Original Article

Serological Detection of Hepatitis B Virus e Antigen and TNF-α in a Dialysis Patient

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Abstract

Background: The hepatitis B virus (HBV) is responsible for all forms of hepatitis (occult HBV infection [OBI]) endangering the health of the public. The growth, division, and activity of immune cells are governed by chemical mediators called cytokines. Evidence suggests that inadequate immune responses contribute to the persistence of HBV. **Objectives:** The goal of this study was to determine the prevalence of hepatitis B e antigen (HBeAg) among HBV surface antigen-positive (HBsAg+) persons by analyzing the association between age and gender and the severity of HBV infection. **Materials and Methods:** Seventy-two individuals from August 2022 to November 2022 were hired: a total of 35 healthy participants and 37 persons with acute or chronic HBV infection. Healthy controls and research participants ranged from 20 to 80 years old, and all of them were analyzed using serum samples (3 mL). The levels of HBV, tumor necrosis factor-alpha (TNF-α), and HBeAg in the blood were determined using enzyme-linked immunosorbent assay (ELISA). **Results:** 37 affirmatives out of 72 Using a double-antibody sandwich ELISA, we determined that our HBV participants met the inclusion criteria. The findings of the HBsAg ELISA Kit indicated that the prevalence of HBsAg was greatest in those 35–49 years old (32.5%) and lowest in those 20–34 years old (21.6%) and 50–64 years old (21.6%). The HBsAg ELISA Kit result showed that the 37 patients who tested positive for HBsAg, 22 were female (59.5%), and 15 were male (40.5%). This suggests that the prevalence of HBsAg infection is higher in females than in males. Dialysis patients have been shown to have increased levels of HBeAg and TNF-α. **Conclusions:** Patients in Babylon province with chronic HBV had significantly higher than average levels of HBeAg and TNF.

Keywords: HBeAg, HBsAg, TNF-α

Introduction

Hepatitis B virus (HBV) infection is a global public health problem. The frequency distribution of HBV genotypes in Iraqi patients was recorded by several authors.[1] The prevalence of this infection has increased among many patients with other diseases.[2-5] Hepatitis B "e" antigen (HBeAg) is a highly conserved protein found in all mammalian orthohepadnavirius that is essential for viral replication. It is created through the posttranslational modification of the precore protein.[6,7] While its precise function in HBV transmission, replication, assembly, and packing is unclear, it is important to highlight that it is not essential for these processes at present. In HBeAg-positive individuals, liver disease has been seen to span a spectrum from complete absence to cirrhosis and hepatocellular cancer (HCC). Consequently, infected persons who tested positive for HBeAg may continue for long periods without experiencing symptoms (with normal serum aminotransferase activity). HBeAg-positive chronic infection is mainly seen in young patients with chronic hepatitis B (CHB) and may last for decades. It is typically a highly-replicative disease state characterized by minimal liver inflammation and normal or near normal histology. On the contrary, HBeAg-positive chronic hepatitis is associated with the presence of necroinflammation and accelerated progression of fibrosis.[8] Those who contract the infection later in life are less likely to exhibit the "immune tolerant" HBeAg+ phenotype, which is

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characterized by high HBV-DNA levels in the blood and normal alanine transaminase (ALT) levels.[9] Previous research has shown that one's HBeAg production rate, and not only HBeAg status, is connected with certain biological traits and clinical symptoms.[7] Production of cytokines is crucial during viral infections because they are fundamental to effective immune responses.[9]

The immune systems of patients on maintenance invasive hemodialysis (HD) therapy for chronic renal failure are negatively impacted by this treatment. HD patients' cellular immunity is lowered as a direct result of the therapy. Normal physiological processes, including the activities of leukocytes, neutrophils, monocytes, and natural killer cells, are altered in these individuals.[10] T-lymphocyte phosphorylation pathways are downregulated and impaired proliferation occurs as a result of HD treatment.[11] HD patients are more likely to get blood-borne viruses such as HBV, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) than healthy individuals.[12] Hepatic inflammation may be triggered by these viruses either on their own or in combination with other infections, which can then progress to cirrhosis and ultimately HCC.[13] Dialysis facilities in different countries have varying rates of HBV infection. The average is 1.6% in the developed world and 1.3% in the developing world. It has been noted that chronic HD patients are prone to viral infections, namely HBV, HCV, and HIV. Its incidence varies considerably across regions and HD facilities.

