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The prevalence of hepatitis D in CHB patients in Baghdad city and its correlation with fibrosis

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***Abstract*---**The aim of the study was to determine the prevalence of hepatitis D for chronic hepatitis B patients and to assess the degree of cirrhosis of hepatocytes. A study of 240 patients with CHB was studied in the laboratories of the Gastrointestinal Hospital, Dubai and Sama Dubai Specialized Medical Laboratories / Baghdad. Confirmatory examinations were conducted for them by conducting HBsAg test to ensure that they are infected with the viruses, HCV and HIV to ensure that there is no viral interference, and HDV IgG test was conducted by ELISA device to identify the male infected was (5) (31.2%) and the females was (11) (68.7%).The results of fibrosis of the patients with hepatitis D viral showed different stages according to the severity of the infection, where it showed (S1). One infection with (6.25%) and the results of fibrosis (4.0) E(KPa), while (S2) the number of patients was (5) (21.25%) and the incision of fibrosis ranged between (7.0 - 8.1) E(KPa), the (S3) showed the number of patients (6) (37.5%) and severity of fibrosis (8.4 -10.1) E(KPa), while (S4) which is considered to have severe fibrosis, showed (4) (25%) (12.1-16.1) E(KPa) which showed a high significant association and the difference between it and fibrosis ($r = 0.482$, $p = 0.001$) within the significance level ($p < 0.01$). Therefore, it is important to determine the infection with hepatitis D when following up on the pathological condition of CHB patients and to conduct a fibroscan test to determine the percentage of hepatocyte fibrosis and its treatment. The conclusion of these results is that the incidence of HDV virus among blood donors is high, and early screening for HDV is necessary to determine the diagnosis and should be based on routine work.

***Keywords*---**CHB, HDV, fibrosis, fibroscan.

Introduction

Delta virus causes severe or clinical signs of hepatitis in people with hepatitis B virus infection as being a co-or super-infection (Pollicino *et al.*,2011). The "delta virus" was proven to infect chimpanzees or woodchucks who was already infected by HBV or woodchuck hepatitis virus (Dastgerdi *et al.*,2012)**.** Hepatic fibrosis, cirrhosis, and also an increase in the risk of hepatocellular carcinoma are all symptoms of HBV/HDV co-infection, which is a critically severe condition (Wedemeyer, 2010). Whereas the method by which HDV induces hepatic tumors is unknown, the infection has been shown to have negative effects on hepatocyte proliferation and survival (Wang *et al.*,2001)**.** Delta virus is a sphere satellite virus with (36 nm) virion size (Tseng *et al.*,2010). Revealed that it has a negatively polarity cyclic RNA which it needed an assistance mechanism to replicate. HBV is usually the one that provides this aid, which shares its envelope proteins (Pollicino *et al.*,2011)**.** Hepatitis delta virus can only spread in people who have to coexist HBV, either because the two viruses are co-infected or because a chronic HBV carrier is super-infected. Hepatitis Delta virus causes an estimated (15 to 250) million individuals worldwide, and also the clinical significance of Hepatitis D infection includes more acute types of viral hepatitis, acute or chronic, as well as a higher risk of cirrhosis and hepatocellular carcinoma (Hadi *et al.*,2017)**.** All of Europe, Africa, the Mideast, and most likely, America are impacted. HDV has been found in almost all of the nations where it has been searched, except for China, Japan and Taiwan, First, at the time, some Far Eastern countries appeared to get a low Delta prevalence **(**Heidrich *et al.*, 2009)**.** Hepatitis D infection produces very severe hepatic diseases than Hepatitis B mono-infection; its illness progresses quickly, causing (cirrhosis, decompensating and hepatocellular carcinoma), as well as a lower five-year survival rate (Abbas&Afzal,2013)**.** The viral response fluctuates with time, as a result, HDVs decrease of Hepatitis B replication does not last throughout the illness **(**Wedemeyer, 2010). There are three stages of chronic Delta virus : (a) Hepatitis D replication and Hepatitis B suppression are active in the first active period; (b) a next moderately active phase characterized by Delta virus decline and Hepatitis B reactivation; and (c) a final phase characterized by the evolution of cirrhosis and hepatocellular cancer as a result of viral replication or recovery as a result of a significant drop in both viruses (Abbas&Afzal,2013)**.** The most frequent way of identifying fibrosis and following disease progression has traditionally been liver biopsy. A liver biopsy, on the other hand, is a painful, expensive and intrusive procedure that has the possibility of negative repercussions (Cadranel *et al.*, 2000). Sampling error and inter-observer variance in staging make it difficult to accurately assess fibrosis with a liver biopsy (Afdhal,2014; Regev *et al.*, 2002)**.** Fibroscan is a name for Transient Elastography (TE). The TE method measures liver stiffness in kilopascals. It is non-intrusive in every way, and it's done using a pen-sized probe implanted near the liver on the skin's surface. Any trained individual in an office or clinic setting can do TE, and the output reading is then analyzed by a qualified health care physician (Brener,2015)**.** TE is a sophisticated technology for measuring TE waves in the liver that was designed to ensure that only high-quality measurements were provided. When the technique was created,

