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RESEARCH ARTICLE

SEROFREQUENCY OF HEPATITIS D VIRUS AMONG HEPATITIS B VIRUS INFECTED PATIENTS ATTENDING TO TALODI HOSPITAL, SOUTH KORDOFAN STATE, SUDAN.

Mokhtar Zakaria Adam Salih[*1], Wafa Ibrahim Elhag[2], Raja Yagoub El Feel[***3], Hamza Abdallh[****4]**

[*]1-Talodi hospital, Laboratory department, South Kordofan, State, Sudan.

[**]2-Assistant professor, Faculty of Medical Laboratory Sciences, Al Neelain University, Sudan.

[***]3-Ministry of Health, Khartoum, State, Sudan.

[****]4- Ministry of Health, Virology lab, Khartoum, State, Sudan.

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ABSTRACT

Background: Hepatitis Delta Virus (HDV) infects only patients that are already infected by hepatitis B virus (HBV) because this is sub satellite virus which depends on and propagate only in the presence of HBV. HDV causes co-infection or super infection with sever complication as compared to only HBV infection. There is no published data in Sudan, therefore, our study aimed to detect serofrequency of hepatitis D among hepatitis B infected patients.

Methods: This is across sectional study included HBV infected patients aged between (11-54) years old with mean 30.4 years old conducted Talodi hospital, South Kordofan, State, Sudan, during February to May 2014.

We performed HDV serum marker, was detected using commercially available enzyme-linked immunosorbent assay kit. Generated data were analyzed by using SPSS program.

Results: A number of 90 patients who were HBsAg positive, attending Talodi hospital, South Kordofan, State, Sudan, were included in this study, their age range (11-54) years old, out of them 24(26.7%), were positive for HDV IgG. From them 11(12.2%) males and 13(14.4) were females. The seropositivity of HDV had associated with risk factors of positive family history, cupping, surgery, and dental surgery. This study shown statistically significant relationship between gender and seropositivity of HDV infection (p=0.03). However, there was no significant relationship between risk factors and HDV.

Conclusion: the seropositivity of HDV among HBsAg is higher in Talodi. Further confirmation and mentoring with large scale specimen is recommended.

Key words: Anti-HDV, Talodi Hospital, South Kordofan State, Serofrequency, ELISA.

INTRODUCTION:

Hepatitis D virus (HDV) is small incomplete negative sense single stranded RAN virus. Its genome is 1,700 nucleotides which it only encode one virus specific protein [1, 2].

HDV is the smallest of known human pathogen and resemble sub viral plant pathogen [3].

HDV occur only in patients infected with hepatitis B virus (HBV) because its genome is encapsulated within protein coat of HBV Ag that allow 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].

In a natural setting only human can acquire HDV either as an acute co infection or as super infection in patient with chronic HBV [2, 4].

Since discovery of HDV in 1977 by Rizetto in Italy 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 [1, 5].

It has been estimated that approximately 5% of HBV carrier are co infected with HDV, leading to an estimated 15 million person infected with HDV worldwide [5].

The transmission routes of HDV are similar to those of HBV and researchers have shown that very little inoculums are sufficient for transmission, these routes include blood transfusion, intravenous drug abuse, sexual contact and nosocomial infection, and there are some

*Corresponding author: Mokhtar Zakaria Adam Salih | E-mail: mokhtarzakaria757@gmail.com

evidences that infection could be transmitted between family members[7].

Several studies have shown that chronic HDV infection lead to more severe liver disease than chronic HBV mono infection with the course of progression of the fibrosis being accelerated, the risk of hepatocellular carcinoma being increased and early decompensation occurring in the established cirrhosis [4].

The most frequent method of diagnosing HDV infection is the measurement of anti HDV (IgM, IgG) in serum by ELISA. PCR can also be used to detect viral RNA in the blood [6].

And because the absence of the epidemiological study of HDV in Sudan, the aim of this study is to estimate the serofrequency of HDV infection in samples of HBV infected patients in Talodi hospital, State of South Kordofan, Sudan.

MATERIALS AND METHODS:

Design

This is across sectional study included HBV infected patients aged between (11-54) years old with mean 30.4 years old conducted Talodi hospital, South Kordofan, State, Sudan, during February to May 2014.

The data was collected by structured questionnaire. Ethical approval was taken from Al Neelain University research ethical board and from patients verbally.

Experimental work

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

Collection of specimens and processing

Three milliliters of blood were collected under aseptic technique into plain container, the sera obtained after centrifugation were kept at -20 until IgG antibodies were qualified by ELISA (DiaSorin, Italy).

All reagents were brought to room temperature before assaying.

Fifty micro liter negative control, positive control and samples were dispensed into their respective wells, then 100µ of diluted enzyme tracer (conjugate) were dispensed into all wells, except for the blank well, then the card board sealer was applied on to microtitters wells to prevent evaporation, and incubated for 3 hours at 37^{o}c.

