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ORIGINAL ARTICLE

Genetic Polymorphism in the Human IL-10 and Human IL-28 B with Increased Risk of Hepatitis B Virus Infection

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ABSTRACT

Background: *A variety of illnesses, including acute and chronic hepatitis, as well as asymptomatic HBV carriers, can result from HBV infection. The host immune system's variability may contribute to the different ways that HBV infection manifests clinically. By regulating HBV replication, cytokines (such as IL-10 and IL-28B) are crucial in reducing acute HBV infection and assisting in the recovery of HBV in many models.* ***Objectives:*** *In this research we aimed to ascertain how IL-10 and IL-28B genetic polymorphism affect the progression of hepatitis B virus infection.* ***Methodology:*** *During the period from August 2023 to February 2024, blood samples were collected from 131 individuals, aged between (6-76) years, and they were divided into two groups, the control group (40) that were collected from healthy people and the patient group (91) samples collected from people infected with HBV. The study groups were divided according to age, sex and residency. The level of interleukins IL-10 and IL-28B in serum was assessed immunologically using ELISA technology in the control and patient groups, and their relationship with hepatitis B infection was determined.* ***Results:*** *The current study revealed that the infection rate in the age groups (21-40) and (50≥) was higher than (20≤) and (41-50), males more than females, and in urban residents more than rural residents, and there was a statistically significant difference between those groups compared with the control group. The Mean ± SE of interleukin-10 in the patient group was higher than in control group with P-value <0.0001, as the current study demonstrated an increase in the level of interleukin-10 when infected with hepatitis B, while the level of IL-28B decreases when infected with hepatitis B, the Mean ± SE in patient group was lower than in control group.* ***Conclusion:*** *After DNA sequence analysis to detect genetic polymorphism in IL-10 & IL-28B, it was found that A/A genotype has the highest frequency in IL-10 gene rs1800896 in the patient group, and in the control group it was A/G genotype, while in IL-28B gene rs8099917, T/T genotype was the highest frequency in both groups.*

INTRODUCTION

Hepatitis viruses namely HAV, HBV, HCV, HDV, and HEV are widely known culprits behind acute hepatitis, a condition that can escalate to acute liver failure.[1]

HBV and HCV infections are particularly concerning as they can develop into chronic conditions, potentially leading to liver cirrhosis and cancer.[2]

Within the hepatitis virus family, HBV stands out as a prominent member of the of Hepadnaviridae family. Infection with HBV can result in various diseases ranging from chronic and acute hepatitis to asymptomatic carriers, liver cirrhosis and primary liver cancer (HCC).[3] The outcomes of HBV infection, whether acute, mild or severe chronic hepatitis or cirrhosis, depend on both viral and host factors.[4]

The body's immune responses to HBV antigens are crucial for clearing the virus during acute infection and can also influence disease progression.[5] Adaptive immune responses are essential for combating HBV, with early innate immune responses paving the way for the development of adaptive immunity.[6]

Cytokines, are vital chemical messengers, play a significant role in regulating immune cell activities. Insufficient immune responses can impact the clearance or persistence of HBV.[7] Important roles for cytokines like IL-10 and IL-28B in the initiation and regulation of immune responses may impact an individual's susceptibility to HBV and the duration of the infection.[8]

IL-10 is produced by various immune cells and is particularly significant in HBV infection, with higher levels observed in patients with chronic hepatitis B.[9] IL-28B, a cytokine receptor ligand, is associated with type I interferons and play a critical role in responding to

viral challenges by inducing interferon-stimulated genes (ISG).[10-11]

Genetic variants in human leucocyte antigen, cytokine and cytokine receptor genes have been linked to HBV susceptibility, persistence and disease severity.[12]

Viral load and the dynamics of hepatic inflammation can be attributed to elevated IL-10 levels and Breg cell counts in chronic HBV.[13] In patients with chronic HBV, polymorphisms close to the IL-28B gene are substantially correlated with both spontaneous viral clearance and a persistent viral response.[14]

METHODOLOGY

All blood samples (5ml) were collected in the Public Health Laboratory in Najaf during the period from August 2023 to February 2024 from 131 individuals, aged between (6-76) years, and they were divided into two groups: 40 samples were considered the control group, which included healthy individuals, and 91 samples were considered the patient group, which included individuals with HBV infection.

