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Role of HBVs Ag and INF-gamma Levels on the Progression of Chronic Hepatitis B type

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Abstract

Over 350 million people in the world are chronically infected with the hepatitis B virus (HBV), and 35–40% of them will develop progressive liver diseases such as liver cirrhosis and eventually hepatocellular carcinoma. All chronic HBV carriers, especially those without clinical symptoms, are permanent sources of HBV infection and impose an immense threat to society. This study aimed to evaluation the levels of HBs Ag, Anti-HB core Ag and INF-gamma and Studying Role of HBVs Ag and INF-gamma Levels on the Progression of Chronic Hepatitis B and study the correlation between HBsAg and INF-γ in chronic hepatitis B. Serum of patients ,and the results showed there is a highly significant difference(P<0.001) in of HBs Ag, Anti-HB core Ag and INF-gamma and control group and the correlation between IFN-γ and HBsAg show there is a significant negative weak correlation between IFN-γ and HBsAg levels and the correlation coefficient was (r=-345, p=0.006). This can be interpreted as the body responding to the infection by increasing IFN-γ production, which helps reduce the viral load.

KEYWORDS: HBV, HBs Ag, Anti-HB core Ag, IFN-γ, ELISA, Cytokines

Introduction

Hepatitis is defined as the inflammatory reaction of the liver parenchyma. It is either acute, which resolves within six months or may be chronic, in response to viral infections is called viral Hepatitis**.(Uwishema *et al.*,2022).**or non-viral causes such as toxins, medications, autoimmune disorder ,bacteria and parasites can also contribute to hepatitis **.(Centers for Disease Control and Prevention, 2019)** .

Hepatitis B is a major global health problem. The burden of infection is highest in the WHO Western Pacific Region and the WHO African Region, where 97 million and 65 million people, respectively, are chronically infected. Sixty-one million people are infected in the WHO South-East Asia Region, 15 million in the WHO Eastern Mediterranean Region, 11 million in the WHO in the WHO European Region and 5 million in the WHO Region of the Americas **(WHO,2024).**

Hepatitis B virus (HBV) infection is a primary contributor to the development of chronic hepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), making it one of the foremost risk factors associated with these conditions. **(li *et al*., 2019)**. Despite the availability of vaccines and drugs, chronic hepatitis B (CHB) is still an incurable disease. HBV is a small, enveloped, hepatotropic DNA virus belonging to the family Hepadnaviridae **(Ganem and Varmus, 1987)** .The virus is highly contagious and can circulate with 108 to 1010 infectious particles per mL in the blood **(Sauerbrei,2014)** . The genome of HBV is remarkably small, approximately 3.2 kilobases in length, and consists of partially double-stranded DNA. This compact structure accommodates four overlapping open reading frames (ORFs). These ORFs encode several key proteins: the polymerase/reverse transcriptase (RT), the capsid-forming core protein and its related secretory precore protein, three related envelope proteins (S protein) and the regulatory X protein **(Hu and Seeger , 2015).**

Four modes of transmission have been identified: (1) parenteral transmission, such as through transfusion, reused syringes, and contact with blood; (2) sexual transmission; (3)vertical transmission; and (4) horizontal transmission, which includes contact with body fluids like saliva, shared toothbrushes, and through secretory skin lesions (**Lee, 1997; di Filippo and Navas, 2023**).In regions with high endemicity, such as Africa and Southeast Asia, HBV infection is predominantly acquired during the perinatal period or early childhood **(Kurbanov etal.,2010 ; Borgia *et al*., 2012).** Approximately 70–90% of infants born to mothers positive for HBeAg, in the absence of active and passive immunization, become chronically infected (**Zou etal.,2012**; **Borgia *et al.*, 2012**). In 1988, Okamoto suggested that HBV could be divided into 4 genotypes based on genome nucleotide variation that is greater than 8%. Since then, at least 10 genotypes (A–J) have been identified. Genotypes A, B, C, D, and F have been further split into subgenotypes with 4% genome divergence**.** In 1988, Okamoto proposed that HBV could be categorized into 4 genotypes distinguished by nucleotide variations in the genome exceeding 8%. Subsequently, at least 10 genotypes (A–J) have been identified. Genotypes A, B, C, D, and F have been further subdivided into subgenotypes characterized by a genome divergence of at least 4%. **(Okamoto etal.,1988; Sousa *et al*., 2018).** Cytokines are a group of proteins which can mediate cell-cell communication in immune system. Based on the structural biology and associated signaling pathways, cytokines and their receptors can be divided into seven major families (**Floss and Scheller, 2019).**

