large three types of viral proteins. The large viral envelope protein contributes to cytoplasmic entry by receptor-mediated endocytosis using the sodium torocholate (NTCP) receptor on the hepatocyte membrane [2]. HBV infection is a major public health concern globally. Chronic hepatitis B (CHB) has reached about 240 million people over the world, while 600,000 people are infected with acute hepatitis B (AHB) each year.

Both hepatitis diseases caused by HBV infection result in more than a million deaths over the world each year [3]. Past reports indicated that hepatitis B and C virus infections reached 350 million infections over the world, which was about 7% of the world population who had been the carriers of the infection. The main reason for such wide spread of the disease is due to the escaping ability of the virus from body immune system, which makes it reaching an infection rate of 3-5% [4]. The high prevalence of HBV infection unquestionably causes a significant negative impact on human health, life, and society, and is related to liver cancer, cirrhosis, fulminant hepatitis, and even death. The most significant pathogen causing blood-borne hepatitis in humans is the hepatitis B virus [5]. Unfortunately, these illnesses don't respond well to the existing treatments once they've been contracted. Precautionary measures are therefore thought to be the best strategy to stop the disease from spreading. Various vaccines have been developed for the infection protection of viruses that have DNA as the main genetic material, which may help people to get the long-lasting immunity [6]. It may be the most crucial HBV preventative measure because someone who has received the vaccine may never become infected [7].

Single nucleotide polymorphisms (SNPs) are part of genetic polymorphisms that could alter the promoter region of a protein encoded gene and therefore change the biological role of the synthesized protein. Also, this variation at the genetic level may affect the increase or decrease in the amount of the corresponding protein production as a result of the translation of this gene [8], which may further change a person's susceptibility to a certain disease [9]. The immunopathogenesis of HBV infection is significantly influenced by cytokines and regulatory molecules. The genetic variation that has been identified at the cytokine genes may affect HBV infection progression [10]. The effects of genetic polymorphism in cytokine genes on illness prognosis as well as individual responsiveness to vaccination and therapy has thus been the subject of numerous recent investigations [11]. Interleukin-10 (IL-10) as a regulatory immune cytokine is an immune protein secreted by many immune cells that play a role in chronic and acute inflammation including monocytes or macrophages, activated T helper (Th-2) cells, and regulatory T cells [16]. It also has a direct effect on the synthesis of tumor necrosis factors (TNFs) (alpha and bata) by controlling inflammatory reactions [12]. SNPs at the promoter region of IL-10 can affect the level of IL-10 gene expression because there are multiple transcription factor binding components in the promoter region, which are directly associated with the control of gene expression [13]. Previous studies had investigated whether certain SNPs in the proximal promoter region of IL-10 gene were related to HBV infection such as polymorphisms on the -592 site [14], -819 site [15], -872 site [16], and -1082 site [17]. This study was to investigate the effects of the genetic polymorphism of the interleukin-10 gene promoter region -1082 (A/G) site on the ability of the HBV infected patients to resist this virus and the development of inflammatory infection.

Materials and Methods

Patient information and blood sample collection

This study included 120 human samples with 90 samples from HBV infected patients (56 males and 34 females) who were diagnosed by a specialist physician based on the clinical symptoms, physical signs, and rapid antibody lab test with age range of 15 to 60 years old. An additional 30 health samples (17 males and 13 females) were collected as the control group. All the patients were from Al-Hilla Teaching Hospital, Babylon Iraq between January and March 2022. The blood samples of each participant were collected by using anticoagulant EDTA tubes after receiving the consent and approval forms from each participant. The whole process was approved by the Ethics Committees of the Ministry of Higher Education Scientific Research and the Iraqi Ministry of Health (approval number: DSM/HO-15314). A total of 5

Table 1. Frequency of HBV and blood samples according to the age stratum.

Age groups (years old)	HBV infected samples		*P* value
	Number of samples	Percentage (%)	
15 - 29	17	18.9	**< 0.05**
30 - 44	45	50.0	
45 - 60	28	31.1	
Total	**90**	**100**	

mL blood was taken from each participant and mixed well with the coagulant substance in the tube slowly before stored at -20°C.

Genomic DNA isolation and genotyping

The genomic DNA of each sample was extracted by using Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Seongnam, Gyeonggi, South Korea) according to the manufacturer's instructions. The primers for IL-10 (-1082 A/G) gene amplification were forward primer 5'- CCT CTT ACC TAT CCC TAC TTC CAC C-3' and reverse primer 5'-GAC AAC ACT ACT AAG GCT TCT TTG GTA A-3'. In addition, the A allele (A) primer sequence was 5'- TGA AGA AGT CCT GAT GTC ACT GA -3', while the G allele (G) primer sequence was 5'- TGA AGA AGT CCT GAT GTC ACT GG-3'. All the primers were prepared in ddH_2O to a working concentration of 10 pmol/uL. The polymerase chain reaction (PCR) amplification was performed by using AllInOneCycler™ PCR system (Bioneer, Oakland, CA, USA) with the program as 94°C for 5 minutes followed by 35 cycles of 93°C for 20 seconds, 62°C for 20 seconds, and extension at 72°C for 30 minutes. The PCR product was checked by agarose gel electrophoresis.

