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Hepatitis D virus infection among hepatitis B virus surface antigen positive individuals in Upper Egypt: Prevalence and clinical features

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ABSTRACT

Background/Purpose: About 248 million people are chronic HBV surface antigen carriers in the world. Hepatitis D virus (HDV) infection present in more than 15 million people worldwide. HDV needs hepatitis B surface antigen (HBsAg) to help its replication. We aimed to estimate the prevalence of HDV infection among HBsAg positive individuals and to determine the clinical, laboratory and virological characters of HDV infected patients.

Methods: This study was prospective cross-sectional analytic one including 186 HBsAg positive cases. Anti-HBc total, IgM and HBV PCR were done for all of these cases. Anti-HDV ELISA analysis was done for all cases. Positive samples for Anti-HDV by ELISA were then tested by HDV PCR.

Results: Of the 186 HBsAg positive cases, 80 were reactive for anti-HDV antibodies, resulting in an overall anti-HDV seropositivity of 43%. Higher prevalence of liver cirrhosis (43.8%), HCC on top of cirrhosis (8.8%) were found in anti-HDV positive compared to anti-HDV negative cases (17.9%) and (3.8%) respectively (p value < 0.001). Portal hypertension and Child-Pugh grade B, C were significantly higher in anti-HDV-positive cases as compared to the anti-HDV-negative ones (47.5% versus 18.9%) and (11.3% versus 6.6%); (16.3% versus 3.8%) respectively (p value < 0.001 for each). HDV RNA was positive in 25 out of 80 anti-HDV-positive cases (31.3%).

Conclusion: Anti-HDV was seropositive in 43% among HBsAg positive cases in Upper Egypt. HDV RNA was positive by PCR in 25 out of 80 anti-HDV-positive cases (31.3%). HDV prevalence using PCR was 25/186 (13.4%) in Upper Egypt.

Introduction

Globally, about 248 million people are chronic HBV surface antigen carriers, and about 5% of them also had hepatitis delta virus (HDV) infection as well [1,2]. The prevalence of HBsAg in Egypt is intermediate (2–7%) [3].

Hepatitis D virus (HDV) is an incomplete RNA virus that needs hepatitis B surface antigen (HBsAg) to help its replication [4]. HDV is considered a subviral particle because it depends on HBV for its propagation [5]. Combined HDV-HBV infection produces more severe liver affection than HBV alone [6].

HDV infection leads to both of acute and chronic liver illnesses [7]. Acute HDV infection can occur at the same time with acute HBV infection (coinfection) or can be superimposed on the top of chronic HBV infection. About 20% to 30% of coinfections of HDV and HBV in humans develop fatal fulminant hepatitis versus 2% of patients with acute hepatitis B mono-infection [8]. Worldwide, Hepatitis D virus (HDV) infection present in more than 15 million people and it is endemic in the Middle East [9]. In Upper Egypt, data about the prevalence, clinical, laboratory and virological characters of Hepatitis D virus-infected patients is rare. This study aims were: 1-To estimate the prevalence of hepatitis D virus infection among HBsAg positive individuals. 2-To determine the clinical, laboratory and virological characters of HDV infected patients.

Patients and methods

This study was a hospital-based, prospective, cross-sectional analytic one. The study was carried out on 186 HBsAg positive cases who were recruited from Tropical Medicine and Gastroenterology

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Department, Al Rajhi Liver Hospital, Assiut University, and Sohag University Hospital during two years.

Inclusion criteria

HBV related liver disorder, aged 18–60 years. HBsAg positive individuals were divided into different clinical categories according to EASL 2012 [10], and we revised this classification according to EASL 2017 [11]. Inactive carrier state (new terminology: HBeAg negative chronic infection); Immune tolerant phase (new terminology: HBeAg positive chronic infection), Acute hepatitis, Fulminant hepatitis, Chronic hepatitis (HBeAg positive and HBeAg negative), Liver cirrhosis, and Primary HCC.

Exclusion criteria

Dual infection with other viruses as HCV and/or HIV, autoimmune or alcoholic hepatitis.

Study procedures

One hundred eighty-six HBsAg positive cases were included. Anti-HBc total, IgM and HBV DNA PCR were done for all of these cases. Anti-HDV ELISA analysis was done for these positive patients. Of these positive HBsAg patients, anti-HDV ELISA positive cases were then tested by HDV PCR. All the samples belonged to different areas of Assuit and Sohag.

