

Full Length Research Paper

Molecular prevalence of Hepatitis B virus infection in Khyber Pakhtunkhwa, Pakistan

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Hepatitis B virus (HBV) infection is a major health problem in the developing countries including Pakistan. This study aimed to investigate various risk factors and prevalence of HBV in different areas of Khyber Pakhtunkhwa province, Pakistan. A total of 1439 individuals (1021 males and 418 females) suspected for hepatitis B infection were screened for HBsAg. All the samples were blindly analyzed for HBV DNA by nested polymerase chain reaction (PCR). Of the total, 49.5% were found positive for HBsAg. Of these HBsAg positive patients, 83.03% were confirmed for HBV DNA. Of the 726 HBsAg negative individuals, 37 (24 males and 13 females) were found positive for HBV DNA. 629 HBV DNA positive individuals include 70.43% male and 29.57% female. Higher prevalence rate (16.53%) was observed in Malakand and lowest (13.35%) in Mardan. Mostly young people with age 16 to 30 years were infected as compared to other age group. Risk factors observed in HBV positive individuals were unhygienic barber practice, blood transfusion, general and dental surgery, unsafe injection and sharing personal items. Trend of sharing personal items was common (20.19%). Extensive vaccination and other preventive measures should be taken to stop the spread of this dreadful disease in the study area.

Key words: Hepatitis B Virus, prevalence, polymerase chain reaction (PCR), risk factor.

INTRODUCTION

Hepatitis B infection is the main health problem throughout the world (Ali et al., 2011; Rauf et al., 2010). Approximately 2 billion people are infected with Hepatitis B Virus (HBV) globally (Zhu et al., 2009; Li et al., 2010; Paraskevis et al., 2002), of which 350 million are chronic HBV carrier (Jose et al., 2012; Ali et al., 2011). Each year approximately 1 to 2 million people die from HBV related complications such as chronic hepatitis, cirrhosis and hepatocellular carcinoma (Khan et al., 2011a).

HBV transmission has been observed by percutaneous or mucosal exposure to infected blood and body fluids (Colin et al., 2006). HBV can transmit through blood, serum, body fluids, semen, saliva and HBV can live for

several days in dried blood on table surfaces, needles, syringes and razors (Workowski and Berman, 2002; Workowski and Berman, 2006). The use of unsterilized dental and surgical instruments, shaving from barber, reuse of needle for nose and ear piercing, reuse of disposable syringes and sharing needles with drugs addicts, sharing personal things such as razors, toothbrushes, and nail cutters, sexual and prolonged close personal contact with infected personnel are also the common ways of HBV transmission (Bukhari et al., 1999).

Pakistan is highly endemic (9 million people infections across the country) (Hakim et al., 2008), with 3% chronic HBV carriers (Noorali et al., 2008; Khan et al., 2011b) and the infection rate is rising day by day (Ali et al., 2011). Generally, the epidemiological studies concerning the prevalence of HBV are restricted to the hospitalized patients (Attaullah et al., 2011), whereas there is very few

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population studies to estimate the exact infected population in different areas.

High prevalence of HBV was observed in geographical areas of low economic status, which underscores the importance in controlling this disease because approximately, 67.5% of the Pakistani population belongs to rural areas of low economic status (Akbar et al., 1997; Alam et al., 2007a). This study was planned with the main aim to determine the molecular prevalence of HBV infection and the risk factors associated with HBV infection in Khyber Pakhtunkhwa Pakistan, as limited data is available about the HBV infection in this region of the country.

MATERIALS AND METHODS

Study area

This study was conducted in the Khyber Pakhtunkhwa province, Pakistan, which includes seven main regions/Divisions; Dera Ismail Khan (D.I.Khan), Bannu, Kohat, Peshawar, Mardan, Hazara and Malakand.

Study samples

A total of 1439 blood samples were collected from both gender, directed by clinicians for diagnosis of HBV in District Head Quarter Hospitals of the respective districts. All the individuals were aged between 01 to 70 years. Informed consent was collected from all the individuals included in this study. Out of 1439 patients, 200 each were from Bannu, D.I.Khan, Kohat, Mardan and Peshawar, 226 from Hazara and 213 were from Malakand Division.

A 3 ml blood sample was collected in a vacutainer from each patient; serum was separated and stored at -20°C in the Molecular Parasitology and Virology Laboratory, Department of Zoology, Kohat University of Science and Technology Kohat, Pakistan for further processing.

HBV screening

HBV screening was carried out with Immunochromatographic (Accurate Diagnostics Canada) for the detection of anti HBsAg.

Biochemical analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed (two readings for each patient) for six months with Microlab 300 (Merck USA) using ALT and AST kit (Diasys Diagnostic System Germany) according to the manufacturer's manual.

HBV DNA detection

HBV DNA was isolated from all 1439 HBsAg positive samples with GF-1 nucleic acid extraction kit (Vivantas USA) according to manufacturer's instructions with minimal alterations.

PCR reactions were carried out in a thermal cycler (Nyxtechnik USA) with 5U *Taq* DNA polymerase (Fermentas USA). The first round of amplification was performed with 5 µl of extracted DNA by

using an outer sense primer and an outer antisense primer specific to the surface gene of HBV. Another round of PCR was carried out with inner sense primer and inner antisense primer.