Statistical analysis

SPSS (version 2023) (IBM, Armonk, NY, USA) was employed in this study for statistical analysis [18]. Dunkin' and one-way ANOVA were applied to exam the probability level with $P < 0.05$ as the significant difference and $P < 0.01$ as the very significant difference.

Results and discussion

General information of hepatitis B patients

The age distribution of HBV infected patients was shown in Table 1 with the highest percentage of 50% at age 30 to 44 years old, followed by 45-60 years old (31.1%) and 15-29 years old (18.9%). There was significant difference among the age groups ($P < 0.05$). The results showed that the most HBV infected cases occurred in the age group of 30-44 years old, which might be caused by the bias of vaccination programs in Iraq with the most HBV vaccination programs targeting the teenagers and males, and therefore, providing unbalanced protection to disease. In addition, most HBV infected patients in this age group were working in hospitals for a long time and their immune systems were already affected by the virus [19]. The gender difference in HBV patients and control groups were shown in Figure 1, where the highest percentage was found in male (62.2%), while the female HBV patients were 37.8%. The statistical analysis results demonstrated the significant difference between male and female HBV patients ($P < 0.05$). The results showed that the infection rate in males was higher than that in females, which compromised with many other studies that males might be more susceptible to infection compared to females, depending on the nature of work and exposure to the virus in the workplace. In addition to the contamination resulting from blood transfusions, shaving tools, dealing with patients in the hospital, drawing blood from patients [19], another study was found that the males were more susceptible than female to HBV infection [20].

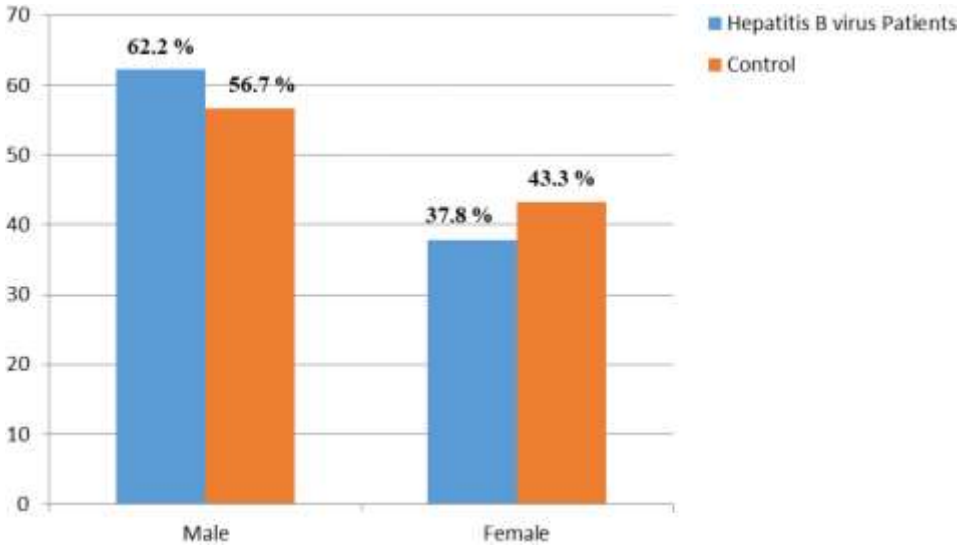

Figure 1. Gender distribution of HBV infected patients.