a high percentage of mistakes were linked to individuals having a lot of visceral fat, which prevented the TE waves from accessing the liver as they should (de Ledinghen *et al.*,2008).

Materials and methods

Two hundreds and forty patients with chronic hepatitis B, they were screened for infection with delta virus by performing an IgG antibody test by ELISA. This study was conducted for patients diagnosed with HBV to the Medical City / Gastrointestinal Hospital / Dubai Laboratories and Sama Dubai Specialized Medical Center on Al Maghreb Street **,** A total of 240 patients with chronic hepatitis B virus were included in the study. These samples was selected after a series of testing, including infection with HBsAg, a positive PCR test for HBV viral load and a negative test for HCV and HIV.

Venipuncture using sterile syringes is used to acquire 5-10 mL of blood from each of the individuals in this study. Blood was collected in disposable plastic tubes and allowed to coagulate at room temperature (18-25°C). Sera were centrifuged for 5 minutes at 3000 rpm, then divided into 250 aliquots and stored at -20°C until further testing. To avoid sample degradation from repeated freezing and thawing cycles, each aliquot of serum was utilized just once. Before using in any of the studies, All sera and reagents were allowed to reach at room temperature before being used .

This ELISA kit detects Hepatitis D Virus (HDV) IgG in human serum and plasma using the indirect-ELISA technique. The method of operation used depends on the procedure (Elabscience, USA). The Fibroscan device (Transient Elastography) was used to examine patients with chronic hepatitis B and measure the degree or level of cirrhosis of the liver by following the correct steps during the examination. The device used is (Compact 530) produced by the Spanish company Echosens/Barcelona. The portable non-invasive solution for efficient liver disease for liver fibrosis and liver steatosis assessment was measured in units E(kPa).

Statistical Analysis

The data were analyzed using the following software: Microsoft Excel, IBM and SPSS V26. The results reported in this study were expressed Pearson correlation coefficient was used to measure the strength and direction of the linear association between two variables.

Results and Discussion

Two hundred and twenty eight positive patients for HBsAg and 12 negative were included, all patients were negative for HCV Ab and HIV Ab. In our study, which was conducted on 240 patients with chronic hepatitis B, they were screened for infection with delta virus by performing an IgG antibody test by ELISA and the result was (16) infected persons (6.6%), distributed among (11) (68.7%) Females and (5) (31.2%) males as shown in Figure (1) .

