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Characterization of the Infectious Agent (Hepatitis B Virus) and its Clinical Correlations

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Abstract

There are many blood borne pathogens, which poses a fatal situations. Hepatitis B virus is one of the most important fatal causative agent for liver cirrhosis and can damage liver. Current study focuses on the various factors associated with the HBV infection. Study reveals about the different HBV Genotypic distribution in a range of HBV DNA Viral Load.

Keywords: Hepatitis; Chronic; Real Time PCR; Hepatocellular Carcinoma; Serology.

Introduction

Hepatitis B virus (HBV) infection is a major cause of morbidity and mortality worldwide. About 2 billion people worldwide have been suffered with Hepatitis B and 350 million are chronic HBV carriers and 600,000 die per year from HBV- related liver disease or hepatocellular carcinoma [1-3]. Liver inflammation is usually known as hepatitis. It is adouble-stranded DNA & enveloped virus of Hepadnaviridea family that causes acute and chronic hepatitis in humans. HBV causes acute and chronic liver disease. The condition can be self-limiting (acute) or can be lifelong (chronic). The one which last longer than 6 months is known as chronic. In children it meant to be silent that means do not show any symptoms and therefore rarely diagnosed. These symptoms may vary from nonspecific flu-like symptoms to fatal liver failure which can cause death. Many adults have symptoms of acute phase such as nausea vomiting abdominal muscle pain stomach ache pain dark colour urine jaundice itching and fatigue etc. Chronic HBV can cause liver damage and scarring in the patients. In India, HBsAg commonness among the population ranges from 2% to 8%, placing India in between HBV endemicity zone and the number of HBV carriers is approximately to be 50 million, forming

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the second largest global pool of chronic HBV infections [4]. HBsAg has been found in fluids of the body including semen, saliva, and serum and the serum is more infectious than other two routes. Perinatal transmission occurs mainly in newborn to HBsAg carrier mothers or mothers with self-limiting hepatitis B during the third trimester of pregnancy or during the early birth of young period. However, epidemiologic proof suggests that most infections occur around at the time of delivery. About 90% of HBeAg positive are mothers, but only 10-15% of HBeAb positive mothers convey HBV infection to their children [5-6]. Based on more than 8% genetic division among HBV strains found worldwide, eight HBV genotypes namely A, B, C, D, E, F, G, and H have been found. Till now, the presence of 5 sub genotypes have been found for each of the HBV genotypes A, B, C and D, while 4 sub genotypes have announce for genotype F. According to the geographic distribution patterns of HBV genotypes, only one or two HBV genotypes have been announce to found in most of the populations studied so far. Hence, most comparative studies on clinical significance of HBV genotypes between apopulations of close nation have endure mainly to two distinct genotypes (genotype B versus genotype C in East Asian countries and genotype A versus genotype D in Europe and India) [7-9]. The aim of this study was to determine the serological & biochemical pattern and molecular characterization of HBV diversity in asymptomatic blood donors in Uttarakhand Population.

Materials and Methods

A total of 50 cases of reactive HBV were collected from Shri MahantIndiresh Hospital and were further sent to Central Molecular Research Laboratory. The whole blood sample were collected in lavender coloured vacutainer tube which ensure mixing anticoagulant with blood to prevent clotting and samples were transported properly in a sealed thermocol box with ice gel pack maintaining 4°C temperature. Viral nucleic acid extraction Hepatitis B virus DNA was extracted from 500 µl serum using High pure system viral nucleic acid extraction kit (Catalogue no. P/N 03 531 376 001) IVD. (Roche, TaqMan) following the manufacturer's guidelines and then extracted DNA was suspended in 75µl of elution buffer. HBV-DNA and viral load; PCR mix was prepared in a total volume of 60µl for samples containing 50µl Master Mix (COBAS TaqMan), 10 µl Mn^{2+} ions.

