Interleukin-17 Gene Polymorphisms and Hepatitis B Virus Infection in a Sample of Iraqi Patients

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ABSTRACT

Hepatitis B, a chronic inflammatory liver condition, constitutes a significant global health issue, with 3 million new cases and over 1 million deaths annually (when combined with hepatitis C). The infection may be acute or chronic, and it transmits horizontally by contact with biological fluids such saliva, blood, semen, tears, vaginal secretions, or perinatal fluids following childbirth, which are transferred from mother to infant. Infections can be easily prevented with immunization, typically administered shortly after birth; nevertheless, if neglected, chronic hepatitis B may lead to severe outcomes, including liver cancer or cirrhosis. Interleukin-17A, a pro-inflammatory cytokine, is essential in the immune response to many infections and is implicated in autoimmune diseases. The cytokine interleukin-17 (IL-17A) is only secreted by activated T cells. cDNA of IL-17 has been isolated and cloned from murine hybridomas (cytotoxic T lymphocyte antigen 8 (CTLA-8)). Cytokine imbalance is a crucial factor in the progression and growth of the hepatitis B virus (HBV). A cross-sectional study was conducted including 180 patients with HBV infection at the Digestive and Liver Diseases Teaching Hospital in Baghdad province, from December 14, 2022, to February 15, 2023. This study comprised two groups: the first group consisted of 40 HBV antibody-positive patients, while the second group included 40 HBV antibody-negative patients, along with a control group of 20 individuals. A blood sample of 5 ml was collected from each patient, divided into 2 ml in an anticoagulated EDTA tube and 3 ml in a gel tube. Whole blood with anticoagulated samples was utilized under optimal conditions for DNA extraction, while serum was isolated from blood for biochemical tests. The study comprised 180 patients, categorized into three groups: HBV Positive, HBV Cleared, and Control. The HBV Positive cohort consists of 99 individuals, comprising 54 males (54.45%) and 45 females (45.45%). The average age in this cohort is 42.52 years, accompanied by a standard deviation of 15.13 years. Of these individuals, 84 (84.85%) are wed. The HBV Cleared group has 45 individuals, including 24 males (53.34%) and 21 females (46.67%). The average age in this cohort is 48.43 years, with a standard deviation of 13.98 years. A predominant 39 persons (86.67%) are wed. The Control group comprises 36 individuals, primarily male, consisting of 24 men (66.67%) and 12 females (33.34%). Genotype distribution for two genetic markers, rs2275913 and rs10484879, among three groups: HBV Positive, HBV Negative, and Control. In the HBV Positive group for the rs2275913 marker, the AA genotype is present in 30 patients, the AG genotype in 36 patients, and the GG genotype in 36 patients. In the HBV Negative group, the AA genotype is observed in 9 patients, the AG genotype in 12 patients, and the GG genotype in 21 patients. Multiple research investigations have concentrated on the role of SNPs in the IL17A gene, particularly rs2275913 and rs10484879, in the susceptibility to chronic HBV infection. The polymorphisms were specifically examined for their possible impact on immune response and disease progression in patients infected with HBV. A study of the Han Chinese population demonstrated a significant correlation between the GG genotype and G allele of rs2275913 and an increased risk of HBV infection, suggesting a causal relationship with heightened susceptibility to viral infection.

1. Introduction

Hepatitis B, a chronic inflammatory liver condition, constitutes a significant global health issue, with 3 million new cases and over 1 million deaths annually (when combined with hepatitis C) (1). The infection may be acute or chronic and is transmitted horizontally through contact with biological fluids, including saliva, blood, semen, tears, vaginal secretions, or perinatal fluids during childbirth, which are transferred from mother to infant. Infections can be effectively prevented through vaccination, typically administered shortly after birth; however, if untreated, chronic hepatitis B may lead to severe outcomes, including liver cancer or cirrhosis. Nearly 40% of hepatocellular carcinoma cases, the second leading cause of cancer-related mortality worldwide, are attributed to chronic hepatitis B. HBV is the archetypal member of the Hepadnaviridae family, consisting of enveloped viruses with relaxed circular double-stranded DNA (dsDNA) genomes encapsulated within virally-encoded capsids. Similar to other members of the Hepadnaviridae family (e.g., duck hepatitis B virus, woodchuck hepatitis B virus), the diminutive genome exhibits a high informational density characterized by significantly overlapping open reading frames (ORFs) (4). Research indicates that HBV can efficiently

infect nearly 100% of hepatocytes (approximately 10^11 cells in the liver of a chimpanzee or human) within 7 to 10 weeks, contingent upon the inoculum dosage, occurring without any evidence of hepatocellular damage. The primary factor influencing the outcome of an HBV infection is the virus's interaction with the host's immune system. The host's antiviral immune response to HBV can damage hepatocytes and induce liver inflammation while eradicating the virus, so facilitating the progression of the disease. The process significantly influences the advancement and result of hepatitis B. The host's immunological response to HBV comprises two fundamental components: innate immunity and adaptive immunity (8).