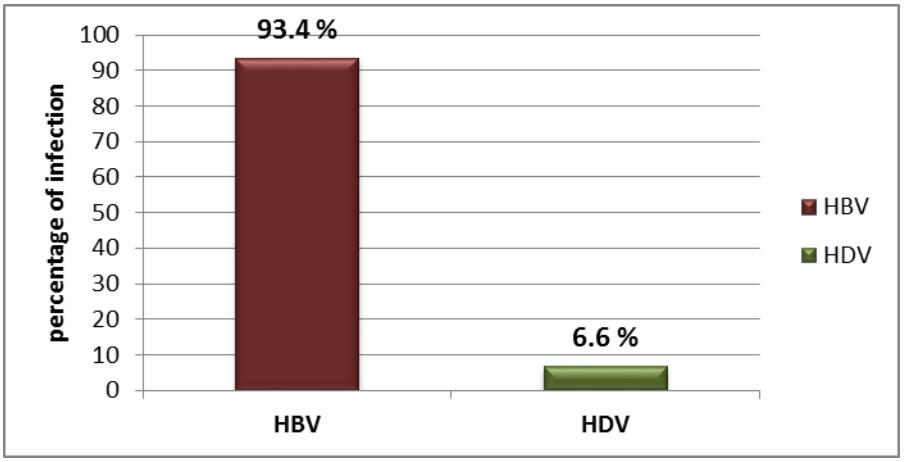

Fig (1) : Distribution of hepatitis D infections among patients with chronic HBV

Hepatitis delta virus patients are more likely to develop Fibrosis than HBV-mono infected patients. Overall, HDV infection progresses to fibrosis within 5 years and to hepatocellular carcinoma within 10 years, on average (Miao *et al.*,2020). When comparing this study with other studies done in the world, In Turkey HDV prevalence in HBsAg-positive patients ranged between <5% in Western Turkey up to >27% in South East Turkey (Degertekin *et al.*, 2008), HDV infection rates highly prevail in Tunisia (15.33%), Mongolia (8.31%), and Niger (5.04%) (Chen *et al.*,2019)

In the studies conducted in Iraq, the results were mixed, as a positive percentage (5.6%) was recorded for HDV IgG (Hadi *et al.*, 2017) and a study conducted by (Alfaham, 2012) showed a percentage (7.4%) and a study **(**Al-Hilli & Al-Ugaidy,2002) showed percentage (8.3%), while another study in Mosul city showed a decrease in the rate of infection with hepatitis D virus, as it showed a percentage of (1.1%) **(**Salim and Abdullah 2014), while (Habib and Al-Obaidi 2006) conducted a study that showed a high rate of hepatitis D by (37%) compared to other studies that showed low rates.

The results of fibrosis of the patients with hepatitis D viral showed different stages according to the severity of the infection , where it showed (S1)one infection with (6.25%) and the results of fibrosis (4.0) E(KPa), while (S2) the number of patients was (5) and by (31.25%) and the severity of fibrosis ranged between (7.0 - 8.1) E(KPa), the (S3) showed the number of patients (6) with a percentage (37.5%) and severity of fibrosis (8.4 -10.1) E(KPa), while (S4) which is considered to have severe fibrosis, showed (4) and (25%) and severity of fibrosis was (12.1-16.1) E(KPa) as in table (1) .

Table (1) Stages of fibrosis of patients with HDV and the severity of fibrosis of liver cells

Stage fibrosis	No.HDV +ve	Percentage	Fibrosis E(KPa)
S1	1	6.25 %	4.0
S2	5	31.25 %	7.0 – 8.1

S3	6	37.5 %	8.4 – 10.1
S4	4	25.0 %	12.1 – 16.1
Total	16	100%	-

The result of fibrosis is measured in kilopascals E (kPa), which ranges from 2 to 75 kPa. Between 2 and 6 kPa, the liver is considered normal, the average normal result is 5.3 kPa, while anything outside the normal range usually indicates liver disease, The gold standard for evaluating liver scarring, indicated that the optimal limit for detecting cirrhosis is approximately 14 kPa.

The stiffness of the liver is assessed by measuring the speed of a vibration wave (also called a "shear wave") generated on the skin. The velocity of the shear wave is determined by measuring the time it takes for the vibration wave to travel to a specific depth within the liver. A graphical representation of this is provided on the screen. Since fibrous tissue is harder than a normal liver, the degree of cirrhosis can be inferred from liver sclerosing. To improve test reliability, a minimum of 10 correct readings are taken, with a success rate of at least 60%, and an interquartile range of -30% of the mean value, with results expressed in kilopascals (kPa).

The report of the final result of the liver cirrhosis examination shows the degree of cirrhosis measured in E unit (kPa), with cross-sectional images showing histological changes in liver cells due to hardening of these cells as in Figure (2) where each image shows a stage of cirrhosis of hepatocytes (F0, F1, F1 F2, F3, F4).

