High prevalence of chronic hepatitis D virus infection in Eastern Turkey: urbanization of the disease

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Abstract

Introduction: Both hepatitis B virus (HBV) and hepatitis D virus (HDV) infection play an increasingly important role in liver diseases. The main objective of this study was to investigate the socio-epidemiological, laboratory and radiological aspects of both HBV and HDV infection near the Iranian border of Turkey.

Material and methods: The study included 3352 patients with HBV and HDV infection. Socioepidemiological, laboratory and radiological aspects of the study subjects were retrospectively examined. Comorbid metabolic diseases were not assessed due to the retrospective design of the study.

Results: Most of the study subjects were HBe antigen negative. No significant difference in terms of HBV-DNA levels or HBe antigen seropositivity was detected between the city centre and rural areas ($p > 0.005$). The mean HBV-DNA level in the anti-HDV-positive group was significantly lower than in the anti-HDV-negative group ($p < 0.001$). The rate of HDV-RNA positivity in women was higher than in their male counterparts ($p = 0.017$). Anti-HDV-IgG was detected in 18.4% of tested subjects who came from an urban area. In contrast, 12.5% of subjects of the rural group had a positive result for anti-HDV-IgG. Among 134 ultrasonographically evaluated delta hepatitis patients, 37.3% had liver cirrhosis. On the other hand, in 1244 patients with hepatitis B monoinfection, there were 90 patients with liver cirrhosis. Radiologically, the rate of hepatic steatosis in delta hepatitis patients was lower than in those with HBV monoinfection.

Conclusions: Hepatitis D virus infection was particularly prevalent among the urban population as well as in female subjects. More broadly, the current observations are the first to suggest an inverse correlation between delta hepatitis and ultrasonography-proven hepatic steatosis.

Key words: hepatitis B virus, hepatitis D virus, hepatosteatosis.

Introduction

Chronic liver diseases are important causes of morbidity and mortality all over the world, and the Mediterranean basin is no exception [1]. In fact,

the "the hepatitis B belt" originates from the Far East and extends to the Middle East, encompassing the Iranian border of Turkey. Hepatitis B-related liver diseases present a serious clinical problem, and extensive research is being conducted to develop new approaches to prevent hepatitis B virus (HBV) infection [2]. Hepatitis delta is the most devastating form of chronic viral hepatitis. Although a number of studies have been conducted in Turkey, socio-epidemiological and clinical aspects of HBV infection are still unknown in the eastern part of Turkey, where hepatitis D virus (HDV)-related liver disease is endemic. Our understanding of the epidemiology of HBV has changed significantly in the past decade. Key papers have defined the impact of socioeconomic status on disease risk, describing the current epidemiology of HBV and establishing a link between natural disasters and HBV infection. Since HDV carries a high mortality rate, the identification of epidemiologic drivers in HDV infection is urgently needed [3]. So, this study aims to identify the socio-epidemiological risk factors associated with HBV infection, to determine the impact of HDV infection on the liver and to estimate the prevalence of HDV infection as well as the rate of missed diagnosis of HDV in current practice. Moreover, this study aims to show the correlation between HDV infection and hepatic steatosis (HS) since, to our knowledge, no study has been performed so far to evaluate the association between HDV infection and hepatic steatosis.

Material and methods

Subjects

We retrospectively analysed the data obtained from 3352 consecutive patients with HBV infection who were referred to our institution between 2012 and 2014. Of these patients, 2133 were male and 1219 were female. Patients were included in this study if information on hepatitis B serology, HBV-DNA, HDV-RNA, anti-HDV IgG, HBe antigen and liver transaminases was available within the 30 days of the first documented visit. Due to the significant changes in HBV distribution following the recent earthquake in Van province (Figure 1), pre-earthquake years (before 2012) were excluded from the analysis. The median age was 59 years (range: 13–89 years) for patients with HBV and 55 years (range: 18–89 years) for those with HDV (n = 700). At diagnosis, 700 (18.7%) patients who had HBV also had an HDV infection. Based on virological examination and demographic data including sex, age and residential area, patients were divided into groups. According to their residence, patients were divided into two groups: urban and rural. In terms of the virological studies, the HBV infected and HDV infected groups were compared. The following parameters were included in univariate and multivariate analysis: age, gender, aspartate transaminase (AST), alanine transaminase (ALT), HBV-DNA, HBe antigen, anti-HDV IgG and HDV-RNA. Regional variations and demographic differences were also analysed. Data obtained from the ultrasound examination of the hepatobiliary system were analysed during the study period. The definition of HS was based on a radiological examination rather than a histologically validated score. A fatty liver on ultrasound examination was considered to be HS. Due to the limitations associated with the retrospective design, HS-related metabolic diseases including diabetes mellitus were not evaluated in the patients.