The choromogen/ substrate was preperd just before the end of incubation, and when incubation was completed, the card board was discarded, and the strips were washed by using automatic washer, after that the strips mouth were turned down on to blotting paper to remove any liquid residue.

Hunderd microliter of chromogen/ substrate solution was dispensed in to all wells and incubated for 30 mimutes at room temprture away from intense light, then 100µ of blocking reagent was dispensed into all wells in the same order and at the same rate as for chromogen/ substrate.

Measurement

The absorbance of specimens were measured with photometer at 450/630 nm within one hour of adding the blocking reagent.

Calculatation and interpretation of result

The result calculated by cut-off value. The cut-off value is determined by adding the mean absorbance for the negative control values (NC) multiplied by 0.5 to the mean absorbance for the positive control values(PC) multiplied by 0.5.

Cut-off value = 0.5 NC + 0.5 PC .

The presence or absence of anti-HD is determined by comparing the absorbance of the unknown samples to that of the cut-off value. The unknown samples with absorbance values less than or equal to the cut-off value should be considered reactive for anti-HD. The unknown samples with absorbance values greater than the cut-off value should be considered non-reactive.

Samples with absorbance values within ±10% of the cut-off value must be retested in order to confirm the initial result. Samples which are repeatedly reactive should be considered positive. Samples which are non-reactive at the second test should be considered negative.

Data analysis

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

RESULTS:

A total of 90 HBsAg positive patients, attending Talodi hospital, South Kordofan State, Sudan, during February to May 2014, were enrolled in this study, their mean age range 30.4 years old, of them 63 (70%) were males and 27 (30%) were females.

Out of the total, 24(26.7%) were seropositive for HDV IgG, (figure1). From them 11(12.2%) were males and 13(14.4%) were females, (figure2).

The seropositive of HDV and age the study reported high positive at age from 20-30 years old, (table1).Based on gender and risk factors the study shown statistically significant relationship (p=0.03) between gender and HDV infection and there were no relationship between infection and risk factors, (table2). Regarding to duration of HBsAg and seropositivity of HDV the duration less than one month, one year, two years, and three years revealed

the same result and there was no significant relation (p=0.912), (table3).

DISCUSSION:

Several researches have been made and reported different results in various countries associated with HDV among HBsAg patients. This study aimed to estimate serofrequency of HDV among HBsAg patients, attending to Talodi hospital, South Kordofan, State, Sudan.

Our study included 90 HBsAg positive patients were investigated for HDV IgG. The overall, seropositive of HDV IgG among HBsAg patients were 24(26.7%), similar to result by Khan *et al* (2011), in Pakistan who found HDV in 190 patients 53(28%) by using molecular technique[5].Other study reported by Popescu *et al*, in Bucharest (2013), they found seroprevlance of HDV (20.4%) in their study[9]. Also, relatively to study conducted by Bakhshipour *et al* (2013), in Zahedan, Iran, they found HDV in 75(17%) patients [1] , However, the lower result were obtained by Ghadir *et al* (2012), in Qom Province, Center of Iran, who detected HDV(2%) in their study[6], and Tahaei *et al* (2014), in Tehran, Iran, who reported 37(7.7%), of HDV from their patients[7], The variation may be due to sample size technique use for analysis. Also, where compared to study conducted in Egypt by Gomma *et al* (2013), who found HDV antibodies in 8 (4.7%), from 170 HBsAg positive healthy individuals[8].

In the present study the relationship between seropositivity and gender demonstrated that 11(12.2) were males and 13(14.4%) were females, and it is significant (p=0.003), it is similar to study which had been reported by Khan *et al* (2011), in Pakistan who found it significantly related with gender (p<0.05) in males 37(69.8) compared to females 16(30.2%) [5]. Regarding age groups the seropositive result was high among 20-30 age range. This agreed to result obtained by Khan *et al* (2011), who found insignificant relationship among age below and above 40 years [5].

However, Popescu *et al*, in Bucharest (2013), they found that Seroprevalence was not gender related, but patients over 40 years were more likely to have anti-HDV antibodies, RR = 1.9 (1.2; 3.0)[9]. There were no relationship between risk factors (cupping, surgery, dental surgery, blood transfusion), and seropositivity of HDV, which was similar to other study done by Ghadir *et al*, in Qom Province, Center of Iran, (2012), were found that there was no significant relationship between tattooing, surgery history, or dental surgery and hepatitis D infection [6], also, Statistically, there was insignificant relationship between seropositivity and duration of HBsAg. In conclusion this study reported high result for serofrequency of HDV among HBsAg positive patients that indicate the important of screening for HDV infection for all patients who had HBV. We recommended to immunized all people who at risk for having HBV. Further confirmation and mentoring with large scale specimen is recommended.

