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Research Article

Hepatitis B-virus Genotype D is Prevalent in a Multiethnic Population of Karachi, Pakistan

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Abstract

The Hepatitis B virus (HBV) remains a major cause of chronic hepatitis and hepatocellular carcinoma (HCC) globally. HBV is broadly classified into ten genotypes (A-J). Each genotype is distinctive due to its geographical distribution, response to certain treatments, and association with disease severity. The investigation of HBV genotypes is worthwhile as it will help predict patient prognosis and ensure effective treatment. Therefore, this study aimed to determine the prevalent HBV genotypes in the multiethnic population of Karachi, Pakistan. A total of 206 patients were enrolled in the study from January 2011 to April 2016. Of the 206 patients that we investigated, a total of 159 patients were positive for HBV infection. Furthermore, genotyping analysis of the positive samples revealed that the most common genotypes were HBV D (59% samples (n=94)), co-infection with genotype A & D (22% (n=35)) and genotype A (16% (n=26)). Most of the HBV-positive patients were males between 21 to 40 years of age. As genotype D is associated with worse outcomes, it is imperative that HBV awareness programs are launched to prevent the further spread of infections in this densely populated city.

Keywords: Chronic hepatitis, HBV genotypes, hepatocellular carcinoma, Pakistan

1. Introduction

Hepatitis B virus (HBV) is a major cause of viral hepatitis worldwide (GBD 2013 Mortality and Causes of Death Collaborators, 2015; Polaris Observatory Collaborators, 2018). Up to 40% of HBV-infected patients can go on to develop serious complications such as cirrhosis, hepatic decompensation, and the formation of hepatocellular carcinoma (Lim et al., 2020). The WHO estimated that in 2019, 296 million people worldwide were living with HBV infection and 1.5 million people get newly infected each year (*Global Progress Report on HIV, Viral Hepatitis and Sexually Transmitted Infections, 2021*). The highest prevalence of chronic HBV infection was reported in the Western Pacific and African regions, while Europe had the lowest reported prevalence (Sheena et al., 2022). One highly endemic country is Pakistan; the actual exposure rate is unknown, but the prevalence is estimated to be 9 million infections, with 3-5% carriers of chronic HBV (Ali et al., 2011). The regional prevalence of HBV ranges between 7 to 20% among the different areas of Pakistan. In rural areas, the prevalence of HBV is found to be higher (2-5%) than in urban areas (ul Haq et al., 2013).

The major risk factors for HBV transmission include a lack of awareness of how HBV is acquired, blood transfusions without prior testing,

poor economic conditions, intravenous drug abuse, sharing and reusing syringes, tattooing and piercing of body parts, inadequate sanitization of surgical equipment and vertical transmission from infected mother to child (Ali et al., 2011).

HBV is a partially double-stranded DNA virus that belongs to the *Hepadnaviridae* family. Its genome is 3.2 kb in length, and it has four open reading frames (ORF) (Karayiannis, 2017). The mutation rate of HBV is higher in comparison with other DNA viruses because its reverse transcriptase, which is used during viral replication, lacks proofreading ability. The higher mutation rate allows it to evade antiviral therapy and persist as a chronic infection (Jeong et al., 2021). Chronic infection with HBV and various selective pressures can cause the emergence of different HBV variants. Due to this variability in its genome, as well as its ability to replicate itself rapidly, HBV can adapt to the hosts it infects (Pourkarim et al., 2014). Using a difference of 8% in the viral genome, phylogenetic analysis classifies HBV into ten genotypes with many sub-genotypes (A to J)(Al-Sadeq et al., 2019). It has been established that the genotype has an impact on the course and outcome of the disease as well as the response to treatment with IFN therapy (Fletcher et al., 2020).

In Western countries with low prevalence, HBV transmission occurs mostly in young adults due to sexual or percutaneous transmission in high-risk groups such as drug users, dialysis patients, and healthcare workers. On the other hand, in regions with high prevalence, such as China, Southeast Asia, and the Middle East, the infection is mostly acquired during infancy or childhood via vertical transmission from infected mothers (Inoue & Tanaka, 2016).

Previous studies have shown that vertical transmission is mostly associated with HBV genotypes B and C infections (Komatsu et al., 2015). Conversely, HBV infections associated with nosocomial spread, intravenous drug injections, tattooing, and blood transmissions are more commonly linked with HBV genotypes A and D (Pourkarim et al., 2014; Tamada et al., 2012), whereas genotype G is associated with men who have sex with men (MSM) (Cornelissen et al., 2016; Pourkarim et al., 2014).