Interleukin-17A, a pro-inflammatory cytokine, is essential in the immune response to many infections and is implicated in autoimmune diseases (9). The cytokine interleukin-17 (IL-17A) is only secreted by activated T cells. cDNA of IL-17 has been isolated and cloned from murine hybridomas (cytotoxic T lymphocyte antigen 8 (CTLA-8)) (10).

Cytokines are tiny, nonstructural proteins with low molecular weights that are crucial in the intricate regulation of inflammation and immunity. The immunological and inflammatory response is believed to involve hematopoietic cells, lymphocytes, diverse pro-inflammatory and anti-inflammatory cells, with cytokines facilitating their complex interactions.(11).

Interleukin-17 (IL-17) is a cytokine produced by a newly recognized subset of helper T cells. The IL-17 cytokine family is associated with several immunological processes, particularly the initiation and facilitation of proinflammatory responses. The proinflammatory activity is evidenced by their involvement in pulmonary inflammatory responses; Th17 cells are distinct from Th1 and Th2 cells (12). IL-17 is high in the lesions of persons with several chronic inflammatory diseases, including lung infections, psoriasis, rheumatoid arthritis, and several chronic viral infections such as HBV and HCV. The Th17 subtype of T helper cells has emerged as a significant biomarker for predicting the progression to cirrhosis in liver diseases. Consequently, cytokines released by Th17 cells, particularly interleukin-17 (IL-17), have been associated with the activation of fibrogenic pathways and the progression to cirrhosis. The Interleukin-17 family comprises six cytokines, with IL-17A being the prototype. The remaining five members are IL-17B, IL-17C, IL-17D, IL-17E (sometimes referred to as IL-25), and IL-17F. IL-17A and IL-17F exhibit greater homology, while IL-17E demonstrates lesser homology (18). Cytokine imbalance is a crucial factor in the progression and development of the hepatitis B virus (HBV) (19). Single nucleotide polymorphisms (SNPs) in cytokine genes may modify protein production and thereby elevate the risk of HBV infection (20). Cytokines are crucial for the progression of hepatic diseases and inflammatory responses.

2. Methodology

Subjects

This cross-sectional study involved 180 patients with HBV infection at the Digestive and Liver Diseases Teaching Hospital in Baghdad province, conducted from December 14, 2022, to February 15, 2023. This study contained two groups: the first group comprised 40 HBV antibody-positive patients, while the second group consisted of 40 HBV antibody-negative patients, along with a control group of 20 persons.

Specimens' Collection:

A five (5) ml blood sample was obtained from patients, divided into two (2) ml in an anticoagulated EDTA tube and three (3) ml in a gel tube. Whole blood with anticoagulated samples was utilized for DNA extraction under ideal conditions, while serum was isolated from blood for biochemical tests. To do this, the blood samples were permitted to coagulate at ambient temperature for 20 minutes, followed by centrifugation at 5,000 rpm for 5 minutes. Serum samples were obtained and processed within two hours post-blood collection. All samples were partitioned into two aliquots and preserved at -20ºC until

utilized for biochemical assessments of liver function (i.e., ALT and AST) and molecular analyses, respectively.

DNA Extraction:

Genomic DNA was extracted from blood samples using the ReliaPrep™ Blood gDNA Miniprep System by Promega for isolation and purification. The process was Data analysis was conducted using the statistical methods prescribed by the manufacturer.

Preparation of primers:

The primers were supplied by Macrogen Company in a lyophilized form. rs2275913-F (TTGACCCATAGCATAGCAGC), rs2275913-R (CTCCATAGTCAGAACCCAGC) and rs10484879-F (TGTAAAACGACGGCCAGTGGGCTAAACCACACAAGAA), rs10484879-R (CAGGAAACAGCTATGACGAGAGAGAGGTTGGAGATTAGA). Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl.

Primer optimization

To examine the optimum annealing temperature of primer, the DNA template was amplified with the same primer pair, (Forward) (Reverse), at annealing temperatures of 55, 58, 60, 63 and 65°C. PCR amplifications were performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X); 1µl for each primer (10pmol); 6µl nuclease free water and 2µl of template DNA. PCR cycling was performed with PCR Express (Thermal Cycler, Veriti, USA) with the following temperature program: denatured at 94ºC for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec; annealing at 55, 58, 60, 63 or 65°C for 30 sec; and extension at 72°C for 30 sec. A final extension incubation of 7 min at 72ºC was included, followed by a 10 min incubation at 4ºC to stop the reactions.

Reaction Setup and Thermal Cycling Protocol

As show in the table 1 the reaction of PCR and step that used in the study.

