ORIGINAL ARTICLE

To Determine Frequency of Occult Hepatitis B virus Infection in Chronic Hepatitis C patients

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ABSTRACT

Background: Chronic Hepatitis B and C are the leading cause of morbidity and mortality worldwide, approximately 2 billion and 350 million world population is infected with hepatitis B virus (HBV) and hepatitis C virus (HCV) respectively.
Aim: To determine the frequency of occult hepatitis B Virus (OHBV) infection in ch.hepatitis C patients.
Place and duration: The study was conducted at Fauji Foundation Hospital, Rawalpindi, from Feb'28 2015 to Aug 28'2015.
Methods: 105 Male and female patients having Anti hepatitis C antibody and Polymerase chain reaction (PCR) for hepatitis - C RNA (Ribo Nucleic Acid) positive presented to emergency room/OPD were evaluated. In all these cases PCR for HBV-DNA (Hepatitis B Virus- Deoxy-Ribo Nucleic Acid) levels were measured by microlab 200 analyzer using end point method.
Results: Mean age (years) was 47.29+9.15 with 24(22.9) male patients and 81(77.1) female patients. 8(7.6%) patients were positive for occult hepatitis B infection in chronic hepatitis C patients.
Conclusion: It is advisable that Physicians and gastroenterologists should perform PCR for HBV-DNA in every HCV positive patient before starting any treatment as a protocol instead of relying only on HBsAg positivity to rule out dual infection.
Keywords: OHBV infection, HBV-DNA, Anti-HCV antibody, real time Polymerase chain reaction

INTRODUCTION

Chronic HBV and HCV infections are the leading cause of morbidity and mortality worldwide, approximately 2 billion and 350 million world population is infected with HBV and HCV respectively. HCV affecting 170 million cases worldwide and accounting 3% of total population, 50% of all these cases become chronic carriers who are at high risk to develop cirrhosis and liver cancer in future[1].

HBV infection either acute or chronic is a major health problemaround the globe, especially in Southeast Asia, Africa, southern Europe and Latin America. HBV is highly endemic in Pakistan, accounts for 9 million infected cases and this number is on steady rise[2]. OHBV is recently identified entity which means presence of HBV- DNA in patients who are hepatitis B surface antigen (HBsAg) negative. Worldwide 5 to 7 million dual infection HBV-HCV have been recognized amongst 350 million HBV carriers. However, it is very likely possible that the exact prevalence rate of dual HBV–HCV infection is often down rated because OHBVinfection is often missed. Reported prevalence of this dual infection ranges from 6.7% to 91.1%, one of possible reason for this prevalence rate may be; that both of these infections share many risk factors and routes of transmission[3]. An other study showed that the inflammatory activity of liver, serum HCV-RNA titers and the degree of fibrosis were significantly higher in HBV-DNA positivepatients than in HBV-DNA negative patents having HCV infection[4]. Another recent study showed that in patients with normal or slightly raised liver enzymes (Alanine transferase) , HBV- DNA was detected in 4(13.3%) patients[5]. Our study will try to find out prevalence of OHBV infection in chronic HCVpatients. If there is a significant association then we have to perform PCR for HBV-DNA in every HCV positive patient, instead of relying only on HBsAg positivity to rule out dual infection. Estimation of true prevalence of dual infection will lead to important management decisions for treatment of such patients in future especiallywho are not responsive to treatment aimed only at Hepatitis C infection.

MATERIALS AND METHODS

This cross sectional study was conducted at Fauji Foundation Hospital (FFH), Rawalpindi, including both outpatient and Emergency Departments from February '28, 2105 to Aug 28, 2015. By using WHO

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calculator sample sizes is 105 with the level of confidence 95%, anticipated population proportion(APP) 13.3% and required absolute precision is 6.5%. The used sampling method is consecutive, non-probability. Both male and female patient between 21-60 years of age who are HCV antibody positive duly verified by real time PCR test and who are negative for HBsAg were included in the study.

Exclusion criteria:

1. Patients with evidence of concomitant liver disease.
2. Patients who have already received antiviral treatment.
3. Patients who have developed hepatocellular carcinoma.
4. Patients who have history of drug induced liver injury or alcohol consumption.
5. Patients with renal failure or on renal replacement therapy.
6. Critically sick patients.

Data collection procedure: An informed consent was taken from all the patients registered for study. Both male and female patients presented to emergency room/outpatient, were evaluated.3ml of blood sample was drawn for PCR. Samples were sent to FFH pathology lab. PCR for HBV-DNA levels were measured by micro lab 200 analyzer using end point method. Results were verified by a consultant pathologist.The study variables were recorded on a proforma.

Data analysis: Data was analyzed by SPSS version 13. Mean and standard deviation was calculated for numerical variable like age in both groups.Frequency and percentages were presented for categorical variable like gender.Odds ratio were calculated. Chi-square test was used to determine the difference. P value < 0.05 was considered significant.

RESULTS

105 patients infected with chronic HCV were checked for presence of OHBV infection. . Distributionof age was calculated in form of mean and standard deviation. Mean age was 47.29+9.15 (Table 1).

Gender distribution was calculated as frequency and percentages of male and female patients. 24 (22.9%) male patients and 81 (77.1%) female patient who were evaluated (Table 2).

