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RESEARCH PAPER

TITLE

CIRCULATION OF HBV STRAINS AMONG HEPATITIS B CHRONIC PATIENTS IN DISTRICT MARDAN OF KHYBER PAKHTUNKHWA, PAKISTAN

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CIRCULATION OF HBV STRAINS AMONG HEPATITIS B CHRONIC PATIENTS IN DISTRICT MARDAN OF KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

Background: Hepatitis B is one of the major health issues around the world, triggered by the hepatitis B virus (HBV) and globally around 3.6 million of the world's populace is chronically diagnosed with HBV. Pakistan is highly endemic and an estimated prevalence rate of 3% has been recorded. Currently, it has been classified into ten diverse variants by the genetic divergence of more than 8% based on the whole genetic makeup of the virus. As different genotypes exhibit different pathogenicity and response towards the medication of HBV, the ongoing study was done with the purpose to inspect the prevailing epidemiological distribution of HBV strains in the Mardan district of Khyber Pakhtunkhwa (KP). **Material and Methods:** Samples were extracted from 700 chronically detected HBV patients and tested by ICT and further ratified for the presence of viral DNA by qualitative PCR. Samples were processed by type-specific PCR to pinpoint the prevalence of specific HBV genotypes. **Results:** All samples were found positive through qualitative PCR and the most predominant genotype identified was HBV type D (88.5%) trailed by genotype A of HBV (11.5%) while other genotypes were not detected in our study in district Mardan. **Conclusion:** The present study shows high

prevalence of HBV genotype D and further investigation is needed to understand both the genetic and geographical diversity of the virus in Mardan.

Key words: Hepatocellular carcinoma, HBV, PCR, genotype

1. Introduction

Despite the accessibility of the Hepatitis B vaccine and large immunization schemes, the disease caused by viral Hepatitis B is still a life-threatening health problem across the globe and around 400 million people are chronically infected while 900,000 deaths occur annually due to hepatic disease-related snags for example cirrhosis of the liver and hepatic cell carcinoma. HBV is a partly, rounded double-stranded (dsDNA) virus of approximately 3.2 kb size that has its place in the family named *Hepadnaviridae*, and is usually made up of major four ORF translating the (preS1, S2 and HBsAg) called envelope encoding genes, X (HBX) and polymerase (HBPol), respectively (Stuyver *et al.*, 2000; MacLachlan *et al.*, 2015; Steo *et al.*, 2018).

2. To-date, HBV has been categorized into 8 different variants A-H and two

new genotypes I and J have been identified recently based on more than 8% of sequence variance of its overall genome. Genotype A is worldwide dispersed and is the major root strain prevalent in European Community, Africa, India, and America in its northern region. Genotypes B and C of HBV have been frequently stated from the East and South-east divisions of Asia (Mahtab *et al.*, 2008). Genotype D is most prevalent in countries mostly including in Mediterranean and Middle East but has been testified across the globe, while genotype E usually exists in the western side or region of sub-Saharan Africa (Mulders *et al.*, 2004; Kramvis *et al.*, 2005). Genotype F typically circulates in Polynesia and US and G also in parts of the US and European Union while one of the presently detected variants H has been itemized from America (Kramvis *et al.*, 2005). Currently seemed one of the HBV strains I have been advised by experts to state as an infrequent variant of the virus, instigated from North region of Vietnam and Laos and just has been testified from the north-west side of both China and India (Huy *et al.*, 2008) and currently from Japan J has been reported (Kao, 2011).

3. Hepatitis B infection is highly endemic in Pakistan and around 9 million people are infected with HBV while 3% of the population is supposed to be chronic carriers (Farzana *et al.*, 2020; Hakim *et al.*, 2008; NoorAli *et al.*, 2008). In a project work supported by Awan and his colleagues where they

defined and explained that the C variant of HBV is one of the utmost currently emerging and evolving genotypes in Pakistani residents with a 27% of incidence rate, which is surely not a positive update regarding HBV as it is typically widespread in cirrhotic patients and reported to be connected with more crucial and dangerous liver infections (Awan *et al.*, 2010). Earlier reported data also stated that variant D has an advanced and more infection rate of liver disease and levels of viral DNA, and a very poor response to the most widely used IFN remedy as compared to other HBV genotypes A and B. This variant also possesses a precise form of genetic sequences that have quite a significant part in the prophecy of long-time-span response to lamivudine treatment (Abbas *et al.*, 2006). It has been specified that variant C of HBV have principally higher and more viral load as related to genotype B, however genotype D have revealed that it possesses and carries much more levels viral load than variant A of HBV (Oommen *et al.*, 2006). As HBV various strains have a dynamic role in development and process of infection and response to diverse antiviral drugs being usually used for viral treatment so prediction of precise and exact HBV strains and variants could be very valuable to pick and selecting a proper treatment in patients diagnosed from chronic hepatic related disease and infection. So, this study is designed with the goal to investigate the prevailing and existing forms of

hepatitis B viral genotypes circulating in the Mardan region of KP as previously no study has been reported regarding HBV genotypes from this part of the province.

4. Materials and Methods

5. Samples collection

6. A total of 700 HBV-positive blood specimens were together and collected from different healthcare units located in Mardan including Mardan Medical Complex and DHQ hospital Mardan. All infected HbsAg-positive patients were registered in the ongoing study while those infected with additional 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 occurrence of HBsAg by ICT (Abbott Lab, US).

