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Short Communication

Prevalence of Hepatitis B Viral Infection in Punjab Province of Pakistan

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ABSTRACT

Hepatitis B viral (HBV) infection is a major health problem globally and more prevalent in Pakistan, which is the main etiological agent of chronic hepatitis B, hepatocellular carcinoma and liver cirrhosis. A total of 659 HBsAg ELISA positive patients out of 16825 patients during July to October, 2013 were screened for HBV by real-time polymerase chain reaction. Out of 659, 522 (79.21%) were found positive for HBV, of which 318 (over all 4.4%) were male, while 341 (over all 3.55%) were female. The HBV PCR positivity rate was 80.18% (n=255) in males and 78.30% (n=267) in females patients. Among different age groups HBV PCR positivity was highest (n=181, 83.41%) in the age group of 31 to 40 years. HBV genome was detected in 79.21% of HBsAg positive samples, showing that HBsAg on ELISA is much more sensitive than PCR as HBV shows chronic conditions and both of the tests are needed against HBV.

According to World Health Organization (WHO) approximately more than 2 billion people are infected with HBV globally, including about 400 million people with chronic stage of infection while every year, approximately 0.6 million die due to HBV associated diseases all over the world (Goldstein *et al*., 2005; Alam *et al*., 2007). Pakistan is an extremely endemic area for HBV with an estimated 9 million people infected with HBV (Noorali *et al*., 2008; Hakim *et al*., 2008). The Intraprovince prevalence of the hepatitis B was very high in Balochistan (*i.e.* 4.3%) while it was 2.5% in Sindh, 2.4% in Punjab and 1.3% in Khyber Pakhtunkhwa (Choudhary *et al*., 2005). Early diagnosis of HBV infection is very important for the prevention of its further transmission. Various serological and molecular based diagnostic techniques are used (Wolters *et al*., 1985), of which ELISA is very common. This technique suffers from an abundance of false positive result due to suboptimal sensitivity and specificity (Mahoney, 1999). Lately, real time PCR (RT-PCR) has been widely used for HBV DNA detection and quantification. The current study was therefore designed to reassess ELISA positive cases by using reverse transcriptase real time PCR (RT RT-PCR) to confirm the specificity and sensitivity of RT-PCR for HBV diagnosis.

Materials and methods

All the HBsAg ELISA positive serum samples were received along with specifically designed data sheets at Chiragh Diagnostic Complex, Lahore, Pakistan during July to October, 2013 from seven collection centers located

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in different districts *i.e.* Lahore, Dipal Pur, Rahim Yar Khan, Liaqat Pur, Pak Pattan, Gujranwala, and Khanpur at Punjab Province Pakistan. The study was approved by the ethical committee of Microbiology department, Kohat University of Sciences and Technology, Kohat.

Quantification of HBV DNA in all HBsAg positive samples were performed by PikoReal 24 Real time PCR system (Thermo Fisher Scientific, Lenixa, KS 66285, USA) using HBV DNA quantification kit AmpliSens, HBV-FRT (Federal Budget Institute of Science, Moscow 111123, Russia) by using the prescribed protocol given in the kit with some modifications. The amplified product was detected by using fluorescent dyes in RT-PCR, which were linked to the oligonucleotide probes to bind specifically with the amplified product and then the fluorescent probes were detected after each replication cycle. The lower detection limit of the assay used was 5.0 ×102 IU/ml while its higher detection limit was 5.0 × 108 IU/ml.

Statistical analysis of the available data was performed by Chi-square test and one way ANOVA test "STATISTIX", version 9.0, Korean made software. The P value less than 0.05 were considered significant.

Results and discussion

The current study was conducted for the prevalence rate of HBsAg in different districts of Punjab Province. Out of a total of 16825 samples, 659 (3.92%) samples were positive for HBsAg. Table I shows 4.4% prevalence rate of HBV in male and 3.55% in the female patients. Likewise patients between 31-40 years of age showed 5.11% prevalence followed by 4.07% in 41-50 years old age group.

Among different regions of Punjab, highest prevalence was detected in Rahim Yar Khan (8%), followed by 6.99%, 5.32% and 5% in Liaqatpur, Pak Pattan and Gujranwala, respectively. The lowest prevalence rate was 2.3% in Lahore and 3% in Dipalpur.

