Original Article

Point Prevalence of Hepatitis B Virus Infection among Adolescents in Visakhapatnam, India

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Introduction: Occurrence of detectable amounts of viral antigen or viral particles in the blood of infected patients with Hepatitis B virus (HBV) is a significant characteristic of HBV infection. Detection of HBV antigen or its DNA among individuals of a community is a crucial factor to know the burden of HBV infection. Hepatitis B surface antigen (HBsAg) is a suitable marker of HBV infection but a poor indicator of infectivity. Hence the tests for the detection of HBV DNA or HBsAg are used. The measurement of HBV DNA in serum has become the main tool to identify viral load, to monitor patients' therapy and to predict whether antiviral therapy would be successful or not. **Methods:** The present study was designated to identify HBV infected individuals among adolescent age group by using combined methods namely, rapid immunoassay technique for HBsAg detection (HEPACARD) and Conventional Polymerase Chain reaction (PCR) analysis for HBV DNA detection. **Results**: Serum samples from 39 patients suspected of HBV infection were tested for the presence of HBsAg and HBV DNA. Eight specimens (20%) were positive for HBsAg as well as HBV DNA using PCR reaction. The ratio of spectrophotometric analysis of the extracted DNA samples was between 1.80-1.92 indicating a highly purified DNA. The gel electrophoresis of amplified PCR products of HBV DNA revealed a single 524 bp band in the test samples. **Conclusion:** Screening for HBV infection among adolescents by HEPACARD and further confirmation by PCR is recommended to monitor the progression of the disease and antiviral treatment. *J Med Microbiol Infec Dis, 2015, 3 (3-4): 48-51.*

Keywords: Hepatitis B Virus, Viral DNA, HBsAg, PCR.

INTRODUCTION

Hepatitis B virus (HBV) is a double-stranded DNA virus belonging to Hepadnaviridae family and is known to cause acute and chronic forms of hepatitis. As per WHO report, about 2 billion people are infected with HBV worldwide, and more than 300 million have a persistent infection [1]. Approximately 600,000 people die each year globally from acute or chronic consequences of hepatitis. The undiagnosed individuals with high HBV viral load are infective and pose a risk to the communities especially as blood donors or health care workers. The level of HBV DNA in serum or plasma has been shown to correlate with biochemical and histological measures of disease, and probably reflects the replicative activity of HBV more accurately [2]. By the introduction of antiviral drugs like lamivudine, close monitoring of patients has become necessary due to the emergence of drug-resistant viral strains or drug withdrawal reaction on cessation of therapy [3]. Acute infection causes liver inflammation; clinical manifestations including loss of appetite, vomiting, and icterus which rarely lead to death. Chronic hepatitis B may eventually cause liver cirrhosis and hepatocellular carcinoma, a fatal disease with a very low response to chemotherapy [4]. The infection is preventable by vaccination. Viral replication in the liver is followed by the appearance of the virus in blood as evidenced by virus-specific proteins and their corresponding antibodies due to host immune response. Blood tests for detection of these proteins and antibodies are used for diagnosis of infection. The hepatitis B surface antigen (HBsAg) is most frequently used in the screening test. It is the first detectable viral antigen to appear during infection, and it may be undetectable later in the course of infection as it is cleared by the host immune system. However, it is a poor indicator of the infection [5]. If the host is capable of clearing infection, HBsAg levels will disappear eventually, and this will be followed by the appearance of IgG antibodies against HBsAg and also against core antigen, (anti-HBs and anti-HBc IgG) [6]. An individual seronegative for HBsAg but seropositive for anti-HBs have either cleared an infection or has been vaccinated previously. People who remain HBsAg positive for at least six months are considered as hepatitis B carriers [7]. The virus carriers possess elevated levels of serum alanine aminotransferase and have inflammation of the liver, as revealed by biopsy. Individuals with high viral loads, characteristically have Ground-glass hepatocytes on liver biopsy [8].

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PCR tests are now available to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment. A variety of molecular technologies are used for quantification of HBV DNA that includes UV spectrophotometry, conventional PCR, Real-time PCR, digital PCR, isothermal amplification methods and biosensors. In the present study, samples from adolescent patients were collected to be tested for HBSAg, viral marker and further detections of viral DNA using molecular techniques like PCR analysis.

MATERIAL AND METHODS

Detection of hepatitis HBsAg. We collected blood samples from 39 suspected young people during eight months, December 2015 to August 2016. The sera separated from the blood samples were subjected to HEPACARD test (J Mitra & Co Pvt. Ltd., New Delhi, India). This screening test used for qualitative detection of HBsAg in the patients' sera is a visual, rapid and sensitive one-step immunoassay. The test uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. An amount of 70 µl of each patient's serum was added to the test card and left for 20 min. After 20 min if two pink colored lines appeared in the test and control region, the sample can be considered reactive for HBsAg. The further confirmatory test for positive samples was performed as mentioned below, as false positive results can be obtained in certain cases due to the presence of other antigens or elevated levels of RF factor. However, the test detects the HBsAg levels at a concentration of 0.5 ng/ml.