All samples were divided and placed in two tubes; 2ml in an EDTA tubes in order to extraction of DNA then amplification by PCR for study of IL-10 rs1800896 and IL-28B rs8099917, and 3ml was collected in a gel tubes and then centrifuged at 4000 rpm for 5 minutes for immunological detection of HBsAg, IL-10 level and IL-28B level in serum.

Immunological detection:

The detection of HBsAg, IL-10 level and IL-28B level in serum was done by using ELISA technique, and the assays was done according to the instructions of the manufacturer.

Molecular detection:

Extraction of human genomic DNA:

The extraction of genomic DNA from whole blood was done by using FavorPrep™ Genomic DNA Extraction Mini Extraction Kit (Blood/Cultured cell) according to the manufacturer's instructions.

Estimation of DNA Purity and concentration:

By using a UV/Visible spectrophotometer and the extraction kit instructions, the purity of human DNA was assessed to be between 4 and 10 µg/ml with an approved absorbance ratio of 1.8 for pure DNA.

DNA amplification:

The gene primers which were used in the current study in the PCR detection of IL-10 (rs1800896) A/G SNP, and IL-28B (rs8099917) G/T SNP are listed in table (1), and PCR mixture was prepared according to the type and target region of DNA by using GoTaq® Green Master Mix, and the test was carried out in compliance with the manufacturer's instructions.

Table 1: The primers sequences that were used in current study

Primer	Direction	Sequence	Product
IL-10 rs1800896	Forward	5'-CTCGCCGCAACCCAACTGGC-3'	159bp
	Reverse	5'-GTAAGGGACCTCCTATCCAG-3'	
IL-28B rs8099917	Forward	5'-CCCACTTCTGGAACAAATCGTCCC-3'	552bp
	Reverse	5'-TCTCCTCCCCAAGTCAGGCAACC-3'	

In the current study we used conventional PCR and the amplification conditions of IL-10 rs1800896 (A/G SNP) & IL-28B rs8099917 (G/T SNP) was as follows; Initial denaturation 95 C° 5 minute, denaturation 94 C° 30 second, annealing 55 C° 30 second, elongation 72 C° 30 second, final elongation 72 C° 5 minute and hold 4 C° 30 minute. Following that, agarose gel electrophoresis was used to examine the PCR products in accordance with the manufacturer's instructions.

Statistical Analysis:

Statistical analysis was made using (graph pad prism version 5) computer software according to T- test, the mean value and standard error (SE) for each value was determined. P. value less than the 0.05 level of significance was considered statistically significant.

RESULTS

In the detection of HBsAg in serum all control group (40) gave a negative results and was examined for genetic polymorphism in IL-10 and IL-28B as control, while the group of patients (91) of a positive result, 40 out of 91 were examined for genetic polymorphism in IL-10 and IL-28B. The samples were divided according to age, sex, and residency. Table (2) provides descriptions of clinical parameter measurements for HBV patients and the control group.

Table 2: Clinical parameters for studied groups

Parameter	Patient (n=91) Mean ± SE	control (n=40) Mean ± SE	P-value
HBsAg (IU/ml)	3.075 ± 0.1038	0.05718 ± 0.00077	< 0.0001
IL-10 (ng/L)	0.1465 ± 0.00916	0.02318 ± 0.00086	< 0.0001
IL-28B (ng/L)	0.1506 ± 0.0089	1.366 ± 0.111	< 0.0001

The average age of HBV patients and control group in the current study was between the (6-76) and (11-66) year respectively.

The current study demonstrates that the age groups (20 ≤), (21-30years), and (41-50 years) are more susceptible to HBV than the other (31-40 years) and (50 ≥), and there were no significant differences between patient age groups (P-value≥ 0.05) compared to control group which there was a significant difference (P-value<0.0001), and for sex group, the male group has the highest percentage of patients, consisting of 55/91 (60.44%), while the female group represents 36/91(39.56%) of patients, the current study demonstrates significant differences of those groups compared to control group (P-value<0.0001), while in residency group, the group Urban show highest percentage 62/91 (68.14%) patients compared with group Rural constituted 29/91 (31.86%). The current study demonstrates no significant differences between Urban and Rural groups (0.3800), while there was a significant difference between residency groups compared to control group (P-value<0.0001), table (3).

