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Evaluating of the Impact the Polymorphisms of IFN- γ (+874 T/A) and the Risk of HBV in Iraqi Patients

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Annotation: In every nation, chronic hepatitis B (HBV) is a serious health issue. A proinflammatory T-helper 1 cytokine, interferon gamma (IFN-γ) has antiproliferative and anticancer properties. Hepatitis B virus (HBV) susceptibility may be influenced by some single nucleotide polymorphisms (SNPs) in the IFN-γ and IFN-γ R1 genes. Here, we study the impact the polymorphisms of IFN-γ (+874 T/A) gene and the risk of HBV in Iraqi patients. The results showed the activities of some interleukins in serum HBV patients, where IL-1β levels indicated a significant (P <0.05) rise in patients (4.26± 0.57 pg/ml) compared to control group (1.09±0.34 pg/ml). IL-17 concentrations in serum of HBV patients indicated a significant (P <0.05) elevated in patients (48.11 ±4.72 pg/ml) compared to healthy volunteers (16.29 ±1.05 pg/ml). In the patient hepatitis infection, the T allele was 44.0%, while the allele T in the control group was 64.0%%. While the allele A in the sample of patients, which is 56.0%, compared to the allele A in control group, which was 36.0%. The frequency of these genotypes among patients was 54.7%, 18.7% and 26.6% respectively, compared with 28.0%, 24.0%

and 48.0% respectively, among controls (Table 5). Statistically, there was a significant difference in the frequency of TT (OR=0.25, 95%CI=0.05-0.57, P=0.001) and AT genotype (OR= 3.43, 95%CI=9.53-0.99, p= 0.001) between patients and controls (table (5). At allelic level, the mutant allele (allele T) was more common among patients than controls (44.0% vs. 64.0%) with a highly significant difference (OR= 0.374, 95%CI=0.43-0.11, p= 0.009). Our outcomes demonstrated a significant association between HBV and genotype frequency, with patients carrying the IFN- γ +874 A/T genotype having a higher risk of hepatitis infection. Therefore, having the TT genotype is linked to a better prognosis for the HBV.

Keywords: hepatitis B, INF—γ, cytokines, polymorphisms.

Introduction

Infection with the hepatitis B virus is a known risk to public health worldwide [1]. Childhood is the most common way of propagation in countries of moderate prevalence (3–10%), whereas early adulthood is the age of infection. In low incidence (3%) areas, such as Europe and America, sexual and percutaneous transfer are the main means of transmission. HBeAg-negative hepatitis, sometimes referred to as the reactivation phase, is a stage of chronic HBV development. 7–25% of patients who test positive for antiHBe (antibody to antigen) develop cirrhosis each year. However, about 2% of chronic hepatitis B patients acquire cirrhosis each year, and about 10% of them develop chronic active hepatitis (HCC), which can lead to liver failure and/or hepatocellular carcinoma [2,3,4]. Hepatitis B virus (HBV), a major cause of end-stage liver disease globally, is the most frequent cause of viral hepatitis [5]. Even though there is a vaccine that effectively prevents HBV infection, new infections still happen because of low vaccination rates as well as the accessibility, cost, and availability of the vaccines in the areas that are most affected [6]. Viral breakout is another source of new infections, and even when infants receive the anti-HBV birth-dose vaccine on time, it can still affect up to 5% of them [7, 8]. The results of HBV infection depend on the interactions of host genetic, environmental, and virological variables [9]. While the cause of persistent HBV infection in some people is unknown, in others, the host response is viral clearance against HBV infection. Single-nucleotide polymorphisms (SNPs) in the regulation area of cytokine genes have been linked to this variation. In addition to influencing illness outcomes and treatment responses, genetic variants like SNPs that alter cytokine composition and production can raise the risk of infection [10]. The immune response to chronic illnesses can be started and maintained by cytokines [11–12]. By altering the amounts or activities of cytokines and their receptors, genetic alterations can impact immune responses. Since single nucleotide polymorphisms (SNPs) occur every 300

