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Research Article

DETERMINATION OF VARIOUS HEPATITIS B VIRUS  
GENOTYPES BY USING GENOTYPE SPECIFIC PRIMERS  
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**Abstract:****Aim:** To investigate the frequency and clinical correlation of hepatitis B for common genotypes with PCR.**Place and Duration:** In the Gastroenterology department of District Headquarter Hospital Sargodha for one year duration from February 2019 to February 2020.**Methods:** The HBV genotype was determined on the basis of 206 serum samples from patients with chronic HBV infection. DNA was extracted from HBsAg and HbeAg positive cases, and their genotypes were determined using species-specific primers.**Results:** All genotypes were specified in patients except genotype E. Genotypes B, C and A, were the predominant ones, appearing in 48 samples (23.3%), 57 samples (27.6%) and 43(20.8%), while genotypes, D and F were appearing in 21(10.1%) and 4(2%) of samples, respectively. However, 33(16.2%) samples were determined as mixed genotype. At the beginning of the study, patients infected with HBV genotype C were generally younger with female dominance at the border. HbeAg to anti-Hbe seroconversion occurs much earlier in genotype B than in carriers of genotype C. Higher levels of HBV DNA were detected in patients infected with genotype C compared to four other genotypes.**Conclusion:** HBV genotype plays an important role in predicting response to various therapies and should be treated as a variable before starting any treatment. Therefore, more detailed research is needed in regions where HBV is hyperendemic.**Key words:** genotype, hepatitis B, hepatocellular carcinoma, liver enzymes, PCR, primers, viruses.**Corresponding author:****Dr. Asma Aslam,**

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## INTRODUCTION:

The hepatitis B virus (HBV) is an important health problem worldwide and places a heavy burden on the healthcare system and an important source of patient suffering. The hepatitis B virus (HBV) is one of the leading etiological factors of acute and chronic liver disease in the world, including lethal fulminant hepatitis, cirrhosis, and is a major cause of hepatocellular carcinoma, one of the cancers<sup>1-2</sup>. Chronic hepatitis B is a serious clinical problem in Pakistan, and over 350 million people worldwide are chronic carriers of the virus<sup>3-4</sup>. HBV is divided into seven genotypes based on phylogenetic analysis of genomic sequences<sup>5</sup>. The first four genotypes (A, D genotypes) were first identified by Okamoto and colleagues. Six years later, two additional genotypes (E, F genotypes) and G and H and genotypes were identified based on nucleotide variants greater than 8% in the genome. Infection is associated with a wide clinical spectrum, from acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carrier status, chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). An estimated 4.5 million people are carriers in Pakistan and Pakistan remains a carrier of 3-4% of HBV coverage<sup>6-7</sup>. The study was conducted to obtain a clear view on the prevalence of HBV infection and the distribution of genotypes in acute and chronic liver disease. We started a study to investigate common HBV genotypes in Pakistan. To this end, we use a species-specific genotyping system, but although nucleotide sequence analysis and subsequent phylogenetic analysis are the result of more reliable and safe genotyping, this is not a suitable large-scale genotyping method<sup>8-9</sup>.

## MATERIALS AND METHODS:

This study was held in the Gastroenterology department of District Headquarter Hospital Sargodha for one year duration from February 2019 to February 2020. The total number of blood samples (n = 1204) for the hepatitis B virus surface antigen (HbsAg) was collected to detect different HBV genotypes. N = 206 HBV DNA positive serum samples were used to assess HBV genotyping. HbsAg was performed by ELISA (DRG Diagnostic, Germany). HBV DNA was extracted from 200 µl serum samples using the Gentra DNA Extraction Kit (Puregene DNA D-5000, Gentra Systems, Minnesota, USA) according to the kit protocol. The extracted DNA pellet was resuspended in DNA hydration solution present in the kit and two rounds of PCR amplifications where the second round primers was hemi nested to the first round PCR at primers were designed for S gene. The typical amplification was performed in 20 µl reaction volume to extracted DNA and Taq polymerase for 35 cycles at 94 °C for 45 seconds, 55 °C for 45 seconds and 72 °C for 1 minute with extension for 10 minutes at 72 °C. All measures were taken

to prevent contamination during PCR, and negative and positive control serum was included in each study. All HBV PCR positive samples were selected for genotype analysis. DNA genotypes HBV A to H were determined using mild modification using HBV genotype specific primers for the Hbs Ag surface gene. In the first round of PCR amplification, the primers used were external primer pairs, and in the second round, internal primer pairs were used depending on the protected nature of the nucleotide sequences in the regions between the S1 to S genes. Regardless of the eight HBV genotypes, two P1 (sense) and S1-2 primers (antisense) were universal external primers (1063 bp). B2 primer was used as an internal (sensory) primer with a combination of mix 1 for genotypes A, B and C. B2R primer was used as an internal (antisense) primer with a combination of mix 2 for D, E and genotypes. Mixture 3 contained a universal primer specific primer specific for G and H genotypes. These primer combinations for the second round of PCR were designed based on differences in genotype dimensions. The first PCR was carried out in a tube containing 20 µl of a reaction mixture made up of the following components: 10pmol of each outer primers, 500 µM of four deoxynucleotides, 2U of Taq polymerase (Promega, USA), 10 x PCR buffer containing 1.5mM Mg Cl<sub>2</sub>. The thermal cycler (Master Gradient PCR System, Eppendorf AG, Germany) was programmed to first incubate the sample for 2minutes for 95°C followed by 35 cycles consisting of 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 1 and half minute with final extension for 7 minutes at 72°C. A second PCR was performed for each sample with a mixture of B2 common sense primers and A to C primers and a universal B2R antisense primer, and 2 primers containing types D to F were mixed. The HBV genotypes for each sample were determined by identifying genotype specific DNA bands. Two different second-round PCR products from one sample were individually electrophoresed on a 2% agarose gel, stained with ethidium bromide and evaluated with a transilluminator. The dimensions of the PCR product were estimated based on the 50 bp DNA ladder migration model (Gibco BRL, Life Technologies). It enables the specific detection of PCR products for mix I, type A, B and C, allows the detection of mix types II, D, E and F. Identification of the different HBV genotypes found in our study.

