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Study some biochemical parameters and interleukin-6 (+174C/G) gene polymorphism in patients with Hepatitis B

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ABSTRACT

The current study was aimed to estimate interleukin-10 concentrations with studying the IL-6 (+174 C/G) gene polymorphism in an individual with Hepatitis B. This study involved 100 with hepatitis B individual. A total of (60) patients with hepatitis B were included in this investigation. The patients were between the ages of 15 and 60. These patients were hospitalized to Kirkuk, Iraq's Al-Jumhuri Hospital between January and March 2022. (2022). 30 healthy individuals served as the control group in this experiment. As compared to the control group, hepatitis B patients' liver enzyme (AST & ALT) and interleukin (4 & 6) activity significantly increased (P≤0.05). For molecular study, the findings exhibited that genotype (CG) had the highest frequency of 25 (41.7%) in hepatitis B individuals versus a lower frequency through normal individuals group 6 (20%), whereas genotype (CC) had the frequency of 24 (40%) in hepatitis B individuals compared to the highest frequency of 20 (66.7%) in normal individuals group. The genotype (GG) was found in 11 (18.3%) hepatitis B individuals compared to 4 (13.3%) in normal individuals group. Therefore, its concluded that the IL-6 (+174 C/G) gene was found to be related with hepatitis B risk in the Iraqi population.

INTRODUCTION

The family Hepadnaviridae contains the tiny, enveloped hepatitis B virus (HBV), which has a genome that is largely double stranded [1]. Despite nearly three decades of effective preventative vaccination, HBV continues to be the root of a number of significant public health issues [2]. Globally, an estimated 248 million people had chronic HBV infection in 2010, and in 2013 over

686,000 people died as a result of consequences from chronic HBV infection [3]. Globally, Hepatocellular carcinoma continues to be primarily brought on by chronic HBV infection, with viremia being the primary risk factor [4]. In order to minimize viral hepatitis by 2030, the World Health Organization has identified a motivated strategy that will result in a 90% decrease in new infections and a 65% decrease in mortality. However, only 10% of HBV patients are aware of their infection [5]. Infusion bags, multiple uses of medication vials, intravenous drug use (IDU), working in a medical facility, blood transfusion or transplantation of organs without HBV examination, acupuncture, tattooing, prolonged international travel, and improperly sterilized surgical equipment are risk factors for HBV viruses [6-7]. The cytokine IL-6 has a wide spectrum of pleiotropic effects that change how different lymphoid cells operate. [8]. IL-6 communicates by membrane-bound and soluble IL-6 receptors, which respectively mediate a conventional signaling pathway. It should be noted that IL-6's pro-inflammatory responses are mediated by trans-signaling, its regenerative or anti-inflammatory actions are mediated by conventional signaling [9]. There has been much written about the functions of IL-6 in HBV disease, involving acute and chronic hepatitis B, and HBV-related illnesses. The sIL-6R makes it difficult to interpret the function of IL-6 in HBV. The level of liver cells damage across the spectrum of HBV-related diseases is closely associated to IL-6 production during HBV infection, which is mediated by a number of different routes [10–12].

MATERIALS & METHODS

Subjects

This experiment includes a total of (60) hepatitis B patients. The patients ranged in age from 15 to 60. Between January and March 2022, these patients were admitted to Kirkuk, Al-Jumhuri Hospital. In this investigation, the healthy group consisted of 30 healthy participants.

Liver enzymes assay

The activity of liver enzymes AST and ALT was measured according to the method of action described by the manufacturer (Biolabo, USA).

Interleukins assay

IL-4 and IL-6 were quantified using the ELISA technique (Bioassay Technology Laboratory, China). The ELISA kits in question were enzyme-linked immunosorbent assays. On the plate wells, antibodies from humans were immobilized, one for each of the two parameters. Cytokines in the specimen were able to bind to coated antibodies in the wells. After adding of the biotinylated human antibody, binding to particular cytokines in the sample also happened. StreptavidinHRP was then added and attached to the biotinylated antibody at that point. Unbound streptavidin-HRP was removed both during and after the washing stage. After the solution for the substrate is added, color appears in a proportion to the quantity of human cytokines. At 450 nm, the absorbence was measured [13-14].

Preparation of sample

The loading procedure into the gel's gaps has already begun after inserting 5 ul of pure DNA for electrophoresis with 3 ul of loading buffer (Intron company/ Korea). To get the tincture to the other side of the gel, a 70 V CM2 electric charge was given for a period of one to two hours. The gel was placed in a pool containing 500 ml of DW and 30 ul of red safe nucleic acid dye before being inspected by a source of UV light with a 336 nm wavelength.

DNA isolation and genotyping

2 mL of blood collected in a tube containing EDTA for Extraction of DNA t o extract DNA, salting out has been utilized Lahiri et al. [15].