Interferons (IFNs) are cytokines with antiviral, immunomodulatory, and antitumor properties. IFNs are glycoproteins with a strong antiviral activity that plays an important role in adaptive and innate immune responses. They are classified into three categories (type I, II, and III) based on the structure of their cell-surface receptors. As an effective drug for controlling chronic viral infections, Type I IFNs are approved to be clinically used for the treatment of HBV infection. The therapeutic effect of interferon will be enhanced when combined with other drugs. IFNs play a biological function by inducing the expression of hundreds of IFN-stimulated genes (ISGs) in the host cells. **(Li, *et al*., 2022).**

MATERIALS AND METHODS

Samples collection

This study included the collection of 85 HBV patients and 20 of healthy individuals as a control group for comparison , HBV patients divided to 47 males and 38 females, ranging from (20- 75) year , with median age 41 year. Patients were outpatients attending Al-Karama Teaching Hospital in AL-Kut City/ Wasit Province / Iraq. This work was carried out in the College of Science, Department of Biology, center gene x , PCR unit at AL-Karama Teaching Hospital in Wasit Province/Iraq ,from December 2022 to June 2024.

Five milliliters of peripheral blood were collected from patients. 2.5 milliliters of blood were used to isolate the serum that was frozen at-40Ċ and which was used in the study of proteins .Patients co-infected with hepatitis C and HIV and other autoimmune diseases, as well as the pregnant women were excluded from our study.

Screen of HBV infection by using ELISA kit for HBsAg and Anti-HB core Ag Serum samples were carried out according to the designation (fortess diagnostics,United Kingdom) on the ELISA worksheet .

Evaluation of Human IFN-γ concentration by ELISA kit

Serum samples were carried out according to the designation (YLBiont,china) on the ELISA worksheet . The kit measurement of IFN-GAMMA in human serum、plasma、tissue homogenates and other biological fluids,

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Interferon-gamma (IFN-GAMMA). Add Interferon-gamma(IFN-GAMMA)to the wells, which are pre-coated with Interferon-gamma (IFN-GAMMA) monoclonal antibody and then incubate. After that, add anti IFN-GAMMA antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Interferon-gamma (IFN-GAMMA) are positively correlated.

Statistical analysis

The collected results were analyzed using SPSS (V.20, IBM); independent t-test and One Way ANOVA test were used as appropriate, by calculating least significant difference (LSD) to find p value between the studied groups. The results were presented as Mean±S.E. and the p value ≤0.05 was considered as significant result. Chi square $\varkappa^2$ test was used for obtaining p value between number and percentage. In addition, Pearson Correlation coefficient (r) test was performed for detecting the correlation between the studied parameters.

Results

1.Detection of HBsAg

As shown in table (1), which illustrated the serum level of HBsAg in the studied groups, it was detected that HBs Ag levels significantly increased ($p<0.001$) in the HBV patients as compared to healthy individuals group (control). The mean of HBsAg levels was (0.19±0.03) in control and (8.72±0.31) ng/mL in the patients.

