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RESEARCH ARTICLE

REAL TIME PCR BASED DETECTION OF HEPATITIS B INFECTION IN SUSPECTED LIVER DISEASE PATIENTS OF DISTRICT MARDAN, KPK

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Abstract

Chronic hepatitis is a common liver infection usually caused by different viruses, where Hepatitis B virus (HBV) has been documented as the major cause and lead to many complications. Globally about 2 billion people are HBV positive and 7-9 million are in carrier state in Pakistan. In this study the prevalence of HBV was confirmed by q-PCR based on age , gender and area. Total 183 serum samples were processed through real-time PCR for detection of HBV DNA and age wise, gender wise and area wise data was analyzed through Chi square test. Genomic DNA of HBV was detected in 99 (54.06%) samples out of total 183 ELISA positive samples of suspected liver disease patients. Out of 99 patients, 66.66% were male and 33.33 % were female patients. Positive cases in age wise people of <20 years was 37.37%, 21-40 years 42.42% and in 41-60 years (16.16%) and in age > 60 years 4.04% was observed. In Tehsil wise distribution, 54.54% HBV DNA positive samples were found in Tehsil Mardan, 32.32% in Takhtbhai and 15.15% in Kattlang. This study concludes that male infectivity rate with HBV is relatively higher then female in District Mardan. People between 21-40 years were found more prone to HBV infection. Among the three tehsil of district Mardan, the highest numbers of HBV infected patients were found in tehsil Mardan with 54 (54.54%) patients.

Introduction

Hepatitis B virus belongs to an enveloped virus family of hepadnaviridae that causes chronic hepatitis (Khokhar et al., 2002; Bu ¨chen, 2003). More than a million people face death annually due to HBV related problems (Lavanchy , 2004; Wright , 2006). About 15 to 40 percent of HBV positive patients develop liver problems such as failure, cirrhosis and hepatocellular carcinoma (HCC) (Lok , 2002). Acute infection causes liver inflammation, vomiting, jaundice, however, seldom fatality occurs (Chang, 2007). Hepatitis B infection in chronic stage may cause liver cirrhosis and hepatic cancer that results in a fatal disease with hard-up response to modern chemotherapy and vaccination is the only hope (Chang, 2007; Pungpapong et al., 2007). The infection of Hepatitis B virus (HBV) is globally concern nowadays and out of 2 billion people 360 million people are in chronic stage (Elizabeth et al., 2011). Chronic hepatitis B is a significant issue in Pakistan (Khan et al., 2002). Transmission of HBV occurs in mucous membrane and skin through blood or blood product transfusion, contaminated surgical equipments, injection of drug, injuries from sharp edges of instrument, sexual exposure and chronically infected person contact to another person in house. Secretions, body fluids and infectious blood are main sources of transmission (Alter, 2006; Ali et al., 2009). Vertical transmission of HBsAg and HBeAg in pregnant women may also occur but with low incidence (Abbas et al., 2003). An average occurrence of hepatitis B antigen in Pakistan was

1.4–11.0% in healthy adults while rate of prevalence in pediatric population is 2.4 percent (Syed et al., 2009).

Material and Methods

A total 183 HBV positive samples using 3rd generation ELISA assay kit (Glibe Diagnostics, ITALY) were received at District Headquarter hospital (DHQ) Mardan, KPK, from different Tehsil (Tehsil Kattlang, Tehsil Mardan and Tehsil Takhtbhai) of district Mardan for HBV detection from October 2012 to November 2012 in the Provincial Program for Hepatitis Control and Prevention. All sera were stored in aliquots at -70 °C for subsequent nucleic acid (DNA) extraction.

Quantitative Detection of HBV RNA

The genomic DNA of HBV was detected using viral DNA extraction kit (Nucleospine, Germany), according to the manufacturer protocol. Quantitative detection of HBV DNA in all patients was performed by SmartCycler II Quantitative PCR (Cepheid, USA) with *Taq* Man technology (Applied Biosystems, Foster City, Calif) using fluorescent probes keeping thermocycler of conditions of 94°C for 45 sec, 50°C for 60 sec, and 72°C for 60 sec for 35 cycles. This detection range of this assay is 5.0×10^2 to 5.0×10^7 IU/mL. Specimens that cross the upper limit were normally diluted 100-fold and retested. The resulted values from diluted samples were multiplied by dilution factor to obtain the actual HBV DNA concentration in international units per mL.

Statistical Analysis & Graphical representation

Statistical Analysis for the calculation of percentage and distribution of the samples in different groups was analyzed through SPSS 16.0 using Chi square test.