Table 3: Mean ± SE, P-value and Classification of HBV patients according to age, sex and residency

Category		Patient group	Control group	P-value
		Mean ± SE	Mean ± SE	
Age	**20 ≤**	3.205 ± 0.47	0.055 ± 0.0047	<0.0001
	21-30	3.386 ± 0.148	0.056 ± 0.0042	
	31-40	2.780 ± 0.234	0.061 ± 0.0070	
	41-50	3.085 ± 0.203	0.057 ± 0.0039	
	50 ≥	2.918 ± 0.241	0.057 ± 0.0038	
Male		3.046±0.1234	0.0573±0.000921	
Female		3.118±0.1845	0.05692±0.00146	
Urban		3.066 ± 0.1265	0.057 ± 0.0093	
Rural		3.093 ± 0.1846	0.058 ± 0.0066	

As for the detection of Human IL-10 in serum the current study showed that the level of IL-10 increases in patients with hepatitis B virus, while the level of IL-28B decreased, table (2). The correlation between IL-10 and IL-28B levels with study groups was illustrated in table (4-a) and table (4-b) respectively.

Table 4-a: The Mean ± SE & P-value of IL-10 in the study groups

Parameter		IL-10 (ng/L)		P-value
		Patient group (no. 91)	Control group (no. 40)	
Age	**20 ≤**	0.0720 ± 0.004	0.0253 ± 0.0054	<0.0001
	21-30	0.1360 ± 0.019	0.0219 ± 0.0042	
	31-40	0.1360 ± 0.012	0.0219 ± 0.0065	
	41-50	0.1178 ± 0.011	0.0224 ± 0.0062	
	50≥	0.1600 ± 0.0187	0.0219 ± 0.0050	
Sex	**Male**	0.1489 ± 0.0104	0.02325 ± 0.00124	
	Female	0.1429 ± 0.0169	0.02307 ± 0.00116	
Residency	**Urban**	0.1451 ± 0.0117	0.02337 ± 0.00089	
	Rural	0.1497 ± 0.0143	0.0221 ± 0.0029	<0.0002

Table 4-b: The Mean ± SE & P-value of IL-28B in the study groups

Parameter		IL-28B (ng/L)		P-value
		Patient group (no. 91)	Control group (no. 40)	
Age	**20 ≤**	0.0830 ± 0.004	0.993 ± 0.486	<0.0001
	21-30	0.1436 ± 0.019	1.457 ± 0.621	
	31-40	0.1330 ± 0.009	1.745 ± 0.662	
	41-50	0.1028 ± 0.004	0.918 ± 0.423	
	50≥	0.2399 ± 0.016	2.159 ± 0.791	
Sex	**Male**	0.1536 ± 0.0101	1.482 ± 0.136	
	Female	0.1460 ± 0.016	1.192 ± 0.183	
Residency	**Urban**	0.1487 ± 0.01128	1.332 ± 0.1175	
	Rural	0.1546 ± 0.0147	1.561 ± 0.3413	

PCR products of gene polymorphism

Two primers for amplified products of IL-10 rs1800896 gene by conventional PCR gave the amplified product of 159 bp. The current study found three genotypes after analysis by DNA sequencing G/G, A/A and A/G in both HBV patients group and control group, figure (1).