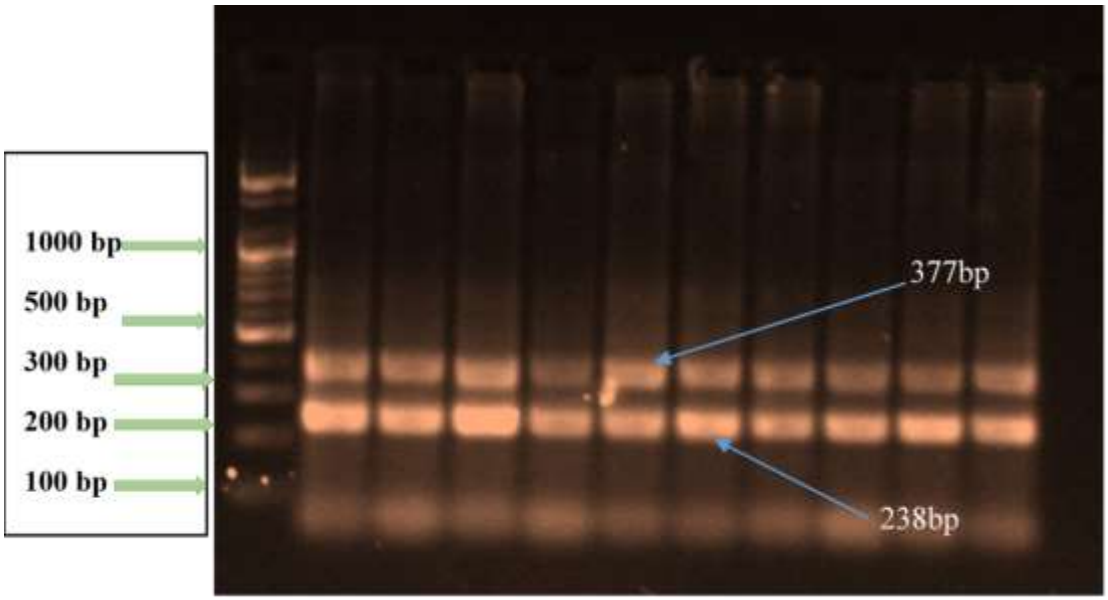

Figure 2. The genetic polymorphisms of IL-10 in HBV infected samples. The 377 bp band represented A allele and 238 bp band represented G allele.

The genetic polymorphism of IL-10 (-1082 A/G) in HBV patients

Many previous studies investigated the relationships between genetic polymorphisms of some biological factors and HBV infection. The genetic variation of interleukin-10 (-1082 A/G) was targeted in this study as a risk factor for HBV infection, especially in resource-limited countries [21]. The results demonstrated that the factors associated with IL-10 (-1082 A/G) positivity were age stratum between 30-44 years old and gender of male, which was consistent with the results of previous research [22]. The PCR results of IL-10 genetic polymorphism (-1082 A/G) showed two alleles, allele A and allele G, with different product sizes of 377 bp and 238 bp, respectively (Figure 2). The IL-10 genotype AG demonstrated the highest frequency of 38 samples (42.2%) in

Table 2. The genotype frequency of IL-10 (-1082 A/G) gene in HBV infected and healthy samples.

Genotype	HBV infected samples	Control samples	Odds ratio (95% confidence interval)	*P* value
AA	34 (37.8 %)	20 (66.7%)	2.000	> 0.05
AG	38 (42.2 %)	6 (20.0%)	1.180 (0.64 - 1.33)	< 0.01
GG	18 (20.0 %)	4 (13.3%)	0.702 (0.45 - 1.13)	< 0.05

HBV infected group compared to that of 6 samples (20%) in healthy control group. The IL-10 genotype AA showed a frequency of 34 samples (37.8%) in HBV infected group compared to that of 20 samples (66.7%) in healthy control group. In addition, IL-10 genotype GG was presented in 18 (20%) HBV infected samples, while only 4 (13.3%) healthy samples presented it in control group (Table 2). IL-10 is produced by blood monocytes and lymphocytes through the regulation of IL-10 gene expression. The production level of IL-10 regulates the immunity of the body and is essential for the control of HBV infection. The severity of HBV infection and body's immune response are both affected by the IL-10 genetic polymorphism. Furthermore, the genetic variants may reduce the production of IL-10 by Immunol cells [23], which explains the different individual immune response and the immune function of the host. Therefore, it is crucial to identify the IL-10 polymorphisms for the prediction of individual vulnerability to HBV infection. Shin *et al*. found that IL-10 (-592C) allele, a high IL-10 production genotype, could accelerate chronic HBV infection [24]. Cheong *et al*. also mentioned in their study that IL-10 (-592A), a low IL-10 production genotype, was more susceptible to HBV [25]. However, IL-10 (-592AA) genotype demonstrated the protection capability against HBV infection [26]. Peng *et al*. suggested that patients' HBeAg seroconversions were caused by IL-10 intermediate producer haplotypes or genotypes [27]. All those findings suggested that the IL-10 gene variation affected different diseases. Through the results of this study, a strong correlation between vulnerability to HBV infected chronic liver disease and IL-10 polymorphism (-1082) was established. The IL-10 (-1082) gene was linked to an increase in the incidence of chronic hepatitis infection. The IL-10 polymorphism was discovered to be essential for human susceptibility to HBV infection. According to the results of Crawley *et al*., IL-10 (-1082), (-819), and (-592) could be related to several haplotypes [28]. While the homozygous GCC haplotype had a high level of IL-10, the homozygous ATA haplotype showed a low level of IL-10. The ACC haplotype, meanwhile, could increase human body's production of anti-HBV surface antigen antibodies nearly twice as much as people without this haplotype, while the (-1082A) allele could reduce the production of anti-hepatitis A virus (antiHAV) antibodies compared to (-1082G) allele in homozygous people [29, 30]. The current study also proved a close association between the immune protein IL-10 (-1082 A/G) and the changes of HBV clinical features. Some medical and non-medical practices such as blood transfusion and tattooing may increase the risks of HBV infection with the possible HBV contaminated blood exposure. Meanwhile, such infection pathways may cause genetic variation in some immune cells that produce immune proteins and reduce the cell defensive effects against pathogens [6]. In addition, contracting infections related to HBV and even HCV may cause a genetic variation in the immune cells, which affects the production and effectiveness of immune proteins.

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