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Detection of the serum levels of some immunological parameters in patients with HBV

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Abstract: *The number of vaccinated people against hepatitis B virus (HBV) infection has increased but the infection remained a major worldwide public health risk that threatens human health and depends on the complex interaction between viral replication and the host immune response the clinical outcome of HBV infection detected. The current study set out to ascertain the serum concentrations of two complement compounds (C3 and C4) and several significant cytokines (IL-1β, IL-12, and IL-17A) in individuals infected with HBV.Ninety patients in all were collected from the Baquba Teaching Hospital during the period 10 July to 20 November, and every diagnosis was made using an ELISA kit. The levels of IL-1β, IL-12, and IL-17A in serum samples from both HBV patients and healthy control groups were measured using an ELISA kit. The patient group exhibited elevated levels of IL-1β, IL-12, and IL-17A in comparison to the control group. A single radial immunodiffusion plate was utilized to compare the patient's levels of C3 and C4 to those of the control group compared to the control group, the IL-1β, IL-12, IL-17A show high levels as (111.2±35.1 vs 99.31±27.01 pg/ml), 78.2±22.3 versus 75.41±19.21 pg/ml), and 61.21±23.21 versus 54.21±21.21 pg/ml), respectively. and the C3 and C4 levels in the patients' serum were lower as 86.21±27.1 versus 112.2±19.21 pg/ml and 22.32±8.21 versus 37.21±11.12 pg/ml, respectively.It has been discovered that measuring the levels of IL-1β, IL-12, IL-17A, C3, and C4 can be useful in determining the extent of liver cell damage and forecasting how long hepatitis will take to proceed.*

.**Keywords: HBV , serum , immunological parameters , ELISA .**

1.Introduction

The majority of instances of viral hepatitis are caused by the hepatotropic, non-cytopathic DNA virus known as the hepatitis B virus (HBV) (12). The innate immune system responds quickly and forcefully, acting as the initial line of defense against HBV insults.

After then, it either directly kills virus-infected hepatocytes or uses non-cytolytic mechanisms mediated by soluble cytokines to initiate the powerful adaptive immune response (4).

One of the main causes of acute, chronic, and occult hepatitis (OBI), the hepatitis B virus (HBV) poses a significant risk to public health. It is well recognized that cytokines are significant chemical mediators that control immune cell development, proliferation, and function. Evidence is mounting indicating the persistence of HBV is caused by insufficient immune responses (7).

Different cytokines play a role in tissue damage and viral clearance during viral infection. Th2 cytokine response results in the establishment of a persistent, lifelong infection, whereas Th1 cytokine profile results in a cell-mediated immunity and is accompanied by remission. The pathophysiology of viral hepatic infections may involve the generation of anti-inflammatory Th2 and pro-inflammatory Th1 cytokines (8).

The human immune system includes the complement system (CS), which is made up of about 30 proteins and is essential for defense against a range of pathogens and illnesses, including viral infections. The complement system is activated through three distinct pathways, namely the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP). Once activated, these pathways combine to form a membrane attack complex (MAC), which damages target cell membranes, ultimately resulting in cell lysis and death (9). Upon activation, all three complement system pathways have the potential to result in viral opsonization and complement system component deposition. The outcome of this response is largely reliant on the infectious agent and can either enhance or reduce viral infection. It can also be dysregulated due to the expression of specific viral proteins (6). In the pathophysiology of acetaminophen-induced hepatotoxicity, the complement system's activation is crucial for the development of injury and hepatic inflammation (7).

2.Methodology

Samples collection

In April 2023, thirty serum samples from healthy control subjects and sixty HBV-positive patients were randomly picked from Baquba Teaching Hospital. Five milliliters of blood were extracted into a vacutainer tube without any anticoagulant. Following clotting, the samples were centrifuged, and the serum-containing supernatants were removed, divided into equal portions, and kept at 70°C. Hepatitis B surface antigen (HBsAg) in human serum was qualitatively detected using a lateral flow chromatographic immunoassay (ELISA) equipment, which allowed for the diagnosis of HBV infection in the patients.