AARMS-PCR detection

A 96-well plate with qTOWER3G was used for qPCR in Shanghai, China. A final volume of 20 l was used for allele-specific qPCR reactions. This volume contained 1 l of template DNA, 1 l of forward primer (0.2 M), 1 l of reverse primer (0.2 M), and 10 l of 2 PerfectStartTM Green qPCR. 8 l of HO, SuperMix, and nuclease-free. The initial activation steps of the qPCR technique are 50°C for 2 min and 95°C for 10 min.

The primers

The primers were solubilized, disintegrated in free ddh20 to a final amount of 100 pmol/ul as a standard solution, and stored at -20 to prepare a work primer suspended concentration of 10 pmol/ul. IDT then performed an experiment with 10 ul of the standard solutions and 90 ul of free ddh water to obtain 100 ul volume.

Table (1): the specific primer of IL-6 (174C/G) gene

Primer	Sequence	Tm (0C)	GC (%)	Product size
C allele Wild type Primer	5’- GCAATGTGACGTCCTTTAGCTTC -3’	72	50.0	388
G allele Mutant type Primer	5’-TTCTTACAACACAAAATCAAATCT-3’	49.4	42.1	
Common Reverse Primer	5’- IF4 TCCCCCTAGTTGTGTCTTCCC	-	-	

Statistical analysis

The data, the levels of parameters in current study, were statistically examined using Minitab, a statistical analysis application, as well as Excel. The data was presented as an mean ± standard deviation. The current study's data were statistically evaluated utilizing the ANOVA test for finding significant variations by comparing the means of the two groups in current study and using Duncan's multiple test [16].

RESULTS & DISCUSSION

Hepatitis prevalence and breast cancer

The total number of patients surveyed was 60, with 39 (48.75%) having hepatitis C and 21 (26.25% having hepatitis B, as shown in the table (2).

Table (2): Prevalence of viral sero-markers in the study population.

Tests	Subjects with hepatitis	Healthy subjects
HCV antibody	39 (48.75%)	0 (0%)
HBsAg	21 (26.25%)	0 (0%)
Total	60	30

Liver enzymes

Hepatitis B patients' liver enzyme (AST & ALT) activity significantly increased (P0.05) when compared to the control group, as shown in Table 2.

Table (2): activities of liver enzymes in the groups of study

Tests	Subjects with hepatitis	Healthy subjects
AST (U/L)	106.31±18.03*	21.74±4.93
ALT (U/L)	91.42±13.71*	16.91±2.59

**: reflects that the two groups are significantly different.*

The acquired results demonstrated the diagnostic use of liver functions in chronic HBV infection, with AST and ALT having the greatest clinical relevance. As a result, the current study suggests using AST and ALT as biomarkers. Among these biomarkers is liver function, which is crucial for assessing the disease's severity and hepatic function in HBV infection patients. It has been demonstrated, as in the current investigation, that HBV infection may change the levels of the liver enzymes in the serum. In this respect, it has been proposed that chronic HBV patients experiencing an acute flare-up have ALT levels that are markedly elevated. An increase in both enzymes is more usually associated with hepatic injury because the release of liver enzymes into the bloodstream is a result of liver cells injure and damage brought on by HBV infection. The level of ALP has been shown to be highly correlated with HBsAg seropositivity [17–19], and the current data confirm this association since all HBV patients had elevated ALP levels and were also HBsAg

seropositive. Similar conclusions can be derived for AST and ALT, and it has been noted that HBsAg-positive patients had higher levels of both enzymes [20].

Interleukins (4 and 6) levels

Table (3) shows a significant increase (P≤0.05) in the levels of each interleukin (4 & 6) in patients with hepatitis B compared to the control group.

Table (3): levels of Interleukins (4 and 6) in the groups of study

Tests	Subjects with hepatitis	Healthy subjects
IL-4 (ng/ml)	26.151±3.27	11.932±1.36
IL-6 (ng/ml)	5.618±1.49	2.193±0.37

**: reflects that the two groups are significantly different.*

According to the findings of the current investigation, patients with viral hepatitis B had higher IL-4 and IL-6 levels. The current study's findings supported those of Al-Dabagh [21], who found that HBV patients had higher serum levels of IL-6 (20.57 1.70 pg/ml) than the healthy control group (17.67 4.88 pg/ml). In addition, there was no appreciable difference in the serum levels of IL-4 between the HBV patient group (26.36 3.43 pg/ml) and the healthy control group (27.00 354.36 pg/ml). Increased levels of IL-6 and IL-4 are thought to be a uniquely proinflammatory cytokine that quickly activates the host defense system to carry out various tasks [22]. During heterosubtypic challenge, early and direct IL-6 signals helped immune responses at the infection site [23]. According to several research, IL-6 rather than WBC or CRP was a more appropriate measure in cases of significant systemic inflammation for individuals who had chronic liver damage [24]. However, liver injury is caused by IL-4 and IL-6 that are overproduced and chronic [25].