Both the development of autoimmune diseases and the host immunological responses to HBV infection depend primarily on the cytokine tumor necrosis factor (TNF)-. Macrophages and T cells generate TNF-α, which limits viral replication by promoting the production of multiple inflammatory cytokines.[14] The proliferation of HBV-specific cytotoxic T cell (Tc) cells, which are crucial for inhibiting HBV replication, needs TNF-α.[15] Therefore, TNF inhibitors (such as infliximab, adalimumab, and etanercept) may depress the anti-HBV immune response, resulting in HBV replication.[16] Individuals with autoimmune diseases who were treated with TNF-α inhibitors had a pooled prevalence of HBVr of 4.2% (95% confidence interval: 1.4%–8.2%).[17]

Renal hemodynamics and nephron transport are altered by TNF, which affects transporter activity and expression. It also plays a mediating role in organ injury by promoting the infiltration and death of immune cells. TNF-alpha (TNF-α) is a pleiotropic cytokine that mediates both rises and drops in blood pressure and is upregulated in chronic inflammatory conditions including hypertension and diabetes. Yet, modest elevations in TNF have been linked to increased NaCl retention and hypertension, whereas high amounts of TNF reduce blood pressure. Different levels of TNF inside the kidney, the subject's physiological condition, or the sort of stimuli inducing the inflammatory response may all play a role in explaining the varying effects seen. Both the activity and expression of transporters are modified by TNF, leading to changes in renal hemodynamics and nephron transport. It also promotes immune cell infiltration and cell death, which contributes to mediating organ damage. We will discuss the existing research and try to explain the disparities we found in the following.[18]

Materials and Methods

The Thi Qar Health Department of the Ministry of Health gave its consent to the research using a tailored approval template. Before beginning sampling, patients gave their permission after being explained the study's goals. A total of 37 people had either a recent or long-term HBV infection.

The sample was collected at Al-Hussein Teaching Hospital, Infectious Diseases Department, the Main Blood Bank of Thi Qar province, and Al-Mousawi Private Laboratory in AL Nasiriya City.

The sample was collected between August 2022 and November 2022.

The sample type was as follows: 37 serum samples from study participants were frozen at −20°C to prevent repeated thawing and freezing.

During examination, an anti-HBV enzyme-linked immunosorbent assay (ELISA) kit was used to determine the concentration of the antibody in the plasma sample. The assay was performed as directed by the manufacturer, and the microplates were read at 450 nm using an ELISA reader (human). The absorbance of the occulter sample might be compared to the cutoff value to identify the presence or absence of HBV surface antigen (HBsAg). The HBeAg ELISA kit was used to extract HBeAg HBV-DNA from 3 mL of serum following the manufacturer's instructions from Sunlong Biotech Co. Ltd. (Hangzhou City, Zhejiang Province, China). For this purpose, we employed SPSS Statistics (Chicago, Illinois, USA). The analysis of variance test was used to analyze the demographics of the HBeAg-seropositive population, and significant differences in prevalence across sexes and throughout the age spectrum were defined as a *P* value of 0.05 or below.

Ethical approval

The Declaration of Helsinki was strictly adhered to ensure that the participants were not put in any unnecessary danger. Patient verbal and analytical permission was sought before the sample collection. The study methodology, subject information, and consent form were reviewed and authorized by a local ethics committee, as shown by document BMS 510/16 (containing the number and the date on June 27, 2022).

Table 1: Distribution of dialysis HBV patients (males and females) by age

Age Stratum	Years	HBV	*P* value
		Number (%)	
	20–34	8 (21.6)	<0.05
	35–49	12 (32.5)	
	50–64	8 (21.6)	
	≥64	9 (24.3)	
Total		37 (100)	

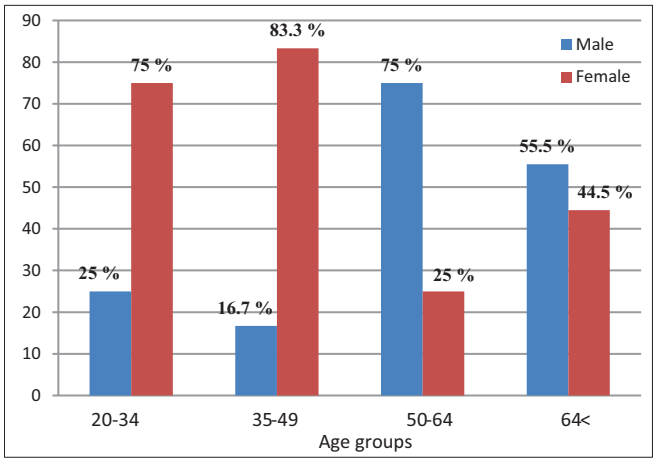

Figure 1: Hepatitis B virus patient demographics by age and gender, by males and females

Results

This cross-sectional study included 37 persons who tested positive for HBsAg and ranged from 20 to 80 years old. A total of 37 of the 72 individuals who had their HBV e antigen status examined had positive results. Females made up more of the sample (n = 22) than males (n = 15) [Table 1].