All patients were subjected to: (1) Clinical evaluation (medical history and physical examination). (2) Abdominal ultrasonography examination. (3) Liver function tests (serum levels of total and direct bilirubin, albumin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase). (4) Prothrombin time estimation. (5) Complete blood count. (6) Anti-HBV Core IgM and total. (7) HBV DNA by real-time PCR for positive HBsAg cases. (8) Anti-HDV by ELISA. (9) HDV RNA by real-time PCR for positive anti-HDV cases.

Sampling

Venous blood samples (10 ml) were collected from all cases under standardized conditions. 2 ml in EDTA coated tube for CBC, two ml for prothrombin time and concentration. Six ml delivered into plain tube and allowed for clotting to prepare serum. Non-hemolyzed serum was separated by centrifugation and was divided into aliquots, was used for determination of liver functions, anti-HBc total and IgM, HBV DNA PCR and the last two parts were stored in aliquots in −20, one for Anti-HDV by ELISA and last part for HDV RNA by PCR.

Methods

1 **Liver function and S. creatinine concentrations** were measured on ABX Pentra 400 chemistry autoanalyzer (Horiba Medical, Irvine, California, USA).
2 **Complete blood count** was measured using ABX Pentra XL 80 five differential automated cell counter (Horiba Medical, Irvine, California, USA).
3 **Prothrombin concentration and international normalized ratio (INR)** by (SysmexR CA-1500 System; Siemens, Germany).
4 **Anti-HBc total**: The qualitative detection of core total antibody to hepatitis B virus in human plasma was done using Chemiluminescent microparticle immunoassay (CMIA) (Gitlin) [12]. It was done on "ARCHITECT $i1000_{SR}$" Immunology analyzer by Abbott.
5 **Anti-HBc IgM**: The qualitative detection of core antibody IgM to hepatitis B virus in human plasma was done using Chemiluminescent microparticle immunoassay (CMIA) (WHO) [13]. It was done on "ARCHITECT $i1000_{SR}$" Immunology analyser by Abbott.
6 **HBV DNA (viral load) by real-time PCR**: DNA viral load detection was performed on 7500 Fast Real-Time PCR System (applied Biosystems ® USA) by QIAGEN G mbH Artus HBV TM PCR kit (24), V1. Lot number 1,40724 Hilden, Germany.
7 **Anti-HDV Screening**: Qualitative anti-HDV determination is a competitive assay, based on the ELISA technique (Enzyme-Linked Immunosorbent), using the methodology described in the manufacturer's protocol. ETI-AB-DELTAK-2 (P2808) (Diasorine SPA) Italy.
8 **HDV RNA PCR**: Extraction of HDV RNA and complementary DNA (cDNA) Synthesis: HDV RNA was extracted from 200 μl serum sample using PROBA-NA RNA/DNA Extraction kit (Proba-NA Technology, Lot No: B14-2 Russia). About 16.5 μl of the extracted RNA was reverse transcribed into cDNA with reverse transcriptase enzyme (Lot No: C0305F Russia). Prepare RT mix (2 μl of RT-buffer, 1 μl of RT mix primers and 0.5 μl of reverse transcriptase). The thermal cycling was performed in a thermal cycler for 30 min at 40 °C, then heat up to 95 °C and leave for 5 min.

Prepare Taq polymerase solution in one tube (10 μl of PCR buffer and 0.5 μl of TECHNO Taq -polymerase) (Lot No: C04-k) then take ten μl from this mixture and add five μl of premixed cDNA reaction mixture for the preparation of cDNA.

For thermal cyclers with active regulation: step 1 on temp. 94 for 5 min for one cycle. Step 2 on temp. 94 in 10 s and 62 in 20 s. For 50 cycles [on Linegene Real-Time PCR by Bioer company Germany (Fully automated detection system, automatically record and analyze the data after amplification)].

Ethics

Informed consents were taken from all participants before enrolment according to National Ethics Committee. The study received approval by the Medical Ethics of Faculty of Medicine at Assiut University. Participants were informed that refusing of participation did not affect having complete benefit from the available hospital medical service and treatment. Data were collected by personal interview with participants taking into consideration data confidentiality. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS- version 17, SPSS Inc., Chicago IL, USA) Software. All data were presented as mean ± SD or frequencies. For statistical evaluation, Chi-squared test was used to determine the proportion, while student T-test was used for comparison between continuous variables. Significance was accepted at $p < 0.05$.

