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RESEARCH PAPER

TITLE

MOLECULAR PREVALENCE OF HEPATITIS B VIRUS (HBV) GENOTYPES IN PESHAWAR

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MOLECULAR PREVALENCE OF HEPATITIS B VIRUS (HBV) GENOTYPES IN PESHAWAR

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Abstract

Background and Aim: Globally Hepatitis B is life-threatening problem including in Pakistan with an estimated incidence rate of 3% in the general population. According to recent findings Pakistan has an enormous burden of HBV infection, but very little attention has been drawn in terms of the molecular epidemiology of HBV. Till now HBV has been categorized into 10 genotypes and different genotypes exhibit different pathogenicity and response towards the treatment, so this research study was planned to evaluate the current status of different HBV genotypes prevailing in Peshawar district of Khyber Pakhtunkhwa (KP).

Method: A collection of 550 blood samples was carried out from chronically infected HBV- positive patients and tested by ICT that was further confirmed for HBV DNA with qualitative PCR and followed by type-specific PCR to identify the prevalence of specific HBV genotypes. Sequencing was performed on the respective genotype and a phylogenetic study was done using the MEGA 6.0 tool.

Results: All samples were diagnosed HBV positive. Genotype D was recorded as the most widely distributed (90%) while 10% were detected positive for genotype C and no other genotype was found in our study in district Peshawar. Phylogenetic analysis of obtained sequences shows close homology with already reported strains of HBV.

Conclusion: The only genotype predominant was genotype D in the District Peshawar region which is the most commonly found genotype in Pakistan and the neighboring countries.

Key words: Hepatitis B, Genotype, HBV, PCR

Introduction

HBV is one of the primary leading causes of hepatic infections and almost 400 million people around the globe are strongly believed to be chronically infected by the virus (McMahon, 2009). Difficulties like cirrhosis and hepatocellular carcinoma (HCC) ascends due to HBV causing fatalities of 1 to 2 million annually and is considered the 10th foremost cause of deaths globally (Yoo *et al.*, 2018; Rooherman *et al.*, 2005; MacLachlan *et al.*, 2005). HBV is an enclosed virus that has its place in the hepadnaviridae family, and has the capability of infecting animals and humans both. It possesses a circular form of DNA genome (3.2 kb) that is double helical in

structure and composed of 4 overlapping open reading frames: pre-core region, pre-surface region, the X coding region and the P coding region. To date it has been characterized into 8 different genotypes A-H and 2 new genotypes named I and J have been recognized recently based on more than 8% of nucleotide divergence of the whole genome. Genotype A of HBV is worldwide distributed and is the major genotype prevalent in European states, Africa, North of the US and India. Genotype B and C of HBV mostly exists in Southeast and East Asia (Mahtab *et al.,* 2008). Genotype D is predominant in the Mediterranean states and the Middle East but it has been described all over the world, whereas genotype E circulates in the West of the sub-Saharan region of Africa (Mulders *et al.,* 2004; Kramvis *et al.,* 2005). Genotype F is mostly identified and reported from US and Polynesia and genotype G of HBV is associated with European countries and North of America whereas one of freshly documented genotypes H has been identified and exists in Mexico region of the US (Kramvis *et al.,* 2005). Genotype I has been currently endorsed by scientists to evolve as a very rare strain, patented from North of Vietnam, Hanoi and Laos and also has been stated from North West of China and North East of India (Huy *et al.,* 2008) and J mostly from regions including Japan has been reported (Kao, 2011).

In Pakistan highly endemic situation of HBV infection has been reported and about 9 million people are diagnosed with HBV while 3% are strongly confirmed to be chronic carriers (Hakim *et al.,* 2008; Khan *et al.,* 2011; Farzana *et al.,* 2020) and the incidence rate of HBV is getting dramatically high day by day (Muhammad *et al.,* 2011). A study reported by Awan et al in Pakistan verified that genotype C is highly emerging and frequently prevailing genotype with a recorded prevalence rate of 27%, indicating an alarming situation that is commonly prevalent in patients diagnosed with more severe conditions of liver infection, cirrhosis and believed to be interrelated with further hepatic complications (Zunaira *et al.,* 2010). Preceding findings also defined that genotype D of HBV have usually higher rate of liver infection and an extra amount of viral DNA, and indicates low response to interferon treatment as associated to other HBV genotypes A and B. Genotype D also carried a very specific arrangement of sequences that might have a vital character in the prediction of extended and long-lasting effect to lamivudine therapy (Zaigham *et al.,* 2006). Also it is believed and reported by different studies that strain C specifically carries more levels of DNA as related to genotype B while

genotype D have greater loads of HBV DNA as compared to HBV genotype A (Oommen *et al.,* 2006). It has been understood that HBV genotypes have a quite an important function in the progression and development of liver infection also including response to antiviral drugs so prediction of an accurate and specific strain of HBV could be very beneficial to suggest a suitable therapy to chronically infected HBV patients. So this study was done with the aim to search out the existing patterns of HBV genotypes circulating in the Peshawar region of KP as previously few studies have been reported yet regarding HBV genotypes from this part of the province.