Figure 1. Van city localization

Serological markers and biochemical evaluation

Liver transaminases were analysed using a commercial biochemistry kit. The presence of HBV and HDV infection was investigated using both ELISA and PCR methods. HBV-DNA was analysed via the Artus HBV-DNA-QS-RGQ kit (Qiagen, Germany). HDV-RNA was analysed using the primer design one step Rt-PCR kit (Primer design, England) and Rotor Gene Q Real time PCR (Qiagen, Germany). Anti-HBe, anti-HDV IgG and HBsAg levels were analysed with ELISA (Cobas 601, Roche, Germany).

Statistical analysis

The statistical evaluation of the data was undertaken using the SPSS 15.0 program. Descriptive statistics for the continuous variables are presented as the median, average deviation, standard deviation, and minimum and maximum values, and the categorical data are presented as a percentage and number. For the continuous variables, the Kruskal-Wallis test were used to compare the groups. The χ^2 test was used to assess the significance of the difference among the ratios in the advanced analysis. P-value < 0.05 was considered to be significant.

Results

The majority of patients (n = 1480; 55.8%) were residents in the rural areas of Van province.

The mean age of patients who came from the rural areas of the region was lower than those who were residents in the urban areas (p = 0.02). The mean age of male patients was also higher than that of female patients (p = 0.03) (Figure 2). When compared to female patients, male patients had higher levels of liver transaminases (p = 0.001). There was also a significant difference in the levels of liver transaminases between the residents of urban and rural areas (p = 0.001). There was no statistically significant difference between the residential areas (urban or rural) in terms of demographics, HBV-DNA levels or HBe antigen seropositivity (p > 0.005). Most of the participants of the current study (2799 patients; 75%) were HBe antigen negative. There were no significant differences between genders according to the HBe Ag seropositivity (p > 0.005). Furthermore, no significant correlation was observed between the HBe antigen status and gender (p > 0.05). One thousand one hundred and eighteen patients were tested for anti-HDV IgG and 478 were tested for HDV. The remainder of the participants (57.3%) did not have delta hepatitis assays. The mean HBV-DNA level in the anti-HDV-positive group was significantly lower than in the anti-HDV-negative group (40 ±13 IU/ml vs. 1490 ±887 IU/ml, p < 0.001). Overall, the delta positivity showed a negative association with HBV-DNA levels. According to the analysis of the serum, 117 of the 743 male patients were anti-HDV IgG positive (15.7%) while 54 of the 375 female patients had anti-HDV IgG seropositivity (14.4%). There was no significant difference between genders in terms of HDV-IgG seropositivity (p > 0.005). Interestingly, the analysis of serum from 330 men showed that 56 (16.9%) were HDV-RNA positive. Of 148 women with hepatitis B, 39 (26.3%) had a positive HDV-RNA result. The HDV-RNA positivity rate in women with hepatitis B infection was 26.3% compared to 16.9% in male patients (p = 0.017) (Tables I and II).

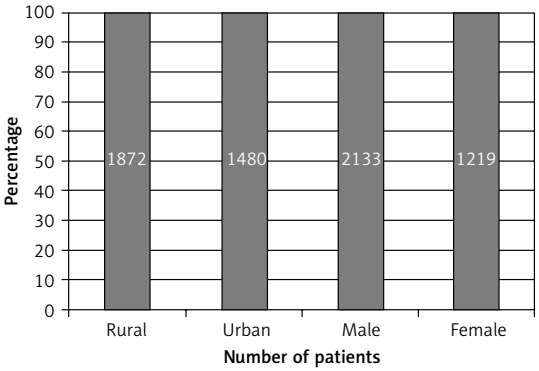

Figure 2. Distribution of according to gender and residential area

Furthermore, we conducted an examination of anti-HDV-IgG on 521 patients living in the urban areas and 597 patients living in the rural areas of Van province. There was no significant difference in terms of the demographic characteristics between the two groups. In contrast, the mean age of HDV-positive patients was higher than their HDV-negative counterparts (48 ±13.3 vs. 39 ±13.9; p < 0.001). In the urban group, 96 (18.4%) patients had a positive result for anti-HDV-IgG. However, only 75 anti-HDV-IgG positive patients (12.5%) were detected in the rural group. There was a statistically significant difference between the residential groups in terms of anti-HDV-IgG seropositivity (p = 0.007). Similarly, patients in the urban group had a higher rate of HDV-RNA positivity than the patients in the rural group (23.4%

