

SEROFREQUENCY OF HEPATITIS D VIRUS AMONG KNOWN HEPATITIS B PREGNANT WOMEN, ALJAZEERA STATE, SUDAN

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ABSTRACT

Background: Hepatitis D is a major global health problem affecting 15 to 20 million individuals worldwide. Hepatitis D virus (HDV) is a small defective RNA virus dependent on Hepatitis B virus (HBV) for its replication and expression. The global prevalence of HDV is poorly known due to lack of studies in several parts of the world. Therefore, our study aimed to detect serofrequency of hepatitis D among known HBV pregnant women.

Methods: This is a cross-sectional study included 90 known HBV positive pregnant women, Aljazeera, State, Sudan, during February to May 2016. HDV serum marker, was detected using commercially available Enzyme-linked Immunosorbent assay, data was collected using direct interviewing questionnaire.

Results: Ninety HBV positive pregnant women were included in the study and their age ranged between 20 and 40 years with a mean age of 30.4 years. The generated data were analyzed by using SPSS program. Out of total 3 (3.3%) women were positive for HDV IgM. The seropositivity of HDV had association with risk factors of positive history of hepatitis and history of abortion. Statistical analysis showed significant relationship between seropositivity of HDV and other factors including age, gravidity and gestational age ($p < 0.05$).

Conclusion: There was insignificant relationship between risk factors and HDV. Further confirmation and mentoring with large scale specimen is recommended.

Key words: Anti-HDV, known HBV, pregnancy, Serofrequency, ELISA, Aljazeera State-Sudan.

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INTRODUCTION

Hepatitis D virus (HDV) is a defective RNA virus presenting similarities to some plant viroids, which requires hepatitis B virus (HBV) as a helper virus for its propagation^[1].

Among 240 million chronic HBV carriers reported worldwide, approx. 15 to 20 million individuals are also infected with HDV^[2-3]. In Africa, of the estimated 65 million chronic HBV carriers, about one fourth of HBsAg-positive individuals show dual-infection with HDV. Chronic HBV/HDV co-infection can lead more often to severe liver diseases, like fulminant hepatitis, if compared to HBV mono-infection^[4,5], whereas HBV/HDV super-infection is associated with chronic infection among 90 % of the virus carriers^[3]

Coinfections of HBV and HDV are usually acute, self-limited infections.

The chronic form of HDV is seen in less than 5% of HBV – HDV coinfecting patients^[2]. Acute HDV infection occurs after an incubation period of 3 - 7 weeks, and a preicteric phase begins with symptoms of fatigue, lethargy, anorexia and nausea, lasting usually 3 to 7 days. During this phase, ALT and AST activities become abnormal.

The appearance of jaundice is typical at the onset of the icteric phase. Fatigue and nausea persist, clay-colored stools and dark urine appear, and serum bilirubin levels become abnormal.

Superinfection is associated with fulminant acute hepatitis D and severe chronic active hepatitis, often progressive to cirrhosis^[26].

HDV is a spherical hybrid particle of ~36 nm in diameter, composed of an outer coat containing hepatitis B surface antigens (HBsAg) and host lipids. The inner nucleocapsid consists of small and large hepatitis D delta antigens (sHDAg and LHDAg) and a single-stranded, circular RNA molecule of ~1.7 kb^[6,7]. The unique open reading frame of the HDV genome encodes the delta antigens. sHDAg is required for HDV genome synthesis while LHDAg inhibits HDV-RNA

synthesis and is essential for HDV particle formation ^[8]. Currently, eight HDV genotypes (HDV1-8) have been described with variable

HDV1 infection is associated with both severe and mild liver disease, whereas HDV2 and HDV3 are associated with mild clinical course and outbreaks of severe fulminant hepatitis, respectively^[9]. However, there is limited information on the clinical course of the other five HDV genotypes.

The HDV prevalence has been reported in various parts of the world. In Africa, the anti-HDV antibody prevalence in HBsAg carriers was reported only in Cameroon (17.6 %) and Gabon (15.6 % to 70.6 %) ^[10,11,12].

Recently, HDV prevalence in sub-Saharan Africa was estimated from 1.3 % to 50 % ^[4].