HBV DNA isolation. The extraction procedure was performed according to manufacturer instructions by using commercially available kits (QIA amp DNA Mini kit, Qiagen, Germany). Finally, the DNA was washed with 1 ml of 70% ethanol and allowed to dry for 15-20 min at 37°C and resuspended in 300-400µl of TE buffer, pH 8.5 and stored at -20°C. UV-visible spectrophotometry was used to find out the ratio of absorbance at 260/280 to determine the quality of the isolated DNA [9, 10].

Detection of HBV DNA in sera by PCR. HBV DNA was quantified by using conventional PCR (GeNei ™ Bangalore, India). The 20 µl reaction mixtures contained 200 µM dNTPs**,** 1x PCR reaction buffer is containing 10mM HCl, 50mM KCl, 1.5mM $MgCl_2$ and 0.001% w/v gelatin, 10 pmoles forward primer (5'-GGT ATG TTG CCC GTT TGT CC-3') and reverse primer (5'-CCC AAT ACC ACA TCA TCC AT-3') each, 2 µl (100ng) template and 1U of *Taq* DNA polymerase. The amplification program included an initial denaturation of 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, elongation at 72°C for 30 s with a final elongation at 72°C for 10 min. Negative patient sera and positive controls were also run along with the test samples.

Gel-electrophoresis of PCR products. The samples were run on a 1% agarose gel, stained with Ethidium bromide and visualized by placing on a uv transilluminator and photographed directly by gel documentation unit. The presence of a 524 bp fragment indicated a positive result. In parallel with samples, 100 bp DNA size marker was also run on the gel to estimate the molecular weight of DNA fragment in the gel.

RESULTS

A total of 39 suspected adolescent patients were studied over a span of 8 months. The HEPACARD test revealed eight adolescents were HBsAg positive. The mean values of optical density A_{260} and A_{280} with uv-visible spectrophotometry were calculated, and the results showed that the ratios were lie in between 1.80-1.92 which indicates that the isolated DNA is in highly purified form. The presence of HBV DNA in serum is a reliable marker of active HBV replication [11]. HBV DNA levels are detectable by 30 days following infection, generally, reach a peak at the time of acute hepatitis and gradually decrease and disappear when the infection resolves spontaneously [12]. In cases of acute viral hepatitis with equivocal HBsAg test results, testing for HBV DNA in serum may be a useful adjunct in the diagnosis of acute HBV infection, since HBV DNA can be detected approximately 21 days before HBsAg typically appears in the serum. HBV DNA levels in serum are useful in determining the status of chronic HBV infection, by differentiating between acute and inactive disease states [13].

For the standardization of PCR, viral DNA obtained was initially subjected to PCR using oligonucleotide primers described in materials and methods section. The best conditions for the first round of amplification were obtained when using 1U *Taq* pol, 2µl DNA and 35 cycles of amplification with a primer annealing temperature of 58°C. For the second round of amplification using 1U *Taq* pol with 30 cycles of amplification and 10 min of extension of the DNA gave the best results. During these investigations, PCR was available to detect HBV DNA sequences in virtually all patients with active viral antigen expression and also in a high proportion of hepatitis patients who were silent for active HBV, but may or may not show signs of a contact with the HBV. HBV DNA was detected in 8 (20%) of 39 patient samples respectively (Figure 1). An ethidium bromide stained agarose gel showing typical results of HBV DNA. The bands correspond to 524 bp were positive. However in the present study few cases were reported as positive among the adolescent age group.

DISCUSSION

In similar study, Compri *et al.* (2012) [14] and Brechot (1993) [15] also investigated the prevalence rate of Hepatitis infection in children. Wang *et al.* (1991) [16] had reported nine plasma positive samples of HBV DNA using PCR using two primer pairs specific for surface and core regions of hepatitis virus. Kaneko *et al.* (1990) found that HBsAg was utilized for the detection of HBV virus infection and HBV DNA detection using PCR has been shown to be a direct measure of complete virions and to be potentially a very sensitive method.