Results

Fifty (50) EDTA blood samples were collected from patients with HBV infection. The patient's whole blood sample collected in lavender colored vacutainer tube which ensures mixing anticoagulant (EDTA) with blood to prevent clotting and then sample is transported in the properly sealed insulated thermocol box with ice gel pack maintaining 4^0C temperature. Out of 50 patients 32 were from OPD (Out Patient Department) where there were 20 males and 12 females tested for HBV while 18 patients were IPD (IN Patient Department) where were 07 males and 11 females. All the patients were HBsAg positive having and 4 male patients were HBeAg positive. The presence of HBe Ag indicates active replication of HBV and people with HBe Ag positive are considered highly infectious for hepatitis B. The ALT is sustained suppression of HBV replication until HBV DNA is undetected. A viral load of > 10000 copies/ml (2000 IU/ml) is strong risk predictor of HCC independent of Hbe Ag status, ALT level and liver cirrhosis (12). Out of 50 patients 13 patients have high viral load ($\geq 10^3$ IU/ml), among them there were 6 males and 7 females while 29 patients have low viral load ($\leq 10^2$ IU/ml). There were 16 male and 13 female patients. There were 8 patients suspected serum sample for hepatitis B virus whose target was not detected among them there were 5 male and 3 female patients. There were 8 females and 6 males whose ALT (Alannine aminotransferase) was higher from the normal range of ALT i.e. 21-72 U/L, out of 50 patients in all. There were 7 males and 8 females whose AST (Aspartate amino transferase) was higher from the normal range of AST i.e. 17-59 U/L. Total Bilirubin was higher in 7 males and 9 females than normal range i.e. 0.2-1.3 mg/dl. Alkaline Phosphate was higher in 7 males and 9 females than normal range which should be 138-126 mg/dl normally Globulin was reported higher in 07 males and 07 females from normal range of 2.30-3.50 g/dl. Amount of phosphorus was lower in 4 males and higher in 1 female from normal range of 2.5-4.5 mg/dl. The potassium was lower in 1 male from the normal range of 3.5-5.1 mmol/L. The sodium was lower in 3 males and higher in 1 male from the normal range i.e. 137-145 mmol/L. Calcium was lower in 5 males while higher in 1 male from the normal range i.e. 8.4-10.2 mg/dl. Total protein was low in 2 males and high in 1 male and 3 female from normal range of 6.3-8.2 g/dl. Percentage of neutrophil was higher for 7 male and 4 female while lower in 1 male and in 1 female from the normal range i.e. 44-68%. Platelet count was

low in 1 female and 0 male while high 2 male and 1 female from the normal range of 150-400 *10^3/µl.

Serum was separated from all the blood samples and further DNA was isolated by High pure system viral nucleic acid extraction kit (Catalogue no. P/N 03 531 376 001) IVD. Further qPCR (Quantitative PCR) was done for all suspected cases with high viral load that is subjected for Hepatitis B surface antigen. Nested PCR used primer for Hepatitis B surface Antigen briefly for a 544- nucleotide (nt) segment of the HBV surface gene, nt 223 to 766 relative to the Hbv prototype (Gene Bank accession no. AB033557), was amplified using primers for the first round of PCRS1 (5'-CCTGCTGGTGGCTCCAGTTC-3') S2 (5' ATACCCAAAGACAAAAGAAAA-3'). For second round, these primers are utilized so that different genotypes will be detected after amplification (13). S3 (5'-GCGGGGTTTTTCTTGTTGAC-3'), S4 (5'-GGGACTCAAGATGTTGTACAG-3'). The genotype was determined on the basis of the amplicon size of the amplified DNA. The genotype A, B, C, D, E, and F has the amplicon size of 68, 281, 122, 119, 167 and 97 respectively.