Table 1. Reaction Setup of PCR

PCR Component Calculation				
No. of Reaction	35	rxn	Annealing temperature of primers	60
Reaction Volume /run	25	µl	Length of PCR product (bp)	934, 425

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
Master Mix	2	X	1	X	12.5
Forward primer	10	µM	0.5	µM	1
Reverse primer	10	µM	0.5	µM	1
Nuclease Free Water					7.5
DNA		ng/µl		ng/µl	3
Total volume					25
Aliquot per single rxn	22µl of Master mix per tube and add 3 µl of Template				

Steps	**°C**	**m:s**	**Cycle**
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	

Extension	72	00:45	
Final extension	72	07:00	1
Hold	10	10:00	

PCR products loading

For PCR product, 5µl was directly loaded to well. Electrical power was turned on at 100 volt/ 50mAmp for 60min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system.

3. Result and Discussion

This study had 180 patients categorized into three groups: HBV Positive, HBV Cleared, and Control. The HBV Positive cohort consists of 99 individuals, comprising 54 males (54.45%) and 45 females (45.45%). The average age in this cohort is 42.52 years, accompanied by a standard deviation of 15.13 years. Of these individuals, 84 (84.85%) are wed. The HBV Cleared group has 45 individuals, including 24 males (53.34%) and 21 females (46.67%). The average age in this cohort is 48.43 years, with a standard deviation of 13.98 years. The majority, 39 persons (86.67%), are wed. The Control group comprises 36 individuals, primarily male, consisting of 24 men (66.67%) and 12 females (33.34%). This cohort is considerably younger, with a mean age of 25.75 years and a standard deviation of 5.08 years. All participants in the Control group are unmarried.

Table 2 delineates the medical histories of patients categorized into three groups: HBV Positive, HBV Cleared, and Control. Every one of the 99 people in the HBV Positive group has a documented history of HBV infection (100%). A substantial percentage of 60 individuals (60.61%) has a surgical history. A minor proportion of 10 individuals (9.90%) possesses a history of tattoos. Blood transfusion history is documented in 69 individuals (69.7%). In the HBV Cleared group, all 45 individuals possess a history of HBV infection (100%). Twenty-seven people, constituting 60%, have received surgical intervention. This category has no persons with a history of tattoos. Nine individuals (20%) possess a history of blood transfusion. In the Control group, none of the 36 participants have a history of HBV infection. Only three individuals (8.34%) have undergone surgery. Six persons (16.67%) possess a history of tattoos, and an equivalent number have a history of blood transfusions (16.67%).

Table 2 Various Histories of the patients

History	HBV POSITIVE (n=99)	HBV CLEARED (n=45)	CONTROL (n=36)
History of HBV	99 (100%)	45 (100%)	0
History of Surgery	60 (60.61%)	27 (60%)	3 (8.34%)
History of Tatoo	10 (9.90%)	0	6 (16.67%)
History of Blood Transfusion	69 (69.7%)	9 (20%)	6 (16.67%)

In the figures 1 and 2 presents the mean and standard deviation (SD) values of ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) enzyme levels in three different groups, respectively, HBV+ (Hepatitis B positive), HBV- (Hepatitis B negative), and Control. For ALT, the mean value in the HBV+ group is 69.24 with a standard deviation of 16.52. In the HBV- group, the mean ALT level is 45.93 with a standard deviation of 8.95. The Control group has a mean ALT level of 30.58 and a standard deviation of 5.5. For AST, the HBV+ group shows a mean value of 58.73 with

a standard deviation of 16.03. The HBV- group has a mean AST level of 34.67 with a standard deviation of 3.75. The Control group presents a mean AST level of 21.67 and a standard deviation of 4.08.