## RESULTS:

The clinical course of HBV infection varies depending on the age of the patients and the immune response, and on the other hand the condition of the disease was based on the results of the ELISA test and divided into different infectious conditions. The total number of samples to detect HbsAg is n = 1204; where n = 829, negative cases and 375 are positive cases, as shown in Table 1.

**Table 1: Active HbsAg infection in ELISA Positive Male & Female patients (n=1204)**

	Male	Female	Total
No. of samples	721	483	1204
Negative	231	360	519
Positive	252	123	375
Percentage	34.95	25.46	31.15

PCR was performed in all cases of Hbs Ag positive and only (n = 205) HBV DNA, Table 2. In the genotype study, all HBV DNA positive cases were analyzed in to determine the HBV genotype using central site specific primers. Data were evaluated and analyzed that genotypes A, B and C are dominant and genotypes D and F in Pakistan only occur in four cases. During this study, G and H genotypes were not identified.

**Table 2. HBV-DNA Detection by PCR done on positive male & female patients (n=375)**

	Male	Female	Total
No. of samples	252	123	375
Negative	135	35	170
Positive	117	88	205
Percentage	46.43	71.54	54.93

### DISCUSSION:

Viruses have traditionally been classified according to antigenic properties, but with the latest advances in molecular biology, genotypic classification through genomic variation analysis. HBV genotyping is important to clarify the pathway and pathogenesis of the virus. The variety of test sequences between different virus isolates is important because variants may differ in serological reactivity, pathogenicity, virulence and treatment response patterns<sup>9-10</sup>. Clinical course of changes in HBV infection; with increasing evidence of age and immune response, and a strain of virus infecting a person. Overall, less than 1% of acute infections have been seen to cause fulminant hepatitis and death. About 10% of infected adults become chronic HBV carriers. Chronic carriers are usually asymptomatic, but there may be histological evidence of liver damage, from mild inflammation to cirrhosis and HCC (hepatocellular carcinoma)<sup>11-12</sup>. Five different genotypes were identified in this study, namely that strains A, B, C, D and F. Genotype A was mainly found in chronic patients. Mayerat et al. Although genotype A has been reported to suggest that it causes chronicity more often because it is more common in patients with chronic hepatitis than genotype D, the reverse was true for patients with acute hepatitis. Karachi is a cosmopolitan city and various immigration movements from various ethnic groups have taken place since the time of Alexander the Great. The presence of different HBV genotypes has been reported to reflect the origin of migrants and other migration patterns at a time when well-known migration waves have occurred in other countries. Bowyer et al. It has been reported that typical A and D genotypes are illustrated by South Africa and are associated with migration from northwestern

Europe, southern Europe and India<sup>13</sup>. The same genotypes in Argentina, A and D, reflect waves of migration from northwestern Europe, Italy and Spain. Although it has some structural features with genotype A strains, it is believed to be the original New Earth genotype. The HBV F genotype was found in four positive cases of Hbs Ag with high viral load. The most divergent F genotype is found in South and Central America. We also found mixed genotypes during this study. In any case, the common genotypes are B and C. In most cases, there were three genotypes. In some cases, both HBV and HCV were detected by PCR. All cases had genotypes A and C. Patients with HDV co-infection had genotypes A, B, C, D and F. In this study, we describe a PCR-specific primer-based genotyping method in which HBV isolates can be divided into A to H genotypes. An analysis was performed to confirm the specificity of the PCR typing results. We confirm the specificity of the results obtained using our phylogenetic genotyping and PCR system in the HBV S1 to S genes<sup>14</sup>. This method is very convenient and will help scientists carry out large-scale epidemiological studies. In fact, Stuyver et al. (20) reported in 2000 that a new HBV genotype has been identified, recently called the G genotype<sup>15</sup>.

### CONCLUSION:

Environmental factors make it difficult to predict results from one geographical region to another. Therefore, more detailed research is needed in regions where HBV is hyperendemic. Viruses have traditionally been classified according to antigenic properties, but with recent advances in molecular biology, HBV genotyping is important to clarify the path and pathogenesis of viruses. It is becoming increasingly clear that the HBV genotype may play a role in predicting response to various therapies and

that it should be treated as a variable before starting any treatment. Genotypes B and C have also been found to be more common in patients with acute liver disease.

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