The various genotypes of this virus have their own geographical distributions. According to multiple studies, genotype A is prevalent in Africa and Europe, genotypes B and C are common in Eastern and Southeastern Asia (Sunbul, 2014), and genotype D is widespread in the Mediterranean region, Western Asia, and the Middle East (Kramvis et al., 2005). Moreover, genotype E is confined to West Africa (Mulders et al., 2004), while genotype F is restricted to Central America. Lastly, genotype G is found in the United States, France, Colombia, and Brazil, and genotype H is prevalent in Central and North America (de Sousa et al., 2018). The more recent genotypes I and J have been reported in Vietnam and Japan respectively (Sunbul, 2014). In Pakistan, the most common genotypes in descending order of frequency are D, mixed D, and A infections, and then A. Genotypes B, C, E, and F were seen only in 1.5% of the cases (Mahmood et al., 2016).

One city in Pakistan, Karachi, is one of the most populated cities in the world and home to people of multiple ethnicities and professions (Ahmed et al., 2019). Epidemiological data on the prevalence of HBV genotypes in this densely populated city will help the authorities to curb the further spread of infections. It will also allow physicians to provide better treatment. Hence, this study was carried out to analyze the current prevalent genotypes of HBV present in Karachi, Pakistan.

1. Materials and Methods

A total of 206 patients were enrolled in the study after taking their informed consent. Serum samples were received and analyzed at the Molecular Pathology section of Dow Diagnostic Reference & Research Laboratory (DDRRL), Dow University of Health Sciences, from January 2011

to April 2016. Data regarding age and gender were also collected.

2.1 DNA Extraction from Serum Samples

HBV DNA extraction was performed from 200µl of serum sample using QiaAmp Mini DNA Kit (Qiagen, Germany) according to the manufacturer's protocols. The DNA was eluted in 80 µl of elution buffer and stored at -80 ºC. Extracted DNA was subjected to Real-time PCR by using Artus HBV PCR Kit (Qiagen, Germany) following the manufacturer's instructions. Amplification was performed in the Rotorgene Realtime PCR instrument, programmed as 1 cycle of 95 °C for 10 min followed by 45 cycles which involves denaturation at 95 °C for 20 sec, extension, and annealing at 60°C for 60 sec. The lower limit of this assay was 3.8 IU/ml.

2.2 HBV Genotype Analysis

All HBV DNA-positive samples were subjected to nested PCR using a commercially available HBV Genotyping Kit (Genekam, Germany) according to the manufacturer's instructions. Nested PCR is a two-step PCR in which the first-round product is used as a template for second-round PCR. Outer primer pairs and inner primer pairs were designed for the most conserved region of the HBV genome.

2.2a Step- 1 PCR

The first round of PCR was conducted in 20ul of reaction mixture containing 2 µl of DNA, 10 µl of buffer, and eight µl of primers and then placed on thermocycler programmed at 95°C for 10min, followed by 40 cycles consisting of 94 °C for 20s, 55°C for 20s, and 72°C for 1 min. This product used inner primers as a template for the second round.

2.2b Step-2 PCR

Two second-round PCRs were performed for each sample with two different PCR mixes. Mix A had primers for types A to C, whereas mix B had primers for types D to F. 1ul of first-round PCR product was added to two tubes containing the inner primer pairs, PCR buffer, and Taq DNA polymerase. The reaction was programmed at 95°C for 10 min followed by 20 cycles consisting 94°C for 20s, 58°C for 20s, and 72°C for 30s. 20 cycles consisting, 94 °C for 20s, 60 °C for 20s, and 72°C for 3s. The PCR products were run on 2% agarose gel.

The HBV genotypes for each sample were identified by their specific DNA bands as seen on an agarose gel stained with ethidium bromide, using 50 bp ladder (Fermantas DNA ladder) under a UV-transilluminator (Figure 1).

