

Frequency of hepatitis B virus infection with comparison of diagnostic markers: A retrospective study

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Abstract

Hepatitis B Virus is a common infectious disease that represent a major health problem worldwide. It is the most common cause of liver failure, liver cirrhosis, as well as hepatocellular carcinoma and transmitted by dental treatment, surgery, injecting drug users, piercing and being with many sex partners. To understand the frequency of HBV infection with comparison of diagnostic markers, we performed a retrospective study from January 2022 to December 2023 in the population of Punjab, Pakistan. The present study was carried out for the sensitivity and specificity of viral biomarker for HBV surface Ag (HBsAg) and HBV enveloped Ag (HBeAg). Sensitivity and specificity of immunoassays was evaluated in samples of HBV patients. Serum levels of liver enzyme were analyzed. Statistical analysis was performed using SPSS version 21. The precision of the Rapid immune-chromatography test were good and sensitivity as well as specificity for the diagnosis of HBsAg were 97.9% and 100% respectively. Out of 190 patients, 100% were tested positive for HBsAg and 91.9% for HBeAg. HBsAg seropositive patients were 48.8% and 48.96% HBV patients shows elevated levels of both ALT and AST respectively, 10%

HBV patients showed elevated level of bilirubin, 33% HBV patients showed elevated level of ALP and 21.04% HBV patients whose creatinine level significantly higher, which is between 1.1 to 1.3 mg/dL. This study revealed that there is an association between ICT results and urine color and indicates a correlation between HBsAg and HBeAg. The information accumulated can provide a better understanding to design appropriate and sensitive diagnostic approaches for treating these patients.

Keywords: HBV surface Ag (HBsAg), HBV enveloped Ag (HBeAg), Hepatitis B Virus, Hepatocellular carcinoma (HCC), biomarkers, early detection

Introduction

Hepatitis B Virus is a common infectious disease that represent a crucial health problem worldwide with substantial mortality and morbidity[1]. Approximately around 257 million individuals are infected with chronic HBV (hepatitis B virus) at globally and more than 887,000 yearly deaths are the consequence of HBV associated outcomes for instance liver cirrhosis and hepatocellular carcinoma[2]. Liver cancer classified as the fourth most lethal type of cancer[3], with an average survival period minimum eleven months[4]. In 2016 Hepatitis B surface antigen was calculated to be approximately 3.9% positive at globally[5]. There are broad regional variability in the frequency of Hepatitis B Virus infection, with rates higher than 8% observed in Asia, regions of Africa and Western Pacific; intermediate regions rates (2-7%) are eastern and southern Europe, whereas in western Europe less than 2% rates are observed, USA and the Australia[6]. It's have been seen that, in sub-Saharan Africa about 70-95% of participants have minimum one marker of HBV infection[7].

In progressed countries prevalence of Hepatitis B Virus infection is high in particular groups, for example immigrants from prevalent areas, dental treatment, man who have masculinity with man, surgery, injecting drug users, piercing and being with many sex partners. In Saharan Africa and China, Hepatocellular Carcinoma correlated with Hepatitis B Virus is one and only preceding

reason of HCC in men.[8, 9]. Nonetheless, currently, migration and population movements are altering the frequency of prevalence in low endemic European countries such as., Italy and Germany, due to the higher prevalence rates of HBsAg in refugees and immigrants from outsider as compared with the native population [10]. In high-risk populations it has not been possible to prevent critical Hepatitis B Virus infection significantly, despite the implementation of universal vaccination strategies[11].

Chronic hepatitis B (CHB) virus infection frequently leads to serious problems and death after era[12] thus, the assessment of therapeutic treatments is constructed on surrogate markers, for example, hepatitis B enveloped antigen (HBeAg) and histology seroconversion[13]. While the therapy of chronic Hepatitis B Virus infection has progressive in the past era throughout enhancement of nucleoside/nucleotide correspondents and immune modulators, there is a considerable need for advancing therapeutic response.

Now, complexes under progress are estimated with liver histology as crucial end point for efficiency. Hepatic histology is vital for evaluation of the etiology and production of liver infections[14]. Though successive liver surgeries are not involvement of the typical management of chronically Hepatitis B Virus-affected patients as they are aggressive, are related with a certain level of difficulties, and could not be executed more commonly than yearly[15].Therefore, there is a necessity for alternative markers that are existing in clinical training and appropriately reliable to evaluate the efficiency of therapeutic involvement over short stages of time.