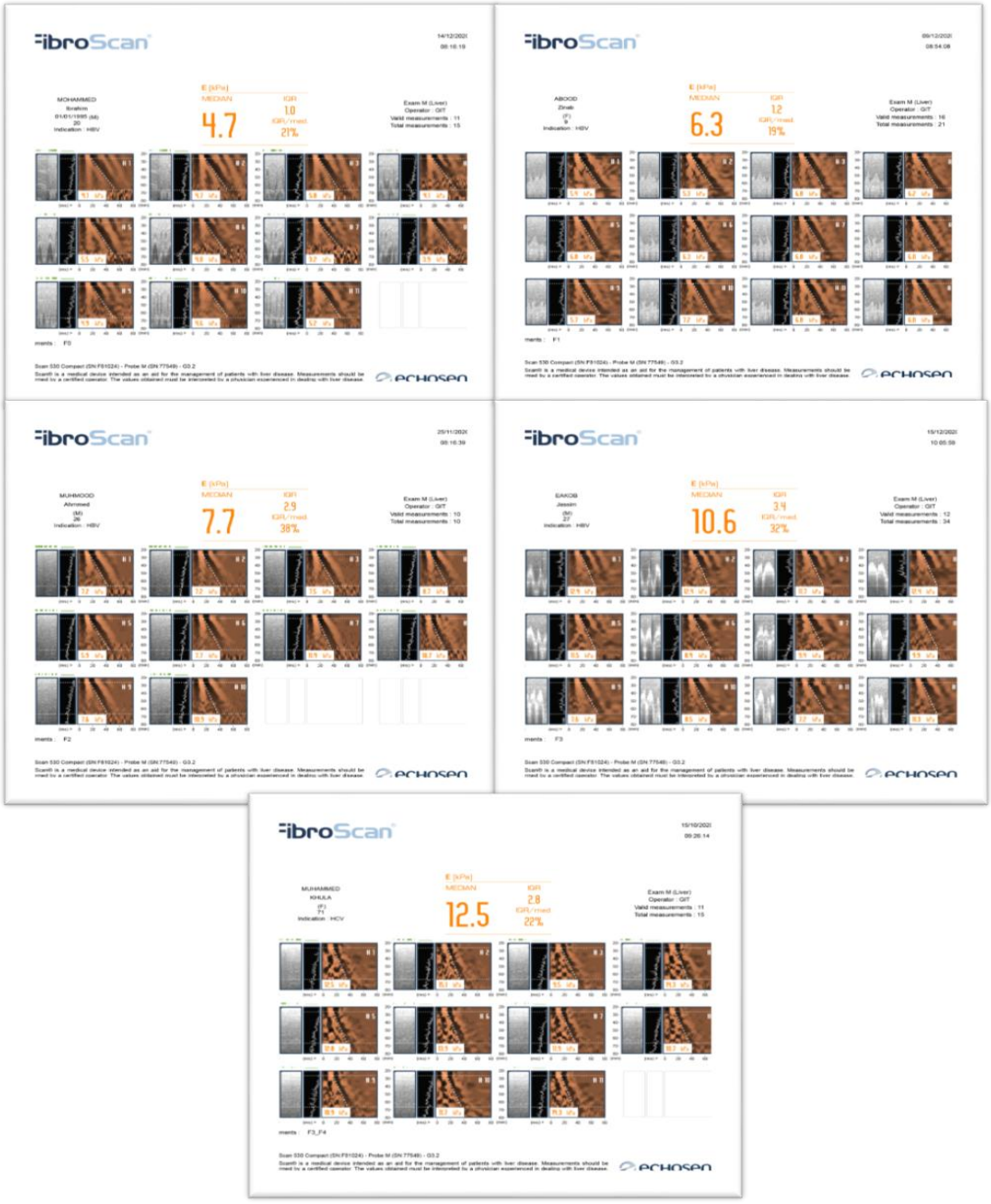

Fig (2) : Fibroscan result report for different stages of fibrosis .

the IQR/Med measurement is 30%, If these numbers are greater than 30% it indicates that there may be high numbers of rib echos or that there are some outlier measurements. Aim for measurement of around 20-25%. If the IQR/Med measurement is greater than 30% and the study is suggesting significant fibrosis recommend that the study is repeated.

Figure (3) shows an illustration of the results of the fibroscan examination, placing the probe in the correct position during the examination between the placement of the probe in the middle of the liver and reading the results correctly, and placing it below or above one space between the ribs, which affects the accuracy of the result.