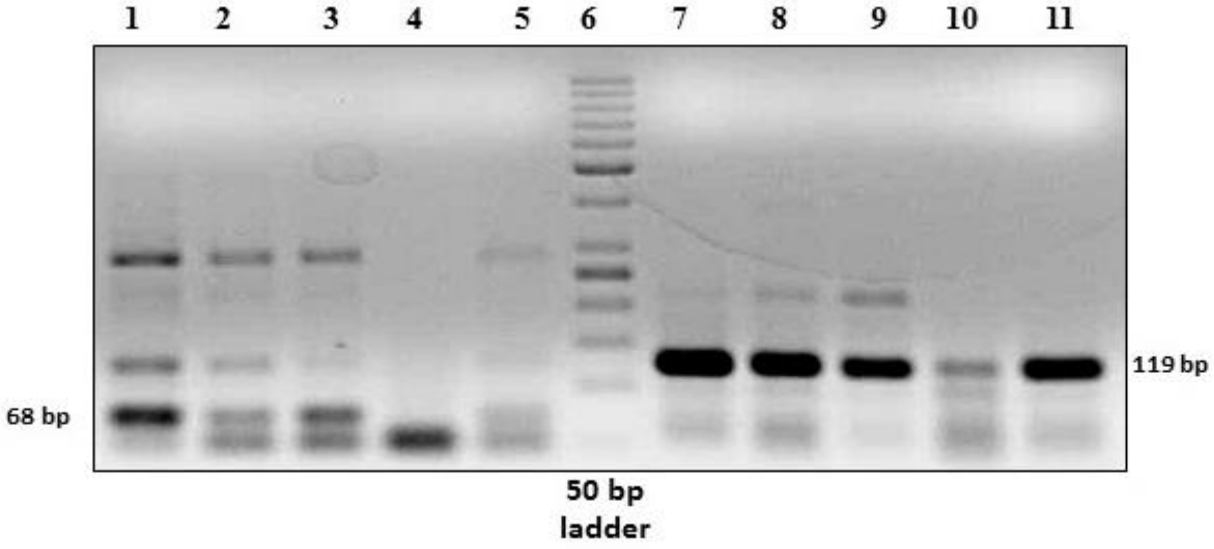

Figure 1. 2% agarose gel electrophoresis where lane 6 shows 50 bp DNA ladder, lanes 1, 2, and 3 show co-infection of A and D, lane 4 shows genotype A, and lanes 7, 8, 9, and 11 show genotype D.

2.3 Statistical Analysis

The descriptive statistics were reported using SPSS statistics 21. The prevalence of HDV was shown as percentages.

2. Results

A total of 206 patients were recruited in the study. 47 were negative for HBV DNA, while 159 were found to be positive for HBV infection. Among these, most (65%) were male. There were more males than females if seen separately within each age group as well. The ages of the participants ranged from 10 to 70 years, with a median age of 30 years. Based on the data collected, patients were divided into four age groups: 1-60 years old, 21 to 40 years old, 41 to 60, and over 60 years of age. Most (n=96) of the HBV-infected patients were between 21 to 40 years old (Figure 2). Out of 159 positive samples, 59% of patients had genotype D, 22% showed co-infection of genotype A & D, 16% had genotype A, while in 3% of cases, genotypes were un-typable by this method (Figure 3).

3. Discussion

HBV is one of the major health problems faced worldwide by children and adults of all ages (Lim et al., 2020; Sheena et al., 2022). Several studies have attempted to delineate its geographical distribution as well as its genotypic prevalence. This is because awareness of the genotype aids in better comprehension of the course and likely outcome of the disease. The way by which the virus was likely acquired and the pathologic features of the disease also vary by genotype (Sunbul, 2014). It is also helpful to know the genotype since it allows for the monitoring of the disease and the provision of efficient treatment, especially in those at risk of developing hepatocellular carcinoma (GUVENIR & ARIKAN, 2020).

