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Research Article

SEROFREQUENCY OF HEPATITIS D VIRUS AMONG HEPATITIS B INFECTED PATIENTS, KHARTOUM STATE, SUDAN.

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ABSTRACT

Background: Hepatitis Delta virus (HDV) is a defective RNA virus dependent on Hepatitis B virus (HBV) infection for its replication and expression. It is estimated that approximately 5% of hepatitis B surface antigen (HbsAg) carriers in the world are HDV infected patients. HBV-HDV co-infection may lead to more severe acute disease and higher risks of fulminant hepatitis, cirrhosis, and hepatocellular carcinoma than those having HBV infection alone. Therefore, our study aimed to detect serofrequency of hepatitis D among hepatitis B infected patients.

Materials and Methods: This was cross sectional study included known HBV infected patients, conducted Khartoum State, Sudan, during March to June 2015. HDV Antigen was detected using commercially available ELISA kit. Generated data were analyzed by using SPSS program.

Results: A number of 90 patients who were HBsAg positive, attending Khartoum state hospitals, Sudan, were included in this study, aged between (20-79) years old, out of them 4(4.4%), were positive for HDV Ag, and all of them were males. There was no association between positive HDV Ag and risk factors such as Hemodialysis, blood transfusion, IV drug abuse, cupping as well as previous history of hepatocellular carcinoma.

Conclusions: Although HDV had a low frequency in our area, it is important for healthcare providers and policy makers to plan preventive strategies for HDV spread as well as HBV prevention programs among high risk population. Further investigations with large scale specimen are recommended.

Keywords: Hepatitis Delta virus, Hepatitis B, ELISA, Serofrequency, Khartoum-Sudan.

INTRODUCTION

Hepatitis D virus (hepatitis Delta) is a defective negative sense single-stranded RNA virus [(1)].The antigenomic strand of HDV is 1,700 nucleotides which encodes the only virus specific protein, hepatitis delta antigen (HDAg), in two molecular-weight forms: small HDAg (S-HDAg) and large HDAg (L-HDAg) [(1, 2, 3)].

HDV requires the HBsAg of Hepatitis B virus to establish infection in humans [(1)]. Because its genome is encapsulated within protein coat of HBV Ag that allows HDV to gain cell entry, so they utilize the same so far unidentified receptor [(2)]. With exception of envelope protein, HDV life cycle is independent of HBV [(2)].

Since discovery of HDV in 1977 by Rizetto in Italy among patients with a severe form of Hepatitis B virus infection it is well documented that HDV is wide spread disease that has affected a large number of populations with HBV infection in the world, but its frequency varies greatly throughout different geographical regions, it is highly endemic in the middle east, in the Mediterranean area, Amazonian region, and several African countries [(2, 4)].

Up to 5% of the world's populations have been infected with hepatitis B virus (HBV), probably at least 5% of the HBV carriers have hepatitis D virus (HDV) super infection. It is estimated that 15 million people are infected with HDV world wild [(4)]. In association with HBV, HDV produces significantly more severe illness than HBV alone [(5)].

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The disease spectrum of HDV infection varies greatly from fulminant hepatitis, rapidly progressive disease, to a subclinical course. Persistent replication of HDV associated with continuous hepatic inflammation and elevated alanine aminotransferase (ALT) levels is a characteristic of chronic active hepatitis D [6].

There are two modes of the HDV infection either as an acute co infection or as super infection in patient with chronic HBV [3, 7].

The routes of HDV transmission are similar to those for HBV, including blood-borne, sexual, percutaneous, permucosal, and perinatal transmission. However, patients having HBV-HDV co-infection may have more severe acute disease and higher risks of fulminant hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) than those having HBV infection alone [8] . Also, HBV infected patients with HDV superinfection have a higher rate of progression to chronic disease and serious complications [9].

Based on phylogenic analysis, HDV isolates collected worldwide have been classified into 8 groups, HDV-1 (former genotype I), HDV-2 (former genotype IIa), HDV-3 (former genotype III), HDV-4 (former genotype IIb), and HDV-5 to HDV-8 [10].

The most frequent method of diagnosing HDV infection is the measurement of anti HDV (IgM, IgG) or HDV antigen in serum by ELISA. PCR can also be used to detect viral RNA in the blood [11].