Although HBV is endemic in Nigeria, data on HDV seroprevalence are limited. A previous study showed that HDV antigen was detectable in 6.5 % of patients with chronic hepatitis B in Southwest Nigeria ^[13]. In addition, another study reported an anti-HDV prevalence of 12.5 % in 96 HBsAg positive patients ^[14]. Moreover, a recent study showed that HDV1 prevails with 53.3 % in Southwestern Nigeria followed by the HDV5 (33.3 %) and HDV6 (13.3 %), which were more restricted to the northern part of Nigeria ^[4]. In association with with HBV, HDV produces significantly more severe illness than HBV alone (Gupta *et al.*, 2005)

The aim of this study is to estimate The serofrequency of HDV sample in HBV infected pregnant woman Aljazeera state, Sudan.

MATERIALS AND METHODS

Design:

This is a cross-sectional study included HBV infected pregnant woman aged between (20-44) years old in Aljazeera State, Sudan, during February to May 2016. The data were collected by structured questionnaire. Ethical approval was taken from Al Neelain University Research Ethical Board and from patients verbally.

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 IgM antibodies were qualified by ELISA (AccuDiag TM China). All reagents were brought to room temperature before assaying.

Test Procedure:

Fifty micro liter negative control, positive control and samples were dispensed into their respective wells, then 100 μ 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 C°.

The chromogen/ substrate was prepared 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.

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

Measurement:

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

Calculation 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 2.1 .

Cut-off value = NC *2.1 . The presence or absence of anti-HDV is determined by if the sample less than one it considered negative while positive when read more than one and when read between 0.9-1.1 it considered borderline.

Data analysis:

The generated data were analyzed by using master sheet and Statistical Package for social Science (SPSS) program. The seropositivity of anti-HDV (IgM), and related to age, gravidity, trimester of pregnancy, history of hepatitis and history of miscarriage were demonstrated by chi-square test and statistically significant relationship was obtained by p- value ($p < 0.05$) and related to age,

gravidity, trimester of pregnancy, history of hepatitis and history of abortion were demonstrated by chi-square test and statistical significant relationship was obtained by p-value($p \leq 0.05$).

RESULTS

A total of 90 known HBs Ag positive pregnant women were enrolled in this study, their mean age was 30.4 years old.

The overall result revealed that HDV antibodies were detected in 3 (3.3%) among the studied group (Figure 1).

The present study showed statistically significant relationship ($p=0.002$ and 0.04) between history of hepatitis infection and history of miscarriage respectively with HDV infection, however, it was insignificant with age, gravidity, and gestational age of pregnancy (p value 0.367 , 0.154 and 0.962 respectively) (table1).

Most of studied pregnant ladies were in 20-40 age range, in trimester (0.962), primary and multi-gravidity (0.154), (0.4) had no past history of abortion or miscarriage.

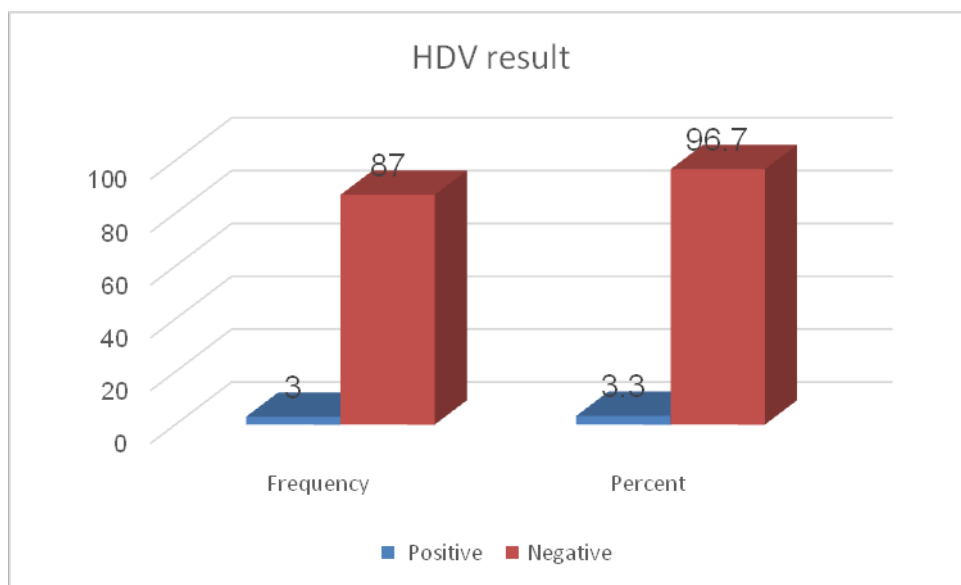


Figure 1: Serofrequency of HDV infection among known HBV pregnant women (n=90).