The cytokines IL-1β, IL-12, and IL-17A were measured in the patient samples using the ELISA kit. Diluting coating antibodies for each cytokine was done, and they were then applied to each well of a 96-well ELISA plate and left to overnight at 4°C. Blocking buffer was added to the plates and they were incubated at 37°C for an hour after being cleaned with PBST. After adding serum, biotinylated detector antibody, and streptavidin-HRP polymer (SPP) conjugate, a mixture of cytokine stabilizing buffer (CSB) and serum was added. Each step was then incubated for one to two hours at 37°C. After adding the substrate (TMB solution), the reaction was halted with the addition of 2M H_2SO_4 after 30 minutes. The ELISA reader was used to determine the ODs. Additionally, cytokine standards were made, and the standard curve was used to calculate each cytokine's concentration (pg/mL). The concentration of C3 and C4 in the patient's serum can also be ascertained using the C3 and C4 Kits single Radial Immunodiffusion plate.

Statistical analysis done by Version 20 of the SPSS statistical program.

3.Results and discussion

As seen in (Figure 1), the study comprised 60 patient samples, of which 38 were male and 22 were female. The control group consisted of 30 samples.

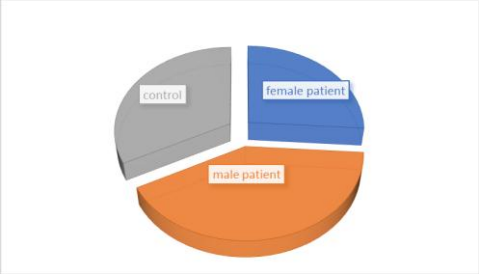

Fig.1 The distribution of the study groups

Table 1 illustrates that when HBV patients were compared to the control group, they had higher levels of IL-1β, IL-12, and IL-17A (126.2±29.01 against 87.1±19.2 pg/ml), (102.5±26.2 versus 78.01±25.4 pg/ml), and (68.3±18.3 versus 42.1±17.3 pg/ml), respectively. The findings demonstrated a significantly significant difference ($p<0.05$) between the patients and the healthy control group.

Table 1:. Serum level mean of IL-1β , IL-12 and IL-17A and healthy control groups.

cytokines		Male	female	p value
		38	22	
IL-1β	mean± SD	111.2±35.1 pg/ml	99.31 ± 27.01 pg/ml	**> 0.05**
IL-12	mean± SD	78.2±22.3 pg/ml	75.41±19.21 pg/ml	**>0.05**
IL-18 A	mean± SD	61.21±23.21 pg/ml	54.21±21.21 pg/ml	**>0.05**

cytokines are essential to the immunopathogenesis of HBV infection and can influence both the infection's natural course and susceptibility to infection (10). cytokines can regulate the host immune response and lessen viral multiplication. One of the potent proinflammatory cytokines, interleukin (IL)-1β is a multifaceted inflammatory cytokine that plays a crucial function in host defense and is a key immunological response regulator (11). A higher level of hepatic necroinflammation may be indicated by a high IL-6 level. As a result, it is a sensitive indicator of the severity and course of the disease (12).

In comparison to female HBV patients, male patients had higher levels of IL-1β, IL-12, and IL-17A (111.2±35.1 vs 99.31±27.01 pg/ml), 78.2±22.3 versus 75.41±19.21 pg/ml), and 61.21±23.21 versus 54.21±21.21 pg/ml), respectively. The findings revealed no significant difference in p-value between the patients and the healthy control group ($p<0.05$). as displayed in table (2).

Table 2:. Relationship between IL-1β , IL-12 and IL-17A and sex in studied groups

cytokines		Patients	Control	p value
IL-1β	N	60	30	**<0.05**
	mean± SD	126.2±29.01 pg/ml	87.1±19.2 pg/ml	
IL-12	N	60	30	**<0.05**
	mean± SD	102.5±26.2 pg/ml	78.01±25.4 pg/ml	
IL-18 A	N	60	30	**<0.05**
	mean± SD	68.3±18.3 pg/ml	42.1±17.3 pg/ml	

Hepatitis B e antigen (HBeAg), a component of HBV, has been shown to regulate a number of mechanisms in chronic HBV infection to limit both the generation and effects of IL-1β. HBeAg attenuates its downstream pathway of NF-κB activation and greatly reduces the LPS-induced NLRP3 inflammasome activation and IL-1β generation (13).