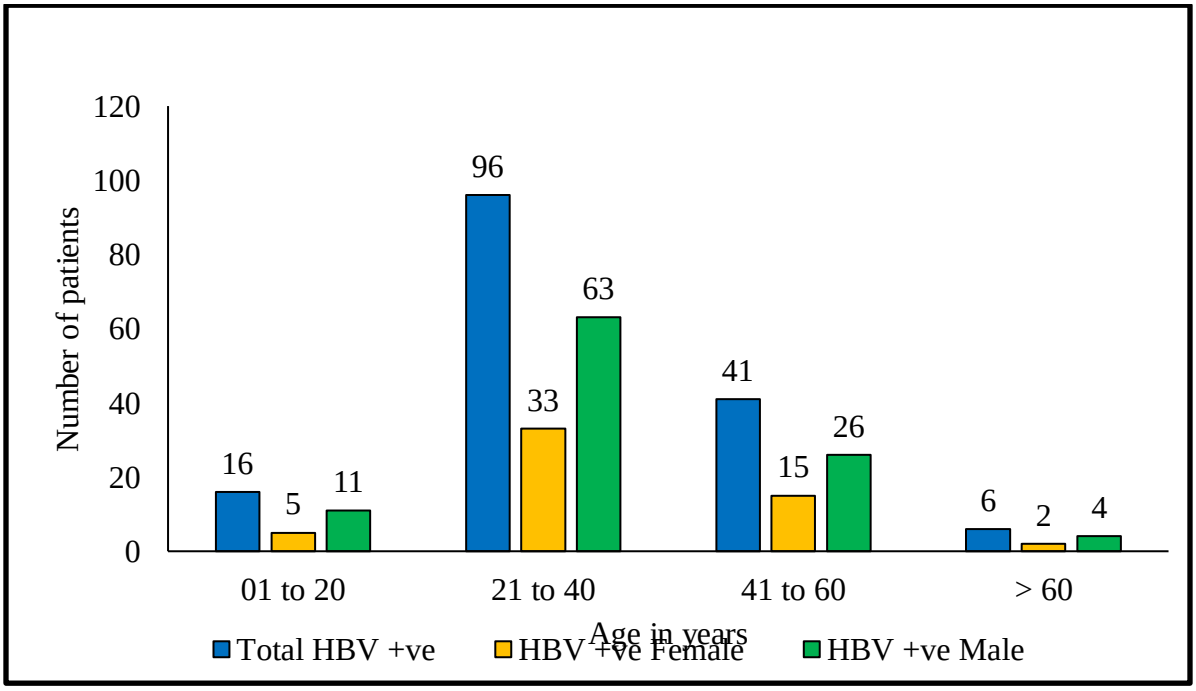

Figure 2. The gender and age-wise distribution of HBV positive patients.

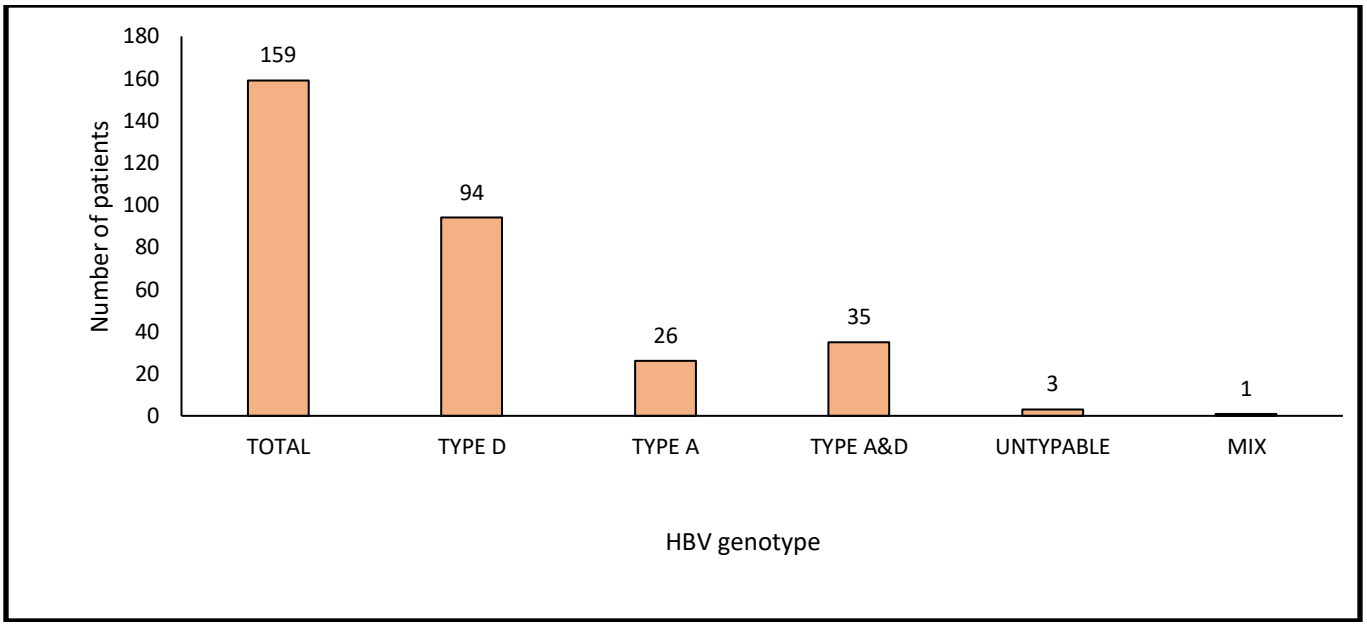

Figure 3. The number of patients with each genotype of HBV.

