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Original Article

Detection of Anti-HBV and Anti-HCV in sera of subjects with evidence of normal or abnormal liver assessment results.

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ABSTRACT

The study was designed to evaluate the prevalence of HBV and HCV infections in cases referred for biochemical laboratory evaluation of liver function status. For this study, 50 serum samples with evidence of liver impairment based on biochemical evaluation and other 50 serum samples without any biochemical evidence of liver impairment, were randomly selected and called 'impaired liver function and normal liver function groups respectively. The sera were screened for the presence of anti-HbsAg, anti-HCV and in-vivo antibody sensitization. The result of the present study showed that Anti-HbsAg was detected in 3 (6%) of the sera of normal liver function group and 5(10%) of the sera of impaired liver function group. However sensitized antibodies and anti-HCV were not present in any of the sera in both groups. The present study revealed an over all 16% prevalent rate of possible HBV infections in cases referred for biochemical evaluation of the liver function. The study showed that hepatitis B viral infection may be responsible for 1 out 10 cases of hepatitis in this area. The incidence of hepatitis B viral infection in cases of normal liver function possibly suggest that when clinical indications suspects hepatic involvement in a subject inclusion of HBV screening in addition to the normal biochemical evaluation may be necessary. The lack of evidence for HCV infection is discussed.

Keywords: Hepatitis B virus, Hepatitis C virus, impaired liver.

INTRODUCTION

Viral hepatitis is a major health problem causing liver disease in most parts of the world where the prevalence rate is high. We have previously reported a prevalence rate of 7% for HBV, 3% for HCV and 2% for co-morbidity amongst blood donors[1]. We have also shown a prevalent rate of 2% for HBV amongst pregnant women[2]. However, HCV infection was not detected among pregnant women in the study. Hence, from our study of both HBV and HCV infections in this locality, it is possible to say that blood donors seemed high risk for reservoir of both infections. It has been estimated that there are more than 350 million infected carriers of Hepatitis B virus (HBV) and about 170 million people are infected with Hepatitis C virus (HCV)[3]. Majority of the cases of the viral infections remains asymptomatic and invasion of the liver by these agents leads to a wide range of symptoms[4,5]. Since infections with HBV and HCV could present with no specific early signs and symptoms, it could be possible to miss such diagnosis especially when the index for hepatic assessments had shown no evidence of impairment. Hence the present study was designed to evaluate the prevalence of HBV and HCV infections in cases referred for biochemical laboratory evaluation of liver function status.

MATERIALS AND METHODS

Samples:

One hundred serum samples referred to the chemical pathology laboratory for the biochemical evaluation (such as alanine and aspartate aminotransferase and bilirubin) of the liver function were randomly selected for the study after all the results for the original requests have been generated. The left over samples were then screened the same day of collection for anti- HbsAg, anti-HCV and antibody sensitization. based on the biochemical results for liver functions tests, the sera were group into "impaired liver function group (ILFG) (n=50) and normal liver function group (NLFG) (n=50). In using the left over samples for the present study, the WHO ethical guide for use of left over sample was applied in the present study.

Hepatitis B surface antigen (HbsAg) screening:

The principle is based on detection of Hepatitis B surface antigen (HbsAg) antibodies in the serum using one step HbsAg strip (Acon Laboratories incorporated USA). One step HbsAg strip is a qualitative, lateral flow immunoassay. The membrane is pre-coated with anti-HbsAg antibodies on the test line region of the strip. The mixture migrates upwards on the membrane chromatographically by capillary action to react with anti-HbsAg antibodies on the membrane and generate a coloured line. The presence of this coloured line in the test region indicates positive result, while its absence indicates a negative result. The procedure is as described by the manufacturer. In brief, the test strips were immersed vertically in the respective sera for 15 seconds. The test strips are then placed on non-absorbent flat surface allowed to incubate for 15 minutes. This allows for reaction between the pre-coated anti-HbsAg antibodies and the HbsAg antibodies present in the sera. For HBV sero-positive sera two distinct lines at the test and control regions of the test strip will appear. However, for HBV sero-negative sera only one distinct line at the control region of the test strip will appear.