As a result, these could encourage the development and upkeep of chronic infection. On the other hand, LPS-induced NLRP3 inflammasome activation and IL-1β production are enhanced by hepatitis B c antigen (HBcAg) (13). Ultimately, the counterbalance is revealed to be the increased expression of IL-1β in serum, peripheral blood mononuclear cells (PBMCs), and primary human hepatocytes cultured in vitro (14,15). Prior research revealed elevated levels regarding IL-1β in CHB (16).

The highest serum levels of IL-12 were accompanied by HBeAg or HBsAg seroconversion in both AHB and CHB patients (17), indicating that serum levels of IL-12 may be a useful marker to assess cellular immunity for HBV infection. Serum levels of IL-12 are also correlated with alanine aminotransferase (ALT) levels. Increased IL-12 improves the antiviral characteristics of HBV specific T cells, including cytotoxicity, polyfunctionality,

and multispecificity, and restores the antiviral function of worn-out HBV specific CD8+ T cells. Moreover, pro-apoptotic molecule Bim is strongly reduced by IL-12, which may contribute to the early attrition of HBV-specific CD8+ T cells (17).

IL-17A is the representative effector cytokine released by Th17. IL-17F is the sixth member of the six family members (IL-17A to IL-17F) of the important proinflammatory cytokine family (18).Each family member is encoded by a different gene. IL-17A inhibits HBV replication in a noncytopathic way and increases the production of the antiviral proteins oligoadenylate synthetase mRNA and myxovirus resistance A(19).

A positive feedback loop that exacerbates the progression of chronic liver disease associated with HBV infection is formed when HBxAg-activated hepatic stellate cells (HSCs) draw in more Th17 cells to the liver. These Th17 cells may then stimulate the proliferation and fibrotic marker secretion of the HSCs through the action of IL-17A and IL-22(20).

Comparing HBV patients to the control group, the low levels of C3 and C4 were found to be 86.21±27.1 versus 112.2±19.21 pg/ml and 22.32±8.21 versus 37.21±11.12 pg/ml, respectively. The findings demonstrated a significantly significant difference ($p<0.05$) between the patients and the healthy control group. as displayed in table (3).

Table 3:. Levels of C3 and C4 in HBV Patients and the control group

cytokines		Male	female	p value
		38	**22**	
C3	mean± SD	64.21±21.21 pg/ml	59.21±19.01 pg/ml	**>0.05**
C4	mean± SD	25.01±21 pg/ml	17.2±11.1 pg/ml	**<0.05**

Male HBV patients had higher levels of C3 and C4 than female patients did (64.21-22.1.21 versus 59.21-19.01 pg/ml) and (25.11-21-21 versus 17.2±11.1 pg/ml), respectively. The findings demonstrated a significantly significant difference ($p<0.05$) between the patients and the healthy control group. as indicated by table (4).

Table 4:. Relationship between C3 , C4 and sex in studied groups

parameters		Patients level	Control levels	p value
C3	N	60	30	**<0.05**
	mean± SD	86.21±27.1 mg/dl	112.2±19.21 mg/dl	
C4	N	60	30	**<0.05**
	mean± SD	22.32±8.21 mg/dl	37.21±11.12 mg/dl	

The earliest responses to viral hepatitis infection is the complement activation and its level has been shown to be reduced in this diseases (1). The complement system serves as the host's initial line of defense against invading diseases, including viruses (21). Complement components cling to the pathogen's surface, facilitating host cell phagocytosis, membrane attack complex formation, pathogen dissolution, and anaphylatoxin release to induce inflammation and aid in pathogen removal (7,23). Patients with CHB have lower serum levels of complement components C3 and C4 (24).

Conclusion

When compared to healthy individuals, we observed that patients with HBV had greater levels of IL-1β, IL-12, and IL-17A. The results imply that these cytokines could influence the development of chronic HBV infections as well as chronicity, hepatic inflammation, and immunosuppression. Additionally, this study demonstrates that patients' C3 and C4 levels are lower than those of control groups.

Ethics

All subjects involved in this study were informed, and the agreement will be obtained verbally from each one before collecting samples.

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