Results

Demographic, clinical data and possible risk factors for HBsAg positive cases

One hundred eighty-six HBsAg positive cases were included, 142 were males (76.3%), and 44 were females (23.7%). The mean age was 40.7 ± 13 years. Fatigue was the commonest complaint (55.4%). Jaundice and ascites were the most common findings (22%) and (12.4%) respectively, followed by splenomegaly and lower limb edema (8.1%) for each. As regard to risk factors for hepatitis among

Table 1
Demographic, clinical data and possible risk factors of HBsAg positive cases.

Gender: male	142 (76.3%)
Age (years)	40.7 ± 13
Marital status: married	146 (78.5%)
Presenting complaints	
Fatigue	103 (55.4%)
Haemorrhage	30 (16.1%)
Nose	4 (2.2%)
Gums	18 (9.7%)
Melena	3 (1.6%)
Both haematemsis and Melena	5 (2.7%)
Arthralagia	26 (14%)
Abdominal pain	40 (21.5%)
Disturbed conscious level	15 (8.1%)
Signs	
Jaundice	41 (22%)
Lower limb oedema	15 (8.1%)
Hepatomegaly	5 (2.7%)
Splenomegaly	15 (8.1%)
Hepatosplenomegaly	2 (1%)
Ascites	23 (12.4%)
Past history	
Jaundice	14 (7.5%)
Hepatitis or contact with patients with hepatitis	35 (18.8%)
Blood transfusion	16 (8.6%)
Surgical operation	44 (23.7%)
Parenteral drug abuse	4 (2.2%)
Schistosomiasis	35 (18.8%)
Dental manipulations	87 (46.8%)
Tattooing	8 (4.3%)
Family history	
Hepatitis B virus infection	41 (22%)

Total number: 186. Data are expressed as number and percentage or as mean ± SD.

Table 2
Baseline laboratory data, serological markers and virological characteristics of HBsAg positive cases.

Total bilurubin (umole/l)	44.9 ± 71.4
Total protein (g/L)	69.9 ± 9.9
Albumin (g/L)	38 ± 9.2
ALT (IU/L)	99.2 ± 263.3
AST (IU/L)	98.8 ± 273.9
ALP (IU/L)	120.4 ± 115.1
Creatinine(umol/L)	83.9 ± 50.9
WBCS ($\times 10^3$/mm ^{3})	5.7 ± 2.5
PLT($\times 10^3$/mm ^{3})	206.3 ± 81.6
HB (g/dL)	12.5 ± 2.2
Positive HBc IgM	14 (7.5%)
Positive HBc (total)	169 (90.9%)
HBe Ag positive	17 (9.1%)
HBV-DNA by real time PCR (Positive)	135 (72.6%)
Real-time PCR (IU/ml)	
Mean ± SD (Range)	$1.2 \times 10^7 \pm 1.3 \times 10^8$ (0–1.8×10^9)
Anti-HDV positive	80 (43%)

Total number: 186. Data are expressed as number and percentage or as mean ± SD

the study cases, dental manipulations, surgical operation, and family history of HBV infection were the common risk factors (46.8%; 23.7%; and 22%) respectively (Table 1).

Baseline laboratory data, serological markers and virological characteristics of HBsAg positive cases

The majority of patients 169 (90.9%) were negative for HBeAg, and (72.6%) of patients were HBV DNA positive by Real-Time PCR. HBV DNA range from 0 to 1.8×10^9 (IU/ml). Only 14 cases (7.5%) were HBc IgM positive. Of the 186 HBsAg positive cases, 80 were also positive for anti-HDV antibodies, resulting in overall anti-HDV seropositivity of 43% (Table 2).

Table 3
Classification of HBsAg positive cases.

HBeAg negative chronic infection	49 (26.3%)
Old terminology: inactive carrier state.	
HBeAg positive chronic infection	8 (4.3%)
Old terminology: Immune tolerant phase	
Acute hepatitis	10 (5.4%)
Fulminant hepatitis	4 (2.2%)
Chronic hepatitis	50 (26.9%)
Liver cirrhosis	54 (29%)
Primary HCC + Liver cirrhosis	11 (5.9%)

Total number:186, Data are expressed as number and percentage.

