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Research article

Hepatitis B virus (HBV) a Severe Health Problem in Mardan, Khyber Pakhtunkhwa: A Molecular Based Study

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Abstract

Hepatitis B is a severe health issue in both developed and developing countries that infects approximately 3.5 billion individuals globally. HBV infection is a more contagious disease than HCV and HIV infection. The current research work was conducted at D.H.Q hospital, Mardan Khyber Pakhtunkhwa (KPK). Only HBsAg positive patients (n=750) were selected and for further confirmation of HBV DNA in all patients, Real Time PCR was performed. Out of a total of 750 patients, 34.6 % (n=260) patients were found to be HBV positive using Real Time PCR method. Prevalence of HBV DNA was high in male patients (p= 0.0151) as compared to female patients and also in patients of older age (>60 years). All patients were divided into three groups on the basis of viral load, that is, low viral load 100-10000 IU/ml, intermediate viral load 100,000-10,000,000 IU/ml, and high viral load >10,000,000 IU/ml. The study showed that 61.15% patients were placed in intermediate viral load category. All the HBV DNA positive patients were also screened for HBeAg and 31.53% were found reactive for HBeAg. This study provides a clear spectrum of molecular epidemiology of HBV in the Mardan region of KPK, Pakistan.

Keywords: GCMBDR, HBsAg, HBeAg, KPK

1. Introduction

Hepatitis B infection is a blood borne disease that is caused by partially double-stranded circular DNA virus (1) of the family hepadnaviridae (2). HBV has been reported to cause liver complications. Around 600,000 HBV related deaths occur globally (3), most of them are the result of the chronicity of HBV infection. Approximately 350 million people around the world are chronic carriers of Hepatitis B virus (HBV) (4), while 2 billion are infected with this virus (5-7). Complications like hepatocellular carcinoma, cirrhosis or chronic hepatitis cause deaths every year. Prevalence of HBV is less in developed countries like Australia, Eastern Europe and America, while it is widespread in Africa, Pacific Islands and Asia (8). It has been reported that more than 40 million people in Pakistan are infected with HBV and 12 million more are positive for hepatitis C virus (HCV).

Mucosal or percutaneous exposure to HBV, contaminated blood and/or body fluids can transmit HBV (9). It can be transmitted through body fluids such as saliva, semen, serum or blood. It can even survive in dried blood on razors, syringes, needles or on table surface (10). Common ways of transmitting HBV include blood transfusion (11), mother to child transmission, tattooing (12, 13), use of unsterilized surgical and dental instruments, reuse of disposable syringes and shaving blade by barber, reuse of needles for ear and nose piercing, sharing nail cutters, razors or

toothbrushes belonging to infected people, sharing needles with drug addicts and unsafe sexual practices (14, 15).

Patterns of HBV vary worldwide. Areas that are known to be highly endemic for HBV infection harbor almost 45% of world population. In such areas, people acquire it during early childhood. About 43% population of the world lives in intermediate endemicity regions where multiple modes of HBV transmission described previously have been observed. In low endemicity regions, HBV infection has been observed in adolescents and adults most likely due to unsafe sex, injecting drugs or blood transfusion. HBV infection is highly endemic and remains a crucial health problem in Pakistan. Almost 9 million people infected with HBV (15, 16) while 3% are its chronic carriers (17, 18). National estimates related to HBV prevalence in Pakistan are not known but data from different selected groups or from different regions have been reported previously. Available data shows its prevalence as 32% anti HBV surface antibodies through natural conservation and 4% carrier rate with a total 38% prevalence (18). HBV assessed by Hepatitis B Surface Antigen (HBsAg) has been observed in 2%-14% blood donors (19, 20) and 7% health professionals (21). Moreover, 2.6% HBsAg positivity was observed in pre-employment screening of healthy individuals in northern Pakistan (22). Furthermore, 78% hepatocellular carcinoma patients and 30-42% chronic liver disease patients were positive for HBsAg (23, 24). Most of these epidemiological studies were restricted to only hospitalized patients. The clearance of HBsAg from the serum of chronic HBV patients have shown a strong association with clinical cure and improved survival. Recent studies have also shown that HBsAg can serve as an effective biomarker for treatment. Likewise, another study shows the significance of quantitated HBeAg and anti-HBe in analyzing the time course of HBsAg positive liver diseases. The serum level of HBeAg was found highest in chronic active hepatitis with lobular distortion and lowest in acute hepatitis in asymptomatic HBsAg carriers.