Materials and methods

Samples collection

A collection of 550 blood samples were done from HBV-positive patients enrolled in different healthcare units located in Peshawar. All infected and HbsAg-positive patients were included in our study while those infected with other hepatic viral strains (A, C, D, E) or co-infection of HBV and HIV were excluded from the study. All samples were processed for the existence of HBsAg by the ICT method (Abbott Lab, US).

HBV DNA extraction

Extraction of viral DNA was carried out from each sample (200µl) using DNA viral kit (Promega) following manufacturer's protocol.

Qualitative Detection of viral DNA and type-specific PCR

All collected positive HBV samples were processed through PCR to detect active infection. Qualitative detection of viral samples was executed by 2 steps of PCR reactions as defined earlier [Norder *et al.,* 2004] which is regarded as a sensitive and specific method as compared to other serological procedures. Each round of PCR was processed with both a positive control and negative control. For the identification of specific HBV strains, Type-specific PCR was performed for each HBV-positive sample as described earlier [Farazmandfar *et al.,* 2012].

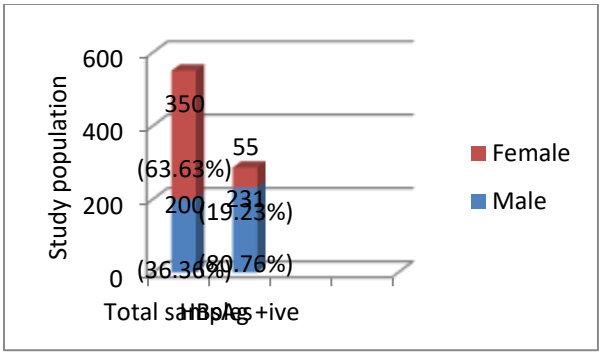
Sequencing of HBV genotypes

To find out the sequences' homology, 3 samples were randomly selected for sequencing and the sequences obtained were aligned by BLAST (Basic Local Alignment Tool). A bio- informatics tool MEGA 6.0 was used for the phylogenetic analysis of sequences. Subject sequences were obtained and retrieved from the online repository NCBI (National Centre for Biotechnology Information), and were aligned and compared with query HBV genotype sequences that were

further used for the construction of a Phylogenetic tree.

Results

A total 550 chronic HBV-positive patients including 200 males and 350 females of age 10-85 years were analyzed in this study. 286 (52%) samples were detected positive for HBsAg (Male: 231, Female: 55) by ICT while viral DNA was detected in all samples by qualitative PCR (fig 1).



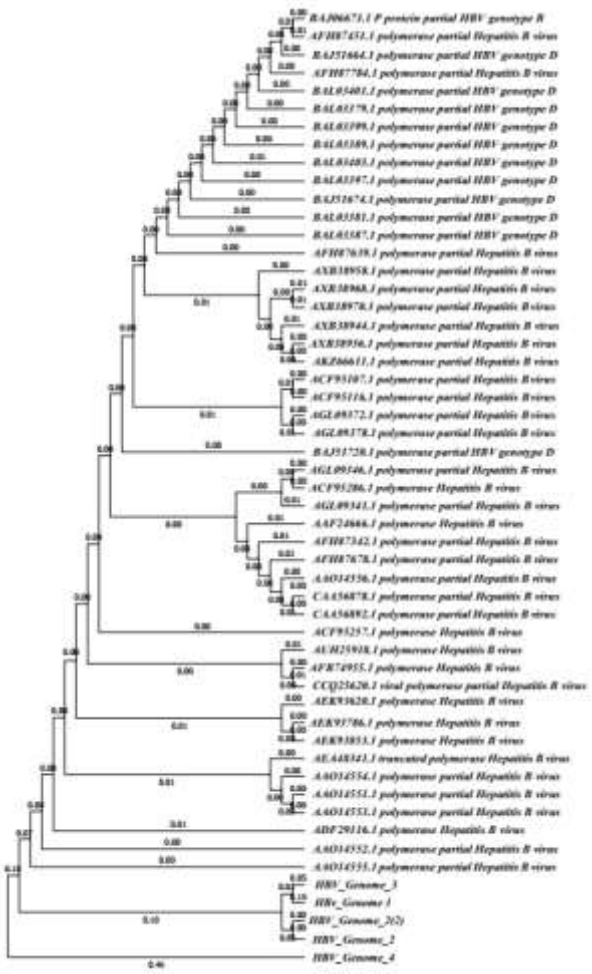
All samples showed successful genotype-specific bands with genotype D as the leading (90%) followed by genotype C at (10%). No mixed infection and other genotypes were detected in our study in the district of Peshawar. Gender-based distribution of viral genotypes showed a high prevalence of genotype D in male patients (84%) as compared to females (74%) while the genotype C ratio remained high in female patients (26%) as compared to males (16%) (Table 1).