Real time PCR detected HBV DNA in 79.21% (522) of the HBsAg positive (659) samples. The current study showed that Lahore had the highest number of true HBV positive cases (n=115, 84.55%), followed by Rahim Yar Khan (80.5%) Liaqatpur (80%) and Khanpur (n=54, 73.79%) (Table I).

The HBV positivity was 80.18% (n=255) in male, and 78.30% (n=267) in the female subjects (Table II).

Moreover, the incidence of PCR HBV positivity was low (n=40, 72.72%) in the age group above 50 years while the highest prevalence (n=181, 83.41%) was reported in the age group of 31 to 40 years (Table I).

In Pakistan, one of the major public health problems is chronic viral hepatitis. HBV prevalence rates have been reported as high as 10% for Pakistan (Malik and Tariq, 1993).

In the past several years, the role of quantified serum HBsAg in the surveillance of treatment efficacy has evinced lot of interest. HBsAg seroclearance confers a favorable prognosis in cirrhosis and HCC (Yuen *et al.*, 2008). The risk of hepatocellular tumorigenesis is lower in the patients with a clearance of serum HBsAg after interferon α-based antiviral therapy (Fattovich *et al.*, 1998). High levels of HBsAg increase the risk of HCC and *vice versa* (Tseng *et al.*, 2012).

Table I.- Prevalence of HBsAg and PCR detection of HBV (%) in both male and female subjects of different age groups from different geographical areas of Punjab Province.

Characte-ristics	Total subject	Positive HbsAg	Prevalence (%)	HBV detected (%)
Sex				
Male	7230	318	4.4	-
Female	9595	341	3.55	-
Age group				
10-20	625	19	3.04	15 (78.94)
21-30	4500	170	3.78	141 (82.94
31-40	4250	217	2.11	181 (83.41)
41-50	4860	198	4.07	145 (73.23)
> 50	2590	55	2.12	40 (72.72)
Total				522 (79.21)
Area				
Lahore	5915	136	2.3	115(84.55)
Dipalpur	2930	88	3	67(76.13)
RY Khan	1287	103	8	83(80.58)
Liaqatpur	1145	80	6.99	64 (80)
Pak Pattan	1823	97	5.32	76 (78.35)
Gujranwala	1640	82	5	63 (76.82)
Khanpur	2085	73	3.5	54 (73.79)
Total	16825	659	3.92	522 (79.21)

Table II.- Gender wise distribution of HBV in the subjects of Punjab.

Gender	Total	Positive	Negative
Male	318	255 (80.18%)	63 (19.82%)
Female	341	267 (78.30%)	74 (21.70%)

(%) for percentage; n, total number; P=.9043 > 0.05.

The current study showed 4.4% rate of infection in the male and 3.55% in females. Moreover, patients of 31-40 years of age had 5.11% prevalence compared to 2.12% in patients above 50 years of age. Ali *et al.* (2012) have reported highest prevalence (*i.e.* 38.88%) of HBV in people belonging to 31 to 40 years age group, while the lowest

rate in the age of less than 10 years old children and 7.14% in above 50 years old people. In another study the highest prevalence rate was 17.16% observed in 16 to 30 years aged people (Awan *et al*., 2012). It was also revealed by the current work that HBV prevalence reported in current study was mostly associated with chronic conditions, since the PCR results were less than HBsAg as shown in Tables I and II.

In another study the 19.58% male were infected with HBV and 10.16% in females (Ali *et al*., 2012). Males were shown to have 6.03% prevalence and females 5.05% (Khan *et al*., 2006).

The reasons behind this rising rate of infection is poor economic background, insufficient health facilities, unscreened blood transfusion, high ratio of intravenous drug users and minimum awareness about communicable diseases in public (Alam *et al*., 2007). In another study the risk factors for possible transmission were observed in HBV positive individuals which were reported as unhygienic barber practices, general and dental surgery, unsafe injection, sharing personal items and blood transfusion (Awan *et al*., 2012).

In the current study it is concluded that real time PCR should be performed coupled with ELISA for accurate diagnosis of HCV in high prevalence areas (Mahmood *et al*., 2016; Kohmoto *et al*., 2003; Garson *et al*., 2005).

Statement of conflict of interest

Authors have declared no conflict of interest.

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