

# FREQUENCY OF HEPATITIS B VIRUS GENOTYPES IN HBsAg POSITIVE PATIENTS IN KHYBER PAKHTUNKHWA

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## ABSTRACT

**Objective:** To determine the different Hepatitis B Virus (HBV) genotypes in HBsAg positive patients .

**Material and Methods:** One hundred and forty three HBsAg positive patients were included in the study. All patients were anti-HCV and anti-HIV negative. All the samples were confirmed for HBV DNA with Rotor Gene™ Real Time Quantitative PCR while HBV DNA positive samples were subjected to type specific PCR for HBV genotyping (A-F).

**Results:** Of 143 samples, 107 (74.82%) were male and 36(25.17%) female, age ranged between 20-40 years. The study demonstrated genotype D in 72 (50.34%) patients as the predominant genotype in KPK population. Genotype B was observed in 37 (25.87%), genotype A in 24 (16.78%) and both genotypes B and D in 10 (6.99%) patients. Genotype C, E and F were not found in any patient.

**Conclusion:** Our study has demonstrated a heterogeneous distribution of HBV genotypes. More extensive studies are required to investigate genetic and geographical divergence and characteristics of the virus in Khyber Pakhtunkhwa (KP).

**Key Words:** Hepatitis B Virus, Hepatitis B Genotypes.

## INTRODUCTION

Hepatitis B Virus(HBV) infection is endemic in many parts of the world. HBV infects nearly 2 billion people worldwide, 400 million of which are chronic carriers and causes approximately 600,000 deaths annually due to acute and chronic consequences<sup>1</sup>. According to WHO, Pakistan falls into the endemic region being a 3% HBV infected country with multiethnic population<sup>2</sup>. HBV is a crucial public health problem in our country and infection rate is increasing day by day. The reason may be lack of proper health facilities or poor economic status and insufficient public awareness about transmission of major communicable diseases including Hepatitis B virus<sup>3</sup>.

The outcome of infection depends upon many factors like host immune status, age at infection, level of viral replication and probably the genetic variability of virus influencing the expression of viral antigens. However, the impact of natural variability of virus on the clinical course of disease has become the focus of research recently<sup>4</sup>.

HBV is classified into eight genotypes from A to H based on an intra-group nucleotide divergence of

the 5-genome sequences or in >8% of the entire genome sequences<sup>5</sup>, which consists of about 3200 base pairs<sup>6</sup>. These genotypes arise during replication as a result of nucleotide mis-incorporation, in the absence of any proof reading capacity by the viral polymerase<sup>7</sup>. HBV exhibits genetic variability with an estimated rate of 1.4-3.2 x 10<sup>5</sup> nucleotide substitution per site per year, which resulted in well recognized sub-type of virus<sup>8</sup>.

A greater demand for genotype of patient strains of HBV is growing as specific clinical associations with each genotype become increasingly apparent<sup>6</sup>. Recent studies demonstrated that a particular genotype may affect clinical manifestations during the course of the disease. HBV genotypic determination is of particular importance for the study of the detection of the virus origin, course of evaluating HBV, the severity and activity of liver disease<sup>9,7,9</sup>, patterns of serological reactivity and replication of the virus, prognosis and response to antiviral treatment<sup>10</sup>. As patients affected by genotype A have a better prognosis, genotype B enhances the possibility of hepatic malignancy, genotype C is associated with faster liver damage than genotype B<sup>11</sup>, alanine transaminase level and the index of inflammatory cellular necrosis in the genotype C is higher than in genotype B, genotype D may develop fulminant hepatitis with high frequency, may be more associated with liver cirrhosis compared to A, whereas genotype F is found to be associated with severe infection and young hepatocellular carcinoma development which is found to be

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associated with higher mortality rates as compared to other genotypes. So this study was conducted to determine and analyze the distribution of HBV genotypes among patients with hepatitis B surface antigen, in Khyber Pakhtunkhwa (KP), for better assessment of severity of complications and of disease.