Table 1: Showing gender-wise distribution of viral genotypes in chronically HBV patients

Gender	Genotype C	Genotype D
Male	32 (16%)	168 (84%)
Female	91 (26%)	259 (74%)
Total	55 (10%)	495 (90%)

Phylogenetic study

Phylogenetic investigation of sequencing data was performed with the help of MEGA 6.0 software. Standard sequences of HBV were retrieved from NCBI. Sequencing results were converted first into FASTA format and were subjected to BLAST and the results obtained were saved and further used for the construction of a phylogenetic tree. The sequences of all HBV genotype D isolates of our study showed closed homology and resemblance of 86%, 87% and 88% with other reported national and international sequences. Though differences with fluctuating time scales were noted. Strain **3382124 3 DR** originated into 2 subclasses of HBV genotype-D **3382125 4 DR** and **3382126 5 DR**.



Discussion

Hepatitis B virus is believed as the most common cause of severe hepatic infectious diseases across the globe especially in evolving countries as well as in Pakistan (Franco *et al.*, 2012). About 4 billion of whole world's population are believed to be chronic carriers of HBV (Sahina *et al.*, 2018). Mostly HBV remains asymptomatic for a long time after infection that can harm and injure the liver silently and so leads to one of the most severe complications known as hepatocellular carcinoma (Samal *et al.*, 2012). Currently Pakistan carries a huge and enormous burden of HBV infection with a 3-5% of prevalence rate mostly in the overall population and in with high risk populations it is recorded as 10-20% (Muhammad *et al.*, 2011). Pakistan is exceptionally endemic and about 9 million people are surviving with HBV, 3% of them are thought to be chronic carriers of the disease (Noorali *et al.*, 2008; Khan *et al.*, 2011) and the infection rate among population is getting worst (Muhammad et al., 2011). This may be due to unstable economic conditions or unhealthy life styles and facilities available to people as well as less awareness regarding the spreading of deadly communicable infectious diseases (HBV, HCV and HIV). We conducted the present study to observe the current patterns of different HBV strains prevailing in the Peshawar district of KP. It has been clarified from different studies that HBV genotypes may affect rates of HBeAg seroconversion, zero clearance of HBsAg, mutational patterns and development of hepatic disease including cirrhosis and development of HCC. Also different strains of HBV predominate in different several parts of the world therefore, heterogeneity of HBV genotypes may have a significant character in disease manifestations and its response towards antiviral drugs or therapy (Chu, 2002)

thus precise information about HBV genotypes is quite essential for the best handling and management of hepatitis B disease. Apart from Pakistan, different epidemiological studies of HBV genotypes described from Asia clarified that genotypes B and C among all genotypes of HBV are the predominant strains occurring in this region. The reason behind this is that majority of studies covering this topic have been conducted and published in China and Japan where it is being proved and concluded that genotypes B and C of HBV are the most dominant strains occurring here. Now presently it has been shown that all 7 HBV strains survive in Asia (Toan *et al.,* 2006). In India genotype A and D are the most prevailing and dominant genotypes (Thaku *et al.,* 2002) while genotype D has been identified in the neighboring country, Afghanistan (Amini *et al.,* 2006). The epidemiological studies related to different HBV strains in various countries of Asia proved the existence of all 7 HBV genotypes occurring in Asia, precisely the major predominant genotype D of HBV. In the current study we have successfully genotyped all of our samples and confirmed the presence of only two strains of HBV, that is genotype C and D with genotype D as the most predominant genotype in Peshawar. Similar patterns and distribution of genotypes were observed when compared to already reported findings that described genotype D of HBV as the most predominant genotype in Pakistan with a slight proportion of HBV genotype A (Noorali *et al.*, 2008, Mohammad *et al.,* 2007; Saeeda, 2009; Mohammad *et al.,* 2013; Majid *et al.,* 2004). Mixed or double infection of HBV genotypes A and D have been claimed by some studies conducted in Pakistan (Zunaira *et al.,* 2010; Zaigham *et al.,* 2006; Mohammad *et al.,* 2007; Saeeda, 2009; Mohammad *et al.,* 2013; Majid *et al.,* 2004). Genotype C identified in our study is believed to be associated and linked with more severe and complex liver diseases and has also been reported by previously published three studies as one of the most prevailing and dominant genotypes in Pakistan (Zunaira *et al.,* 2010; Muhammad *et al.,* 2013; Abbas *et al.,* 2001)Recently, ratios of HBV genotype B, E and F have also been found in a study conducted in Islamabad (Irum et al., 2009) while few previous studies also reported the existence of these genotypes in the Pakistani population (Zunaira *et al.,* 2010; Mohammad *et al.,* 2007) as the existing patterns of HBV genotypes distribution is still not clear so further study is needed that targets different regions of the country to confirm the accurate prevalence of HBV genotypes.

Conclusion

It has been clinched from results that strain D of HBV is the most prevailing genotype (90%) found in Peshawar followed by genotype C 10% while no other genotypes were detected. As genotype D demonstrates a meager and slow response to viral interferon therapy and usually genotype C is the root cause agent of severe liver infection as compare to other viral strains so genotyping of patients' blood samples should be mandatory before starting suitable treatment by practitioners.

Conflict of interest

There is no any conflict of interest among the authors.

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