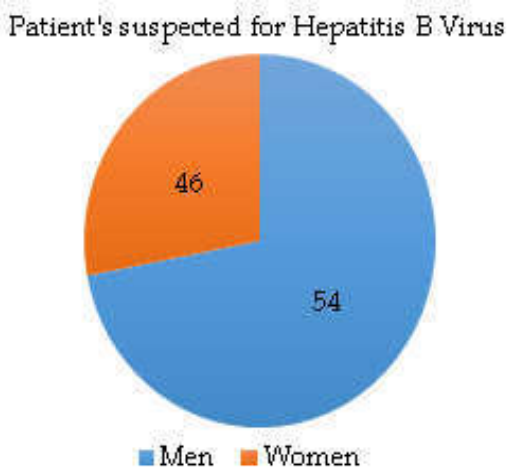

Fig. 1: Percentage of male and female patients suspected for hepatitis B virus.

Table 1: Different age groups with hepatitis B virus infection.

Age group (years)	Number of Cases		
	Male	Women	Total
≤10	NA	1	1
11-21	2	1	3
22-45	10	12	22
46-65	8	6	14
66-75	5	2	7
≥76	2	1	3

Table 2: Showing different viral load for hepatitis B virus

Viral load spectrum for hepatitis B virus	Female	Male	Total
≤ 10^1 IU/ml	07	12	18
10^2 IU/ml	04	04	08
10^3 IU/ml	02	NA	02
10^4 IU/ml	02	02	04
10^5 IU/ml	NA	01	01
10^6 IU/ml	NA	01	01
10^7 IU/ml	01	NA	01
≥10^8 IU/ml	04	02	04
Target not detected	03	05	11

Table 3: Result interpretation for HBV Genotyping [Amplified DNA by using primers (P)]

Sr no.	Amplified DNA by using primers(P)	Patients viral load for hepatitis B virus	HBV Genotype
1.	A_1	>1.10*10^8 IU/ml	D
2.	A_2	4.03*10^4IU/ml	--
3.	A_3	6 .92*10^5 IU/ml	D
4.	A_4	1.11*10^6 IU/ml	D
5.	A_5	9.96*10^7 IU/ml	D
6.	A_6	>1.10*10^8 IU/ml	--
7.	A_7	>1.10*10^8 IU/ml	D