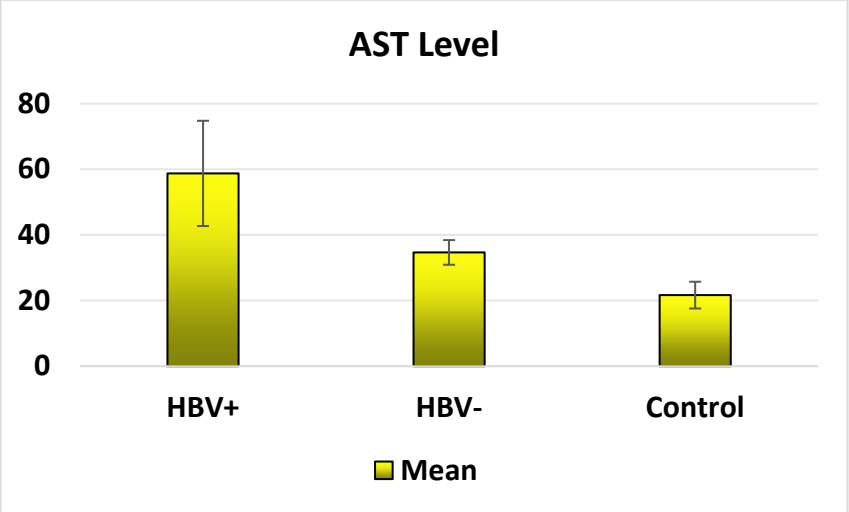

Figure 1 AST level in each group

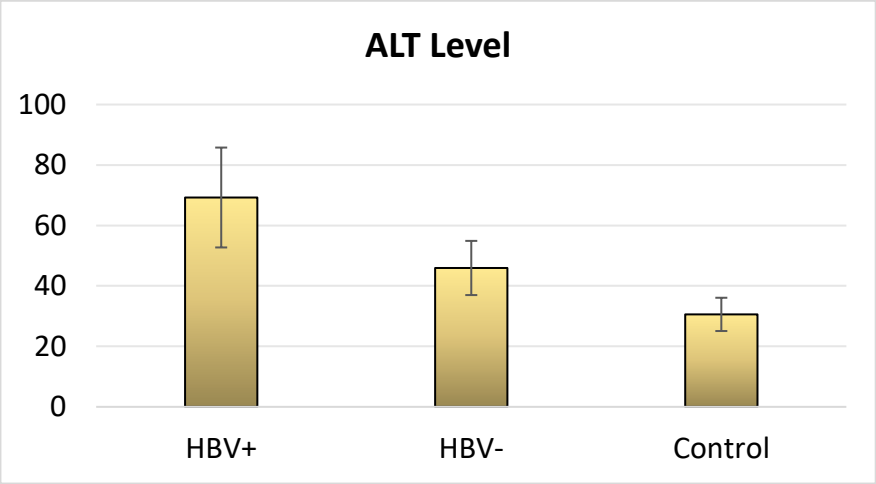

Figure 2 ALT level in each group

Distribution of genotypes of IL-17A:

Figure 3 illustrates the genotype distribution for two genetic markers, rs2275913 and rs10484879, among three groups: HBV Positive, HBV Negative, and Control. In the HBV Positive group for the rs2275913 marker, the AA genotype is present in 30 patients, the AG genotype in 36 patients, and the GG genotype in 36 patients. In the HBV Negative cohort, the AA genotype is observed in 9 patients, the AG genotype in 12 patients, and the GG genotype in 21 patients.

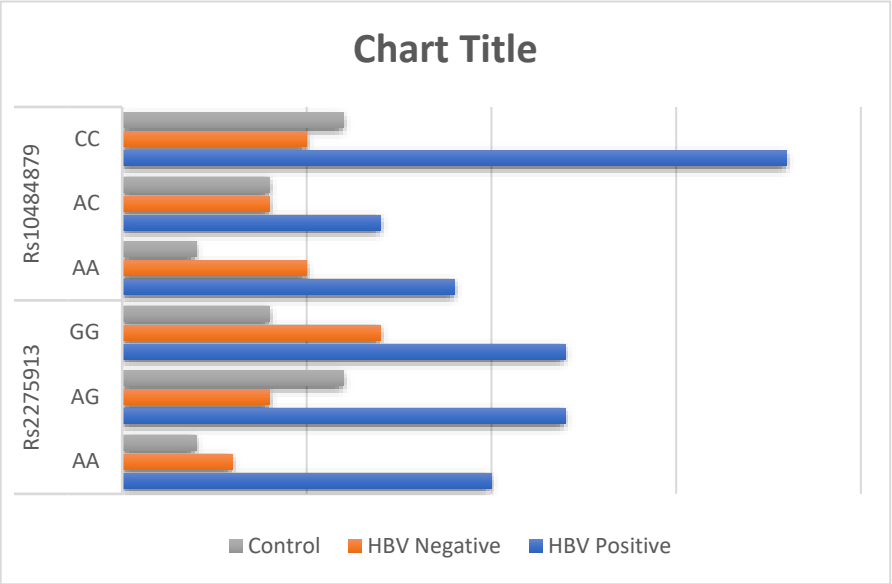

Figure 3 : Distribution of alleles

The association between SNPs of IL-17A with the group of study:

Chi-square test outcomes for the rs2275913 gene across the groups. The Pearson Chi-Square statistic is 2.176 with 4 degrees of freedom (df) and a P-value of 0.703, signifying no significant connection between the groups for the rs2275913 gene. The Likelihood Ratio is 2.133 with 4 degrees of freedom and a P-value of 0.711, indicating no meaningful correlation. The Linear-by-Linear relationship value is 0.362 with 1 degree of freedom and a P-value of 0.547, further confirming no significant linear relationship between the groups for the rs2275913 gene. As demonstrated in Table 3.