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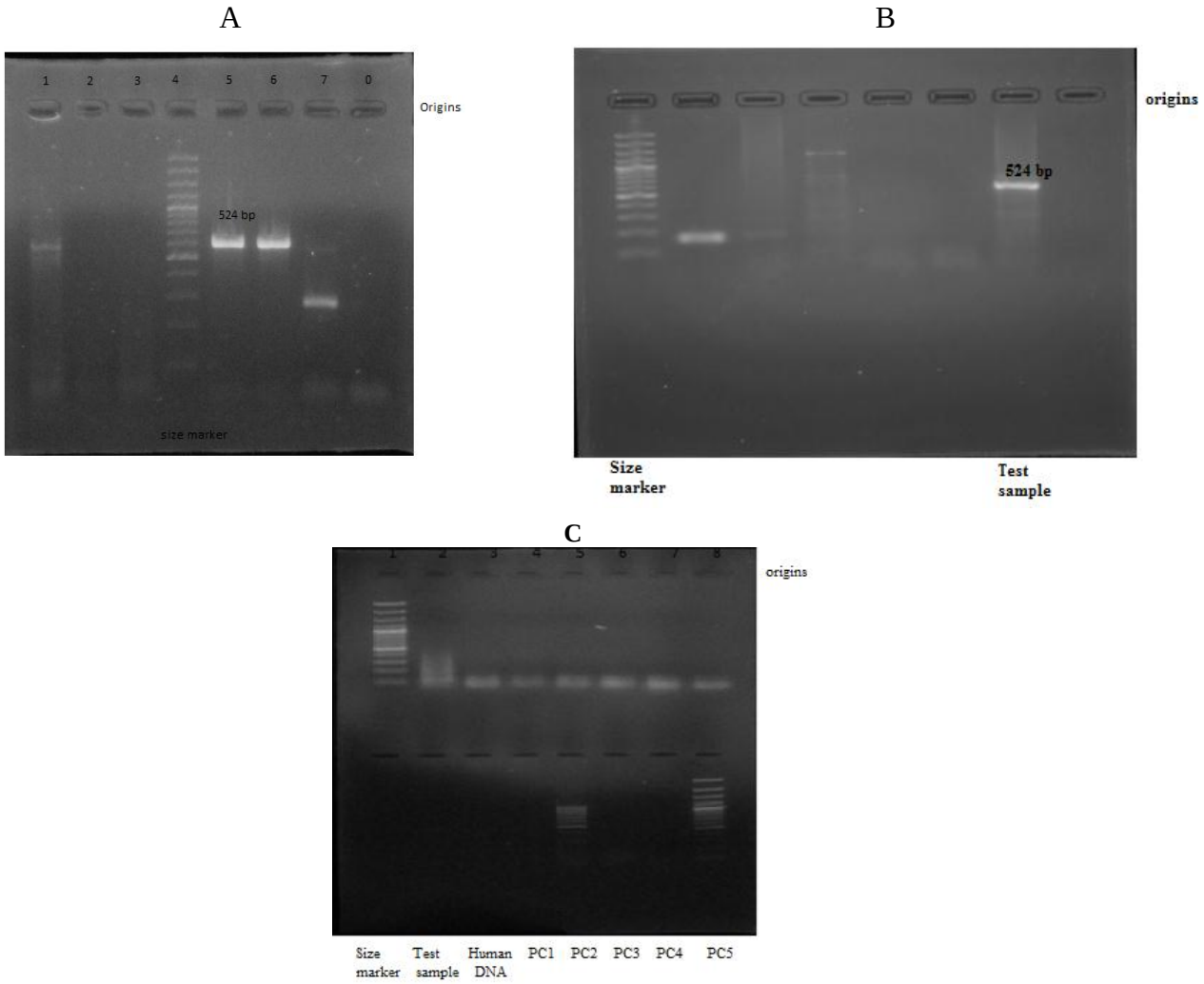


Fig. 1. HBV-DNA PCR. An ethidium bromide stained agarose gel showing typical results of HBV-DNA PCR screening of collected samples which were HBsAg positive. A: Lanes 1, 2, 3, 5, test samples; lane 4, DNA ladder; lane 6, positive control (HBV DNA in negative serum) showing the expected 524 bp fragment; lane 7, recombinant PUC m-T-HBV plasmid control; lane 0, negative control. B: Representative agarose gel electrophoresis of PCR products. Lane 7, positive test sample. C: A representative agarose gel electrophoresis of the following PCR products. Lane 1, molecular size marker; lane 2, positive test sample; lane 3, human DNA sample; lane 4, *Shigella* bacteria DNA; lane 5, *Listeria* DNA; lane 6, *Mycobacterium tuberculosis* DNA; lane 7, *Salmonella* DNA; lane 8, *Staphylococcus* DNA.

They studied the relationship between HBsAg detection and HBV DNA assay by PCR. Their results showed that, out of 29 serum samples, 3 out of 11 chronic hepatitis B patients lost HBsAg were positive for HBV DNA in serum, while all of the patients who recovered from acute viral infection were negative for HBV DNA in serum [17].

PCR is a procedure of high sensitivity, more reproducibility, wide linear range, time-saving and provides an accurate detection of HBV DNA from HBV genotypes of patient's serum samples. Screening of HBV infection among adolescents by HEPACARD and further confirmation by PCR is recommended to detect active HBV infection.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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