2. Materials and Methods

2.1. Description of the Study Area

The local population of Mardan, Khyber Pakhtoon Khwa (KPK) was selected for this study. The study was conducted from January 2014 to January 2015. KPK, formerly named as North-West Frontier Province (NWFP), is one of the four provinces of Pakistan with a population of 26.9 million. Peshawar is the largest city and the capital of KPK, followed by Mardan. The current study was conducted at the Department of Medicine, Mardan Medical Complex, Mardan, KPK. Patients from various remote areas of the province who visited Mardan Medical Complex were recruited for the study. Only HBsAg positive patients were enrolled. For further confirmation of HBV DNA by Real Time PCR, serum samples of the patients were sent to Genome Centre, Lahore Pakistan.

2.2. Blood Sample Collection

Samples were received from different collection centers of KPK. All samples were collected according to the standard procedures. Samples were stored at -20°C till further process. All patients were carriers of HBV and were found HBsAg positive by ICT chromatography and ELFA (Enzyme Linked Fluorescent Assay) method.

2.3. HBV DNA Isolation

HBV DNA was extracted from 150 µl of each sample using Macherey-Nagal nucleic acid extraction kit (USA) which is designed for rapid extraction of highly pure viral nucleic acids from biological samples.

2.4. Real Time PCR

Qualitative and quantitative analysis of the samples was done using Smart Cycler II Real Time PCR (Cepheid, USA). Amplification was done using real time amplification kit prepared by Sacace Biotechnologies, Italy. Total reaction volume was 25 µl, containing 12.5 µl HBV amplification mix and 12.5 µl extracted DNA. For precise checking of the reaction, negative and positive controls were also included with the run. Sensitivity of the assay was 20 copies per ml per blood sample. Specificity of the assay was about 99% (25).

2.5. Statistical Analysis

Statistix 9.0 software was used to analyze and summarize the data. Chi-square test was used for the analysis of categorical variables. A value less than 0.05 was considered as significant (26).

3. Results

A total of 750 HBsAg positive samples comprising 400 (53.33%) males and 350 (46.66%) females were tested for HBV DNA using Real Time PCR. Out of the total 400 male patients, 148 (37%) were found positive for HBV DNA by Real Time PCR, while in females HBV DNA positivity rate was 32% (n=112). Out of the total 750 HBsAg positive samples, HBV DNA was found positive in 260 patients (Table 1). All individuals were categorized into four groups according to their age, that is, 1 to 20 years, 21 to 40 years, 41 to 60 years and above 60 years, respectively. The prevalence of HBsAg was found higher in elderly population.

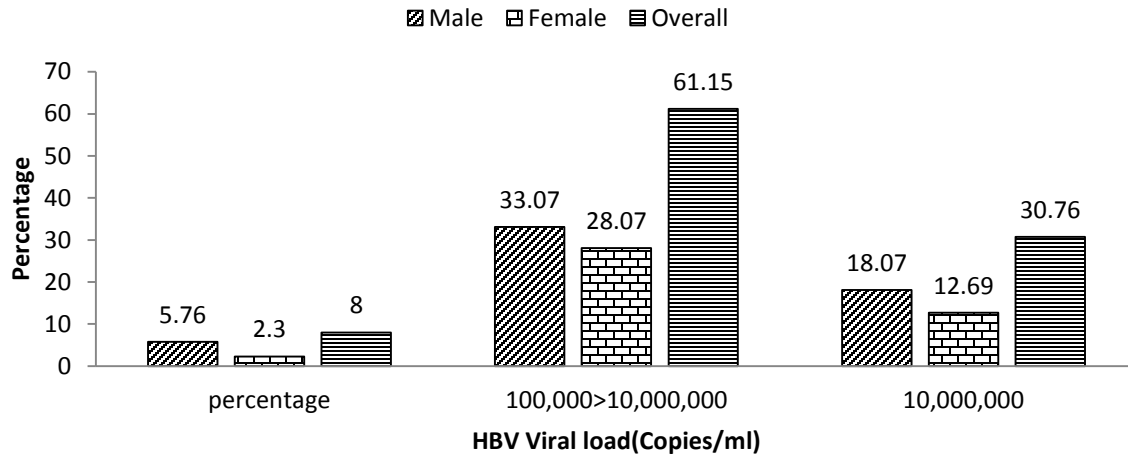
Table 1. Gender-Wise Distribution of HBV