Table 3 Chi-square findings of rs2275913 gene between the groups

	Value	df	P-value
Pearson Chi-Square	2.176	4	0.703
Likelihood Ratio	2.133	4	0.711
Linear-by-Linear Association	0.362	1	0.547

Discussion

Multiple research investigations have concentrated on the role of SNPs in the IL17A gene, particularly rs2275913 and rs10484879, in the susceptibility to chronic HBV infection. The polymorphisms were specifically examined for their possible impact on immune response and disease progression in patients infected with HBV. A study of the Han Chinese population demonstrated a significant correlation between the GG genotype and G allele of rs2275913 and an increased risk of HBV infection, suggesting a causal relationship with heightened susceptibility to viral infection [21]. A separate investigation aimed at elucidating its precise involvement in disease development revealed that the rs2275913 GG genotype was more common in HCC patients compared to those with chronic hepatitis [22]. However, certain studies have revealed contradictory results in different populations. The rs2275913 polymorphism has been identified in a Kazakh population-based investigation as having no significant correlation with vulnerability to chronic viral hepatitis or the advancement to liver cirrhosis [23]. In a meta-analysis study, the rs2275913 AA genotype, despite being found associated with reduced risk against a HBV infection in Asians, did not have any such association in the overall comparison [19]. Although comparison was made to an even lesser extent, the IL17A rs10484879 polymorphism has also been evaluated for its significance in chronic HBV infection. In this respect, an Iranian study proposed a protective role in the progression toward chronic infection since the AC genotype of rs10484879 was found significantly associated with a lower risk of HBV chronicity [24]. On the other

hand, it has been shown that the rs10484879 polymorphism might modulate host immunity against HBV infection, decreasing the risk of chronic infection. The rs2275913 polymorphism has a population-dependent effect, as some studies have reported an increasing risk and severity with the disease, while others have found no such association. On the other hand, the rs10484879 polymorphism seems to exert a protective effect against chronic HBV infection in certain populations. The findings also indicate the complexity of the genetic factors in HBV infection and further research necessary to reveal mechanisms by which these polymorphisms influence the outcome of the disease. Elucidating the genetic associations may lead to the development of targeted therapies and personalized treatment strategies in HBV-infected individuals.

In this Iraqi study with a sample size of 180, we had demographic patterns running distinct across HBV Positive, HBV Cleared, and Control, which agree with literature available on hepatitis B virus epidemiology and transmission dynamics. The HBV Positive had 99 individuals, out of which 54 were males constituting 54.45%, while 45 were females constituting 45.45%. This finding is in accord with other studies indicating that chronic hepatitis B infection is more common in men than in women, often resulting in a male-female ratio of about 2:1 in most parts of the world. This type of gender disparity mirrors higher infection rates in the male population and is probably caused by biological, behavioral, and social factors that raise exposure risks [25], [26]. The mean age was 42.52 years with a corresponding standard deviation of 15.13 years. The age distribution, as expected, signifies that HBV infection is common with increasing age, especially in endemic regions where ages of over 40 years frequently typify infected individuals [27], [28]. Eighty-four subjects, corresponding to 84.85%, were married (a demographic feature that largely influenced the dynamics of HBV transmission). It has been postulated that married people would have a higher exposure rate due to stable sexual partnerships, particularly where HBV prevalence is high [25], [28]. The HBV Cleared group comprises the same number of 45 subjects, with similar gender distribution to the HBV Positive group—24 males (53.34%) and 21 females (46.67%). Approximatively 90% of immune-competent adults experience a spontaneous clearance of the virus and this kind of clearance does not seem to be significantly influenced by gender [27], [28]. The average age in this group was slightly higher, at 48.43 years, with a standard deviation of 13.98, which may indicate that age can affect the possibility of viral clearance—that is, the older the subject, the lesser the chances of spontaneous clearance due to several immune-related issues [26], [29]. Additionally, 39 individuals (86.67%) in this group are married, and therefore there are risks of exposure; a stable relationship may contribute to the transmission of HBV even after clearance has occurred [25]. The Control group consists of 36 individuals, mostly men, since it includes 24 males and 12 females, accounting for 66.67% and 33.34% respectively, as recently illustrated by Tripathi and Mousa [25], [28]. This point toward a greater representation of males within the study, which generally mirrors the trends of HBV studies on epidemiology, in which males are disproportionately impacted. The age for this group is much more skewed towards younger age, with a mean of 25.75 years with a standard deviation of 5.08 years. Its younger age relates directly to the lower chronic HBV infection rate in this control group, probably stemming from vaccination programs and increased awareness of transmission risk in the young population [27], [28]. None of the subjects in the Control group were married, which is unsurprising, given demographic trends that show that younger individuals are less likely to be married, and their marital status may correlate with their lower exposure to HBV [25], [26]. Therefore, these novel findings underline the important role of demographic factors in HBV infection, its clearance, and control, with age, gender, and marital status as key variables for targeted public health interventions to effectively manage and prevent HBV transmission.

