



## RESEARCH ARTICLE

# Molecular profiling and prevalence of hepatitis B virus (HBV) in clinical isolates and its importance

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## Abstract

Hepatitis B virus (HBV) DNA quantification provides insight for treating and curing HBV and hepatic cell carcinoma (HCC). This study deals with the molecular profiling of HBV DNA viral load and the molecular investigation of the HBV DNA genotypes (Revill et al., 2019). The distribution of various genotypes among the population has been studied in this research. Total 102 EDTA serum samples were taken from suspected HBV reactive patients from the different Departments, including OPDs and IPDs of Shri Mahant Indresh Hospital, Dehradun, Uttarakhand, India. After DNA extraction real-time quantification was performed. Nested PCR technology did the HBV genotypic characterization. Out of 102 samples collected for the serological test, 60 samples were found positive for HBsAg and further biochemical profiling was performed. The HBV viral load was quantified in the samples and their titer values were observed between 34 IU/mL to  $1.10 \times 10^6$  IU/mL in which, 16.7% (10 cases) were in which the target was not detected and 83.3%, i.e., 50 cases were with high viral load. Then genotyping of these 50 samples with high viral load was performed by Nested PCR. Genotype d of HBV was found to be most prevalent in 65.62% of cases i.e. 32 cases in total. The presence of HBV genotype varies within the different geographical conditions because of the differences in the major causative factors.

**Keywords:** Hepatitis B virus, genotype, Viral load, HBsAg level, hepatic cell carcinoma, cirrhosis

## Introduction

The hepatitis B virus (HBV), which is ubiquitous in Southern Europe, Africa, Asia, Pacific Islands, and Latin America, is one of the most prevalent viral diseases in people. Between 2% and 20% of the global population in various nations have chronic HBV infection (Lavanchy and Kane, 2016). Many clinical symptoms of HBV infection exist, including the inactive carrier, chronic hepatitis, liver cirrhosis, and

hepatic cell cancer (HCC). In the long run, 15 to 40% of HBV carriers run the risk of developing cirrhosis, liver failure, or HCC (Lavanchy and Kane, 2016). With a 3200 bp genome, HBV is the smallest known human DNA virus. Reverse transcription allows for the transcription of pregenomic RNA in which covalently closed circular DNA (cccDNA), serves as the template for negative-strand DNA and fully double-stranded DNA through DNA polymerase inside the nucleocapsid, respectively, before being assembled with envelope protein to form mature HBV virions (Lin and Kao, 2015). The high rate of spontaneous mutation in reverse transcriptase enzyme causes the genomic DNA of HBV to evolve at a rate of nucleotide substitution that is predicted to be between 1.4 and 3.2  $10^{25}$  sites per year. This exclusive replication strategy results in the occurrence of numerous recombinants, mutations, subtypes, genotypes, and even quasispecies (Echeverría et al., 2015). Recent developments in molecular biology methods have allowed for a greater understanding of the genotypic divisions of HBV and their racial/geographical distributions. There is substantial evidence to support the association between HBV genotype and HBV endemicity, method of transmission, and clinical consequences (Lin and Kao, 2015; Echeverría et al., 2015). The HBV genome's naturally occurring mutations also have clinical and epidemiological repercussions. A number of

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HBV variants, including pre-core/core promoter mutations and pre-S/S deletion mutations, have been linked to developing HCC and advancing liver disease. In this work pathogenicity of hepatitis B, its genotypic distribution and clinical relevance have been studied.

## Materials and Methodology

### Sample Collection

102 EDTA blood serum samples were taken from symptomatic and suspected HBV patients. Samples were collected from different departments, including OPDs and IPDs of Gastroenterology, Pediatric, Tuberculosis, Medicine, Gynecology, Chest and Surgery at Shri Mahant Indresh Hospital, Dehradun Uttarakhand, India. The samples were categorized based on the age of the patient.

### Serological Profiling

Serological profiling was performed by VITROS 3600 Immuno diagnostic system. Using a commercially available HBsAg ES reagent kit, the third-generation enzyme immunoassay (EIA) method was used to determine the serological status (Fernando *et al.*, 2015).

### Biochemical Profiling

Biochemical profiling was performed by VITROS 3600 Biochemical analyzer in which liver function tests such as ALT, AST, alkaline phosphate, total protein, GGT, Albumin, Globulin and Bilirubin were performed.

### DNA Extraction & Quantification

All 60 samples were subjected to serum separation and DNA extraction. DNA was extracted using DNA Minikit by Qiagen. HBV DNA quantification was performed by Rotor-Gene Q 5 Plex Real-Time PCR machine. The viral load was estimated in the 60 clinical samples. The Artus amplification Kit from Qiagen was used to prepare the master mix for the measurement of HBV DNA (Kumar *et al.*, 2021). The 240 bp portion of the 5'-3' untranslated region (UTR) of the HBV genome was reverse transcribed and specifically amplified using Hepatitis B Virus RG Mater A and B reagents and enzymes (Verkuil *et al.* 2008; Fletcher, 2010). HBV DNA viral loads more than 500 IU/ml were taken into account for HBV genotyping, while HBV DNA viral loads between 34 IU/ml and 1.00 10<sup>8</sup> IU/ml were measured.

