INTRODUCTION

Hepatitis may be caused due to various etiologies such as viral infections, autoimmune diseases, ingestion of toxic substances (alcohol), non-alcoholic fatty diseases, particular medicines such as paracetamol and can be of cryptogenic nature. However viral infection is one of the most common causes of hepatitis (Niesters, 2002; Kumar et al., 2013). Diagnosis of hepatitis is made based on clinical, biochemical and serological markers. After a person gets infected by Hepatitis B virus, first viral antigen to appear in patients’ blood is Hepatitis B surface antigen or HBsAg (within 1-2 weeks) (Rysgaard et al., 2012). Serological marker HBs Ag is hence used as hallmark for diagnosis and identification of hepatitis B patients. In patients who generally recover from acute Hepatitis B infection, HBsAg becomes undetectable after 1-2 months and rarely persists after period of 6 months (Longo, 2012). If the HBs Ag is persisting in the patient’s serum even after 6 months, then these cases fall under the criteria of chronic hepatitis B (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4825166/).

Other marker like HBe Ag is used as a secondary marker to indicate active HBV infection. Moreover HBs Ag can be more useful measure when compared to HBeAg for measuring replicative status of virus as some of HBV variants may continue to replicate at a very high level without even secreting HBeAg (Mommeya-Marin, 2003). Therefore monitoring of HBV DNA in serum of these patients is as important as serological markers for predicting the clinical outcomes of infection as it remains positive during the entire course of active disease. Quantification of HBV DNA can be used as more direct method for assessing efficacy of antiretroviral therapy than routinely used serological markers (Brunetto, 1989). Hybridisation technique was one of the commonest older method used prior to introduction of real time PCR for quantification of viral load (Kuhns, 1989)With development of PCR based methods, sensitivity of HBV DNA detection has increased drastically (Kessler, 2000). Recently developed molecular diagnostic methods like real time PCR is now being used for quantification of viral load. This apart from having diagnostic importance also plays an important prognostic role (Baker et al., 1991). Real time PCR has further improved the ease with which HBV DNA can be monitored and has also increased the range over with such levels can be determined (Yeh, 2004; Kohmoto et al., 2003).

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CORRELATION BETWEEN HBV DNA LEVELS, HBEAG AND LIVER FUNCTION TESTS IN UNTREATED CHRONIC HEPATITIS B PATIENTS


Maharastra University of Health Sciences, India

ABSTRACT

Chronic hepatitis B is a condition where various prognostic markers guide in therapy with antiviral agents. In this study we identified all the untreated chronic hepatitis ‘B’ patients, quantified hepatitis B virus DNA in serumand compared these HBV DNA levels with HBeAg AST, and ALT. Serum samples collected from 75 patients were subjected to tests for detection of HBsAgand HBeAg by ELISA, for estimation of AST and ALT levels by fully automated analysers. Quantification of HBV DNA was done using real time PCR. Correlation between HBV DNA levels, liver enzymes and HBeAg was studied and analyzed. We found thatHBeAg positive patients had statistically significant elevated ALT levels as compared to HBeAg negative patients, whereas there was no significant difference found in the AST levels between these two groups. Significant correlation was found between ALT and HBV DNA levels (p<0.05) in HBeAg positive patients and no significant correlation in AST and HBV DNA level. In resource poor setting HBeAg and ALT can guide in antiviral therapy. HBV DNA quantitation by real time PCR has a very significant role in determining the activity of HBV infection in addition to the markers such as HBe Ag and AST, ALT.

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High HBV DNA copies represents active disease and it is one of main individual risk factor as compared to HBeAg and other liver function tests (Chen, 2009; Fan et al., 2008). Several studies have shown that HBV DNA levels may not always be associated with the hepatic tissue damage (Papatheodoridis et al., 2008; Chen et al., 2010). Therefore, measurement of serum HBV DNA in the liver and its correlation with liver damage and thus serum HBV DNA levels may guide clinicians for the starting and ending the treatment. This study was conducted to evaluate the relationship between serum and liver hepatitis B virus DNA levels with other parameters like liver function tests, HBe antigen and HBs antigen in chronic hepatitis B.

MATERIALS AND METHODS

We conducted a cross sectional study over a period of 6 months in which 75 patients of chronic hepatitis B, not started on antiviral therapy, were included. All patients who were having HCV and HBV co-infection or other hepatotropic virus co-infection with HBV were excluded. Ethical clearance from institutional ethics committee was taken before the commencement of the study. Blood samples were collected from 75 patients suffering from chronic hepatitis B and were subjected to detection of HBsAg, HBeAg by ELISA (DSI, Italy) and levels of aspartate aminotransferase (AST) & alanine aminotransferase (ALT) by fully automated analyser (EM 360, Transasia, India). HBV DNA extraction was done by using fully automated Nuclisens nucleic acid extraction machine. HBV DNA quantification by real time PCR on COBAS TaqMan-48 using the proprietary COBAS Taqman real time PCR kit. The data was imported onto SPSS software version 22 for further statistical analysis. Comparison between categorical variables was performed using Fisher’s exact test. Correlation between HBV DNA levels and HBsAg, HBeAg, AST & ALT were analyzed by spearman’s correlation test. P value less than 0.05 were considered statistically significant.