In this study, we found that the age distribution patterns across the HBV Positive, HBV Negative, and Control groups revealed meaningful insights into the epidemiology of hepatitis B virus (HBV) infection, which are consistent with existing literature. The HBV Positive group exhibits a wider age range and higher variability in age compared to the other groups. This pattern aligns with findings from a study conducted in Turkish population, where the highest prevalence of HBV infection was observed in the 31-40 years age group, with a rate of 30.4% among HBsAg-positive subjects [30]. This wider

age distribution in our study suggests that HBV infection may affect a broad spectrum of the population, potentially due to varied exposure risks and the chronic nature of the disease, which can persist undetected for many years. The HBV Negative group, on the other hand, has a slightly older average age and less variability compared to the HBV Positive group. This observation is consistent with global trends reported by the World Health Organization (WHO), which states that new hepatitis B infections are most common among people aged 30-59 years. The older average age in this group may reflect a cohort of individuals who, despite being at risk due to their age, have either cleared the infection or avoided exposure, possibly due to vaccination or other protective factors [31]. The Control group in this study is the youngest on average and exhibits the least variability in age. This is an expected finding, as control groups typically consist of healthy individuals without known liver disease or hepatitis B infection. The younger age and lower variability suggest a selection of individuals at a lower risk of HBV exposure, likely due to effective vaccination programs and increased awareness of transmission risks in this demographic. These findings highlight the importance of targeted vaccination and screening efforts, particularly for high-risk age groups, to reduce the burden of HBV infection [25], [27]. The differences in age variability between the groups also underscore the need for tailored public health strategies that account for the specific age-related risks and behaviors associated with HBV transmission.

The age distribution pattern across HBV Positive, HBV Negative, and Controls reveals some epidemiological information regarding hepatitis B virus infection in our study, very consistent with the literature available. Compared to the other groups, the HBV Positive group indicated a much broader age range with higher variability. This trend is in agreement with the observation of a peak prevalence of HBV infection in the 31-40 years age bracket, with a rate of 30.4% among HBsAg positive subjects in a Turkish population study [30]. This therefore puts our study at a wider age bracket, suggesting that HBV infection may affect a broad spectrum of the population, probably due to varied exposure risks and the chronic nature of the disease, which can go undetected for a long time. In contrast, the HBV Negative group had a slightly older average age with less variability compared to the HBV Positive. This creates correspondence with global trends reported by the WHO, indicating that new hepatitis B infections are most common among people aged 30-59 years. Thus, this older average age may indicate a cohort of individuals who are at risk due to their age but have either cleared the infection or avoided exposure, probably as a result of vaccination or other protective factors against the infection [31]. The average age of the control group in this study was the lowest, and there was the least amount of variability. This would be expected, as control groups are usually composed of healthy subjects with no known liver disease or hepatitis B infection. Lower age and lesser variability may indicate the selection of subjects at low risk for HBV exposure, likely due to efficient vaccination programs and enhanced awareness of transmission risks in this age group. These results support the requirement for targeted vaccination and screening programs in high-risk age groups to reduce the burden of HBV infection. These differences in age variability across groups again point to the importance of tailoring public health strategies to individual age-related risks and behaviors associated with HBV transmission.

Some important patterns concerning the history of HBV infection and other associated risk factors were identified from our risk factor analysis for the HBV Positive, HBV Cleared, and Control groups, thus establishing consistency with previous studies. In the HBV Positive group, 99 patients had a history of hepatitis B virus infection, which represents 100%. The universal infection history in the HBV Positive group outlines the chronic nature of the disease [32]. A high percentage of this group, 60 patients (60.61 percent), is also traced to have a surgery background. It concurs with the general trends of epidemiological trends, as surgical procedures belong to the risk factors for HBV transmission, especially in areas of high infection rate [33], [34]. However, in this category, 10 subjects (9.90%) reported to have a history of tattoos. Although the proportion is much lower, this is expected, considering that in most studies, tattooing is one of the predisposing factors for hepatitis B transmission, especially in highly prevalent populations [33], [35]. Also, 69 subjects (69.7%) in this group had a history of blood transfusion, which is supported by studies in high-prevalence areas where a significant number of HBV infections have been attributed to unsafe blood transfusions [33]. In the

HBV Cleared group, all 45 subjects have a history of HBV infection, and 100% have a common pattern of HBV natural history, where persons who clear the virus often have documented prior infection. Of this study group, 27 have undergone surgery, further corroborating the notion that surgical interventions are a major risk factor for HBV transmission, at least in endemic countries [33], [34]. More interestingly, there have been no reports of tattoos among the subjects of this study group, thus further ascertaining the validity of this study based on the fact that persons who clear HBV usually do not go through high-risk behaviors like tattooing. Only 9 subjects in this group, however, had a history of blood transfusion, and this is according to studies showing that the risk of HBV transmission by blood transfusion is lower in those who have cleared the virus, probably due to less exposure to contaminated blood products [36].