Objective was to determine the frequency of OHBV infection in chronic HCV infected patients. Out of 105 patients 08 (7.6%) patients were positive for OHBV infection, whereas 97(92.4%) patients were found negative (Table 3).

Effect modifier like age (years) was stratified and compared with OHBV infection. There were 05 (62.5%) patients who have ages ranges between 21–40 years were found OHBV infection. Similarly, there were 03 (37.5%) patients who have age ranges between 41–60 years and were found OHBV infection. Chi-square test was used to compare age stratification with occult hepatitis B infection, P value 0.041 (Table 4).

Effect modifier like gender of patient was also stratified and compared with OHBV infection. There was a single male patient who found positive for OHBV infection in his serum whereas there were 7(87.5%) female patients who were found OHBV infection positive. Chi-square test was used to compare gender stratification with OHBV infection, P value 0.468 (Table 5).

Table 1: Descriptive statistics of Age (yrs) of patients (n=105)

Minimum	22
Maximum	58
Mean	47.29
Std. deviation	9.159

Table 2: Distribution of Gender of patients

Gender	Frequency	%age
Male	24	22.9
Female	81	77.1

Table 3: Frequency and percentage of Occult Hepatitis B Infection

	Frequency	%age
Positive	8	7.6
Negative	97	92.4

Table 4: Effect modifier like Age stratification with Occult Hepatitis B Infection patients

Age group (years)	Occult hepatitis B infection	
	Positive	Negative
21-40	5(62.5%)	27(27.8%)
41-60	3(37.5%)	70(72.2%)

P value 0.041

Table 5: Effect modifier like Gender stratification with Occult Hepatitis B Infection patients

Gender	Occult hepatitis B infection	
	Positive	Negative
Male	1(12.5%)	23(23.7%)
Female	7(87.5%)	74(76.3%)

P value 0.468

DISCUSSION

As per definition OHBV infection means presence of HBV-DNA in blood of patients who are negative for HBsAg in serum, irrespective of presence or absence of serological markers for HBV infection. Absence of HBsAg may be due to rearrangements of genome of HBV which interferes with expression of genome, or presence of production of antigenically modified S proteins. The molecular evidence of OHBV infection is the persistent presence of covalently-closed-circular DNC (cccDNA) in hepatocytes. Various host immunological and egi-genitic factors are responsible

for suppression of replication and gene expression in other wise replication-competent HBV in patients infected with OHBV infection[6].

On the basis of presence or absence of antibodies, OHBV infection is classified into sero positive and sero-negative groups. In seropositive OHBV infection Hepatitis B core (HBc) or s antibodies are present while in seronegative OHBV infection all the serologic markers of HBV infection are negative, only the low titer of HBV-DNA (<200 IU/mL) is present.

It has been postulated that seronegative OHBV infected patients may have gradual but progressive loss of HBV specific antibodies either after recovery from acute infection, or very mild type of infection fail to cause development and maturation of antiviral responses, thus analysis of hepatic HBV-DNA extracts is gold standard method for detection of OHBV infection.Serum samples can be used only in absence of hepatic samples[7]. It is therefore recommended that in any case real time PCR for various genomic regions of HBV should be performed to diagnose OHBV infection[8].

In Mediterranean region about 1/3rd of chronic HCV patients are suffering from OHBV infection possibly because both of these infections share common risk factors and routes of transmissions[9].
In comparison to Selimet al[5] who found in their study that the patients mean age in years was 43.4±8.6. 45 (75%) were male patients and 15 (25%) were female patients while in our study, mean age (years) was 47.29+9.15 and male patients were 24 (22.9%) whereas 81 (77.1%) female patients.

The outcome of our study shows that out of 105 HCV positive patients, 7.6% patients were positive for OHBV infectionwhich is an evidence of co-infection while the available literature suggest that such dual-infection may be as high as up to 91%. Though the overall percentage affected in our study is relatively low but still projects a significant value and it is important to pick such cases because the line of management of chronic HCV mono-infection is different fromchronic HBV-HCV dual -infection. Not only management but the progress of disease if untreated is also different in these two groups. In case of dual-infection progression of disease (fibrosis) is much faster and risk of complications is much higher as compare to mono-infection, thus the outcome and prognosis of disease also vary. Similarly if dual infection will be treated on lines of mono infection, the response to treatment will not be as good and cure will be in question. It is thereforerecommended to conduct more such studies at a larger sample size. Special emphasis should be given to those population areas where prevalence of HBV infectionis relatively high or response to therapy against chronic hepatitis C mono-infection is not satisfactory.

CONCLUSION

The study concludes that OHBV infection is present in chronic hepatitis C patients.Physiciansneed to perform PCR for HBV-DNA in every HCV infected patient as a protocol instead of relying only on HBsAg positivity to rule out dual infection. It is important to pick cases of OHBV infection in chronic hepatitis C patients because if remain untreated, the progress of cirrhosis and development of complications is much faster in case of dual infection as compare to hepatitis C mono infection, thus this identification has prognostic value as well. Not only this but prevalence of OHBV in chronic hepatitis C patients leads to the important management decisions for treatment of such patients in future who are not responsive to treatment aimed only at Hepatitis C mono-infection.

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