It was discovered which age groups were the least and most influenced by the seropositivity rate. The following tables show the total number of people in each age bracket. Table 1 displays the prevalence of seropositivity by age group: 21.6% in those 20–34 years old, 32.5% in those 35–49 years old, 21.6% in those 50–64 years old, and 24.3% in those 65 and beyond. To further clarify this, the prevalence might be provided as a percentage of total tests [Figure 1].

The age range of 34–64 years was the one with the highest prevalence of HBeAg seropositivity [Figure 1].

Discussion

HBV is divided into eight genotypes (labeled A–H) based on nucleotide sequence differences of 8% or greater; these genotypes are found all over the world.[19,20] Nigeria is one of several West African countries where the E genotype predominates.[21] Similarly to how clinical prognosis and

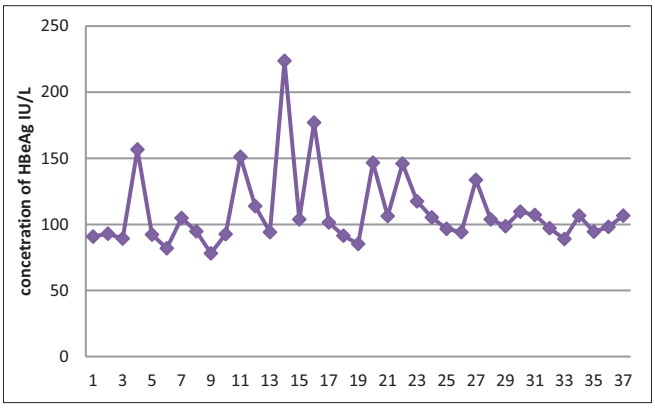

Figure 2: Hepatitis B virus infection is associated with increased HBeAg expression

treatment response have been linked to different genotypes in various demographic groups, HBeAg expression may vary depending on genotype.[22] Because the presence of HBeAg in the serum of HBV patients indicates that the virus is actively replicating in hepatocytes, it is regarded as a surrogate marker for the presence of HBV DNA.[23] Those who are at high risk of developing liver cancer may benefit from HBeAg as well.[23] Results showed that among HBsAg-positive persons, the frequency of HBeAg in the province of Thi Qar was 51.38%. This demonstrates a highly infectious population in Thi Qar that promotes viral transmission and evolution, portending a future increase in the prevalence of liver cancer caused by HBV [Figure 2].

Among 763 HBeAg+ individuals (56% female), of all ages and HBV genotypes, qHBeAg levels were consistently higher in women.[7] High viral replication and infectiousness are linked to e antigen positivity (HBeAg+) in CHB virus infection. In addition, HBeAg-positive CHB is linked to a broad range of liver disease severity.[7]

We found a high incidence of HBV in HD patients on dialysis for more than 5 years, and we found a statistically significant positive connection between HBV positivity and dialysis duration, consistent with a previous study from Karachi, Pakistan. Our results are concerning when compared to those of other research done in Pakistan along these lines. Research in Karachi[24] indicated that 10% of HD patients were HBV positive; research in Islamabad[25] found that 12% of HD patients were HBV positive; and research in Quetta[26] found that 16% of HD patients were HBV positive. Many other investigations revealed the same thing we did: that HD patients with longer dialysis periods had a greater prevalence of HBV positivity. The World Health Organization classifies regions with a carrier prevalence of roughly 5% for HBV as being highly endemic.[27,28] Nonetheless, between 4% and 6% of Pakistan's population is infected with hepatitis B.[28] There is a large number of delta-positive people in numerous areas in Sind, Punjab, and Baluchistan,[29-31] and