Hepatitis C Virus (HCV) Screening:

The principle is based on detection of HCV antibodies in the serum using HCV rapid test device (Core Diagnostics UK). HCV rapid test device is a qualitative membrane based antigen-antibody immunoassay. The membrane is coated with anti-HCV antibodies on the test line region of the device. The procedure was as described by the manufacturer. In brief, 0.1ml of serum sample was added into appropriately labeled sample well of the rapid test device and allowed to incubate for 10 minutes. This allowed for complete reaction between the pre-coated anti-HCV antibodies and the HCV antibodies in the serum. For sero-positive HCV samples two distinct lines appeared on the control and test regions respectively while only one distinct red line at the control region is seen for sero-negative samples.

The Antihuman Globulin Screening Test:

Blood group O Rhesus positive red cells from seven different blood bags were pooled. This was washed several times with normal saline and 5% suspension made. 4 volumes of the test serum were mixed with one volume of the 5% suspension of red cells. The set up was incubated for two hours at 37°C. After incubation, the cells were washed four times with normal saline and re-suspended to 5% strength. One volume of this 5% suspension of cell was mixed with one volume of the anti-human globulin and incubated for 5minutes at room temperature. The result was then read both macroscopically and microscopically. For the validation of the test reaction control tests were set up. This consists of weakly reacting positive cells, strongly reacting positive cells and non reacting cells.

Statistical Analysis: The variables were expressed in percentage.

RESULTS

Anti-HbsAg was detected 3(6%) of the sera of normal liver function group and was detected in 5(10%) of sera of impaired liver function group. No anti-HCV and no sensitized antibodies were detected in both groups of sera. See table 1.

Table 1: result of screening tests for anti- HbsAg, anti-HCV and antibodies sensitization in sera of NLFG and ILFG.

Screening	n	positive	negative
Anti- HbsAg		( )*	
NLFG	50	3(6)	47(94)
ILFG	50	5(10)	45(90)
Anti-HCV			
NLFG	50	0(0)	50(100)
ILFG	50	0(0)	50(100)
Antibodies sensitization			
NLFG	50	0(0)	50(100)
ILFG	50	0(0)	50(100)

Key: NLFG = normal liver function group
ILFG = impaired liver function group
( )* = percentage in parenthesis

DISCUSSION

Viral hepatitis is a primary infection of the liver commonly caused by Hepatitis B virus (HBV) and Hepatitis C virus (HCV). In the present study we did not observed HBV and HCV co-infections in any of the groups studied. However, the detection of anti- HbsAg in both groups represents an interesting finding that showed 16% prevalence in patients with high clinical indication for hepatic involvement.

The study showed that 1 out of 10 cases of possible hepatitis are caused by HBV infection in this locality. However, the 6% prevalence of possible HBV infection in cases of normal liver function suggest level of asymptomatic infections that could be missed out if clinical conditions suggest hepatitis function evaluation but excludes HBV screening.

The detection of anti- HbsAg may not only suggest recent infections which could be asymptomatic[4] or progressive infection especially in the presence of hepatitis but may also suggest burden of infection in the locality since specific antibodies could still be detected few months after infection[6].

The prevalent rate of HBV in these cases referred for biochemical evaluation of liver functions was high than those previously reported in this locality among pregnant women and blood donors[1,2]. However, unlike the study involving the blood donors where HBV and HCV co-infections were observed, this was not the case in the present study, although one would have suspected that there will be high prevalence of HBV and HCV co-infections in the present study.

Elsewhere co-infection of HCV and HBV has been reported among drug addicts, patients on heamodialysis, patients undergoing organ transplantation and HIV infected subjects[7-10]. This possible suggest that factors such as the above if present may predispose to consistent incidences of co-infections involving both viral infections.

The present study thus concludes that it may be necessary to include HBV screening in requests for biochemical evaluation of the liver when there is high indication for it.

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