Case Study

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A CASE STUDY OF HEPATITIS B GENOTYPES OF URBAN POPULATION OF INDIA WITH SPECIAL REFERENCE TO ORISSA

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Abstract: Hepatitis B surface antigen (HBsAg) prevalence among general population ranges from 2-7% putting India in intermediate endemic region having 50 million Hepatitis B Virus carriers. Both Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are a etiological agents of acute and chronic liver disease existing throughout the world .The high genetic variability of HBV and HCV genome is reflected by eight genotypes (A to H) and six genotype (1 to 6), respectively. Each genotype has a characteristic geographical distribution, which is important epidemiologically. Previous studies reported that genotypes D and A as well as D and B are prevalent HBV genotypes, and for HCV genotypes 3a and 3b to the dominant. The objective of this study is "Hepatitis B & C Viruses causing Hepatitis in subjects attending hospitals and Genotyping of Identified Virus "in Bhubaneswar Orissa.

Keywords: Hepatitis B surface antigen (HBsAg), Genotypes, Orissa

INTRODUCTION

Hepatitis B virus (HBV) causes acute and chronic hepatitis and the chances of becoming chronically infected depend upon age (Protto *et al*, 2004). About 90% of infected neonates and 50% of infected young children will become chronically infected. In contrast, only 5% to 10% of acutely infected adults will develop chronic hepatitis B 53. In some individuals who become chronically infected, especially neonates and children, the acute infection will not be clinically apparent 52, 53. Acute hepatitis B can range from subclinical disease to fulminant hepatic failure in about 2% of cases.

Hepatitis B virus (HBV) is one of the major causative agents of acute and chronic liver disease worldwide and is believed to be responsible for a million deaths annually. It is known that different methods of control, cleaning and disinfection of the hemodialysis membranes, machines, instruments and environmental surfaces may interfere with determined prevalence (Busek et al 2002). On the basis of a comparison of complete genomic sequences, HBV has been classified into eight genotypes A-H which show a geographical distribution. Some genotypes are associated with different clinical outcomes. Identification of HBV genotypes is important to begin and follow up the treatment (Milani *et al* 2009). A total of 55 blood donors with mean age 37.5±11.14 yr including 46 men and 9 women age ranged (22 to 66) were investigated in this HBV genotyping study. Occult HBV infection is a world-wide diffused entity although its distribution may reflect the general prevalence of the HBV in the various geographical areas and in the various populations (Raimando et al 2007).

In addition to the serological classification of HBV isolates according to the antigenic determinants of their HBsAg, a genetic classification based on the comparison of complete genomes or S gene sequence has defined eight genotypes of HBV (A-H) (Mojiri *et al*., 2008). When genotypic classification of HBV came into use, the first reports were based on complete genome Sequence (Okamoto *et al* 1987). Gradually the sequence of single genes or parts of genes was used in order to facilitate the comparison of a larger number of strains (Okamoto *et al.*, 1988). According to (Sayan M et al, 2012) Genotyping variation in HBV are critical in the pathogenesis of liver disease and there are considered that the different genotypes may significantly differ in their responses to therapeutic intervention in chronic hepatitis B (CHB)

Genotyping based on complete genome sequence is in ideal method, but sequencing is costly and cannot be easily carried out in clinical diagnostic laboratories for large scale (Thakur *et al.,* 2005). Currently, PCR-RFLP is the most widely used method because it is less complicated compared to direct sequencing and sequence analysis (Mizokami *et al*., 1995). He studied the prevalence of HBV genotypes among HBsAg positive blood donors in Tehran. Many blood donors were unaware that they were infected with HBV until they were found positive for HBsAg during routine screening. Hu *et al*, 2007 evaluated the risk of hepatitis B virus (HBV) transmission via dental hand pieces and the effects of an anti-suction device in preventing HBV contamination.

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Hepatitis B virus infection is a global public health problem, with approximately 400 million people chronically infected (Aggarwal and Ranjan, 2004). Each year it causes more than 500 000 deaths worldwide. Outcome of acute hepatitis B virus infection ranges from asymptomatic subclinical infection (70%) and symptomatic acute hepatitis (30%) to fulminant hepatic failure (0.1-0.5%). A proportion of people infected with hepatitis B virus (5%-10% among adults) progress to chronicity, defined as persistence of infection for more than six months. The rate of chronicity is much higher among neonates and children.

