

Article

Correlation of IL10 and IL28B in Iraqi patients infected with HBV infection

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Abstract

Hepatitis B is a liver infection caused by the hepatitis B virus. The infection can be classified as acute (severe and short-term) or chronic (long-term). Hepatitis B can cause chronic infection and promotes people's risk of death from cirrhosis and liver cancer.

The present study aims to detect the levels of IL-10 and IL-28B in hepatitis B virus patients and their biochemical role in early detection of the disease.

During the period from August 2023 to March 2024, blood samples were collected from 153 individuals in the Public Health Laboratory in Najaf and Al-Hakeem General Hospital, aged between (5-70) years, and they were divided into two groups, the control group, which consisted of 40 samples (24 men and 16 women) that were collected from healthy people and the patient group, which consisted of 113 (68 men and 45 women) samples collected from people suspected of being infected with hepatitis B. HBsAg was immunologically detected in all samples using ELISA technology to verify infection with hepatitis B virus. The results have been indicated that the control group (40) were all negative, while in the patient group, 22 of them were negative and 91 were positive. The samples were distributed according to age, gender, and residence, and the results were compared with the control group. It has

been found that there were statistically significant differences between the two groups (the patient group and the control group).

The levels of IL-10 and IL-28B in sera were evaluated immunologically using ELISA technology in the control and patient groups, and their relationship with HBV infection was determined. The Mean \pm SE of IL-10 in the control group was (0.02318 ± 0.00086 ng/L) and the patient group was (0.1465 ± 0.00916 ng/L) with a high significant difference between the two groups (P-value <0.0001). Also, the current study has reported an increase in the level of IL-10 when infected with hepatitis B type, while the level of IL-28B decreases when infected with hepatitis B, the Mean \pm SE was (0.1506 ± 0.0089 ng/L) in the patient group and (1.366 ± 0.111 ng/L) in the control group and there was a significant difference between the two groups (P-value <0.0001).

Key words: Hepatitis B, IL-10, IL-28B

Introduction

The definition of hepatitis is an inflammation or damage to the hepatocytes that lasts for less than six months [1]. Hepatitis B virus (HBV), the archetypal member of the Hepadnaviridae family of hepatitis viruses, is endemic in many populations and can induce lifelong infection. It is distributed throughout the world. Numerous illnesses, including liver cirrhosis, acute and chronic hepatitis, asymptomatic HBV carriers, and primary hepatocellular carcinoma (HCC), can result from HBV infection [2]. One of the possible causes of the different clinical manifestations of HBV infection could be variations in the host immune response [3]. In this study we investigate the relation of some cytokines in hepatitis B infection. Cytokines play an important role in appropriate immune response to viral infections [4].

The replication of virus and expression of viral protein is related to specific and nonspecific immunity stimulated by virus. Immune cells and its relevant cytokines are

closely related to the pathogenesis and chronicity of hepatitis B [5]. Interleukin (IL)-10 is a potent immunosuppressive cytokine secreted in response to HBcAg by peripheral blood mononuclear cells (PBMCs) from patients with chronic HBV infection [6], while IL-28B constitute a new member of subfamily within the IL-10–interferon family, it is produced by virtually any nucleated cell type particularly dendritic cells following viral infection [7].

Methods

Samples collection

All blood samples were collected from the Public Health Laboratory in Najaf during the period from August 2023 to February 2024.

Patients group

The total number of samples was 113, which collected from people suspected of being infected with hepatitis B virus and ranging in age from 6 to 76 years, 22 of them tested negative for HBsAg, and 91 of them tested positive for HBsAg and other tests were conducted for them.

Control group

The control group was composed of 40 individuals who were asymptomatic and appeared healthy. Their ages were ranged from 11 to 66 years. The control group gave seronegative for immunological examinations.

Both groups were tested for IL-10 and IL-28B levels in sera by ELISA.

Samples collection

Five ml of blood were withdrawn from both control and patients group,

3 mL of which was placed in an EDTA tube in order to extraction of DNA then amplification by PCR to diagnosis of HBV-DNA.

The remaining 2 mL was collected in a gel tube and then centrifuged at 4000 rpm for 5 minutes to isolate serum in an Eppendorf tube. The samples were frozen at a temperature of -20 for subsequent serological examinations HBsAg, IL-10 and IL-28B. All samples were labeled with patient's name and a serial number.

- The detection of Hepatitis B virus antigen

The detection of antibody specific for HBV in serum was performed via ELISA technique by using HBsAg ELISA kit from ADVANCED, and the assay was performed according to the manufacturer's instructions.

- DNA extraction of Hepatitis B virus

The extraction of DNA from serum was performed by AccuPrep® Genomic DNA Extraction Kit, and the assay was performed according to the manufacturer's instructions.

- The quantitative detection of HBV-DNA

The Quantitative detection of Hepatitis B Virus in human plasma and simultaneous detection of a HBV-specific Internal Control (IC), by dual color detection, was accomplished by using 7500 RT-PCR, and the procedure was performed according to the manufacturer's instructions.

- The detection of IL-10

The detection of human interleukin 10 (IL-10) in sera was done via ELISA technique by using IL-10 ELISA kit from BT LAB, and the assay was performed according to the manufacturer's instructions.

- The detection of IL-28B

The detection of human interleukin 28B (IL-28B) in sera was done via ELISA technique by using IL-28B ELISA kit from BT LAB, and the assay was performed according to the manufacturer's instructions.