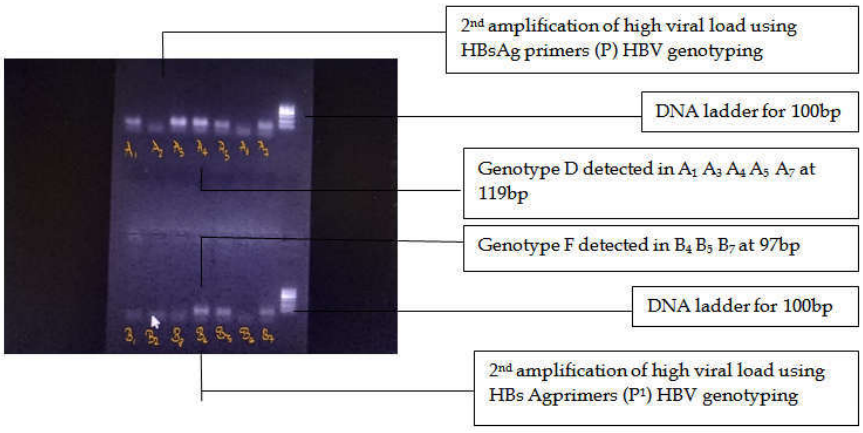

Fig. 2: Agarose gel electrophoretic image for HBV genotyping

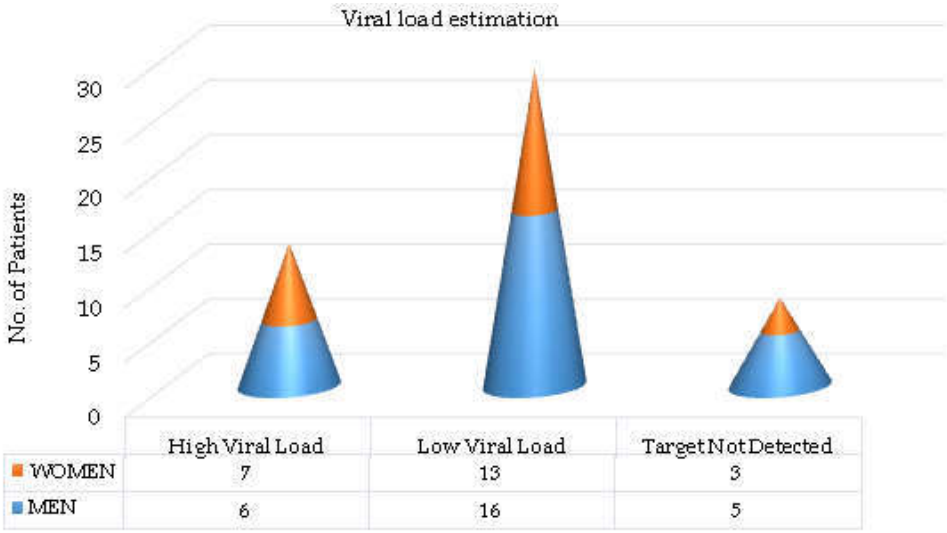

Fig. 3: Cases diagnosed for Hepatitis B Virus

Table 4: Result interpretation for HBV Genotyping [Amplified DNA by using primers (P^1)]

Sr no.	Amplified DNA by using primers(P1)	Patients viral load for hepatitis B virus	Genotype
1.	B1	>1.10*108 IU/ml	--
2.	B2	4.03*104IU/ml	--
3.	B3	6 .92*105 IU/ml	--
4.	B4	1.11*106 IU/ml	F
5.	B5	9.96*107 IU/ml	F
6.	B6	>1.10*108 IU/ml	--
7.	B7	>1.10*108 IU/ml	F

Out of 50 samples, 07 samples came with D and F genotype for HBV. D genotype was detected in 5 and F in 3 serum. 3 patients have both D and F genotypes. From these 7 patients there were 4 females and 3 males (Table 4).

Table 5: Percentage of different genotypes of patients having high viral load out of total 7 tested samples.

Patients	Number of patients	Genotype	Percentage
Male	03	D	42.85%
Male	02	F	28.57%
Female	01	F	14.28%
Female	02	D	28.57%
Total number of patients	03	D and F both	42.85%
Male	01	D and F both	14.28%
Female	02	D and F both	28.57%

Discussion

Even though hepatitis B virus (HBV) infection is a preventable disease through vaccination, an estimated 2 billion people are HBV infected, with more than 350 million HBsAg positive and considered as carriers or actively infected. In this study, we have taken a total no. of 50 symptomatic specimens for HBV examination. There are conflicting reports on the relationship between the biochemical markers of inflammation alanine transaminase (ALT), histological degree of inflammation, and serum HBV-DNA levels by reverse transcription (RT)-PC.

Glucose-6-phosphate dehydrogenase (G6PD) is the most common metabolic disorder of red blood cells as the most widespread form of acute hemolytic anemia, while Hepatitis B Virus infection is a major mother to child transmission of disease [10-12]. The result showed that females aged 20-49 with low G6PD had higher risk of Hepatitis B Virus infection and this might exacerbate ALT elevation in Hepatitis B Virus infected females.

Pathogenicity of HBV infection is mainly immune-mediated, resulting from the host-virus interactions but also from the complexity of HBV (integration, mutation, occult replication), explaining the polymorphism of chronic HBV infection.

It includes immune tolerance, inactive carriage of HBs antigen but also immune elimination with chronic active hepatitis which may lead to cirrhosis. Cirrhosis may result in complications of portal hypertension and liver failure or hepatocellular carcinoma which explain 80% of morbidity and mortality of HBV. HBV chronic infection is a problem of public health, particularly in developing countries, evidence in the need for universal HBV vaccination [12-14].

Monitoring of antiviral therapy: Monitoring during antiviral therapy could serve different purposes: (1) Estimation of the response based on early viral kinetics; and (2) Early recognition of the development of viral resistance with an increase in the viral load after initial reduction or by mutation monitoring. Long term antiviral treatment with nucleotide analogs (NUCs) suppressors HBV replication, delays disease progression and also contributes to resolution of fibrosis [14].