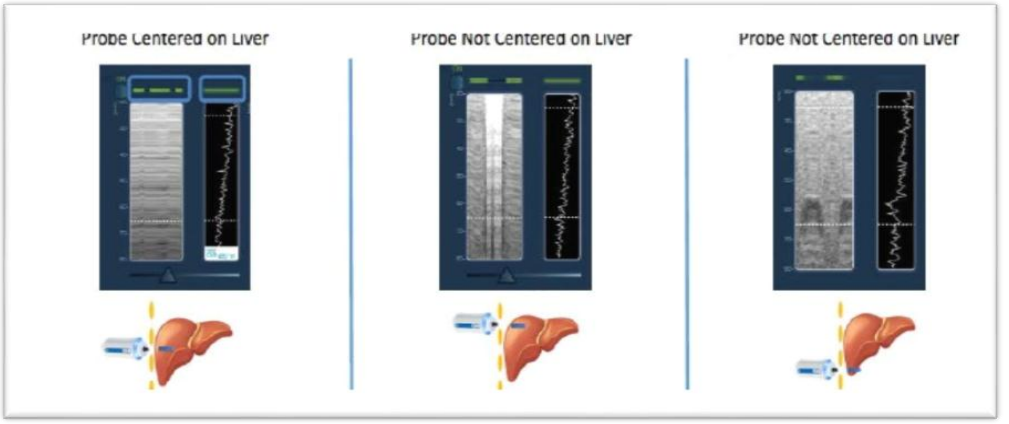

Fig (3) : probe in the right place

Figure (4) also shows the effect of shear wave speed, where when shear wave speed overestimated is non-parallel margin shear wave, also when shear wave speed correctly estimated is parallel margin shear wave .

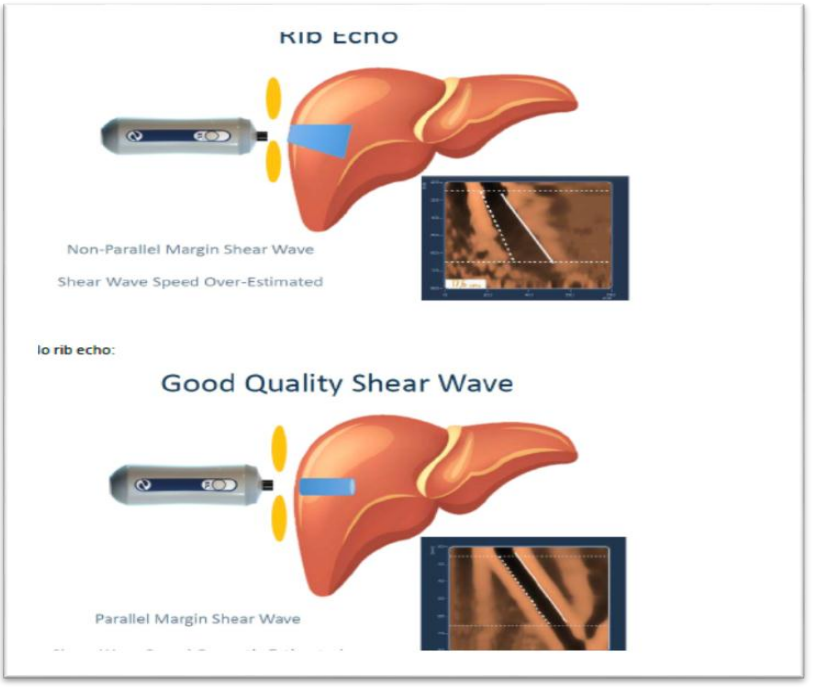

Fig (4) : shear wave speed effect

The total study samples were (240) chronic hepatitis patients, (16) patients were identified with hepatitis D by doing HDV IgG test for all patients who all showed Fibrosis of hepatocytes at different stages(S1,S2,S3,S4). Table (2) showed a high significant association and the difference between it and fibrosis (r=0.482, p= 0.001) within the significance level (p < 0.01).

Table (2) : Liver Fibrosis Association with HDV

Fibrosis stage		Hepatitis Delta Virus
	r	0.482
	P	0.001**

*, ** significant (P<0.05), and highly significant (p< 0.01) respectively

There are many studies that agreed with the results of our study, which showed a high correlation between people with hepatitis D and the occurrence of cirrhosis of their liver. For example, a study of (Braga *et al.*, 2014) showed a positive trend of HDV with the stage of fibrosis and inflammatory activity, another study (Miao *et al.*, 2020) confirmed that HDV infection progresses to fibrosis within 5 years and to hepatocellular carcinoma within 10 years, on average.

One-third of individuals with hepatitis C infection had advanced coronary heart disease with advanced fibrosis, and a study of Mongols with HBV-HDV infection showed that they were more likely to have an advanced liver disease with more severe Fibrosis, lower HBV DNA level, and higher levels of ALT (Fung *et al.*, 2021). Therefore, it is important to determine the infection with hepatitis D when following up on the pathological condition of CHB patients and to conduct a Fibroscan test to determine the percentage of hepatocyte fibrosis and its treatment.

Previous studies suggest that an HBV vaccination coverage above 80% is sufficient for the eventual eradication of both HBV and HDV infection (Goyal & Murray, 2014)**.** The conclusion of these results is that the incidence of HDV virus among blood donors is high, and early screening for HDV is necessary to determine the diagnosis and should be based on routine work.

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