Table (1): ELISA screen test for HBsAg among patients and control

Parameter	Groups	Concentration (Mean±S.E.)	P value
HBsAg (ng/mL)	Control	0.19±0.03	<0.001**
	HBV Patients	8.72±0.31	

** Significant differences

In this study, approximately 98% of the samples from patients with chronic hepatitis were positive for HBsAg, For people with chronic hepatitis B, it is crucial to investigate and measure the amount of HBsAg (hepatitis B surface antigen) in their blood for a number of reasons. First, HBsAg levels are a crucial sign of viral activity. Elevated levels raise the possibility of liver injury since they indicate that the virus is actively reproducing within liver cells. Furthermore, numerous studies have demonstrated a close correlation between raised HBsAg levels and a higher risk of major consequences, including cirrhosis, fibrosis, and hepatocellular carcinoma (HCC). **(Yang *et al*.,2018)** show in his study elevated levels of HBV DNA and HBsAg are associated with increased risks of liver cancer. Chronic HBsAg carriers may be suggested to simultaneously lower the viral load to therefore, measuring these levels allows healthcare professionals to assess the likelihood of these complications occurring.

The findings of Our study were consistent with a previous study conducted in Iraq by Hanash *et al*., 2020 ; Abbood and Al-Mhanah.2019, which reported that all patients tested positive (100%) for HBs Ag. Additionally, a study by Ahmed ,2013 determined that all patient groups, including chronic hepatitis B patients and inactive HBV carrier groups, tested positive for HBsAg. Furthermore, Al-Suraifi *et al*. (2016) documented that all patients tested positive for HBs Ag. However, previous studies conducted by Iraqi researchers (Al-Hmudi, 2011; Salim and Abdullah, 2014) revealed that 44.52% and 49.7% of patients infected with HBV tested positive for HBsAg, respectively. Furthermore, Al-Aboudi and Al-Hmudi (2015) reported that 28.57% of patients tested positive for Ag HBs. therapies, allowing for adjustments as needed (Yuen et al., 2016). (Pateron *et al*., 2017) report that HBsAg levels are crucial for **determining disease stages**, helping classify patients into different categories such as "immune tolerance" or "immune-active". Additionally, (He *et al*., 2012) show that measuring HBsAg plays a vital role in **tracking progress and relapses**, facilitating early intervention in case of disease progression or any relapses. Furthermore, (Terrault et al., 2016 documented that a decline in HBsAg levels can **predict HBsAg loss**, which is a positive indicator of an immune response against the virus. (Boshkoska *et al*., 2020).monitoring HBsAg levels is essential for **assessing treatment needs**, helping to identify patients who require more intensive treatment . Overall, measuring HBsAg is a vital tool for monitoring the health status of patients with chronic hepatitis B and guiding appropriate treatment plans.

2.Anti-HB core Ag

Interestingly, the current study observed that the serum level Anti-HB core Ag was decreased significantly ($p<0.001$) in HBV patients in comparison to control group (Table 2). The mean of Anti-HB core Ag level was (2.81±0.11) in control and (0.11±0.02) in the HBV patients.

Table (2): ELISA screen test for Anti-HBc Ag among patients and control

Parameter	Groups	Concentration (Mean±S.E.)	P value
Anti-HB core Ag	**Control**	2.81±0.11	<0.001**

(ng/mL)	**HBV Patients**	0.11±0.02	

**** Significant differences**

The HBV core antigen is not found as a discrete protein in the serum but it is produced in the hepatocyte cytosol during HBV replication, surrounding the viral genome and the associated polymerase. It is then packaged within an envelope before secretion from the hepatocyte (Hassanein , 2009).According to other results, chronic hepatitis B infection is believed to be common in Iraq and this was coincide with Present results (Abd-Ali et al., 2013). Present results are compatible to several studies about this subject accomplished in Europe and United States (Ganem and Prince, 2004) and in Iraq (Hanash *et al*.,(2020) ; Al-Zubaidi and Al-Rubaye, 2019; Al-Suraifi et al., 2016; Al-Azzawi, 2007) .The development of CHB infection is determined by a complex set of interactions between the host (e.g. age, sex, immune status, and other underlying infections) and the virus (e.g. infective dose, co-infection with other viruses, and viral genotype) (Chang , 2014). Hepatitis B viruses (HBV) are well recognized as the causes of chronic hepatitis, cirrhosis, and HCC (Lu et al., 2012).