Classification of HBsAg positive cases:

Of the 186 HBsAg positive cases, 54 (29%) had liver cirrhosis, 11 (5.9%) had HCC, 50 (26.9%) had chronic hepatitis, and 49 (26.3%) were inactive carrier (new terminology: HBeAg negative chronic infection) (Table 3).

Relation between HDV seropositivity and different stages of HBV infection

Increased prevalence of liver cirrhosis (43.8%), HCC on top of cirrhosis (8.8%) were found in positive cases for anti-HDV compared to negative cases (17.9%) and (3.8%) respectively (p value < 0.001) (Table 4).

Comparison between Anti-HDV positive and Anti-HDV negative cases according to demographic and clinical data

The mean age was higher in the anti-HDV-positive cases as compared to the anti-HDV-negative ones (43.5 ± 13.1 versus 38.6 ± 12.7) and (p value = 0.010). Portal hypertension and Child-Pugh grade B, C were higher in anti-HDV-positive cases as compared to the anti-HDV-negative ones (47.5% versus 18.9%) and (11.3% versus 6.6%); (16.3% versus 3.8%) respectively (p value < 0.001 for each) (Table 5).

Comparison between Anti-HDV positive and Anti-HDV negative cases according to laboratory results

The mean value of total protein, albumin and hemoglobin were significantly lower in anti-HDV-positive cases (p value = 0.009, 0.019, and 0.012) respectively. In contrast, positive HBc IgM was significantly higher in anti-HDV-negative cases as compared to the anti-HDV-positive ones (12.3%) versus (1.3%) (p value = 0.004). Also, the mean value of HBV DNA was higher in anti-HDV-negative cases (p value = 0.042). HDV RNA was positive in 25 out of 80 anti-HDV-positive cases (31.3%)(Table 6).

Comparison between HDV RNA positive and HDV RNA negative cases

There was no difference between HDV RNA positive and HDV RNA negative cases except the higher percentage of arthralgia was found in HDV RNA positive cases, and a lower percentage of platelet count was found in HDV RNA positive cases. Also, the mean value of HBV DNA by Real-Time PCR was higher in HBV Mono infection when compared with combined HBV and HDV infection, but the difference did not reach the statistical significance (p value > 0.05) (Table 7).

Table 4
Relation between HDV seropositivity and different stages of HBV infection.

Variable	Anti-HDV positive (n = 80)	Anti-HDV negative (n = 106)	p. Value
HBeAg negative chronic infection Old terminology: inactive carrier state	15 (18.8%)	34 (32.1%)	<0.001
HBeAg positive chronic infection Old terminology: immune tolerant phase	2 (2.5%)	6 (5.7%)	
Acute hepatitis	1 (1.2%)	9 (8.5%)	
Fulminant hepatitis	0 (0%)	4 (3.8%)	
Chronic hepatitis	20 (25%)	30 (28.3%)	
Liver cirrhosis	35 (43.8%)	19 (17.9%)	
Primary HCC	7 (8.8%)	4 (3.8%)	

Data are expressed as number and percentage; n = number.

Table 5
Comparison between Anti-HDV positive and Anti-HDV negative cases according to demographic and clinical data.

Variable	Positive Anti-HDV (n = 80)	Negative Anti-HDV (n = 106)	p. Value
Gender: male	62 (77.5%)	80 (77.5%)	0.747
Age (years)	43.5 ± 13.1	38.6 ± 12.7	**0.010**
Marital status: married	64 (80%)	82 (77.4%)	0.664
Presenting complaints			
Fatigue	48 (60%)	55 (51.9%)	0.270
Haemorrhage	19 (23.8%)	11 (10.4%)	0.014
Arthralagia	10 (12.5%)	16 (15.1%)	0.674
Abdominal pain	20 (25%)	20 (18.9%)	0.369
Disturbed consciousness	6 (7.5%)	9 (8.5%)	1.000
Signs			
Jaundice	15 (18.8%)	26 (24.5%)	0.377
Lower limb oedema	9 (11.3%)	6 (5.7%)	0.184
Hepatomegaly	2 (2.5%)	3 (2.8%)	0.503
Splenomegaly	9 (11.3%)	6 (5.7%)	0.503
Hepatosplenomegaly	1 (0.5%)	1 (0.9%)	0.503
Ascites	15 (18.8%)	8 (7.5%)	0.070
Portal hypertention	38 (47.5%)	20 (18.9%)	**<0.001**
Child-Pugh grading			
A	21 (26.3%)	11 (10.4%)	**<0.001**
B	9 (11.3%)	7 (6.6%)	
C	13 (16.3)	4 (3.8%)	

Data are expressed as number and percentage except age as mean ± SD; n = number.
Bold values represent significant p value.