Systematized inspection would be help not only influence the particular feature of infected participants but also in quantifying the infection load on a population levels, and thus inhibit further transference and allocating most suitable treatment. This is especially important for crowded countries that had been formerly characterized as highly regional for CHB infection such as Indonesia , China, Nigeria and parts of Asia and Africa, where an massive numbers of people alive with the virus[16]. In this research we find out the rate of HBV in Pakistan population to explain the patterns of biomarkers of the HBV diagnosis.

Methodology

This is a retrospective study conducted from January 2022 to December 2023 at the University of Gujrat, Department of Biochemistry and Molecular Biology, Punjab, Pakistan. This study was designed to find out frequency of HBV infection along with comparison of diagnostic markers in Punjab, Pakistan and to investigate the clinical risk factors related to HBV including occupation, age, sex, blood transfusion. For this study, the patients were selected who came directly to Aziz Bhatti Shaheed Hospital, Gujrat, pathology lab UOG and Divisional Headquarters hospitals in Punjab Pakistan. Information was collected from the 192 patients. This study included sample collection, demographic risk factor data collection, liver function test and ELISA.

This study consisted of three parts. First part was collection of fresh serum samples of Hepatitis B positive patients to perform ALT, AST, total bilirubin, and alkaline phosphatase. Second part was collection of demographically data. Third part was urine collection for the urine analysis.

Immuno-chromatography (ICT) and Strip test were carried out for the screening of HBV confirmation. VIKIA® manufactured by bioMérieux South America, CE-marked regulatory version was used for this analysis. Biochemical Test LFT, ALT, AST Test were performed for analysis of liver function and chemistry analyzer by SELECTRA was used to perform this analysis. Serological analysis After the confirmation, the samples with positive results were analyzed by ELISA test for HBeAg VPK-5003 Hepatitis B “e” Antigen ELISA Kit USA was used., HBsAg DS-EIA-HBsAg-0.01, USA was one-step “sandwich” analyze based on a shortest, noneconomic solid-phase enzyme immunoassay with antibodies marker.

Statistical analysis by using SPSS version 21, statistical analysis was performed. Pearson Chi-square was used to find the significance association between different parameters. Pearson correlation was used to find out the correlation between HBsAg and HBeAg. Mann-Whitney test was used for comparison of sensitivity and specificity of HBsAg and HBeAg. If *P-Value <0.01 is considered significant statistically. If *P-Value >0.01 is considered non-significant statistically

Results

Out of 192 patients the peak of the HBV positive patients was noticed at the age between 9 to 25, which was 52.5%. While the lower peak of HBV positive patients were observed at the age 25 to 45 which was 14.8%. Whereas the number of the patients with Hepatitis B positive at the age 45 to 60 was 32.5%. the number of the male with Hepatitis B were 53% whereas the number of female patients were 47%. the number of patients with Hepatitis B were significantly higher in rural area which were 65% as compared to urban area which were 35%. the higher prevalence of HBV in labor which is 50%; predominantly were large vehicle drivers (30%). While the number of Hepatitis B was lower in students group which is 15%. Whereas the number of housewives with Hepatitis B was 35%. In house wives 10% were pregnant women. HBV patients who did not take injections frequently was 91% as compared to those who received injections frequently was 9%.

Only 15% HBV patients were blood donors whereas the number of hepatitis B patients who were no blood donors are 80%. Therefore, these results show blood donation have association with transmission of HBV infection. The number of blood transfusion was 22%, while the number of those who did not transfused blood was 78%. The consequence of this research shows the number of patients whose partner were not suffering from HBV infection is 85%. The patients who suffered from Hepatitis B virus did not undergo dental treatment were 75%. Whereas the number of hepatitis B virus patients who undergo dental treatment were 25% while 53% HBV positive patients had not undergone surgery, the number of those Hepatitis B virus patients who had undergone surgery were 47% as 10% of HBV patients were suffering from jaundice. 48% HBV patients have piercing 10% HBV patients have their bodies piercing.

while those HBV patients who did not have piercing and tattooed were 52% and 90% respectively, 46% HBV patients whose pH was acidic while 17% HBV patients pH was alkaline Whereas the number of patients whose pH were neutral 37%. HBsAg seropositive, 48.8% and

48.96% HBV patient's shows elevated levels of both ALT and AST respectively, 10% HBV patients shows elevated level of bilirubin, 33% HBV patients shows elevated level of ALP, 21.04% HBV patients whose creatinine level significantly higher, which was 1.1-1.3 mg/dL While 51.5% HBV patients whose creatinine level is 0.8-1 mg/dL whereas 27.08% HBV patients whose creatinine level was 0.61-0.8 mg/dL. sensitivity and specificity of HBsAg and HBeAg were not same. Sensitivity of HBsAg was higher which was 100% as compared to sensitivity of HBeAg which was 91.9%. Whereas specificity of HBeAg was high which was 94.791 as compared to HBsAg, which was 90.625%.