nucleotides and can influence an infectious disease's susceptibility, they are considered biological markers [13]. One cytokine that promotes inflammation is interferon gamma (IFN-γ). Through lymphocytes, including cytotoxic CD8+ T cells, this T-helper 1 (Th1) cytokine can stimulate cellular immunity [14]. HBV risk has been linked to the IFN-γ +874T/A polymorphism, especially in East Asians [15]. So, the aim of investigation was study the impact the polymorphisms of IFN-γ (+874 T/A) gene and the risk of HBV in Iraqi patients.

Materials and method

Subjects

75 individuals between the ages of 20 and 60years who were visiting Kirkuk Teaching Hospital in Kirkuk were involved in this study. Every patient was informed of the goal of these studies and consented to the protocol. For comparison, 75 healthy participants were included in the current study. The participants were split up into two study groups, with each group consisting of:

- Group (1): 75 healthy subjects as control group.
- Group (2): 75 patients with HBV infection as second group.

Inclusion and exclusion criteria

All individuals with a proven hepatitis B virus infection were included in the study, but those with other chronic illnesses were not.

Blood samples

A 5 ml of venous blood was taken from each subject for measurements of some cytokines levels and IFN-γ (+874A/ T) polymorphism.

Extraction of DNA

DNA was extracted using the Wizard® Genomic DNA Purification Kit. The DNA samples were stored at -15 °C until they were needed after extraction.

PCR Premix kit

According to the Korean company BIONEER's accompanying paper, an AccuPower® PCR PreMix kit was used for ARMS-PCR technology research.

Primers

The IFN-γ mutant gene was identified using three of the unique primers, according to [16], where the antisense was:

F (T allele): TTCTTACAACACAAAATCAAATCT ARMSPCR

F (A allele): TTCTTACAACACAAAATCAAATCA

R: TCAACAAAGCTGATACTCCA

The recognition of IFN-γ T/A +874

The IFN-γ+874 gene was identified using the polymerase chain reaction method of the ARMS-PCR replication system, and a minor deviation from the procedure was noted in the master mix preparation [16]. In compliance with the BIONEER protocol, the ARMS-PCR technology investigations were carried out utilizing an AccuPower® kit. Specific A and the antisense primer were used to identify the A allele, while specific T and the antisense primer were used to identify the T allele. To obtain the optimal conditions for interaction, the instrument software was modified and the samples were placed in a thermocycler to replicate the DNA. the optimum condition of detection recognition of IFN-γ T/A +874 were appeared in table (2).

Table (1): the optimum condition of detection recognition of IFN-γ T/A +874

Phase	Tm (0C)	Time	No. of cycle
Initial denaturation	95 °C	15 sec	1 cycle
Second denaturation	72 °C	50 sec	30 cycle
Initial Denaturation	96 °C	30 sec	
Second Denaturation	95 °C	50 sec	
Initial Annealing	65 °C	50 sec	
Second Annealing	95 °C	50 sec	
Initial Extension	72 °C	40 sec	
Second Extension	55 °C	50 sec	

Statistical analyzes

To identify whether averages differed significantly, PCR product data were compared using Fisher's at P <0.05 using the SPSS statistical software. Genotypes, allele frequencies, odds ratios (OR), and confidence intervals (CI) were assessed using the Compare 2 Ver.3.04 software, which was created by J. H. Abramson between 2003 and 2017 [17-18]. According to the www.had2know.com website, the results were examined using the Hardy-Weinberg equilibrium rule.

Results & Discussion

Interleukins

Table (2) show the activities of some interleukins in serum HBV patients, where IL-1β levels indicated a significant (P <0.05) rise in patients (4.26± 0.57 pg/ml) compared to control group (1.09±0.34 pg/ml). IL-17 concentrations in serum of breast cancer patients indicated a significant (P <0.05) elevated in patients (48.11 ±4.72 pg/ml) compared to healthy volunteers (16.29 ±1.05 pg/ml).