Table 6
Comparison between Anti-HDV positive and Anti-HDV negative cases according to laboratory results.

Variable	Positive Anti-HDV (n = 80)	Negative Anti-HDV(n = 106)	P. Value
Total bilurubin (umole/l)	52.9 ± 79.9	38.9 ± 64.8	0.188
Total protein (g/L)	67.7 ± 10	71.5 ± 9.5	**0.009**
Albumin (g/L)	36.2 ± 8.4	39.4 ± 9.6	**0.019**
ALT (IU/L)	84.9 ± 146	109.9 ±325.3	0.522
AST (IU/L)	79.4 ± 126.8	113.4 ± 345.7	0.404
ALP (IU/L)	129.9 ± 163.8	112.9 ± 52.5	0.324
Creatinine(umol/L)	90.7 ± 56.9	78.8 ± 45.4	0.113
WBCS ($\times 10^3$/mm ^{3})	5.8 ± 2.8	5.7 ± 2.3	0.611
PLT($\times 10^3$/mm ^{3})	205.5 ± 94.9	206.9 ± 70.3	0.912
HB (g/dL)	12 ± 1.9	12.8 ± 2.3	**0.012**
Prothrombin time (Second)	14.9 ± 4.3	14.5 ± 3.5	0.376
Positive HBc IgM	1 (1.3%)	13 (12.3%)	**0.004**
Positive HBc (total)	76 (95%)	93 (87.7%)	0.123
HBe Ag positive	6 (7.5%)	11 (10.4%)	0.611
Positive HBV-DNA	56 (70%)	79 (74.5%)	0.788
Real-time PCR for HBV DNA (IU/ml) (Mean ± SD)	$5.4 \times 10^5 \pm 2.2 \times 10^6$	$2.4 \times 10^7 \pm 1.9 \times 10^8$	**0.042**
HDV RNA by PCR	25 (31.3%)	–	NA

Data are expressed as (number/percentage) or as mean ± SD.
NA = Not applicable; n = number.
Bold values represent significant p value.

Discussion

Our study disclosed some important observations. First, HDV prevalence among HBsAg individuals was 43% using Anti-HDV antibodies (80/186). Second, HDV RNA was positive in 31.3% (25/80) of anti-HDV-positive cases. HDV prevalence using PCR was 25/186 (13.4%). Third; liver cirrhosis and HCC on top of cirrhosis were found in a higher percentage in anti-HDV positive cases compared to anti-

Table 7
Comparison between HDV RNA positive and HDV RNA negative cases.