Table I. Serological features of the subjects according to residential areas

Area	HBeAg *n* (%) *p* = 0.267		Anti-HBe *n* (%) *p* = 0.671		Anti-HDV *n* (%) *p* = 0.007		HDV-RNA *n* (%) *p* = 0.051	
	–	+	–	+	–	+	–	+
Urban	721 (85.4)	123 (14.6)	216 (24.8)	655 (75.2)	425 (81.6)	96 (18.4)	180 (76.6)	55 (23.4)
Rural	822 (83.5)	162 (16.5)	248 (24)	787 (76)	522 (77.5)	75 (12.5)	203 (83.5)	40 (16.5)

Table II. Serological features of the subjects according to gender

Gender	HBeAg *n* (%) *p* = 0.207		Anti-HBe *n* (%) *p* = 0.224		Anti-HDV *n* (%) *p* = 0.555		HDV-RNA *n* (%) *p* = 0.017	
	–	+	–	+	–	+	–	+
Male	1018 (85.2)	177 (14.8)	292 (23.5)	952 (76.5)	626 (84.3)	117 (15.7)	274 (83)	56 (17)
Female	525 (82.9)	108 (17.1)	172 (26)	490 (74)	321 (85.6)	54 (14.4)	109 (73.6)	39 (26.4)

Table III. Ultrasonography-based HS patterns of the subjects according to delta hepatitis status

	Hepatitis B *n* (%)	Delta hepatitis *n* (%)	*P*-value
HS Grade (USG based):			
0	926 (74.5)	112 (84.8)	0.002
1	188 (15.1)	8 (6.1)	0.001
2	83 (6.7)	12 (9.1)	0.353
3	46 (3.7)	0 (0)	
Liver cirrhosis:			
0	1154 (92.8)	84 (62.7)	0.001
1	90 (7.2)	50 (37.3)	0.001

vs. 16.4%; $p = 0.051$) (Table I). Of the 134 hepatitis delta patients who were radiologically evaluated, 50 (37.3%) had liver cirrhosis, while of the total of 1244 hepatitis B patients only 90 (7.2%) had liver cirrhosis. The presence of anti-HDV IgG in the participants of the study was related to liver cirrhosis ($p = 0.001$) (Table III).

Another objective of this study was to compare the hepatosteatosis in patients with and without delta hepatitis. The Kaplan-Meier analysis revealed significantly higher incidence of hepatosteatosis in the non-cirrhotic hepatitis B patients than in the non-cirrhotic HDV group (25.5% vs. 15.2%; $p = 0.001$) (Table III).

Discussion

To our knowledge, this is the first hospital-based study to determine the prevalence of HDV infection in an entire area of a defined geographic region. Results from the current study can be generalised since the demographics of Van province closely resemble the demographics of the eastern part of Turkey. HDV is the smallest human virus. The presence and reproduction of HDV heavily depends on hepatitis B surface antigen. HDV infection is associated with a higher risk of cirrhosis and hepatocellular carcinoma compared with those who only have chronic HBV infection. Thus, early diagnostic intervention in HDV infected patients may be the right approach to avert end-stage liver disease and the development of cirrhosis [4].

Currently, the approved therapy for HDV infection includes interferon α 9 MU three times a week or pegylated-interferon α 180 µg on a weekly basis. In this study, the end of treatment and sustained virological responses of patients with HDV-related liver disease were only 32% and 23%, respectively. Oral antiviral agents do not have an impact on HDV replication. So, the presence of HDV leads to higher mortality and a greater economic health care burden in the population [5].

HDV infection is still endemic in certain parts of the world. In Vietnam, the prevalence of HDV infection in chronic hepatitis B patients has been reported to be as high as 15.4% [6]. Previous studies have also demonstrated higher rates of HBV infection in some areas of the Middle East as well as the eastern part of Turkey. The prevalence of delta hepatitis in the eastern part of Turkey is up to 10% [7]. On the other hand, a recent study conducted in blood donors in western Turkey showed that only 3.4% and 2.3% of the HBs antigen-positive patients also had a positive result for anti-HDV IgG and HDV-RNA, respectively [8]. But in contrast to HBV infection, the epidemiological aspects of delta hepatitis are still unclear. Furthermore, despite the recent increase in public awareness and the adoption of national HBV vaccination programmes, chronic hepatitis D (HDV) infection in the eastern part of Turkey remains poorly understood and recognised. Currently there are also no definitive guidelines for the detection of chronic HDV in Turkey.

In the current study, 171 of the 1118 (15.2%) HBsAg-positive patients were total anti-delta antibody reactive. Furthermore, 95 of the 478 (19.8%) HBsAg-positive patients were also HDV-RNA positive. These rates were approximately 3–5 fold higher than those detected in neighbouring countries such as Greece [9], Egypt [10], and Iran [11]. Interestingly, higher rates of anti-HDV IgG seropositivity were observed in patients who came from an urban area compared to patients who resided in rural areas. The accumulation of delta hepatitis in the urban area was unexpected and contrary to the clinical trial data [12]. This result can possibly be explained by the socioeconomic turmoil due to the recent Van earthquake [13]. This phenomenon may also be linked to the geo-political crisis in the region. However, additional investigation into these cases of delta hepatitis is warranted and may provide further insight into the underlying risk factors.