7. Extraction of HBV DNA

8. DNA was mined from 200µl of each sample using a viral QIA amp DNA kit (QIA gene GmbH, Hillden, Germany) which is designed for rapid extraction of highly pure nucleic acid of biological samples.

9. Qualitative Detection of HBV DNA and type-specific PCR

10.
11. To diagnose the ongoing active viral HBV infection, all samples were tested serologically by immune chromatography (ICT) and further investigated through PCR for confirmation of infection. Qualitative detection of samples were executed by 2 different step PCR reaction that have

previously described [16] which is sensitive and specific method in comparison to other serological procedures. Two rounds or disks of PCR were preferred and performed for increasing the specificity and sensitivity as well as precision. All the reactions of PCR were performed by means of both positive and negative controls. For the identification of specific HBV genotypes, type-specific PCR was performed for each positive sample as described earlier (Farazmandfar et al., 2012).

12. Results

13. A total 700 chronic HBV patients including 389 males and 311 females between ages 10-85 years were analyzed in this study. 364 (52%) samples were noticed positive for the occurrence of HBsAg (male: 327, female: 37) by ICT while HBV DNA was screened and present in all samples (table 1).

14. Table1: HBsAg positive samples

S.No	Gender	HBsAg positive	HBV DNA
1	Male	327 (90%)	389 (55.57%)
2	Female	37 (10%)	311 (44.42%)
3	Total	364 52%	700 (100%)

15.
16. All samples showed successful genotype-specific bands with genotype D as the most prevalent genotype 88.5% in this region followed by HBV genotype A 11.5%. No mixed infection and other genotypes were detected in

our study in district Mardan. Gender-wise distribution of HBV genotypes exhibited a high frequency of genotype D in female (92.28%) as compared to male patients (85.60%) while the ratio of variant A remained high in males (14.39%) as compared to females (7.71%) (table 2)

17. Table 2: Gender-wise distribution of HBV strains in chronically HBV patients

18.

19. Discussion

20. The present study was conducted to investigate the existing patterns of HBV genotypes circulating in the Mardan district of KP as earlier no study has been reported from this part of the province. It has been demonstrated that HBV genotypes may influence rates of HBeAg seroconversion, sero-clearance of HBsAg, mutational patterns and progression of liver-related diseases including cirrhosis and establishment of HCC. Also, different genotypes of HBV predominate in various regions and areas of the world therefore, heterogeneity of HBV genotypes may have a significant part in disease manifestations and response to antiviral therapy (Chu *et al.*, 2002) thus accurate and precise knowledge of HBV variance is quite compulsory for the proper handling of disease. Apart from Pakistan, different studies regarding the prevalence of HBV genotypes from diverse regions of Asia exposed that B and C are widely circulating strains of HBV in this

region. One of the reasons for such an existing situation may be that most of the studies were described from Japan and China where these both HBV strains are the most predominant. Here and now, it has been exposed that all of the seven HBV genotypes can be seen in Asia (Toan *et al.*, 2006). Genotype A and genotype D of HBV are the most recurrently prevailing strains in India (Thakur *et al.*, 2002) while HBV genotype D is in the neighboring

Gender	Genotype A	Genotype D
Male	56 (14.39%)	333 (85.60%)
Female	24 (7.71%)	287 (92.28%)
Total	80 (11.42%)	620 (88.57%)

country Afghanistan (Amini *et al.*, 2006). The epidemiological data regarding different HBV strains in various Asian regions approved the occurrence of all seven genotypes mingling in Asia, explicitly the most widespread type of genotype D. In our study we have successfully genotyped all our samples and confirmed the presence of only two strains of HBV, genotype A and D with D genotype being most predominant and prevalent genotype in Mardan. Similar patterns and distribution of genotypes were observed in previously reported findings describing genotype D of HBV as the most predominant genotype in Pakistan with a slight proportion of HBV genotype A (NoorAli *et al.*, 2008; Alam *et al.*, 2007; Baig *et al.*, 2009; Hanif *et al.*, 2013; Mahmood *et al.*, 2016). Mixed infection of HBV genotypes A and D has also been claimed by some studies

conducted in Pakistan (Awan *et al.*, 2010; Abbas *et al.*, 2006; Alam *et al.*, 2007; Baig *et al.*, 2009; Hanif *et al.*, 2013; Mahmood *et al.*, 2016). Genotype C which is believed to be associated and linked with the more complex situation of liver disease has also been reported by previous studies as one of the most prevalent genotypes in Pakistan (Awan *et al.*, 2010; Idrees *et al.*, 2004; Abbas *et al.*, 2001). Low ratios of HBV genotypes B, E and F have also been found in a recent study conducted in Islamabad, capital of Pakistan (Masood *et al.*, 2019) while a few studies had also reported the existence of these genotypes in Pakistani population (Awan *et al.*, 2010; Alam *et al.*, 2007). Since the scenario of HBV genotypes distribution in Pakistan is still unclear therefor, further studies covering different regions of the country to describe accurate prevalence of HBV genotypes are needed.

21. Conclusion

22. This is among the very few studies that provide a detail description of the prevalence of HBV strains in Mardan the second big city of KP. Our results showed that HBV genotype D is the most predominant genotype (85.5%) in this region followed by genotype A (11.5%) while no other genotypes were found in this study. As genotype D possess a meager and slow response as compared to other genotypes to interferon antiviral therapy so genotyping of patient samples must be

made compulsory by physicians before initiating the prescribed treatment.

23. Conflicts of interest

24. No conflict of interest exists among the authors.

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