Variable	Positive HDV RNA (n = 25)	Negative HDV RNA (n = 55)	p. Value
Gender: male	22 (88.0%)	40 (72.7%)	0.158
Age (mean ± SD) (yrs.)	44.4 ± 11.8	45.6 ± 12.6	0.815
Marital status: married	21 (84%)	43 (78.2%)	0.764
Presenting complaints			
Fatigue	18 (72%)	30 (54.5%)	0.140
Haemorrhage	8 (32%)	11 (20%)	0.267
Arthralagia	7 (28%)	3 (5.5%)	**0.009**
Abdominal pain	6 (24%)	14 (25.5%)	0.889
Disturbed consciousness	4 (16%)	2 (3.6%)	0.073
Signs			
Jaundice	7 (28%)	8 (14.5%)	0.216
Lower limb oedema	5 (20%)	4 (7.3%)	0.129
Hepatomegaly	2 (8%)	1 (1.8%)	0.231
Splenomegaly	4 (16%)	6 (10.9%)	0.231
Ascites	5 (20%)	10 (18.2%)	0.198
Portal hypertention	13 (52%)	25 (45.4%)	0.833
Child-Pugh grading			
A	7 (28%)	14 (25.5%)	0.565
B	2 (8%)	7 (12.7%)	
C	6 (24%)	7 (12.7%)	
Total bilurubin (umole/l)	52.0 ± 76.2	37.3 ± 52.0	0.712
Total protein (g/L)	68.4 ± 11.8	69.2 ± 9.9	0.912
Albumin (g/L)	34.6 ± 10.5	37.2 ± 9.1	0.261
ALT (IU/L)	51.9 ± 48.6	47.4 ± 71.4	0.510
AST (IU/L)	59.3 ± 69.6	49.8 ± 59.2	0.884
ALP (IU/L)	115.3 ± 34.1	112.2 ± 46.4	0.393
Creatinine(umol/L)	97.8 ± 71.8	78.6 ± 29.9	0.199
WBCS ($\times 10^3$/mm ^{3})	4.9 ± 1.5	5.9 ± 2.9	0.199
PLT($\times 10^3$/mm ^{3})	167.6 ± 77.3	212.5 ± 93.6	**0.046**
HB (g/dL)	11.7 ± 2.3	12.1 ± 2.1	0.411
Prothrombin time (Second)	16.0 ± 6.8	15.0 ± 2.9	0.783
Positive HBc IgM	0 (0%)	1 (1.8%)	1.000
Positive HBc (total)	24 (96%)	52 (94.5%)	1.000
HBe Ag positive	2 (8%)	4 (7.3%)	1.000
Positive HBV-DNA	18 (72%)	39 (70.1%)	0.547
Real-time PCR for HBV DNA (IU/ml)	$3.1 \times 10^5 \pm 1.2 \times 10^6$	$6.9 \times 10^6 \pm 5.1 \times 10^7$	0.342

Data are expressed as number and percentage or as Mean ± SD; n = number.
Bold values represent significant p value.

HDV negative cases. Fourth, there was no difference between HDV RNA positive and HDV RNA negative cases except the higher percentage of arthralgia was found in HDV RNA positive cases and a lower percentage of platelet count was found in HDV RNA positive cases.

In the present study, there was male predominance (76.3%) among HBsAg positive cases and in delta positive cases 77.5% were males as well with no difference between HDV positive or negative patients regarding gender. These findings were similar to the results previously reported by Gish et al. [14] outside Egypt as well as Ahmed et al. [15] and Fouad et al. [16] inside Egypt. Our results and the previous results may be explained by increased risk factors for viral infection among males.

The majority of HBsAg positive cases in the current study (169/186, 90.9%) were negative for HBeAg. Similarly, Zaky et al. [17] on their study on eighty-three HBsAg-positive patients in Upper Egypt found the majority of the patients (78/83; 94%) were HBeAg negative, presumably indicating pre-core or core promoter mutations in HBV Genotype D. Also, Fouad et al. [16] found that (95/121; 81.9%) of HBsAg positive patients were HBeAg negative and 90.9% of delta patients were HBeAg negative.

In this study, HDV infection was more common in the fourth decade of life and this similar to Zaidi et al. [18] and near the results of Ziaee and Azarkar [19], whose patients were in the fourth decade.

In the current study, HDV prevalence was 43% by Anti- HDV and HDV RNA was positive in 25 (31.3%) out of 80 anti-HDV-positive cases. HDV prevalence using PCR was 25/186 (13.4%). Previous studies in Egypt reported different results. El Zayadi et al. reported HDV prevalence of (47.7%, 21/44) among HBsAg positive chronic liver disease patients in Egypt, and (8.3%, 4/48) among HBsAg carriers [20]. Abdel-fattah et al. [21] in their study on 45 chronic liver diseases patients (24 liver cirrhosis and 21 chronic hepatitis). They reported HDV prevalence of 8.9%. On the other hand, HBsAg was detected in 53.3% of all cases. Also, IgG anti-HDV was detected in 4.2% of positive cases for HBsAg.

Darwish et al. [22] showed positive HDV antibodies in 16.94% of acute HBV patients, 23.53% in chronic HBV patients and 21.9% among HBsAg carriers. Another study was conducted on Egypt including 45 children with chronic hepatitis B (age range from 2 to 15 years), IgG anti-HDV was positive in four children (8.9%) [23]. Zaki et al. [24] detected twenty positive samples for anti-HDV from total 100 cases (20%). None of the cases with acute hepatitis B or HBsAg carriers had Delta antibodies. Anti-HDV was positive in (46.7%), (25%), (14.3%) among schistosomal hepatic fibrosis, drug abusers, hepatocellular carcinoma group respectively.