Interleukin-6 (+174C/G)

Interleukin-6 PCR optimization for +174C/G detection was shown in (Figure 1). The annealing temperature was 62°C. Table (3) exhibited that genotype (CG) had the highest frequency of 25 (41.7%) in hepatitis B individuals versus a lower frequency through normal individuals group

6 (20%), whereas genotype (CC) had the frequency of 24 (40%) in hepatitis B individuals compared to the highest frequency of 20 (66.7%) in normal individuals group. The genotype (GG) was found in 11 (18.3%) in hepatitis B individuals compared to 4 (13.3%) in normal individuals group.

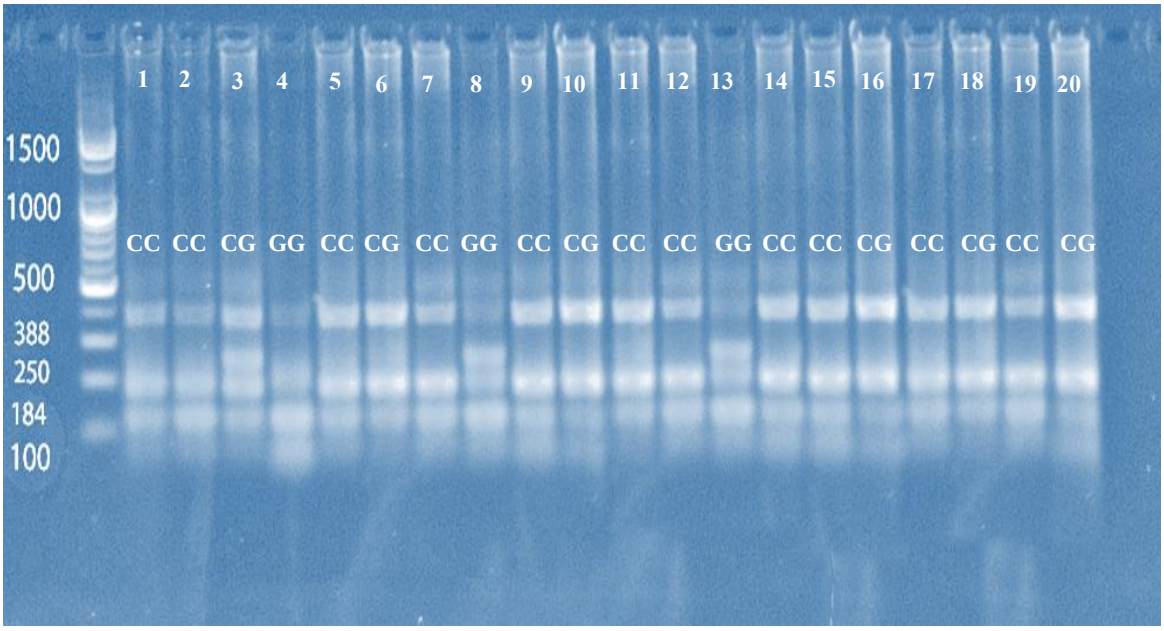

Figure (1): The IL-6 (174C/G) gene electrophoresis showed the two alleles C and G in infected subjects

Table (3): The genotype frequency ranges for IL-6 (174C/G) gene in hepatitis and healthy individuals

	Genotype	Infected (60)	Control (30)	(95%CI) OR	P value
IL-6 (174C/G)	CC	24 (40%)	20 (66.7%)	2.00	0.081 NS
	P.F				
	CG	25 (41.7%)	6 (20%)	1.18 (0.64-1.33)	0.003*
	E.F				
	GG	11 (18.3%)	4 (13.3%)	0.702 (0.45-1.13)	0.047*
	E.F				
*= P≤0.05					

In the current findings, IL-6 levels can be impacted by HBV, and it was also maybe to establish a link between cytokine levels and the IL6-174G/C, similar to what was shown for viral load. Patients with less severe disease, as measured by the METAVIR scale, showed higher amounts of cytokines. These findings imply that increased IL-6 levels may favor the development of chronic hepatitis B; the correlation was seen in the population studied and was linked to the IL6-174G/C polymorphism. Viral elements appear to favor the development of the illness and may aid in the spread of the infection [26]. The wild-type genotype of the IL6-174G/C appears to represent a component that facilitates the propagation of the HBV infection since it was associated to both increased IL-6 levels and a greater viral load. IL-6 promotes the development of CD4+ T cells into Th17 cells while inhibiting the differentiation of Treg cells [27–28]. These T cell subpopulations may not, however, constitute the most efficient form of response in battling HBV and may even encourage the persistence of infection. This is despite IL-6 functioning as a potent pro-inflammatory cytokine on T cells by encouraging Th17 development. As a result, it is possible to consider the CC genotype to be a significant factor in the intensification of the chronic hepatitis B inflammatory process.

CONCLUSION

According to the current findings, the IL-6 (+174 C/G) gene polymorphism was found to be associated with hepatitis B infection risk in the Iraqi population.

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