Table (2): interleukins concentrations in studied groups

Groups Parameter	Control (35)	Patients (80)	P-Value
IL-1β pg/ml	1.09±0.34	4.26± 0.57*	0.001
IL-17 pg/ml	16.29 ±1.05	48.11 ±4.72*	0.001

One significant pleiotropic cytokine implicated in HBV infection is the inflammatory cytokine IL-1β. Chronic liver disorders are thought to be accelerated by IL-1β [19, 20]. The same observations were made in the current study. Watashi et al. [21] discovered, however, that pretreatment with TNF-α and IL-1β significantly decreased host cell sensitivity to HBV infection. We propose that the impact of cytokines or chemokines on the onset and course of disease may be influenced by the early or late stage of infection. According to this study, IL-17 levels in each HBV group were statistically significant higher than those in the control group, indicating a close relationship between IL-17 and liver inflammation. Although the exact mechanism is unknown, IL-17 is a pro-inflammatory cytokine that has been identified as a key mediator in autoimmune illness and immune responses against certain particular pathogens [22]. The current findings are in line with those of [23,24], who showed that HBV patients had noticeably higher serum levels of IL-17 than a control group. According to studies by Yang et al. [25] and Yang et al. [26], IL-17's impact on the immunopathology of chronic HBV was linked to the levels of ALT and AST. Moreover, IL-17 can raise the risk of developing liver cirrhosis [27] by helping to attract immune cells to the liver and promoting inflammation in response to the HBV infection, which can cause liver damage and fibrosis.

Molecular Assays

Hardy Weinberg Equilibrium (HWE) was well-aligned with the genotype distribution in both polymorphisms for both patients and controls. As illustrated in Figure (1), AS-PCR revealed that

this polymorphism manifested in three genotypes in both patients and controls: TT, AT, and TT. The allele A in the control group was 64.0%, but the A allele in the patient's hepatitis infection sample was 44.0%. In contrast, the allele T in the control group was 36.0%, whereas the allele T in the patient sample was 56.0%, displayed in table (4).

Table (4): showed the repeats of two alleles A and T.

Gene	Type of allele	LC women (75)	Healthy women (75)	OR (95%CI)	P value
IFN-γ	*A*	33(44.0%)	48(64.0%)	0.374(0.43-0.11)	*0.009
	P.F	49.07%			
	T	42(56.0%)	27(36.0%)	3.15(1.35-6.03)	
	E.F	38.94%			

PF = Preventive faction, CI = Confidence Intervals, EF = Etiological faction, * = significant difference, OR = Odds ratio.

These genotypes were more common in patients (54.7%), controls (18.7%), and patients (26.6%), respectively, than in controls (Table 5). According to statistics, patients and controls differed significantly in the frequency of AA (OR=0.25, 95%CI=0.05-0.57, P=0.001) and AT genotype (OR= 3.43, 95%CI=9.53-0.99, p=0.001) (table (5)). At the allelic level, patients were more likely than controls to carry the mutant allele (allele A) (44.0% vs. 64.0%), with a highly significant difference (OR= 0.374, 95%CI=0.43-0.11, p=0.009), displayed in table (4).

Table (5): The frequency of IFN-γ mutant gene genotypes

Gene name	The genotype	LC samples (80)	healthy samples m(35)	OR (95%CI)	P value
IFN-γ	AA	41(54.7%)	21(28.0%)	0.25(0.05-0.57)	*0.001
	P.F	45.9%			
	AT	14(18.7%)	36(48.0%)	3.43(9.53-0.99)	*0.001
	E.F	17.5%			
	TT	20(26.6%)	18(24.0%)	1.89(0.9-5.63)	0.284
	E.F	28%			

EF = Etiological faction, CI = Confidence Intervals, OR = Odds ratio, PF = Preventive faction.