Sr. No.	Gender	Total HBsAg Positive patient	Total HBV DNA positive patients	Percentage	P-value
1	Male	400	148	37	<0.05
2	Female	350	112	32	
		750	260	34.6	

In the current study, patients were categorized into three groups on the basis of viral load, that is, low viral load (100-10000 cps/ml), intermediate viral load (100,000-10,000,000 cps/ml), and high viral load (>10,000,000 cps/ml), respectively. HBV viral load in both genders is shown in Figure 1. Among genders, 61.15% (n=159) were found with an intermediate viral load followed by a high viral load (n=80) at a rate of 30.76%, while 8.0% (n=21) had a low viral load. Our study also analyzed low, intermediate and high viral loads in various age groups. The current study showed that 54.23% (n=141) of the HBV DNA positive patients of different age groups had an intermediate viral load, followed by 30% of patients with a low viral load (n=78), while 15% of patients had a high viral load. In age group 1-20 years, 3.8% (n=10) had a low viral load, 5%

(n=13) had an intermediate viral load and 2.69% (n=07) had a high viral load. In patients of age group 21-40 years, 16.5% (n=43) had a low viral load, 38.84% (n=101) were carrying an intermediate viral load, while 5% (n=13) had a high viral load. In the age group 41-60, low viral load and intermediate viral load was observed in 8.07% (n=21) and 5.0% patients, respectively.

Figure 1. Distribution of viral loads among both genders of HBV positive patients.



Analysis of HBV in different age groups showed that the prevalence of HBV was higher in elderly population. Distribution of HBV in different age groups is shown in table 2.

Table 2. Prevalence of HBV in Various Age Groups

S.No	Age	Total HBsAg Positive Patients	Total HBV DNA positive Patients	Percentage	P-value
1	1---20	81	30	37.03	0.1208 >0.05 Non-significant
2	21---40	503	157	31.21	
3	41---60	130	51	39.23	
4	>60	36	22	61.11	
Total		750	260	34.66	

Figure 1 shows the analysis of HBV viral load in both genders. We observed that overall viral titre of HBV was higher in males as compared to females.

Both genders were categorized on the basis of viral load, that is, 20-10000 IU/ml, 100,000 to 10,000,000 IU/ml to 10,000,000 IU/ml, respectively. Viral load among different age groups is analyzed and presented in Figure 2. The majority of patients had an intermediate viral load that was 100,000 to 10,000,000 IU/ml.

Figure 2. Distribution of HBV viral loads among different age groups.