MATERIAL AND METHOD

Blood donor recruitment and sample collection:

Blood sample collected from CAPITAL HOSPITAL and KIMS HOSPITAL, in BHUBANESWAR, ORISSA. Infection can be transmitted from 25 patients to staff and from staff to patient during the blood-taking procedure. All recruited donors were unremunerated volunteers from either urban or rural areas. They were medically assessed and via a questionnaire denied any known risk factors for viral infection. Donors found to be HBV carriers were also asked to give follow-up blood samples for further study.

HBV serological marker determination:

Testing for HBV serological markers, including HBs, anti- HBs, anti-HBe, HBe and anti-HBs, were performed by ELISA using an automatic enzyme detection system (Tecan, Swiss) and a commercial kit (HBsAg, Anti Hbs, Hbc and Anti Hbc ELISA TEST KIT (SURASE B-96, TMB) GENERAL BIOLOGICALS CORP. TAIWAN) according to the manufacturers' protocols. For the quantitative detection of the markers, serum from blood donors was applied to AXSYM MEIA (Abbott Diagnostics). To measure the ALT level, serum was separated and run through an automatic biochemistry analyzer (Hitachi, Japan) using Kit (Shanghai Fousun Long March Medical Science.Co.Ltd. China).

DNA analysis

DNA isolated from 100 donor blood samples according the manufacturer's protocol (Qiagen, Germany) and following the manufacturer's instructions (Qiagen, Germany). If a positive reaction was observed. If there was a second positive test, each individual sample was tested. After that, quantitative PCR was employed for to quantify viral load. As Katsoulidou et al. described, positive samples were genotyped using nest-PCR. Briefly, the first-round PCR primers (outer primer pairs) and second-round PCR primers (inner primer pairs) were designed on the basis of the conserved nature of nucleotide sequences in the regions of the pre-S1 through S genes. At the end, agar electrophoresis was employed to discern genotype.11 HBV DNA reactive samples were randomly picked out From these samples, HBV DNA was extracted from 1.0mL of serum using a kit (Qiagen GmbH, Germany), according to the manufacturer's instructions. Then, sequence analysis, beginning from the S region of HBV genome, was performed the final product is loaded on the Sequenced in Automated DNA Sequencer (Genetic Analyzer, ABI 3130XL) in a 96 well plate Ltd., using an ABI sequencing system.

HBV genome phylogenetic analysis was performed by multiple sequence alignment using the Clustal W v1.83 program. For this purpose, HBV sequences from the donors and reference sequences from the GenBank database http://www.ncbi.nih.gov were aligned. The generated Electrophorogram was read and the Sequences were matched with Standard Genotype by the help of MEGA Version 4 Software and the data were analyzed. Phylogenetic analysis of S gene was done by constructing phylogenetic Tree by using computed among genotypes of HBV, using Kimura 2 parameter matrix and never going method.

RESULTS AND DISCUSSION

Out of the total 25 HBV samples, HBsAg positive were 40%, Anti HBc positive were 32%, Anti HBs positive were 8%. Specially based on ELISA TEST of HBsAg, 15 patients were HBsAg Negative, 1 Patient was in HBsAg Borderline, and 2 Patients were in HBsAg Lower Positive and 7 Patients in HBsAg Higher Positive. And only one of the samples was HBsAg DNA Positive. This study shows a single HBV genotype D infection.

Serological Test Result:

After collection of Samples from different hospitals Serological tests were conducted. Three types of ELISA were performed HBsAg, Anti HBc and Anti Hbs.