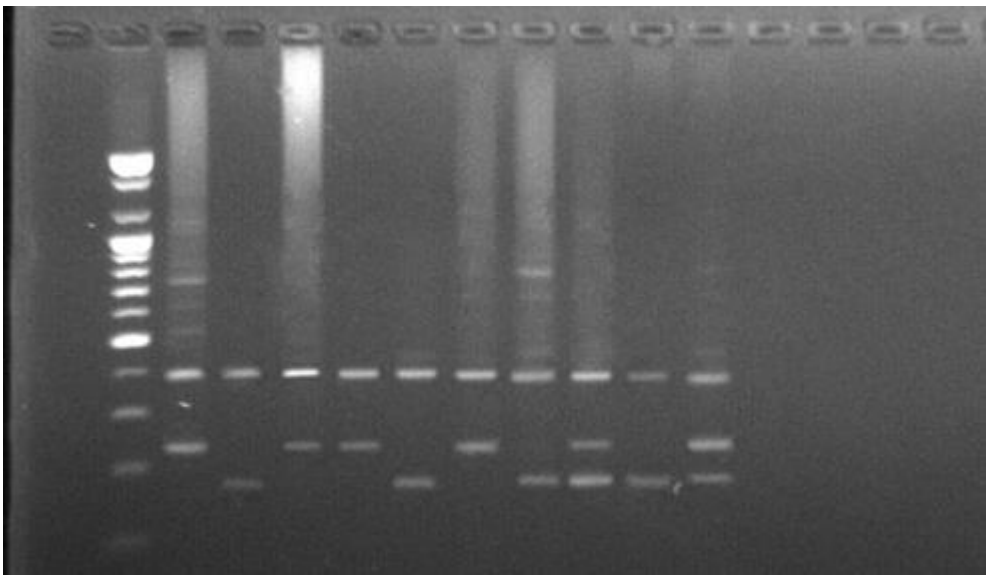

Figure (1): The IFN-γ gene electrophoresis to demonstrates the presence of both alleles, A and T, in HBV samples.

Immune mediators called cytokines alter the character of infections. Different ethnic groups' inflammatory responses may be impacted by differences in their cytokine genes [28, 29]. In this work, we investigated the potential connection between IFN-γ (+874T/A). In this investigation, we found a clear correlation between the risk of HBV and the allele distributions and genotype of +874T/A. According to our research, bearers of the T allele are less likely to become infected, and allele T raises the risk of sickness. Our results differ from those of previous research [30,31,32] and are in agreement with some [29,30]. Previous research has shown that the genotypes TT, AA, and TA are associated with high, low, and intermediate levels of IFN-γ secretion, respectively. This SNP is found in nuclear factor kappa-light-chain-enhancer of activated B cells' transcription factor binding site. Sequences with the +874T allele have been shown in studies to bind to this factor [33]. In contrast to the AA genotype, our results suggested that the TT and TA genotypes may lower the risk of chronic HBV infection. Furthermore, compared to the T allele, there was a clear correlation between the +874A allele and the severity of the HBV infection. To put it another way, the T allele might offer some protection against HBV infection. The findings of Sharhan et al. [29], who identified the +874A allele as a risk factor for chronic hepatitis B infection, contradicted our findings. Although the same geographic region (Asia) may account for similar findings in our study and the work of Sharhan et al. [29], the high sample size boosts the study's power. Sun et al. [30] found that the +874T/A SNP raises the incidence of HBV-related illnesses, which is consistent with our findings. They found that the AA genotype is linked to a 1.350-fold increased incidence of HBV-induced liver lesions, particularly in Asians.

Conclusions

Our outcomes demonstrated a significant association between HBV and genotype frequency, with patients carrying the IFN- γ +874 A/T genotype having a higher risk of hepatitis infection. Therefore, having the TT genotype is linked to a better prognosis for the HBV.

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