Rapid ICT test appropriately identified 190 of the 192 HBsAg-positive samples. Thus, the rapid test has good sensitivity (97.9%) and specificity (100%) for the diagnosis of HBsAg. (Table 1)

Table 1. Sensitivity and Specificity of HBsAg

ICT	HBsAg		HBsAg	
	Positive	Negative	Sensitivity	Specificity
	190	2	97.9%	100%

By the comparison of sensitivity and specificity among HBsAg, HBeAg, through urine it shows the significance value for HBsAg was 0.968, HBeAg was 0.853 which was greater than 0.05 value so its showed sensitivity and specificity of HBsAg, HBeAg and urine analysis were not same. (Table 2)

Table 2. Comparison of Sensitivity and Specificity Among HBV Marker

	HBsAg	HBeAg
Mann-Whitney U	812.000	795.500
Asymp. Sig. (2-tailed)	0.968	0.853

*P-value >0.05 is not considered statistically significant.

While checking the association of immune-chromatography test and urine color, our level of significance value was 0.012 which was less than 0.05, it showed there was association between ICT results and urine. (Table 3)

Table 3. ICT Associated with Urine Color

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.308 ^a	1	0.012

*P-Value<0.05 is considered significant statistically

The current results of table 4 shows that HBsAg and HBeAg have perfectly positive correlation. Co-efficient is 0.571, which shows linear positive moderate correlation between HBsAg and HBeAg. Our level of significance was (P = 0.000) which was less than significance value 0.01(P = 0.01) it shows there was enough evidence to say correlation exists between HBsAg and HBeAg. (Table 4)

Table 4. HBsAg Correlated with HBeAg

Correlations			
		HBsAg	HBeAg
HBsAg	Pearson Correlation	1	0.571**
	Sig. (2-tailed)		0.000
HBeAg	Pearson Correlation	0.571**	1
	Sig. (2-tailed)	0.000	

** *Correlation is significant at the 0.01 level (2-tailed)*

*P-Value <0.01 is considered significant statistically.

Discussion

The aims of this research was to evaluate the epidemiology, clinical demonstration, treatment, and prevention of Hepatitis B Virus associated risk factors. Hepatitis B virus infections are prevalent from one population to another population in a same country or from one region to another region in different parts of the world[17]. Notably, the number of the male with Hepatitis B virus were 53% whereas the number of female patients were 47%. likewise, the study of [18, 19]. In our study the individuals age 9 to 25 showed the higher prevalence of HBV infection. While other study results were little varied. But the age group 20 was remained dominant in terms of prevalence in the study of United State [20], this difference can be due to different geographical places. In this study, rural area of residence, labor occupation, injection frequency, surgeries, and infection in partner act as risk factors for HBV. Whereas very low percentage of HBV patients with tattoo, piercing and blood donor, blood transfusion was observed. Similarly,

the study of Oman showed [21] these were the associated risk factors for the prevalence of HBV infection. Our significant finding was the frequency of HBV infection through piercing was very low. It might be due to Islamic region and secondly there were no trend of tattooing to be observed in Punjab, Pakistan population. These risk factors can be avoided to reduce the incident rate of HBV. Healthy individuals should try to avoid contaminated needles, blood-related product, and treatment from quack.

This study revealed that hepatic enzyme shows elevated level of ALT, AST, ALP, bilirubin, and creatinine in HBV positive patients. This elevated level of hepatic enzyme act as a biomarker for the diagnosis and prognosis of HBV at an early stage. The previous studies also showed liver enzyme tests, ALT, AST, ALP and LFT that include bilirubin were being used as diagnostic biomarkers for the evaluation of HBV infection[22]. Moreover, this study also showed the presence of HBsAg and HBeAg after HBV infection act as a diagnostic marker for the detection of HBV. However, the sensitivity of HBsAg and HBeAg was not same but showed the significant correlation between HBsAg and HBeAg. Previous study also revealed Antibody of HBsAg is the only marker which is present after vaccination and remains positive in both chronic and acute hepatitis B virus infection [23]. Similarly, antibody of HBeAg may persist years to decade after the resolution of HBV infection[24]. The information accumulated by this study will help provide a better understanding to design appropriate diagnostic approaches for treating these patients, so they can recover and lead normal lives.

Funding statement

This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

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