Table 1: Relation between HDV and other factors among known HBV pregnant women (n=90)

N o	Risk factor		Count no.	HDV		P-value
				Positive	Negative	
1	History of hepatitis	Yes	15	2	13	0.002
		No	57	1	56	
		Unknown	18	0	18	
2.	History of miscarriage	Yes	19	3	16	0.04
		No	67	0	67	
		Unknown	4	0	4	
3	Gravidity	Prim-gravidity	36	0	36	0.154
		Multi gravidity	54	3	51	
4	Gestational age	1st trimester	31	1	30	0.962
		2nd trimester	30	1	29	
		3rd trimester	29	1	28	
5	Age groups	20-25	26	0	26	0.367
		26-35	42	2	40	
		36-45	22	1	21	
Total			450	15	435	

*P < 0.05 is significant

DISCUSSION

HDV remains a major health problem in underdeveloped areas with uncontrolled HBV spread. The present study results revealed that only 3 (3.3%) of pregnant women with known HBsAg were positive for HDVAg. The result of our study is low than the results of study conducted in Gabon by Maria Makuwa *et al*, which published in Journal of Clinical Microbiology in 2008 Feb, few data on the prevalence of HDV in the general population in central Africa are available, although one study⁽²⁷⁾ showed a prevalence of 8.5% in three villages in a rural area of Gabon. In the study, the overall prevalence of HDV among pregnant women was (15.6%).

Because it is regarded as a serious disease, a number of studies were conducted in HDV in several countries. One of these studies was published in 1988 and assessed the prevalence of HDV in pregnant women in Bandung, a densely populated area of Indonesia. Out of the 926 pregnant women included in the study, only 2.8% (26) were carriers of HBsAg, among which, none tested positive for delta antibodies in spite of the fact that in this Indonesian population, HbsAg was frequent (Vranckx *et al.*, 1988). Accordingly, the results of this study are almost similar to the results of the study conducted by the researcher. The variation in our result and other may due to variation in sample size, race and method used for detection.

This result is lower than results of a local study conducted by McCarthy *et al.*, in Eastern Sudan who reported that 9% Of hepatitis B positive patients infected with HDV⁽²²⁾. Also agrees with that obtained in Oman by Aghanashinikar *et al.*, who reported that according to study conducted in 1991²¹, but disagree with that reported in central Sudan by Hyams *et al.*,. Who reported that about 27.8% of hepatitis B positive patients infected with HDV.

In a regional study conducted in Burkina Faso ⁽²⁾ which was published in Journal of Clinical Microbiology in May 2014, almost 2,300 blood serum samples from Burkina Faso ($n = 1,131$), Nigeria ($n = 974$), Chad ($n = 50$), and the Central African Republic ($n = 118$) were screened for HBV and HDV. Among 743 HBsAg-positive serum samples, 74 were positive for HDV antibodies and/or HDV RNA, with considerable differences in prevalence, ranging from <2% (pregnant women from Burkina Faso) to 50% (liver patients from Central African Republic)

The result of this study was lower than the result of the study conducted by researcher.

These differences may be due to HBV vaccination camping which resulted in decline in hepatitis D virus prevalence.

Generally, the results of our study 3(3.3%) considerably lower than results of studies conducted 0in other groups than pregnant women in Cameroon (17.6 %), Gabon (15.6 % to 70.6 %) ^[10, 11, 12], and higher than sub-Saharan Africa 1.3 % to 50 % ^[4] and this value is in good agreement with previous study reported a HDV antigen prevalence of 6.5 % in Nigerian ^[13].

Also it was similar to result were obtained by Ghadir *et al.*, (2012), in Qom Province, Center of Iran, who detected HDV (2%) in their study⁽¹⁸⁾ 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⁽²⁰⁾ The variation may be due to sample size technique use for analysis.

and Tahaei *et al.*, (2014), in Tehran, Iran, who reported 37(7.7%), of HDV from their patients⁽¹⁹⁾,

Our results showed significant statistical relation between anti HDV and history of hepatitis infection and history of abortion this result was different to study carried out by Opaleye *et al.*,^[14] and Mhalla *et al.*,^[15].

Gestational age, gravidity and age did not contribute significantly to increase HDV infection in this study.

CONCLUSION

HDV infection was frequent in HBV pregnant women. Therefore, practitioners and health care providers should be made aware of the risk of dual infection with HBV and HDV so as to reduce the risk by providing treatment and employ stringent preventive measures to decrease the chances of spread of these infections.

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