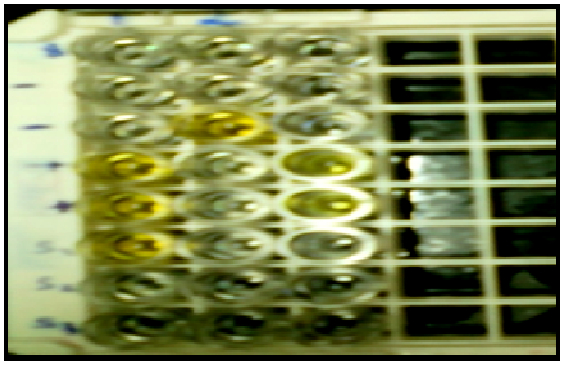


Figure - ELISA Test of HBV

Samples referred from Hospital;

Name of the hospitals	Patient ID
Capital hospital ,BBSR	RMRC 1-120
Municiple hospital , BBSR	RMRC 120-180
KIIMS, BBSR	RMRC 181-225

Serological Test Result

Clinical Data: The etiology of the HBV cases is illustrated in the total rest of the samples,

Percentage of POSITIVE CASE of HBV

HBsAg positive – 40%
Anti HBc positive – 32%
Anti HBs positive- 8%

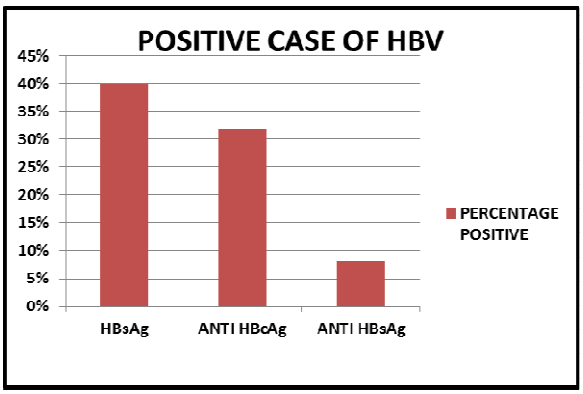


Figure - Percentage of POSITIVE CASE of HBV

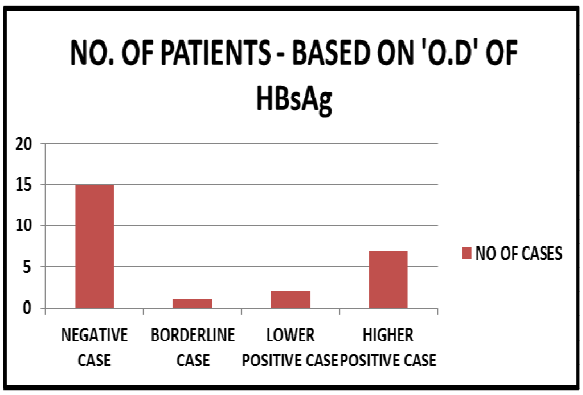


Figure - No of Patients based on "OD" of HBsAg

No. of Patients based on 'O.D' of HBsAg

HBsAg Negative– 15 Patients.
HBsAg Borderline– 1 Patient.
HBsAg Lower Positive– 2 Patients.
HBsAg Higher Positive – 7 Patients.

In the 25 samples, the 10 samples were HBsAg positive. The Optical Density of these 10 samples were, cut off value= 0.048

Sample 1- 2.778	Sample A1- 1.003
Sample 2- 0.052	Sample A2- 1.003
Sample 4- 2.503	Sample A3- 1.823
Sample 5- 0.099	Sample A4- 2.918
Sample 16- 0.060	Sample A5- 2.945