This study was carried out to identify which HBV genotypes are prevalent in the city of Karachi, Pakistan. It was found that the most prevalent HBV genotypes in Karachi were genotype D (59%), followed by A and D (22%) and genotype A (16%). This is in agreement with other studies that suggested that genotype D is the most common one in Pakistan (Ali et al., 2011; Mahmood et al., 2016).

More than half of the study participants that tested positive for HBV were male. Even after separating HBV-positive patients into four age groups, the males outnumbered females in all groups. This gender disparity has also been reported in previous studies (Ayano et al., 2018; Baig, 2009). *Awan et al*. also reported a higher percentage of males (58.3%) affected by HBV than females (41.7%). This consistently higher infection rate in males may be explained by the higher risk of exposure men face. The social, cultural, and religious influences present in Pakistan mean males are employed and visit barbershops etc., more frequently, whereas, in comparison, females tend to stay home more.

Moreover, most of the HBV-positive patients in this study were 21 to 40 years old. Another study conducted in the province of Punjab in Pakistan also reported that the highest HBV positivity was in those aged 21 to 40 years, whereas the prevalence of infections in the very young or elderly was only 1.49% and 1.65%, respectively (Punjabi et al., 2016). This supports the notion that most HBV infections in Pakistan are present in young adults, perhaps again due to greater exposure in this age group as compared to the others.

The geographical pattern of HBV genotypes correlates with their different modes of transmission. Studies have shown that genotypes B and C spread via vertical transmission, genotypes A and D transmit via body piercing/ tattooing, reuse of syringes, blood transfusion, unprotected sex, and poor facilities for hair cutting, dental clinic etc., whereas genotype G is transmitted mainly among men who have sex with men (Datta, 2008; Schaefer, 2005). Furthermore, occupation, lifestyle, socioeconomic status, and cultural attitudes in various ethnic groups significantly influence the route of HBV transmission (Smolle et al., 2012). Pakistan is a country where poor healthcare facilities exist, and sharing of needles, blood transfusion without

prior testing, unprotected sex, and lack of awareness of HBV transmission and HBV vaccination are all common; these might be some of the reasons that explain the prevalence of HBV genotypes A and D in Karachi.

Various studies have been carried out in different parts of Pakistan to identify the country's most prevalent genotypes of HBV. According to one such study, genotype B is found in Punjab and Baluchistan, genotype C in Khyber Pakhtunkhwa, and genotype A is seen in Sindh (Awan et al., 2010). However, the most common one found in Pakistan is genotype D (Harris et al., 2017; Masood et al., 2019). This is concerning because genotype D is known to have a higher association with serious consequences, such as the development of cirrhosis and hepatocellular carcinoma (Fletcher et al., 2020; Thakur et al., 2002). Hence, there is an urgent need to increase awareness and control the further spread of HBV infections in Pakistan.

4. Conclusion

Genotype D is the predominant HBV genotype in Karachi, Pakistan, followed by co-infection with genotypes A and D. Genotype D is mostly spread through horizontal transmission routes and is associated with more severe outcomes, so people must be aware of how it can be acquired. Karachi is a large city with people from various ethnic groups and belonging to different occupations; therefore, HBV awareness programs should be organized urgently so that people take precautionary measures and are more careful when going about their lives. Suitable treatment and proper diagnostic facilities should also be set up to reduce complications and mortality from HBV infections.

Conflict of Interest

All contributing authors declare no conflicts of interest.

Funding

The study did not receive any external funding.

Study Approval

This study was approved by the Institutional Review Board of the Dow University of Health Sciences (IRB-339/DUHS-12).

Consent Forms

Yes, consent was taken from the participants.

Authors Contribution

FK, MZ conceptualized the study and wrote the final manuscript, MZ, AL helped in the analysis and writing the first draft, did the experimental analysis, and NR supervised the whole project and wrote the final manuscript.

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