RESULTS

The mean age of population in this study is 34 yrs with male preponderance (Table 1). This can be explained that the study was carried out in an Army hospital and the dependent clientele are mainly the serving soldiers. The mean AST level in chronic hepatitis B patients was 43.81 IU/ml (normal 10-40 IU/ml) and The mean ALT level was 61.13 IU/ml (normal 7-56 IU/ml). The mean HBV DNA level in these patients was 2096140.683 IU/ml. On HBV DNA level estimation by real time PCR, 42 of 75(56%) patients showed detectable levels of HBV DNA. In the patients, who showed detectable HBV DNA (n=42), the mean AST levels was 44.95 IU/ml, the mean ALT level was 65.07 IU/ml, and the mean HBV DNA level was 835102.52 IU/ml. Correlation was tried to establish between serum AST and ALT levels and HBV DNA levels in HBeAg positive patients (Table 5). The ALT level showed significant correlation p=0.001(p<0.005). Whereas, no correlation could be established between AST and HBV DNA. Of all the 9 patients who were HBeAg positive, all had detectable HBV DNA levels (100%), five had HBV DNA levels greater than 20,000 IU/ml indicating very high viral replication. HBV DNA was detected even in 46.96% of HBeAg negative patients. 10 patients out of 66 HBeAg negative patients showed HBV DNA level greater than 20,000 IU/ml(15.15%). Both mean AST and ALT level were higher in HBeAg positive patients.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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<tr>
<td>AGE</td>
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<td>20</td>
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</tr>
<tr>
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<td>228</td>
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</tr>
<tr>
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Correlation was analyzed between the two data

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<th>PARAMETER</th>
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<th>Std. Deviation</th>
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<tr>
<td>AST</td>
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<tr>
<td>ALT</td>
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<td>29.110</td>
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<td>HBV DNA</td>
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</table>

p value was 0.858 which is not significant.(p<0.05)

Correlation between the two was analyzed

p value turned out to be 0.537 which was not significant(p<0.05)
DISCUSSION

Chronic Hepatitis B (CHB) patients are regularly monitored for HBeAg, liver function tests and HBV DNA levels. These parameters have a role in their prognosis and in management of CHB. Presently various therapies are available such as PEGylated interferon, antiviral therapy for preventing the long term complications in CHB patients. In our study we have done quantitation of HBV DNA using real time PCR and tried to see the correlation between HBV DNA levels with various other factors such as serological marker (HBeAg)and Liver function tests (AST, ALT). In our study we found that the HBeAg positive patients had statistically significant elevation of ALT levels as compared to HBeAg negative patients. There was no significant difference found in the AST levels between these two groups.

The studies on the biochemical and serological markers reported higher levels of AST and ALT levels in HBeAg-positive CHB patients compared to HBeAg-negative chronic hepatitis B (Papatheodoridis et al., 2008; Chen, 2010). In our study we found no significant correlation in AST and HBV DNA level, however significant correlation was found between ALT and HBV DNA levels (p<0.05) in HBeAg positive patients. Previous studies have reported similar findings that HBeAg-positive Chronic hepatitis B patients had higher HBV copies than HBeAg-negative cases (Kohmoto et al., 2003; Chen et al., 2009). In our study we found that higher mean HBV DNA level (20, 77, 971.89IU/ml) was seen in HBeAg positive case group as compared to HBeAg negative cases (248069.08 IU/ml).

The study by Chu et al. has shown that, in chronic hepatitis B patients, HBV DNA level of more than 20,000 IU/ml was detected in 96% of HBeAg-positive cases (Fan et al., 2008). In our study, only 55.55% showed HBV DNA levels higher than 20,000IU/ml. We found that HBV DNA levels were higher in HBe antigen positive patients and also in patients showing elevated ALT levels (signifying greater liver damage). HBV DNA were also elevated in patients negative for HBe antigen but showing high ALT levels. Hence we conclude that HBV DNA level estimation is a better modality in determining activity of Hepatitis B virus. The HBV DNA quantitation by real time PCR is a very significant role in determining the activity of HBV infection in addition to the markers such as HBeAg and AST, ALT. In our study we found significant correlation of raised ALT in HBeAg positive patients who also had increased HBV DNA levels. Compared to the serological and biochemical markers, doing HBV DNA quantitation is a better marker in selection of patients for treatment, and also see the efficacy of antiviral therapy, and in identifying the development of HBV drug-resistant strains.

Limitations

The patients who were included were predominantly young serving male personnel and few veterans, hence they do not represent general population. Sample size is less and further evaluation needs to be done on a larger population.

Table 3. Mean ALT level in HBV DNA positive patients

<table>
<thead>
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<th>PARAMETER</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
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<tr>
<td>HBV DNA</td>
<td>835102.52</td>
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<tr>
<td>ALT</td>
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Aknowledgment

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Conflict Of Interest

None to declare.

REFERENCES


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