Materials and methods:

Design

This was across sectional study included HBV infected patients, conducted Khartoum State hospitals, Sudan, during March to June 2015. The data was collected by structured interviewing questionnaire. Ethical approval was taken from Al Neelain University research ethical board and consent from patients verbally.

Experimental work

Serum specimens were collected from known HBsAg positive patients, and screened for hepatitis D Antigen using enzyme linked immune sorbent assay (ELISA) technique at research laboratory\AL Neelain University.

Collection of specimens and processing

Five milliliters of blood were collected under aseptic technique into plain container, the sera obtained after centrifugation were kept at -20 until serologic test was performed by using ELISA Kit (Diagnostic, USA) . And the results obtained were interpreted according to manufacturer's instructions.

Assay procedure

All reagents were brought to room temperature (20-25°C) before running the assay.

Six wells were reserved for blank and controls, three wells for negative control, two wells for positive control.

Fifty µl of negative control, positive control and samples were dispensed into their respective wells, and then 50 µl of Extraction Solution were dispensed into all wells, except for the blank well.

The microplate was covered with adhesive sealer to prevent evaporation, and incubated for 30 minutes at 37°C, when incubation was completed, adhesive sealer was discarded, and the strips were washed (for 5 more times) by using automatic washer, after that the strips mouth were turned down on to blotting paper to remove any excess liquid from wells. Then 100 µl of HRP-Conjugate Reagent were added into each well except the blank, and mixed gently. Then the plate was covered and incubated for 30 minutes at 37°C. When incubation was completed, the adhesive sealer was discarded, and the strips were washed as above.

Fifty µl of Chromogen A and 50 µl Chromogen B solutions were dispensed into each wells including the Blank and mixed by tapping the plate gently. Then the plate was incubated at 37°C for 10 minutes away from light.

By Using a multichannel pipette 50 µl of Stop Solution was added into each well and mixed gently.

Measurement: The plate reader was calibrated with the Blank well and the absorbencies of each well were measured at 450nm/630nm within 5 minutes of adding the Stop Solution.

Calculation and interpretation of results

The result calculated by cut-off value. The cut-off value is determined by multiplying 2.1 to the mean absorbance values of the negative controls.
Cut-off value = ***Nc × 2.1**.
***Nc = (**the mean absorbance values of the three negative controls)

Interpretations of the results

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1) : Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis D virus specific antigens have been detected with this HDV–Ag ELISA kit.

Positive Results :(S/C.O.≥1) : Samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HDV virus specific antigens have been detected using this HDV–Ag ELISA kit.

Borderline (S/CO =0.9-1.1) : Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline. Retesting of these samples in duplicates is recommended. Repeatedly reactive samples could be considered positive for antigens to HDV. Follow up and supplementary testing any positive with other HDV tests or analytical systems (e.g. PCR, WB) is required to establish the infection state.

Data analysis

The generated data were analyzed by using master sheet and Statistical Package for Social Sciences (SPSS) program. The seropositivity of HDV Antigen, and related to gender, age, and risk factors, were demonstrated by chi-square test and statistical significant relationship was obtained by p-value ($p \leq 0.05$).

Results:

A number of 90 patients who were HBsAg positive, attending Khartoum state Hospitals, Sudan, during March to June 2015 were included. Their age range between (20-79) years old, 70(77.8%) were males and 20(22.2%) were females. 66(73.3%) were undergoing hemodialysis, 16(17.8%) were previously diagnosed with hepatocellular carcinoma, were included in this study.

Out of the total, 4(4.4%) were seropositive for HDV Ag, (figure 1). And all of them were males 4(4.4%) (figure2).

The seropositivity of HDV Ag and age groups range shown in (table 1), there was no association between positive HDV Ag and risk factors such as Hemodialysis, blood transfusion, IV drug abuse, cupping as well as hepatocellular carcinoma (table 2).

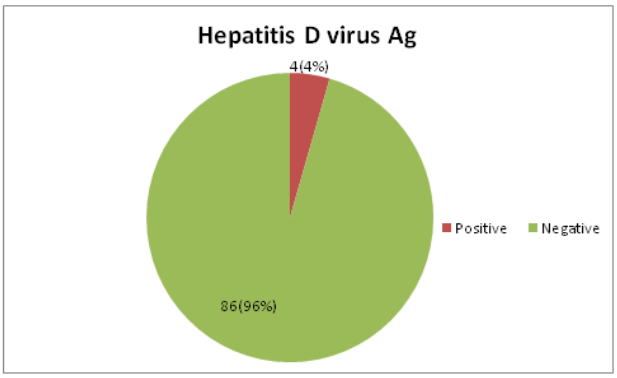

Figure 1: Serofrequency of Hepatitis D Virus Ag among known HBsAg patients (n=90).