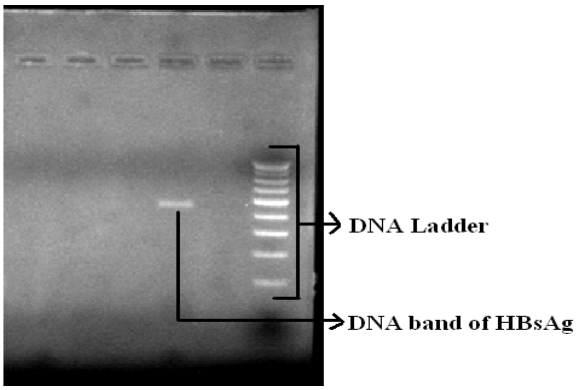


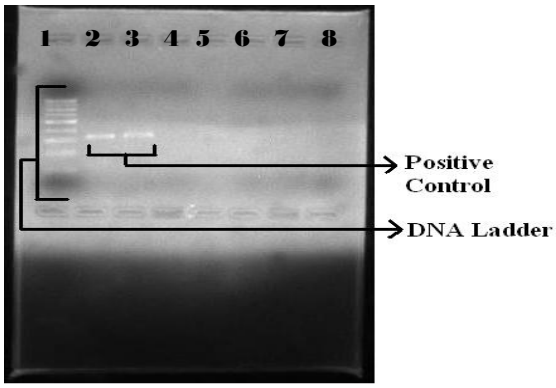
Figure - Checking for the presence of HBV DNA after electrophoresis

Molecular Test Result: In the total rest of 25 samples, only 10 samples were positive of HBsAg, which were taken and checked for the presence of viral DNA. It was seen the patients positive for HBsAg showed positive results for viral DNA.

The result is shown in the following:

Lane 1- Ladder
Lane 2- Negative Control
Lane 3- A3
Lane 4- A4
Lane 5- A5
Lane6- A2

100bp DNA Ladder was used as a marker. And the expected DNA band was around 429bp compare to the DNA Ladder.



Lane 1- DNA Ladder
Lane 2- Positive Control
Lane 3- Positive Control
Lane 4- Sample 1
Lane 5- Sample 2
Lane 6- Sample 4
Lane 7- Sample 5
Lane 8- Sample 16
Lane 9- A1
Lane 10- Negative Control

Quantitative measurement of hepatitis C viral load

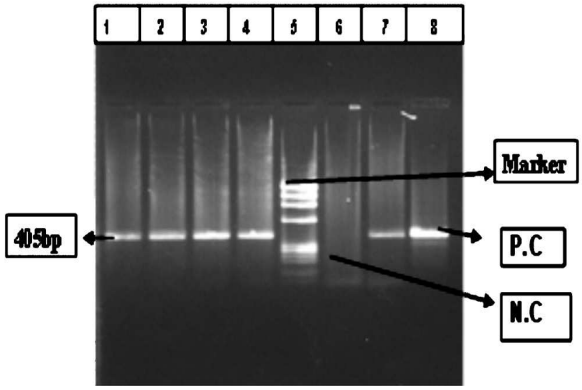


Figure- HCV virus cDNA band of 405 bp.

Lane 1- Sample 1
Lane 2- Sample2
Lane 3- Sample3
Lane 4- Sample 4
Lane 5- cDNA Ladder
Lane 6- Negative Control
Lane 7- Sample 7
Lane 8- Positive Control

HBV genotypes have distinct geographical distributions. In India, genotype A and D are the circulating genotypes with genotype D being the predominant genotype circulating in western India**.** A recent study among patients with chronic liver disease from New Delhi reported predominantly genotype D followed by genotype A12. HBV isolates from one of the primitive tribes of the Andaman and Nicobar Islands showed predominance of genotype D like mainland India with minimal difference in isolates suggesting the likelihood of the introduction of HBV from mainland India 20. In our study we found the predominance of genotype. A followed by genotypes C and D. HBV has 6 different genotype distributed in different parts of world. Genotype influence activity & outcome of HBV associated chronic liver disease as well as response to antiviral therapies therefore it is essential to know the circulating genotype for taking better therapeutic control. In India genotype A & D have been reported in different parts of country. In North India genotype A & D widely distributed (Ref.). In a another study in south India has been reported genotype A, D & C (Borkakoty *et al*. 2006) in these study they were found A(41.6%) followed by genotypes C (27.8%) and D (11.1%) seven isolated (19.9%) could not be genotyped.

CONCLUSION

ELISA, Diagnostic and Nested PCR proved to be a suitable method for diagnosis of HBV infections in the subjects attending hospital. Genotype D HBV appears dominant genotype prevalent in Bhubaneswar Orissa. In our study we find that the gene which is responsible for the Hepatitis B in the subject is Partial S gene. In our study are one HBV DNA was detected in 25 person which is of genotype D may be due to population movement.

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