The Control group had 36 individuals with no record of HBV infection, a result consistent with the profiles of control groups in HBV studies where the effect of proper screening and vaccination is evident in the lack of infection. In this group, only three patients (8.34%) have had a surgical operation, a result consistent with the low risk of HBV transmission in uninfected individuals. In addition, six have a history of tattoos and an equal number also have a history of blood transfusion, 16.67% for each. These are general population risk factors and identification of these activities does not indicate a strong association with HBV infection as they do among groups where knowledge of the infection caused by the virus is known. Such patterns underline the need for understanding the risk factors in HBV acquisition and, to be specific, explain the surgical history and/or exposure to potentially contaminated blood products that may affect HBV clearance. This study cements the need to level targeted interventions, as HBV leads to a proportionately higher disease burden in a high-risk population requiring rigorous screening and vaccination, to decrease the risk of HBV transmission. The level of liver enzymes was analyzed in the HBV Positive, HBV Negative, and Control groups for this research. The findings are very useful to denote clearly the effect of HBV infection on liver function, which has been well validated by existing literature on the subject [37].

In the HBV Positive group, the ALT level has a mean of 69.24 with the standard deviation of 16.52. This conforms to reports in the literature that illustrate heightened ALT levels among patients with acute hepatitis B disease, which, as in the case, exceeds1000 IU/mL during acute flare-ups from the disease. This indicates rampant inflammation and damage to the liver [38]. It indicates persistent hepatic injury in active HBV infection people, with these high levels seen in this group. For the HBV Negative group, the mean ALT level is 45.93 with a standard deviation of 8.95. This finding is typical in patients with chronic HBV, where despite significant liver injury, often the ALT levels remain persistently normal according to Sarin and Kumar [39]. This is most likely not a strict HBV effect but a region where HBV is more endemic, meaning that the use of ALT alone is likely not a good surrogate for liver health in these participants [40]. Variability in ALT within the HBV Negative group is a driver for the challenges in using ALT as the sole maker of assessing liver injury. The mean ALT level in the control group was 30.58 ± 5.5, which is within normal limits for healthy subjects. Normally, ALT levels should register below 30 IU/L in males and 19 IU/L in females. This is a value considered within the expected range in a population free of liver disease and thus proves that there is no serious hepatic injury in the control group. In this group, the AST levels were 58.73 ± 16.03. In HBV Positive patients, high levels of AST are usually an indication of liver cell necrosis and typically lower than AST levels in cases of chronic hepatitis [39], [41].The pattern indicates the hepatic damage inflicted by HBV, where ALT is usually higher than AST.

The mean AST in the HBV Negative group is 34.67 with a standard deviation of 3.75. Even if AST levels are within normal values, they could probably flag some underlying liver problems when associated with HBV DNA levels. This would mean that AST, like ALT, cannot reflect the extent of liver damage in patients with chronic HBV infection — a point reported by other studies as well [42], [43]. In a Control group, the average AST level was 21.67, with a standard deviation of 4.08, which is completely normal and acceptable for healthy populations. This is indicative to support the consensus that the control group, free of liver disease, exhibits the enzyme level one would expect. The enzyme

levels, in aggregate, are highest in the HBV Positive group, followed by HBV Negative, with the Control group having the lowest levels. This is in agreement with the background that HBV infection causes marked elevation of liver enzymes due to the destruction of hepatocytes, with ALT often more elevated than AST in active HBV infections [44]. The elevation in liver enzymes, prominently observed in the HBV Positive group, underlines the importance of monitoring these biomarkers for liver inflammation and in guiding treatment decisions for patients with chronic hepatitis B. However, the comparison for viral load across the groups does not show a significant difference for both the F-value, which is 0.811, and the P-value, which is 0.450. This point has no significant variation of viral load between the groups. This finding concurred with researchers' contributions whose findings show nonsignificant relationship of viral loads with the distribution of the genotypes of HBV-infected patients, suggesting that viral load may not be helpful to elicit information on genotypic outcomes across population [45]. Despite this, however, this study reappears to show again that the ALT and AST level remain invaluable tools in assessing the extent of the damage in the liver and the HBV infection progression. Several conclusions can be derived from the analysis of the rs2275913 and rs10484879 genetic markers when considering expression in HBV Positive, HBV Negative, and Control groups, which are all in concurrence with published literature, while at the same time indicating that more investigation needs to be conducted in the future.