## MATERIAL AND METHODS

A total of 143 patients with HBsAg found positive by 3<sup>rd</sup> Generation ELISA and further confirmed by Rotor Gene™ Real Time Quantitative PCR, were included in this study. Written informed consent was taken and approval by the hospital ethical committee was obtained. All the blood samples were collected from the patients attending Medical Outpatients Department in Khyber Teaching Hospital, Peshawar and Rehman Medical Institute, Peshawar, Khyber Pakhtunkhwa (KP) which were later analyzed at Ibrahim Hospital, Dabgari Gardens, Peshawar for HBV Genotyping. Patients having added Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) Infection were excluded from the study. Also excluded were the patients having domiciles outside Khyber Pakhtunkhwa (KP).

## RESULTS

Of the total 143 samples, 107 (74.82%) were male and 36(25.17%) female, age ranged between 20-40 years. This study demonstrated that genotype D in 72 (50.34%) patients is the predominant genotype circulating in KPK population. Genotype B was observed in 37 patients (25.87%) followed by genotype A in 24 (16.78%), both genotype B and D in 10 (6.99%) patients. Genotype C, E and F were not found in any patient.

## DISCUSSION

Hepatitis B virus (HBV) infection is a global health problem with its continuously increasing morbidity and mortality in the developing countries like Pakistan. Despite the presence of Hepatitis B vaccine, new Hepatitis B virus infection remains common worldwide. Of 350-400 million people who are estimated to be persistently infected with hepatitis B virus, three quarters reside in Asia<sup>12</sup>.

This study demonstrates that Hepatitis B virus genotype D is the main genotype circulating in the affected population of Khyber Pakhtunkhwa (KP). Previously, several studies have been conducted which report Pakistan among many regions with immediate to high HBV sero-prevalence, with D being the most prevalent genotype<sup>13</sup>. Previous studies have shown that distribution of genotype / HBV have clear geographical characters. The pattern of genotype prevalence found through this study is in line with the

study from south East Asia, especially the countries sharing borders with Pakistan and confirms the high proportion of genotype D in South Asia<sup>14</sup>.

HBV genotypes have different biological and epidemiological behaviour. Since they influence the activity and outcome of HBV- associated chronic liver disease, as well as the response to anti viral therapies, their detection and monitoring is more than just academic but also medically significant. Therefore, HBV genotype should become a routine exercise in clinical medicine and molecular epidemiology. Detection of HBV genotypes is also very important to clarify the pathogenesis, route of infection and virulence of the virus.

Reports have shown that genotype A is most common in Northwest Europe and United States, that genotype D is most frequently found in the Mediterranean region, and that genotype F predominates in Central and South America. Genotype E, G, H have undefined characteristics of geographical distribution. Just like the genotypes, the distribution of HBV sub genotypes is also distinctly geographic<sup>16,17</sup>. This study will provide useful information for treatment and management of HBV infection in KPK, where no such molecular based study of the disease has been done before. An epidemiologic study with sufficient participants in a wide age range is necessary to address this issue.

This current study reveals that males are more frequently positive for HBV infection as compared to females. Also HBV infection is found to be significantly higher in persons with age between 20-40 years. The reason may be that men are more exposed to the risk factors. In Khyber Pakhtunkhwa (KP), people are less educated and there is poor awareness about the infection and especially its mode of spread. Males mostly work outside their homes or in agricultural lands while women are mostly involved in household activities due to Pukhtoon culture, traditional and religious preference and influences, therefore, it is well understood to analyse the difference of frequency of infection among the gender category. Males visiting barber shops, donating blood and IV drug abuse increases their risk of getting infection.

The 143 samples analysed for genotyping indicated the highest prevalence of genotype D 72 (50.34%) followed by genotype B and A. Genotype C, E and F were not found in any patient indicating absence of these strains in the region, but genotype B and C are more prevalent in Japan and China. A study by Toan et al. reveals that all seven HBV genotypes can be found in Asia<sup>19</sup>. The predominant HBV infection in Afghanistan was found to be genotype D. The epidemiological data about HBV genotypes in various Asian Countries revealed the presence of all seven genotype in Asia, particularly the pre-dominance

of genotype D<sup>20</sup>. In our study 6.99% patients were infected with genotype B & D. Genotype mixture in HBV patients is also common in Thailand.

## CONCLUSION

We have reported the various genotypes prevalent in Khyber Pakhtunkhwa (KP) for the first time and also the patients infected with more than one HBV genotype. Large multicenter trials across the province are needed for better understanding of the various HBV genotypes prevalent in local population.

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