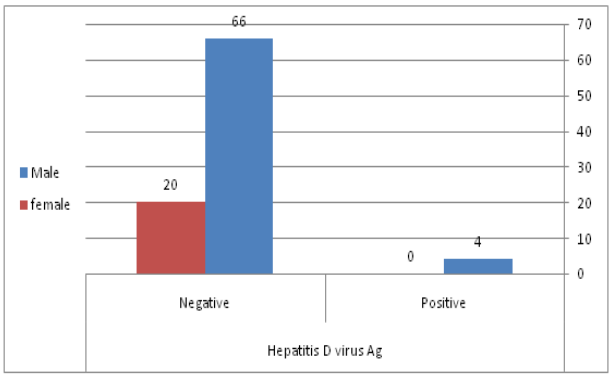

Figure 2: Serofrequency of HDV among study population according to gender.

Table 1: Serofrequency of HDV among study population according to their age

Age group range/ years	Hepatitis D virus Ag		Total n (%)
	Positive n (%)	Negative n (%)	
20-29	0(0.0%)	14(15.9%)	14(15.9%)
30-39	2(2.2%)	17(18.9%)	19(21.1%)
40-49	1(1.1%)	19(21.1%)	20(22.2%)
50-59	1(1.1%)	15(16.7%)	16(17.8%)
60-69	0(0.0%)	11(12.2%)	11(12.2%)
70-79	0(0.0%)	10(11.1%)	10(11.1%)
Total	4(4.4%)	86(95.6%)	90(100.0%)

Table 2: Relation of Serofrequency of HDV and risk factors

	Hepatitis D virus Ag		
	Positive n (%)	Negative n (%)	P value
Hemodialysis	4(4.4%)	62(68.9%)	.217
Blood transfusion	0(0.0%)	6(6.7%)	.585
IV drugs users	0(0.0%)	0(0.0%)	-
Cupping	0(0.0%)	0(0.0%)	-
Hepatocellular carcinoma	0(0.0%)	16(17.8%)	.341

Discussion:

This study as used descriptive cross sectional study planned to determine the serofrequency of Hepatitis D virus among HBV infected patients. Our study included 90 known HBsAg patients were investigated for HDV Ag. the serofrequency of HDV was 4(4.4%) which is similar to results in study conducted in Egypt by Gomma *et al* (2013), who found HDV antibodies in 8 (4.7%), from 170 HBsAg positive healthy individuals [(12)]. Also compared to result obtained by Ghadir *et al* (2012), in Qom Province, Center of Iran, who detected HDV(2%) in their study [(11)].

Our result considered low compared to high seroprevalance reported by Tahaei *et al* (2014), in Tehran, Iran, who reported 37(7.7%), of HDV from their patients [(13)], also study by Alizadeh *et al* (2010), found Anti-HDV IgG was found in 14 (17.3%) individuals. Only one participant was positive for anti-HDV IgM [(14)].

The only similar published study in Sudan done by Mokhtar Z, *et al* (2015), who found 24(26.7%), were positive for HDV IgG [(15)].

The variation may be due to use of difference techniques in detection, sample size or technique use for analysis.

In this study HDV positive patient was in middle age, similar to study of Alizadeh *et al* (2010) who was found that the cases of seropositivity is highest with adults age [(14)].

in the present study, statistically, there was insignificant relationship between risk factors (cupping, hemodialysis, IVdrug abuse, blood transfusion), and seropositivity of HDV, which was similar to study done by Ghadir *et al*, in Qom Province, Center of Iran, (2012), who found that there was no significant relationship between tattooing, surgery history, or dental surgery and hepatitis D infection [(11)].

In conclusion although this study reported low result for serofrequency of HDV among HBsAg positive patients, it is important for healthcare providers and policy makers to plan preventive strategies for HDV spread as well as HBV prevention programs among high risk population. Further investigations with large scale specimen are recommended.

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