For the rs2275913 marker, the HBV Positive group shows an almost equal distribution of genotypes, with 30 patients having the AA genotype, 36 with AG, and 36 with GG. In fact, the result of the present study, in line with that of a study conducted on the Iranian population, shows that no significant association of rs2275913 genotype was observed under conditions of chronic HBV infection, and therefore, the genetic variant could not have been a critical factor for HBV susceptibility among certain populations [24]. In contrast, the HBV Negative group less frequently demonstrated the AA genotype in 12 patients, while higher numbers showed the AG genotype in 12 and GG in 21 patients. This result is opposite to multiple studies like [46] suggesting that the AG genotype is associated with a decreased risk of chronic HBV infection, begging the question of possible genetic variation in susceptibility between different study populations or cohorts. The Control group is also characterized by a heterogeneous distribution of genotypes, including 6 patients with the AA genotype, 18 patients with AG, and 12 with GG. Such a general relatively balanced distribution in all three groups may suggest that, although rs2275913 could not have any strong association with HBV status [19], genetic variability is still possible, hence further investigation into this SNP required. For the rs10484879 marker, the CC genotype was more frequent in the HBV Positive group. Literature, however, evidences that the AC genotype in this polymorphism was significantly associated with a decreased risk of HBV chronicity [24], showing a protective effect. The predominance of the CC genotype in the HBV Positive group may suggest population-specific differences in genetic susceptibility to HBV. In the HBV Negative group, there was an equal distribution for genotypes: 15 patients had the AA and CC genotypes and 12 had the AC genotype. This distribution is in agreement with studies [24], [46] that have frequently observed the CC genotype among HBV-negative individuals, therefore one can also say that this genotype is common among non-infected populations. Again, in the Control group, the most prevalent is the CC genotype, found in 18 patients, then the AC genotype in 12 patients, and lastly the AA genotype in 6. Other studies like [46] have reported the prevalence of the CC genotype in healthy controls, thus showing its commonness in non-infected populations. Genotype distributions in the HBV Positive, HBV Negative, and Control groups differed significantly, suggesting that these genetic markers may mean different things in different populations and should be further researched to clarify the association.

Moreover, the results of the Chi-square test on rs2275913 and rs10484879 genes do not show any significant association between groups for these genetic markers in the Iraqi population. The Pearson Chi-Square is 2.176 with df=4 and a p-value=0.703, hence no association for the rs2275913 gene. This finding is further supported by a likelihood ratio of 2.133 with df=4 and p-value 0.711, together with the linear-by-linear association value of 0.362 with df=1 and p-value 0.547, all consistently giving an inference of no significant linear relationship between groups in relation to the rs2275913 gene. Here,

the chi-square tests' results for the rs10484879 gene are 2.167 with df = 4, yielding a p-value of 0.705, hence once again, there is no significant association between the groups. The likelihood ratio, thereby, having a Likelihood Ratio of 2.219 and df = 4 and p-value = 0.695, and the Linear-by-Linear Association value of 0.039 with df = 1 and p-value = 0.844 also formed a very poor significant linear association for the rs10484879 gene. Chi-square tests on rs2275913 and rs10484879 genes in the Iraqi population, and regional studies, resulted in showing that these genetic markers do not correlate considerably and variate within and between groups, which means there is no genetic differentiation at the concerned loci in the population under study. In summary, our study has provided several important findings into demographic, risk factor, and genetic profiles in HBV infection, clearance, and control. The gender distribution of both HBV Positive and HBV Cleared groups indicated a slightly higher prevalence among males, which agreed with reports in existing literature. The mean age of these groups highlights a greater susceptibility in older people to chronic HBV infection, likely due to the risk of prolonged exposure and a possibly reduced immune response. In addition, a high proportion of married individuals in both HBV Positive and Cleared groups underlines the role of stable sexual partnerships in HBV transmission and, therefore, the need for targeted interventions in these populations. On the other hand, our analysis of risk factors confirms a history of surgical procedures as an independent risk factor for HBV infection, with a significant proportion of people in both groups: HBV Positive and Cleared having undergone surgery. The association of tattoos with HBV infection, while less prominent, agrees with broader epidemiological findings. This is further supported by a significantly high prevalence of blood transfusion history in HBV Positive subjects, thus proving the already well-documented risk of HBV transmission through contaminated blood products. Therefore, stringent infection control measures in medical and cosmetic procedures could avert HBV transmission risks. Value increases in ALT and AST in the HBV Positive group were concordant with literature and reflect persistent liver inflammation and damage, thus warranting regular follow-up of these biomarkers in chronic HBV subjects. Observed discrepancies in enzyme levels of the HBV Positive, HBV Cleared, and Control groups reiterate the fact that assessment through liver function tests is only an indirect indicator of HBV infection. Insignificant differences in viral load across groups may indicate that, although this is an important parameter, it will not be in step with transaminases or the clinical outcome in every instance. Therefore, a comprehensive approach to HBV management should be undertaken. The research was mainly focused on the polymorphisms of the gene IL17A, rs2275913, and rs10484879. The results obtained could be of great importance in the investigation of the association of these markers with HBV susceptibility and progression. Individual differences in the effect of rs2275913 in various populations indicate that there may be genetic predispositions toward the infection and progression of HBV, which are dependent on ethnic and environmental factors. That the rs10484879 polymorphism confers protection in some populations further underscores the complexity of host-pathogen interactions involved in HBV infection. The findings add to a growing literature on the genetic factors contributing to HBV pathogenesis and illustrate the potential for individualized medicine approaches in managing HBV infection. Our findings bring forth the multifaceted nature of demographic variables, driven risk behaviors, liver enzymes, and genetic predispositions in the epidemiology and clinical outcome of HBV infection. The results suggest that targeted public-health interventions based on specific demographic and genetic profiles could be made to improve the effectiveness of HBV prevention and treatment strategies. Furthermore, genetic associations open other avenues for research in the development of personalized therapies based on individual risk genes

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