Two recent studies from Lower Egypt on chronic HBV infected patients demonstrated HDV prevalence of 14.8% (32/216) using total HDV antibodies [15] and 8.3% by using anti-HDV IgG and 9.9% by RT-PCR [16].

The change in the prevalence of HDV infection among HBsAg positive cases in our study and previous studies in Egypt may be related to the followings: fist, difference in patient's categories as our study included all clinical forms of HBV disease (HBeAg negative chronic infection, HBeAg positive chronic infection, Acute hepatitis, Fulminant hepatitis, Chronic hepatitis, Liver cirrhosis, HCC with more than 60% of them in the last 3 categories). However, some

of the previous studies included one category as HBeAg negative chronic infection (Inactive carrier state) or chronic hepatitis, and others included children only in their studies. Second, we used total anti-HDV to detect HDV infection, but some of the previous studies used IgG anti-HDV. Third, the patients mean age in the current study was 40.7 ± 13 years. HDV infection was more common in the fourth decade of life. However, the mean age of patients in Fouad et al. study was 33.7 ± 10.6 [16].

In addition, (65.1%) of patients in Fouad et al. [16] study in Lower Egypt had F0-1 and (34.9%) had F2 using Transient Elastography, However, more than 60% of patients in the current study had chronic hepatitis, liver cirrhosis or HCC which may be another explanation for the difference in HDV prevalence between both studies in the same Country.

Studies from the Middle East have shown different results. In Saudi Arabia, 13.6% out of 81 HBV carriers had positive anti-HDV [25]. The prevalence of HDV infection in HBsAg-positive in Lebanon is 1% [26]. The seroprevalence rate of HDV antibody among HBsAg positive asymptomatic carriers was reported to be 2%, 31%, 5.2% in Jordan, Kuwait, and Turkey respectively [27].

In our study, a higher percentage of liver cirrhosis (43.8%) and HCC on top of cirrhosis (8.8%) were found in anti-HDV positive cases compared to anti-HDV negative cases (17.9%) and (3.8%) respectively. This agrees with the results of Rizzetto et al. [28] and Carmo-Fonseca, [29] who reported that individuals having HBV-HDV combined infection more liable to develop liver cirrhosis and hepatocellular carcinoma (HCC) than those had HBV mon-infection.

In our study, portal hypertension and Child-Pugh grade B, C were significantly higher in anti-HDV-positive cases as compared to the anti-HDV-negative ones (47.5% versus 18.9%) and (11.3% versus 6.6%); (16.3% versus 3.8%) respectively. Also, the mean value of total protein, albumin and hemoglobin were significantly lower in anti-HDV-positive cases. In addition, there were no significant differences between HDV RNA positive and HDV RNA negative cases except arthralgia was significantly higher in HDV RNA positive cases and platelet count was significantly lower in HDV RNA positive cases.

In our study, the mean value of HBV DNA by Real Time PCR was higher in HBV mono infection when compared with combined HBV and HDV infection, and this means that HDV infection led to decreasing HBV replication and this was similar to data obtained by Latika and Summaiya [30] as well as Fouad et al. [16] Also, Gish et al. [14] demonstrated that 56% of HDV infected patients were negative for HBV DNA by PCR.

The current study has three limitations. First, the sample size was small. Second, our study included all clinical categories of HBV infection, and some of these categories had a very small number of patients, and this makes the comparison between the positive anti-HDV group and the negative anti-HDV group in each category was difficult. Third, we used total anti–HDV in the detection of HDV prevalence.

Conclusions

Anti-HDV was seropositive in 43% among HBsAg positive cases in Upper Egypt. HDV RNA by PCR was positive in 25 out of 80 anti-HDV-positive cases (31.3%). HDV prevalence using PCR was higher in Upper Egypt 25/186 (13.4%) than in Lower Egypt 9.9%.

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Competing interests

The authors declared that they had no competing interest.

Ethical approval

The study received approval from the Medical Ethics of Faculty of Medicine at Assiut University.

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