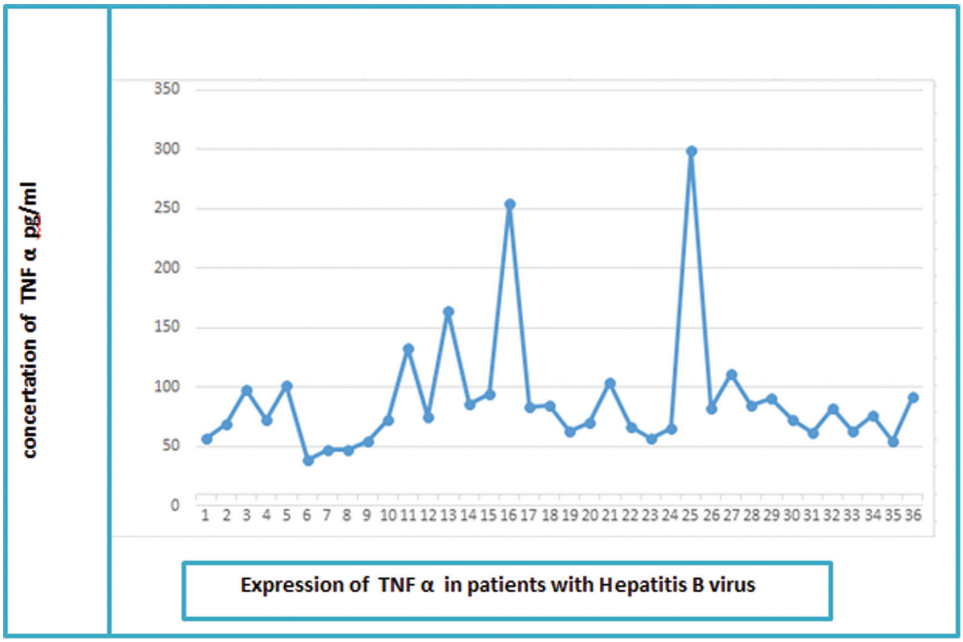

Figure 3: TNF levels in hepatitis B virus patients

Table 2: The correlation between HBV and TNF α according to ANOVA

TNF	Sum of squares	df	Mean square	F	Sig.
Between groups	60,233.079	2	30,116.539	16.611	0.000
Within groups	97,905.571	54	1813.066		
Total	158,138.650	56			

ANOVA: analysis of variance, df: degree of freedom, F-value = variation between sample means/variation within the samples, Sig = significant differences among group means are calculated using the F statistic, which is the ratio of the mean sum of squares (the variance left over)

Table 3: According to studies, there were notable distinctions between HBV and TNF

TNF	N	Mean	Std. deviation	Std. error	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
Male	15	85.8519	55.05118	14.21415	55.3655	116.3382	38.52	254.81
Female	22	92.5421	50.21130	10.70508	70.2796	114.8045	54.07	298.89
Control	20	21.9415	11.54407	2.58133	16.5387	27.3443	7.32	41.07
Total	57	66.0094	53.14042	7.03862	51.9093	80.1094	7.32	298.89

Expanded Programme on Immunization (EPI) coverage for hepatitis B immunization is low, so the spread of the virus is expected. Considering the already high prevalence of HBV in the general population and, in particular, immune-compromised chronic renal disease patients, it is probable that the high frequency of HBV in HD patients is linked to this. The widespread reusing of previously used dialysis machines and the accompanying disregard for established operating procedures guiding the preservation of clean equipment are also key contributors. TNF-α is expected to suppress HBV replication by stimulating cytotoxic T cells. By blocking these cytokine-producing cells, this explains the increase in TNF with HBV in Figure 3.[32] Table 2 shows degree of freedom between groups and within groups where the results were degree of freedom (df) 2 between group and 54 within groups. In the current study, serum TNF levels were found to be significantly (P = 0.05) higher in the HBV patient group compared with the control group [Table 3].

TNF levels in hepatitis B virus patients

TNF is a pro-inflammatory cytokine that is secreted in response to pathogenic infections. TNF-converting enzyme separates soluble TNF from transmembrane TNF (tumor necrosis factor-alpha converting enzyme [TACE]). TNF exerts its functions through the activation of two distinct receptors (TNF receptor 1 [TNFR1] and TNF receptor 2 [TNF2].[33-36] Because of TNF-α's widespread role as a pro-inflammatory agent, biologics that inhibit TNF-α and associated cytokine pathways

have been used to treat a variety of inflammatory and autoimmune diseases.[37-39] The fact that TNF-α is used to treat a wide range of illnesses demonstrates its importance in HBV reactivation. It was previously thought that these cytokines had a minor impact on the adaptive immune system, which was in charge of the HBV immunological response. Few studies have looked at the cytokine production profile in occult HBV infection (OBI) patients, and the exact mechanism causing liver damage is still unknown. Other research has suggested that hepatocyte damage may be caused by the production of cytokines such as TNF-α and INF in response to HBV covalently closed circular DNA persistence and transcription in hepatocytes.[40,41] TNF-α is required for the development of an effective antiviral immune response.[42,43] TNF-α is a pleiotropic multifunctional cytokine that contributes to the maturation of the body's defensive systems by increasing Th-1 cellular immune response, phagocytic cell activity, and cytotoxic cell production, in addition to its role in the regulation of immune-inflammatory processes. In the current study, serum TNF levels were found to be significantly (P = 0.05) higher in the HBV patient group compared with the control group (1–3). These findings are consistent with those of other authors, and it is concluded that monitoring TNF levels is indicative of liver damage even when liver enzyme levels are normal.[5,44] TNF-α levels in the blood are significantly higher in people with any degree of liver inflammation, suggesting that it could be used as a predictor of liver inflammation.[45]

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Conflicts of interest
There are no conflicts of interest.

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