Figures and tables

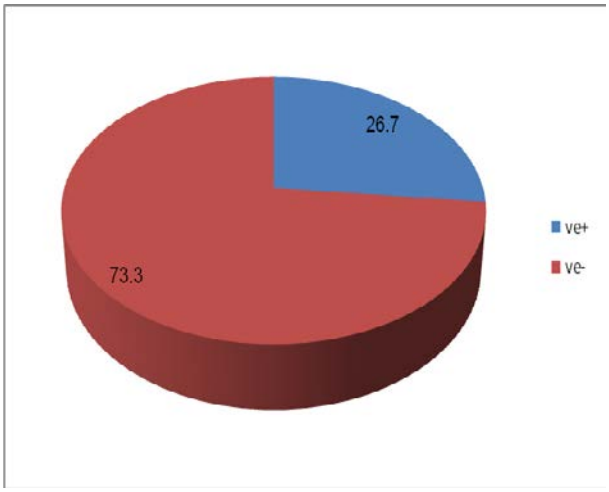

Figure 1: Serofrequency of HDV IgG among known HBsAg patients.

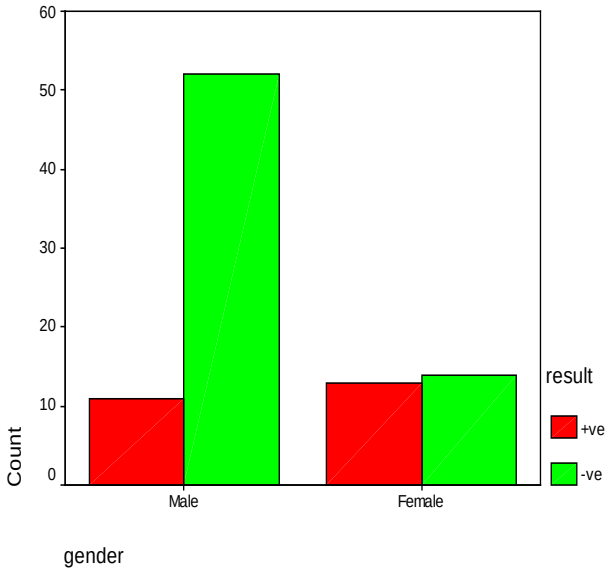

Figure 2: Serofrequency of HDV among study population according to their gender.

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

	Positive, No, (%)	Negative, No, (%)	Total, No, (%)	P value
Age range/years				0.845
10-20	2 (2.2)	10 (11.1)	12 (13.3)	
20-30	11 (12.2)	24 (26.7)	35 (38.9)	
30-40	8 (8.9)	23 (25.6)	31 (34.4)	
40-50	3 (3.3)	8 (8.9)	11 (12.2)	
50-60	0 (0.0)	1 (1.1)	1 (1.1)	
Total	24 (26.7)	66 (73.3)	90 (100)	

*P < 0.05 is significant.

Table 2: Serofrequency of HDV among HBsAg patients in relation to demographic data and risk factors.

	HBsAg positive, No. (%)	HDV-IgG Ab positive, No. (%)	P value
Gender			0.03
Males	63 (70)	11 (12.2)	
Females	27 (30)	13(14.4)	
Positive family history			0.312
Yes	60 (66.7)	18 (20.0)	
No	30 (33.3)	6 (6.7)	
Cupping			0.445
Yes	20 (22.2)	4 (4.4)	
No	70 (77.8)	20 (22.2)	
IV drugs abuse			-
Yes	0.00)(0	0.00) (0	
No	90 (100)	24 (26.7)	
Health worker			-
Yes	0 (0.00)	0 (0.00)	
No	90 (100)	24 (26.7)	
Surgery			0.613
Yes	10 (11.1)	2 (2.2)	
No	80 (88.9)	22 (24.4)	
Dental surgery			0.450
Yes	2 (2.2)	1 (1.1)	
No	88 (97.8)	23 (25.6)	
Blood transfusion			0.097
Yes	7 (7.8)	0 (0.00)	
No	83 (92.2)	24 (26.7)	
Not identified			0.259
Yes	19 (21.1)	7 (7.8)	
No	71 (78.9)	17 (18.9)	

*P < 0.05 is significant.

Table 3: Cross tabulation between seropositivity of HDV and duration of HBsAg among study population.

	Positive, No, (%)	Negative, No, (%)	Total, No, (%)	P value
Duration				0.912
Less than one month	6 (6).7	12 (13.3)	18 (20.0)	
1 year	6 (6).7	15 (16.7)	21 (23.3)	
2 years	5 (5.6)	19 (21.1)	24 (26.7)	
3 years	6 (6.7)	16 (17.8)	22 (24.4)	
4 years	1(1.1)	4 (4.4)	5 (5.6)	
Total	24 (26.7)	66 (73.3)	90 (100)	

*P < 0.05 is significant.

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