Amplified product was subjected to electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under UV illuminator.

RESULTS

Blood samples from 1439 HBV suspected patients (1021 males and 418 female) were collected from the various District Head Quarter Hospitals of Khyber Pakhtunkhwa, Pakistan. Of the total, 49.55% (489 males and 224 females) were found positive for HBsAg and 50.45% were found negative for HBsAg. All the samples including HBsAg negative were further analyzed for the detection of HBV DNA by PCR. Among the 713 HBsAg positive patients, HBV DNA was confirmed in 592 (83.03%) patients and in 121 (16.97%) patients, (70 male and 51 female), HBV DNA was not detected. Among the 726 HBsAg negative patients, 37 patients (24 males and 13 females) were confirmed for HBV DNA and the rest of all were found negative for HBV DNA.

Division based prevalence of HBV infection

629 (43.71%) samples were positive by PCR for HBV infection. High prevalence of HBV infection was reported from Malakand division (16.53%) and D.I.Khan division (14.63%) as compared to Hazara division (14.15%), Peshawar division (14.15%), Kohat division (13.67%), Bannu division (13.5%) and Mardan division (13.35%) (Table 1). Gender-wise prevalence among the HBV DNA positive samples showed that males were more affected than females. In this study the PCR positive HBV samples included 443 (70.43%) males and 186 (29.57%) females (Table 1). Male to female ratio was found to be 2.38:1.

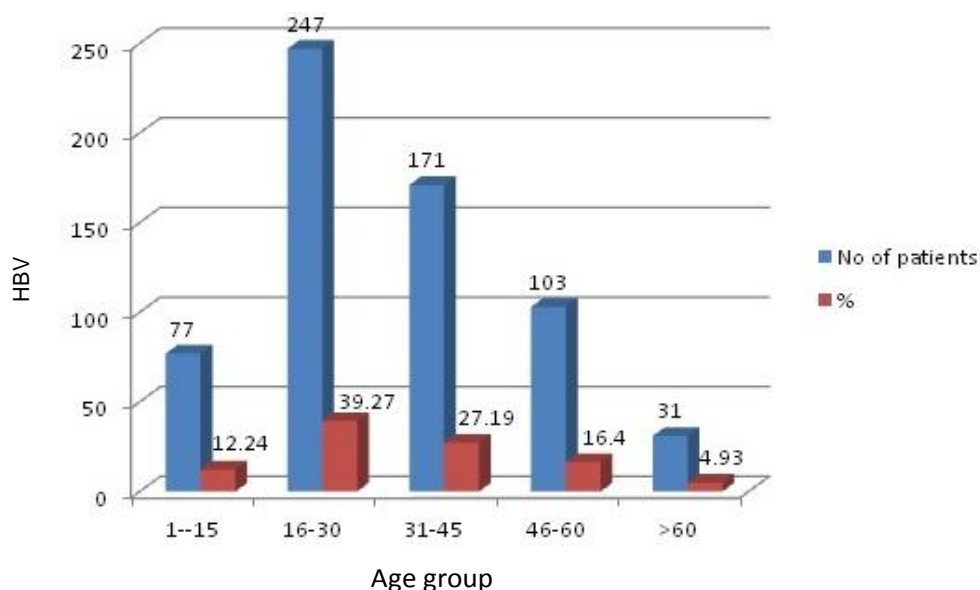
Age-wise prevalence was observed in all the PCR positive samples which were categorized into five age groups. The highest infection rate of 39.27% was observed in the age group of 16 to 30 years while a lower infection rate of 4.93% was observed in the age group of more than 60 years (Figure 1).

Risk factors associated with HBV

Patients were interviewed for the various risk factors to find out the possible modes of transmission. Over all HBV DNA positive cases showed high trend of sharing personal items (20.19%). Other risk factors identified were shaving from community barber (10.65%), blood transfusion (9.54%), dental procedures (16.06%), general surgery (9.38%), history of injection (16.69%), sexual contact with hepatitis B positive partner (9.7%) and skin tattooing (4.45%) (Table 2).

Table 1. HBV Prevalence in the different Divisions of Khyber Pakhtunkhwa (n = 1439).

Division	HBsAg positive		HBV DNA positive		Total HBV positive n (%)
	Male	Female	Male	Female	
Bannu	65	35	68	17	85 (13.5)
D.I.Khan	71	29	61	31	92 (14.63)
Hazara	58	42	59	30	89 (14.15)
Kohat	82	18	70	16	86 (13.67)
Malakand	91	22	74	30	104 (16.53)
Mardan	55	45	56	28	84 (13.35)
Peshawar	67	33	55	34	89 (14.15)

**Figure 1.** Prevalence of HBV among various age groups.**Table 2.** Risk factors associated with HBV transmission.

Risk factor	HBV positive (n = 629)		Total observed, n (%)
	Male	Female	
Barber risk	67	-	67 (10.65)
Blood transfer	43	17	60 (9.54)
Dental risk	74	27	101 (16.06)
G. surgery	38	21	59 (9.38)
History of injections	68	37	105 (16.69)
Sexual contact	40	21	61 (9.7)
Skin tattooing	08	20	28 (4.45)
Sharing personal items	105	22	127 (20.19)