### Genotyping by Nested PCR

For HBV genotyping characterization nested PCR technique was used. This method uses PCR with type-specific primers and is easier, faster, and more precise for genotyping HBV. In order to target conserved areas of various HBV genotypes (Naito *et al.*, 2001; Datta *et al.*, 2014), a master mix was prepared to utilize specific pairs of HBV primers. Regardless of the six HBV genotypes, the first-round PCR primers (outer primer pairs) and second-round PCR primers (inner primer

pairs) were developed based on the conserved nucleotide sequences in regions of the pre-S1 through S genes. P1 was a universal outside primer for sense, and S1-2 was for antisense (1,063 bases). For genotypes a, b, and c, B2 was utilized as the inner primer (sense) in a mixture known as mix A. Antisense primers BA1R (type a specific), BB1R (type b specific), and BC1R were included in Mix A. (type c specific). For genotypes d, e, and f, B2R was utilized as the inner primer (antisense) in a mixture known as mix B. Mix B contained the sense primers BD1 (for type d), BE1 (for type e), and BF1 (for type f) (Naito *et al.*, 2001). Gel electrophoresis analyzed amplified products, including HBV genotypes a, b, c, d, e, f, g, h of amplicon size 68, 281, 122, 119, 167, 97, respectively (El-Mowafy *et al.*, 2017).

## Results

### Sample analysis on the basis of age

It was found that out of 60 patients, 5 were below 25 yrs, 6 were between 25 to 40 yrs, 22 patients were between 40 to 55 yrs, 2 were between 55 to 70 yrs and 6 patients were above 70 yrs (Fig. 1).

### Serological analysis

Out of 102 samples evaluated in the laboratory for serological profiling, 60 samples were HBsAg reactive & 40 samples were HBsAg Non-Reactive. Further, 60 HBsAg reactive samples were evaluated in the laboratory for biochemical profiling, molecular profiling and genotypic characterization.

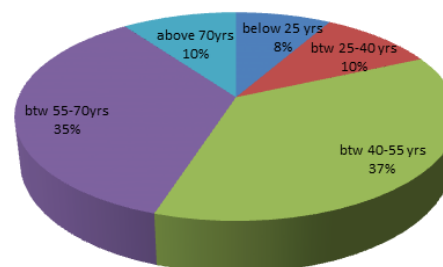
### Biochemical analysis

In biochemical profiling, it was found that 24 samples reported higher ALT levels, 22 samples reported higher AST levels, 23 samples reported higher alkaline phosphate levels, 14 samples reported higher total protein levels, 21 samples reported higher GGT levels, 23 samples reported higher albumin levels, 22 samples reported higher Globulin levels & 19 samples reported higher bilirubin levels.

### DNA Quantification analysis

In the DNA quantification, 50 samples were found with high viral load, and 10 samples target not detected (TND). The

**Hepatitis B Infected Cases Based On Patients' Age**



**Figure 1:** Data chart showing age contribution in HBV cases

DNA Quantification for other samples was also done and the results are given in the amplification plot (Figure 2) (Table 1). After that, samples with high viral load were selected for genotyping by nested PCR technique. Genotyping Results were analyzed on an agarose gel electrophoresis unit (Du *et al.*, 2007) as shown in Figure 3 and 4 (Table 2).

**Table 1:** Detection of viral load in clinical samples

Total Cases	High Viral Load	Target Not Detected
60	50 (83.3%)	10 (16.7%)

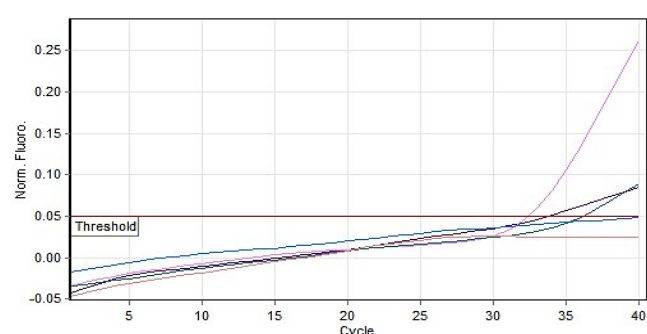
### HBV Genotyping analysis

**Table 2:** Amplification Plot for HBV DNA

S.N.	Color	Sample	Type	Ct
1	■	PC	Unknown	28.56
2	■	CASE 1	Unknown	28.43
3	■	CASE 2	Unknown	30.40
4	■	CASE 3	Unknown	29.6
5	■	NTC	Unknown	NEG