Result and Discussion

Pakistan is in the intermediate HBV prevalence area with a carrier of 7 to 9 million people (Ali et al., 2011; Andre, 2000). Another study has shown that in northern areas of Pakistan the overall prevalence of HBV was 37% (Manzoor et al., 1997). The HBV prevalence in healthy children was 3.6 percent in Pakistan (Manzoor et al., 1997). Out of 183 patients examined 99 patients were found with HBV infection. Out of these 99 positive patients 36.1% were male and 18.03% were female as given in Figure 1. Similar results were also shown in previous study which demonstrates that HBV infection in male is 63% as compared to female 47% (Lewis-Ximenez et al., 2002). The main reason behind this may be due to high use of drug and sexual partnership is men as contrast women. Prevalence of HBV infection was extensively higher in Tehsil Mardan (54.54%) as compared to Takhtbhai (32.32%) and Kattlang (15.15%) as shown in Figure 2. Earlier studies demonstrated that about 67.7% of HBV infection in Pakistan belongs to poor people and rural areas (Alam et al., 2007). Patients studied on the basis of their ages showed that HBV infection was significantly higher (42.42%) in patients between age group 21-40 years followed by in patient with age <20 (37.37%), 41-60 years (16.16%) and >60 (4.04%) as given in Figure 3. The prior study explained that HBV acute infection is more prominent in young people less than 30 years versus chronic and past infection ((Lewis-Ximenez et al., 2002). Similar outcomes were also found in another study which reports that people with age 21-30 years are more prone to HBV infection and infection rate is higher in male versus female (Adoga et al., 2010). According to previous study detection of HBV DNA in serum varies from state to state mean low in asymptomatic patient while high in anti-HBe positive patient (Jardi et al., 2001)

In Pakistan the prevalence of HBV constantly increased due to lack of proper laboratory facilities for diagnose of the disease (Shah et al., 2001). The overall HBV prevalence in District Mardan was (54.06 %). This rate is high as compared to other study due to lack of proper screening facilities or expertise in screening blood and blood products for possible HBV infection. Public sector hospitals are partly contributing towards the spreading of the disease in District Mardan (Najib et al., 2011).

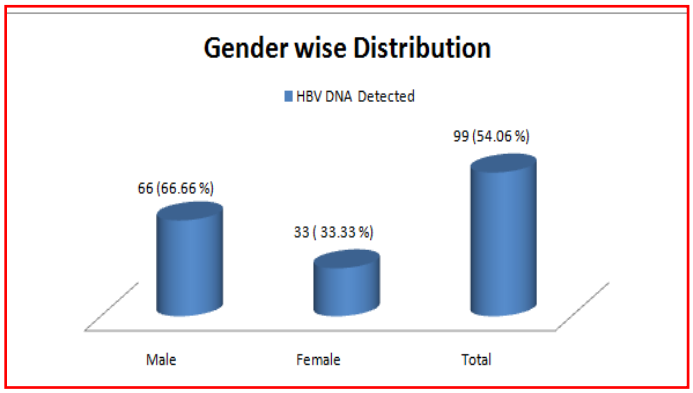

Fig 1: Rate of HBV infection in male and female patients of District Mardan

Prevalence of HBV infection in different Tehsils was observed in which Tehsil Mardan 52.52%, 32.32% and 15.15 % in Tehsil Takhtbhai and Tehsil Kattlang were positive respectively. We showed that HBV infectivity in Tehsil Mardan is higher than other

Tehsils of district Mardan. The present study might be helpful to attract the attention of both local and international organizations towards these areas. The Mardan District was taken as model because all its districts have very low literacy rate, far-off from basic health services and are affected by tedious and natural disasters (e.g. floods).

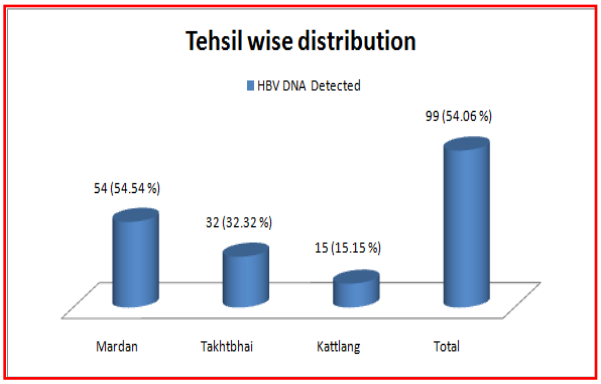

Fig 2: Rate of HBV infection in different Tehsils of District Mardan.

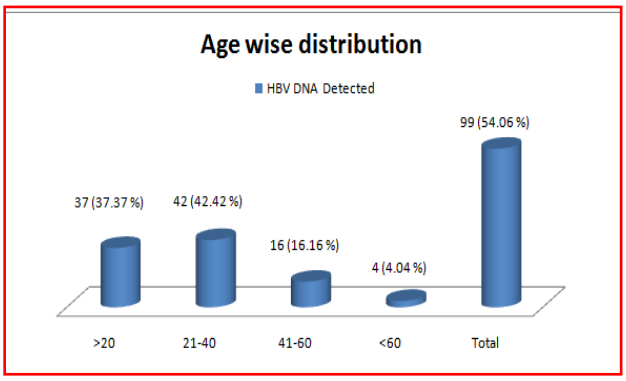

Fig 3: Age wise distribution of HBV infection in District Mardan

Conclusion

This study concludes that the most specific, sensitive and reliable test use for detection of HBV is q-PCR. Male infectivity rate with HBV is relatively higher then female in District Mardan. Age group 21-40 years was found the most susceptible group to HBV infection. Among the three tehsil of district Mardan, the highest number of HBV infected patients was found in tehsil Mardan with 54 (54.54%) patients.

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