After acute hepatitis B, most persons achieve complete immune clearance of virus, yielding lifelong immunity. How ever upto 90% of infants, 30% of children fewer than 5 years of age, and between 5% and 10% of adults develop chronic infection. The chronic infection is confirmed by the presence of hepatitis B surface antigen (HBsAg) in serum for at least 6 months and is characterized by several clinical phases: the immune tolerant, immune active, or clearance and inactive phases.

The immune tolerant phase usually follows perinatal infection and is characterized by the presence of hepatitis B e antigen (HBeAg), high levels of HBV DNA, normal levels of alanine aminotransferase (ALT), and minimal or no liver inflammation. The immune clearance phase is characterized by high levels of the HBV DNA, elevated ALT levels, and active liver inflammation. Initially, those in the immune clearance phase will be HBeAg positive and most will eventually clear HBeAg and develop antibody to hepatitis B e antigen (anti–Hbe). A minority of patients who clear HBeAg will continue to have elevated ALT and HBV DNA levels either continuously or intermittently and have chronic hepatitis B. The majority of those who clear HBeAg will involve the inactive HBsAg phase. This phase is characterized by the absence of HBeAg, presence of anti–Hbe, normalized ALT levels, low or undetectable HBV DNA, and improvement or resolution of hepatic necrosis and fibrosis. The first Risk Evaluation of Viral Load Elevation and Associated Liver Disease/ Cancer-Hepatitis B Virus (REVEAL-HBV) cohort study revealed that HBV viral load is a strong predictive factor for the risk of cirrhosis and HCC and baseline serum HBV DNA levels > 2000 IU/ml may increase the risk of cirrhosis and HCC in adult HBV carriers [14-15].

Conclusion

Antiviral therapy using newer nucleotide analogues with lower resistance rates such as entecavir or tenofovir could suppress HBV replication, improve liver function, and delay or obviate the need for liver transplantation in some patients. Antiviral therapy before LT (liver transplant) may prevent HBV recurrence after LT by reducing the level of viremia to extremely low levels. Patients with minimal disease whether in the immunotolerant phase or with inactive infection, should not be treated. However, if it is confirmed that the risk of HCC is 10% within 10 years for patients with more than 10^6 viral loads, these patients should receive antiviral treatment irrespective of the activity of their liver disease. In patients who are HBeAg positive, the primary goal of antiviral therapy is to obtain HBe seroconversion. If the patient is young and has predictive factors of favourable response, a finite course of PEGylated interferon should be tried as a first line option in genotype A and B patients. Long-term therapy is probably required in patients who are HBeAg negative. Nucleoside analogues are better tolerated than PEGylated interferon, but the therapeutic choice must take into account the risk of drug resistance. In patients with severe liver disease, i.e. decompensated liver cirrhosis or HBV recurrence on the liver graft, one might consider combining nucleoside analogues lacking cross-resistance from the start to provide the best chance of long-term control of viral replication and disease progression. Finally, it is recommended that physicians should be brought back into the position of prescribing licensed drugs, even if they are only licensed for another treatment, when there is evidence for superiority of such an approach. One such example is tenofovir, which has been licensed for HIV and displays higher efficacy and a better safety profile than adefovir. Hepatitis B virus (HBV) is a major cause of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma, particularly in Asia-Pacific countries. The major complications in HBV carriers are hepatocellular carcinoma (HCC), liver failure and oesophageal varices following the progression to cirrhosis, while some develop HCC without cirrhosis. The progression to liver fibrosis and these other complications could be prevented by treatment with nucleus (t) ide analogues (NUCs); however, NUCs must be continuously administered for a long time. Peg interferon could lead to HBV surface antigen loss. It is difficult to use peg interferon in HBV-infected patients with decompensated cirrhosis. Acute liver failure due to HBV infection and acute exacerbation of chronic hepatitis B could be treated by NUCs. Universal vaccination programs against HBV could prevent new HBV infections globally [16-21].

Conflict of interest: None

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