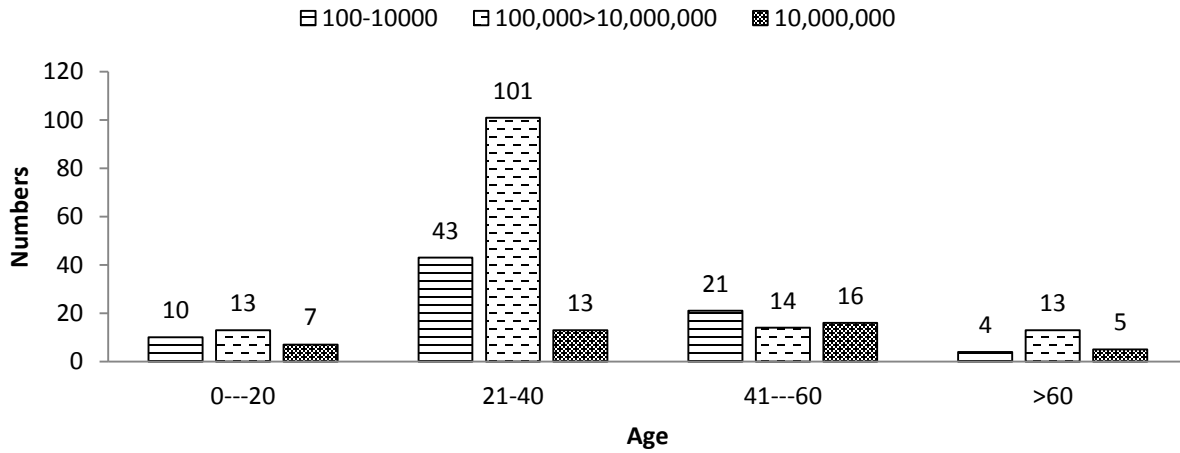


Table 3 shows HBeAg reactivity in HBV DNA positive patients which shows that 32% of patients with a high viral load, that is >10,000,000, were found reactive for HBeAg. We observed that 82 (31.53%) were HBeAg reactive.

Table 3. Hbeag Positivity among HBV DNA Positive Patients

HBV markers	HBV Viral load(Copies/ml)			Total
	100-10000	100,00>10,000,000	>10,000,000	
HBeAg Reactive	04(5.1%)	42(29.78%)	36(87.80%)	82(31.53%)
HBeAg Non-Reactive	74(94.87%)	99(70.21%)	05(12.1%)	178 (68.46%)

P=0.0000 < 0.05, Significant

4. Discussion

The current study analyzed the prevalence of HBV among the population of KPK. Out of the total 750 samples, 260 were detected for HBV DNA with a prevalence rate of 34.6%. The prevalence of HBV DNA was found higher in males as compared to females. This is in line with several other studies conducted in various areas of Pakistan as well as other countries which also showed that the prevalence of HBV is higher in males as compared to females (25, 27-30). For this study, the patients were categorized in various age groups that are 1-20 years, 21-40 years, 41-60 years, and > 60 years, respectively. High prevalence rate (61.11 %) was found in the age group > 60 years,

followed by age group 41-60 years with a prevalence rate of 39.23%. Patients of age group 21-40 years showed low prevalence rate at 31.21%, while patients of age group 1-20 years showed a prevalence rate of 37.30 %.

According to Munir et al. (2013), overall prevalence of HBV was found 3.0% in Mardan and it was higher in females (3.2%) as compared to males (2%) (31). Likewise, another study by Khan et al. showed the dominance of HBV over HCV in Mardan, KPK Pakistan (32).

A systematic analysis of HBV prevalence in Pakistan conducted in 2011 revealed that the percentage of HBV infection was 4.3318%, among healthcare persons (3.25%), among military recruits (4.276%), among pregnant women (5.872%), among healthy blood donors (3.93%), among patients with liver diseases ($27.54\% \pm 6.385\%$), among patients with cirrhosis (28.87%), among patients with hepatitis ($15.896\% \pm 14.824\%$), among patients with HCC ($22\% \pm 2.645\%$), among multiple transfused patients (6.223%), among users of injectable drugs (14.95%), among ophthalmic patients (3.89%), among surgical patients (7.397%), among prisoners (5.75%), among ophthalmic patients (3.89%), and among users of injectable drugs (14.95%) (33).

The next most important aspect of the current study is that all the HBV DNA positive patients were checked for HBeAg reactivity. About 88% of patients with a high viral load were found reactive for HBeAg, followed by patients with an intermediate viral load, that is, 42 (29.78%), while 04 (5.1%) of patients with a low viral load were found reactive for HBeAg.

5. Conclusion

It is concluded that HBV DNA positivity rate was higher in males as compared to females. It is also confirmed that HBV DNA positivity rate is high in age group >60 as compared to other age groups and patients with a high viral load were mostly reactive for HBeAg.

Competing interest

None.

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