Results

-The detection of HBsAg in serum:

The HBsAg in serum was determined immunologically by ELISA for both groups, where the control group (40) gave a negative result, while the group of patients (113) 22 of whom gave a negative result and 91 of whom had a positive result, 19 out of 91 were tested for HBV-PCR, 17 of them the result was positive (detected) and 2 were negative (undetected), the Mean \pm SE of patient and control groups was (3.075 \pm 0.1038), (0.05718 \pm 0.00077) respectively and there was a significant difference between both groups (P-value<0.0001). The study groups were divided according to age, sex, and residency. The average age of HBV patients and control group in the current study was between the (38.36 \pm 16.34) and (33.25 \pm 14.51) year respectively. The current study has confirmed that the age groups (20 \leq), (21-30), and (41-50) are more susceptible to HBV than the other (31-40) and (50 \geq), and there were no significant differences between patient age groups (P-value \geq 0.05) compared to control group which there was a significant difference (P-value<0.0001), as for sex groups (Male and Female), 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 has reported significant differences of those groups compared to control group (P-value<0.0001), while there were no significant differences between Male and Female groups (0.3692), as for residency groups (Rural and Urban) the group Urban show highest percentage 62/91 (68.14%) of patients compared with group Rural constituted 29/91 (31.86%), the current study has shown 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 4-1 represents the descriptions of age, sex and residency for the studied groups.

Table (4-1): Mean ± SE, P-value and classification of samples according to age, sex and residency

Parameter		Patient group (no. 91)	Control group (no. 40)	P-value
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	
Sex	Male	3.046±0.1234	0.0573±0.000921	
	Female	3.118±0.1845	0.05692±0.00146	
Residency	Urban	3.066 ± 0.1265	0.057 ± 0.0093	
	Rural	3.093 ± 0.1846	0.058 ± 0.0066	

-The detection of Human IL-10 level in serum

The current study has confirmed high significant difference (P-value<0.0001) in IL-10 levels between patient groups and control group, Mean ± SE (0.1465 ± 0.00916), (0.02318 ± 0.00086) respectively, where the level of IL-10 was increased in patients with hepatitis B virus.

-The correlation between IL-10 level for the studied groups according to age

The current study has revealed a significant difference (P-value <0.05) in the serum level of IL-10 between the age group (20 ≤) and other age groups in sera of individuals while there were no significant differences between the rest age groups (P-value>0.05) compared to control group which there was a significant difference (P-value<0.0001).

Regarding the sex groups, there were no significant differences between Male group and Female group (0.3742) but there was a significant difference compared with control group (P-value<0.0001), as well as for the residency groups of patients (Urban and Rural) there was a significant difference (P-value<0.0001) between them and control group, while there was no significant difference (0.4081) between Rural and Urban patient groups, table (4-2) represents the P-value and Mean ± SE of IL-10 level sera of patients and control groups.

Table (4-2): The Mean ± SE & P-value of IL-10 in studied 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	<0.0002
	Female	0.1429 ± 0.0169	0.02307 ± 0.00116	
Residency	Urban	0.1451 ± 0.0117	0.02337 ± 0.00089	<0.0002
	Rural	0.1497 ± 0.0143	0.0221 ± 0.0029	

-The detection of Human IL-28B level in serum

The current study has shown high significant differences (P-value <0.0001) in IL-28B levels between patients and control groups, Mean \pm SE (0.1506 \pm 0.0089), (1.366 \pm 0.111) respectively, where the level of IL-28B was decreased in patients with hepatitis B virus.

-The correlation between IL-28B level for the studied groups according to age

The current study has revealed a significant difference (P-value <0.05) in the level of IL-28B in sera between the age group (\leq 50) and other age groups, while there were non- significant differences between the rest age groups (P-value>0.05) compared to control group which there was a significant difference (P-value<0.0001).

Regarding groups, there were no significant differences between Male and Female groups (0.3418), while there was a significant difference compared with control group (P-value<0.0001), as well as for the patient residency groups (Urban and Rural) there was a significant difference (P-value<0.0001) between them and control group, while there was no significant difference (0.3800) between Rural and Urban patient groups, table (4-3) represents the P-value and Mean \pm SE of IL-28B level in sera of patients and control groups.

Table (4-3): The Mean \pm SE & P-value of IL-28B in the studied groups

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

Discussion

According to a study, the age group of 25 to 44 years old had the highest incidence of HBV in Europe, followed by that of 15 to 24 years old. In contrast, at the age of 2 years in West Africa, 30% of children had HBV infection and 15% had persistent infection; by the age of 10 years, 90% of children had infection and 20% had become chronic carriers [8]. In another study, 2,460,218 births with maternal HBV status were screened in order to assess the impact of an HBV-prevention program on mother-to-child transmission (MTCT). The yearly rates of HBsAg and HBeAg seropositivity among pregnant Native women aged 15–49 years dropped from 12.2% to 2.6% between 2008 and 2022 [9]. The current study is agreed with a study which has shown that the frequency of IL-10 -producing B and T cells are dramatically increased in

chronic hepatitis B patients [10]. Regarding IL-28B the current study agreed with a study revealing that the levels were significantly lower in chronic hepatitis B [11].

conclusion

The current study has reported a high rate of infection with hepatitis B virus among patients, and there was a significant increase in interleukin 10 in patients with hepatitis B compared to the control group, while there was a significant decrease in interleukin 28B levels in patients with hepatitis compared to the control group.

The infection rate among males was relatively higher than that of females, while the age groups (20 ≤), (21-30), and (41-50) have the highest frequency of HBV infection than the other (31-40) and (50 ≥), there were no significant differences between Urban residents and Rural residents in infection with the HBV.

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