3.IFN-γ

The statistical analysis revealed that there is a high significant increasing ($p<0.001$) in the serum level of IFN-γ in HBV patients in comparison to control group (Table 3). The means levels of IFN-γ were (73.04±6.85) and (313.89±29.97) ng/mL in the control and HBV patients respectively.

Table (3): Evaluation of Human IFN-γ concentration in patients and control

Parameter	Groups	Concentration (Mean±S.E.)	P value
IFN-γ (ng/mL)	**Control**	73.04±6.85	<0.001**
	HBV Patients	313.89±29.97	

**** Significant differences**

The results of study agreed with study of **Hillaire, *et al*.,(2023**) in his study will focus on the crucial role of IFN-γ in inhibiting HBV infection. Interferon's (IFNs) are a group of cytokines that are secreted by host cells with the reaction to viral or bacterial infection. IFN-γ has several functions with the potential to augment Presentation of antigens on major histocompatibility complex (MHC) molecules, activation of immune cells, induction of apoptosis, Cellular proliferation. The pharmacological mechanism of IFNs is associated with their capacity to selectively attach to particular targets. Receptors, present in both infected and uninfected cells, serve to trigger the transcription of IFN-stimulated genes (ISGs).

Also Rizvi *et al*.,(2012) also agree with result by showed high serum levels of IFN-γ were significantly elevated in patients of HBV compared to controls ($p < 0.05$).Also Wang *et al* .,(2020) HBV specific IFN-γ

producing T cells are associated with viral clearance Also Xiong *et al*.,(2007) explain in his results production of IFN-γ (interferon-gamma) in samples from healthy individuals and carriers of Hepatitis B Virus (HBV).

The Correlation between IFN-γ and HBsAg

The correlation between the studied parameters was analyzed to detect the relationship between the studied parameters. As shown in table (4), it was noticed that there is a significant negative weak correlation between IFN-γ and HBsAg levels and the correlation coefficient was (r=-345, p=0.006).

Table (4): Correlation between IFN-γ and HBsAg

Parameter		**IFN-γ**
HBsAg	**Pearson Correlation (r)**	-0.345**
	Sig. (2-tailed)	0.006

**** Significant differences**

The inverse relationship between **IFN-γ** and **HBsAg** is attributed to the role of **IFN-γ** in activating the immune response, which helps reduce the spread of the virus and eliminates infected cells, thereby decreasing **HBsAg** levels in the body **The result of study show** significant negative weak correlation between IFN-γ and HBVsAg levels the result agree with **Wang, et al., 2019 who show in his study** negative correlation between S-specific IFN-γ producing cells and HBsAg level. Patients with HBeAg/HBsAg loss after flare showed higher frequency and dominance of HBV-specific IFN-γ producing CD4 T cells, compared to patients without HBeAg/HBsAg loss. Both the frequency and the dominance of HBV S-specific IFN-γ producing CD4 T cells were positively correlated with the decrease of HBsAg during flare. A differentiation process from TNF-α producing cell to IFN-γ producing cell in HBV-specific CD4 T cells was observed during flare.

Conclusion

Findings of the current study showed that IFN-γ were different significant increasing ($p<0.001$) in the serum level of in HBV patients in comparison to control group and IFN-γ played a Crucial rule in the Fight Against HBV Infection

Studying the correlation between HBsAg and INF-γ in chronic hepatitis B. cases can provide several valuable insights. it can help determine how the immune system responds in chronic cases, shedding light on factors that may lead to response failure. Additionally, the study may identify different immune patterns among patients, which could assist in recognizing subgroups that might benefit from specific treatments

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