### Discussion

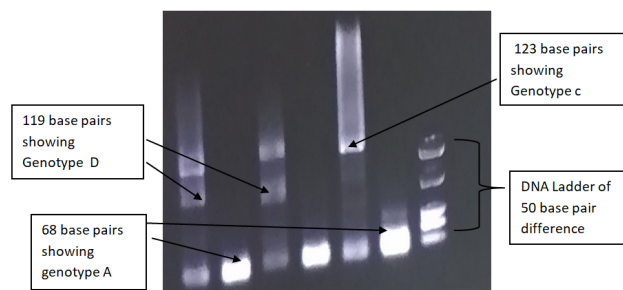
HBV DNA Quantification shows high HBV DNA in the age group 40 -55 yrs (40 %). Further, HBV Genotyping done by nested PCR shows the high prevalence of HBV genotype d with 22 (44%) cases, followed by HBV genotype a is 10 (20%) cases, genotype b is 5 (10%), genotype c is 6 (12%) cases, genotype e is 2 (4%) cases & genotype f is 5 (10%)



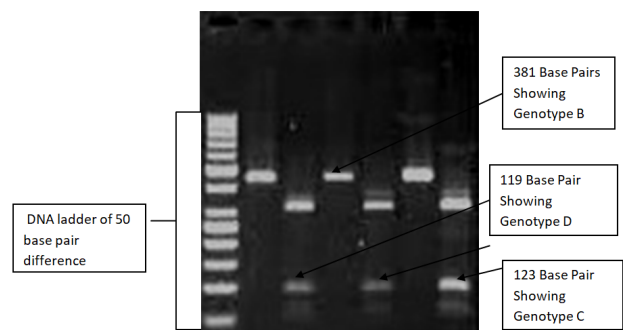
**Figure 2:** Amplification Plot for HBV DNA

**Table 3:** Amplification Plot for HBV DNA

Range hbv dna titer (iu/ml)	No. of cases	Hbv genotypes detected							
		A	B	C	D	E	F	G	H
1.00×10 <sup>1</sup> - 1.00×10 <sup>3</sup>	30	5	2	5	14	1	3	-	-
1.00×10 <sup>4</sup> - 1.00×10 <sup>7</sup>	18	5	3	-	7	1	2	-	-
>1.00×10 <sup>8</sup>	02	-	-	1	1	-	-	-	-



**Figure 3:** Agarose Gel Electrophoresis showing DNA ladder in first well and showing genotype A (i.e. 68 bp) in 2<sup>nd</sup>, 3<sup>rd</sup> & 6<sup>th</sup> well, genotype D (i.e. 119 bp) in 5<sup>th</sup> & 7<sup>th</sup> well & genotype C (i.e. 123 bp) in 3<sup>rd</sup> well



**Figure 4:** Agarose Gel Electrophoresis showing DNA ladder in first well and showing genotype B (i.e. 381 bp) in 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> well, genotype D (i.e. 119 bp) in 1<sup>st</sup>, 2<sup>nd</sup> well and genotype C (i.e. 123 bp) in 6<sup>th</sup> well

cases. genotype g & genotype h are not detected. These results highlighted the possibility of the maximum presence of Genotype d & absence of Genotype g & Genotype h in Uttarakhand. As we studied in previous research papers it was found that genotype d and genotype a has the high possibility in Northern India and our research strengthened the previous research (Tapias *et al.*, 2002).

Respectively, it was seen that out of 60 cases (SGPT) ALT level estimated showed increased levels in 24 (40%) cases, also (SGOT) AST levels showed an increase in 22 (36.6%) cases, alkaline phosphatase levels showed an increase in 23 (38.3%) cases, total protein levels showed increase in 14 (23.3%) cases, albumin levels showed increase in 23 (38.3%) cases, globulin levels showed increase in 23 (38.3%) cases, GGT levels showed increase in 22 (36.6%) cases, bilirubin levels showed increase in 19 (31.6%) cases. As shown in previous research, genotypes has relation with biochemical parameters (Tapias *et al.*, 2002; Rastogi *et al.*, 2011) and in our study we found the same correlations, so our study strengthens the previous research. The correlation of HBV DNA viral load and its Genotyping revealed high viral load with HBV Genotype d. On correlating biochemical parameters with genotyping it was found that a person infected with HBV Genotype d have higher ALT, AST & Alkaline phosphatase levels.

## Conclusion

The above studies concluded that the prevalence of HBV Genotyping and its clinical isolates has a significant correlation with elevated biochemical parameters such as SGOT (AST), SGPT (ALT), bilirubin, alkaline phosphate, albumin, globulin & total protein and higher HBV DNA Viral load is of utmost importance.

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