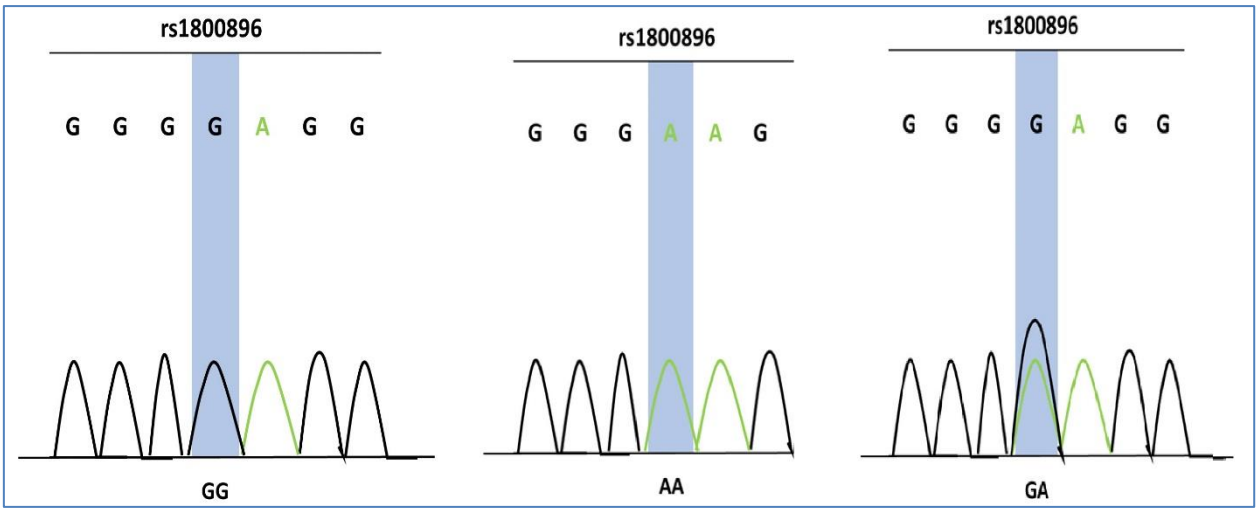

Fig. 1: Sequence of nucleotide polymorphisms of IL-10 rs1800896

The distribution frequencies of different genotypes of IL-10-1082 were analyzed among HBV patient group and control group table (5-a). The genotype frequencies of IL-10-1082 among these two groups met the H-W equilibrium.

Table 5-a: Genotype frequencies of IL-10-1082 in Patient and control groups

Genotype	Patient group		Control group	
	No.	%	No.	%
G/G	2	5	1	2.5
A/A	34	85	17	42.5
A/G	4	10	22	55
Total	40	100	40	100
G allele	8	10	24	30
A allele	72	90	56	70
Total	80	100	80	100

The current study demonstrates that the genotype A/A of IL-10-1082 was the highest frequency in HBV patient group, and genotypes A/G and G/G had a low frequency, while in control group the genotype A/G had the highest frequency followed by A/A genotype, but G/G genotype had the lowest frequency. In current study the polymorphism of -1082 in the IL-10 promoter region was identified and found a correlation of these polymorphism with HBV patient group.

As for IL-28B rs8099917 gene, forward and reverse primers set gave the amplified product of 552 bp. The current study found three genotypes after analysis by DNA sequencing G/G, T/T and G/T in both HBV patients group and control group, figure (2).