DISCUSSION

Hepatitis B infection is an international health concerned problem with its continuously increasing burden in

developing countries like Pakistan (Alam et al., 2007a). Representing all the geographical regions of Khyber Pakhtunkhwa, no study on HBV prevalence is available. The current study was conducted with the main aim to

find out the molecular prevalence and the risk factors in all the seven divisions of Khyber Pakhtunkhwa. We found that 43.71% of the subjects were infected with HBV. Almost all the individuals were referred to the D.H.Q. hospitals of the related division/district for HBV diagnosis and medications.

High prevalence (16.53%) of HBV was observed in Malakand division as compared to other divisions of the province and lowest (13.35%) rate of infection was observed in Mardan division. The high prevalence of HBV in Malakand division may be due to low literacy rate, low economic status, far off from basic health facilities, frequent exposure to risk factors and affect of this area by tiresome and natural disasters (for example, earthquake, floods). Male (70.43%) were found to be more infected with HBV than female (29.57%) and male to female ratio of HBV positive individuals was 2.38:1. In these areas, barber shaving, involvement in blood transfusion practices, drug use and homosexuality are very common which strengthens the arguments for such high prevalence of HBV in males. This study is in line with other studies (Naz et al., 2002; Khan et al., 2011a), who reported that more male were infected with HBV than female. Similarly in another study of hepatitis B virus infection among different sex and age groups in Pakistani Punjab, Khan et al. (2011b) reported high prevalence in male (68.15%) than female (31.85%). Nwokediuko (2010) also reported a significantly higher (79.2%) infection rate in male as compared to the female (20.8%). Zubair et al. (2010) determining the frequency of hepatitis B virus among children with chronic liver disease also find out a high 54% prevalence in male than females 46%. Moosa et al. (2009) and Awan et al. (2010) reported a high (59.1, 58.3%) prevalence in males than females (40.9, 41.7%) respectively. In earlier studies in Pakistan, high prevalence results of HBV in males compared to females have been observed by Alam et al. (2007b) and Usman et al. (2003). In Bangladesh, similar results of high prevalence rate in males (67.86%) as compared to females (32.14%) have been observed by Mahtab et al. 2008. This high prevalence of HBV in male reflects the increased frequency of high risk behavior as compared to females.

This study showed that almost all the age groups were affected by HBV. The prevalence rose from 5.35% in children of age 1 to 15 to a peak of 17.16% in people aged 16 to 30 years. After this, it declined to 12.16 and 7.16% in people aged 31 to 45 and 46 to 60 years respectively. While very old >60 age group were very less frequently 2.15% infected by HBV infection. This means that there was an age effect on the prevalence of hepatitis B infection. This high prevalence among the young age groups may be attributed to the more frequent exposure to risk factors and prolonged HBV infection and may be due to their greater exposures and interaction in society as compared to children and aged peoples. Our study also identified many risk factors for HBV infection.

The barber risk was markedly higher (10.65%) in male individuals. Khan et al. (2011a) also reported 32% barber risk in males as they routinely shaved with community barbers. The previous studies showed that barber risk was the most frequent risk factors contributing to the transmission of HBV infection because of the reuse blades. But during the last few years the trend has changed substantially and barbers now use disposable blades and razors (Janjua and Nizamy, 2004). Because of the presence of HBsAg in the saliva of HBV patients, the contaminated dental instruments also play an important role in HBV infection (Fox, 1996).

Our study identified 16.06% dental risk factors in the individuals. This may be due to low economic status of the patients which were attended by local practitioners which using equipments without any sterilization or autoclaving techniques. These results showed low percentage of dental risk as compared to previous studies of Khan et al. (2011b) (41%) and Usman et al. (2003) (60%). This decrease in the rate of dental risk may be due to awareness and education of the peoples. Sexual contact with hepatitis positive partner (9.7%) and skin tattooing (4.45%) contribute in the spread of HBV infection. Risk factors with history of injection and previous history of surgery contribute 16.69 and 9.38% respectively. This may be due to the lack of awareness about the possible risk factors among the healthcare providers and the population. Blood play significant role in the transmission of HBV infection (Alavian et al., 2007). During this study 9.54% of the respondents had the history of blood transfusion which is supported by the results of 4.04% by Khan et al. (2011a). Similar results of potential source of HBV transmission in Pakistan were reported by Castolo et al. (2001), Ali et al. (2009) and Qureshi et al. (2009).

Sharing personal items (20.19%) have also been identified as major risk factor for Hepatitis B virus infection. Tanveer et al. (2008) also reported the high habit (37.5%) of sharing personal belongings in the infected persons that could account for the high risk of infection.

Conclusions

It is concluded that HBV infection is still prevalent in the Khyber Pakhtunkhwa Province. Massive awareness programs, extensive vaccination and other preventive measures should be taken to stop the spread of this alarming disease in the Khyber Pakhtunkhwa, Pakistan.

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