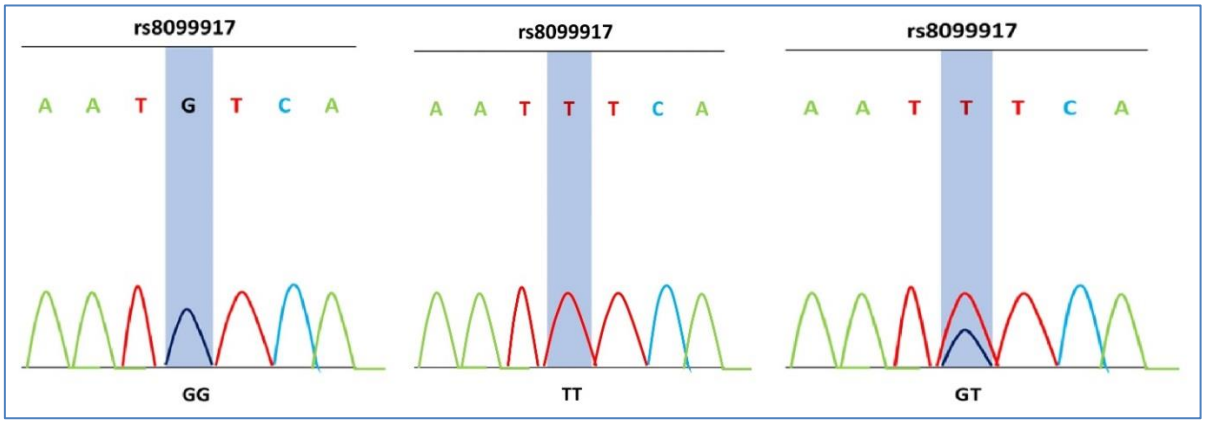

Fig. 2: Sequence of nucleotide polymorphisms of IL-28B rs8099917

The distribution frequencies of different genotypes of IL-28B were analyzed among HBV patient group and control group table (5-b). The genotype frequencies of IL-28B among these two groups met the H-W equilibrium.

Table 5-b: Genotype frequencies of IL-28B in Patient and control groups

Genotype	Patient group		Control group	
	No.	%	No.	%
G/G	1	2.5	9	22.5
T/T	25	62.5	16	40
G/T	14	35	15	37.5
Total	40	100	40	100
G allele	16	20	33	41.25
T allele	64	80	47	58.75
Total	80	100	80	100

The current study demonstrates that the genotype T/T of IL-28B was the highest frequency in HBV patient group followed by the genotype G/T and G/G genotype has the lowest frequency, while in control group the genotype T/T had the highest frequency followed by G/T and G/G genotypes. In current study the polymorphism in the IL-28B promoter region was determined and found a relation of these polymorphism with HBV patient group.

DISCUSSION

A research study supported the findings that the highest prevalence of HBV in Europe occurs in the 25 to 44-year old age bracket, followed by the 15 to 24 years old age group, in West Africa, about 30% of children are infected with HBV by the age of 2, with 15% developing persistent infection. By age of 10, 90% of children are infected, with 20% becoming chronic carriers.[15] The study also indicated that males are more susceptible to exposure to chronic HBV infection, along with complications like cirrhosis and hepatocellular carcinoma compared to females.[16]

Another study conducted in Crete Island reported that the prevalence of HBsAg was 3.3% in general population, 3.4% in the semi-urban areas and 3.2% in the remote and rural regions.[17]

The current study concurred with research linking elevated IL-10 levels to HBV serum levels, liver inflammation, fibrogenesis processes, and disease progression.[18]

A recent research showed that ageing leads to significant immune dysfunction, affecting vaccine responsiveness due to immune system defects.[19] Additionally, another study suggesting that greater IL-10 production in males is not the main reason for sex-related differences in immune function.[20]

In one study, IL-28B levels were lower in HBV patients compared with healthy individuals, aligning with previous research on chronic HBV infection.[21] Genetic studies revealed that the genotype A/A of IL-10-1082 was mostly prevalent, while genotypes A/G and G/G were less common.[22] The study also found that the rs8099917 TT genotype was predominant.[23]

For the association with liver diseases some studies indicated that single-nucleotide polymorphisms(SNPs) on IL28B are linked with liver diseases.[24] In our study, the T allele was more prevalent than the G allele in both study groups, with the T/T homozygous genotype being dominant in HBV patients and control group, this aligns with previous findings showing that the TT genotype had the highest frequency.[25]

In summary, our research findings corroborate various aspects of HBV infection, genetic factors, IL-10 levels, and immune responses.

CONCLUSIONS

The current study demonstrates that there is a high rate of infection with hepatitis B virus among patients. Interleukin 10 was significantly higher in hepatitis B patients than in the control group, although interleukin 28B levels were lower in hepatitis patients than in the control group. The infection rate among males was relatively higher than that of females, the age groups (20 ≤), (21-30), and (41-50) has the highest frequency of HBV infection than the other (31-40) and (50 ≥), and there were no significant differences between Urban residents and Rural residents in infection with the HBV.

The genotype A/A of IL-10-1082 was the highest frequency in HBV patient group and control group, while in IL-28B the genotype T/T was the highest frequency in HBV patient group and control group. The polymorphism of -1082 in the IL-10 and in the IL-28B promoter regions was determined and reported a relation of this polymorphism with HBV patient group.

Ethical approval

Protection of human and animal subjects, the authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data, the authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent, the authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Use of artificial intelligence for generating text, the authors declare that they have not used any type of

generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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