Seropositivity of anti-HDV-IgG is currently considered to be the gold standard for HDV screening. However, some patients cannot be detected using this technique, and further examination including HDV-RNA should be performed in patients with HBV infection [14]. The current findings also indicate that less than 50% of patients in the study group were screened for HDV infection through anti-HDV IgG and HDV-RNA assays. This suggests that half the participants were sent home without testing for anti-HDV. Failure to test for delta hepatitis may be due to the lack of laboratory equipment as well as lack of awareness about delta hepatitis.

Although HDV infection rates have been declining in the western world, less is known about HDV prevalence and risk factors in women. Therefore, in the current study, we used HDV-RNA positivity to compare the HDV infection rates between genders, and found significant differences. The highest HDV prevalence was observed in female patients, implying that in coming years HDV infection will be more prevalent in this population. The confirmation of these results and the role of potential confounding factors such as earthquake-related conditions require further study.

HDV infection is generally characterised by low levels of HBV-DNA due to the suppressive impact of HDV on HDV viral replication [15]. Similarly, the results of the current study showed a higher prevalence of HDV infection in older patients.

Compared with HBV mono-infected patients, patients with delta hepatitis are older and also have a higher risk of liver cirrhosis. According to the data obtained from delta hepatitis patients, both a higher rate of cirrhosis and a higher prevalence of HDV infection were found in older patients, similar to the results of previous studies [16].

Ultrasound, computed tomography and magnetic resonance imaging are imaging modalities frequently used in the diagnosis of HS. Despite their high sensitivity, none of these modalities distinguish between non-alcoholic steatohepatitis and other types of non-alcoholic fatty liver disease or determine the degree of fibrosis [17]. Hepatic steatosis causes increased echogenicity on ultrasound, which can be contrasted with the lower echogenicity of the spleen or renal cortex. A similar pattern can be seen with diffuse fibrosis, giving rise to the term "fatty-fibrotic pattern", although the echo shadows tend to be coarser in the presence of pure fibrosis. The sensitivity of ultrasound decreases with mild fatty infiltration, and the sensitivity for the detection of steatosis progressively decreases as the body mass index (BMI) increases [18].

Unlike in chronic hepatitis C virus infection, the relationship between chronic HBV infection and HS is not clear. In a recent study reported from the USA, biopsy-proven HS was found to be 55% in patients with HBV infection. Furthermore, a high prevalence of HS was mostly detected in male patients with an Asian background [19]. In another study, HS was detected in one-third of patients with HBV-related chronic liver disease [20]. However, the impact of HDV on HS is still poorly understood. The current study suggests that the presence of HS was negatively associated with HDV infection. The lower rate of HS in delta hepatitis patients even in older age compared with patients who only have chronic HBV infection may possibly be due to the diagnosis being made at advanced stages of the disease.

This study has several strengths. To our knowledge, it is the first reported study to examine the association between delta hepatitis and HS. Furthermore, this study also presents extensive information on the demographic features of delta hepatitis patients. The current study has two limitations. First, this study includes a relatively small number of HDV-positive patients, and there need to be more data to determine the outcome. Second, since this study was not designed to evaluate the liver biopsy results, there was a lack of histopathological examination results.

There have been conflicting reports about the association between hepatotropic viral infections and hepatic steatosis. Yet, hepatic steatosis in patients with HDV infection has not been studied before. Our results suggest that lack of hepatic steatosis in the liver ultrasonography is an important parameter for HDV infection, possibly representing a novel relevant diagnostic strategy in hepatitis B patients. On the other hand, hepatic steatosis is mostly detected in patients with diabetes mellitus as well as metabolic syndrome defined as the presence of three or more of the following: central obesity (waist circumference more than 40 inches in men and more than 35 inches in women); a fasting triglyceride level of 150 mg/dl (1.69 mmol/l) or more; an HDL cholesterol level of less than 40 mg/dl in men or less than 50 mg/dl in women; blood pressure of 130/85 mm Hg or higher; and a fasting glucose level of more than 110 mg/dl (6.11 mmol/l). Due to the retrospective design of the study, we have not been able to obtain data of the metabolic status [21]. Taken together, our data also suggest that the lower rates of hepatic steatosis cannot be explained by HDV infection alone and that factors such as diabetes mellitus and metabolic status might play key roles in the pathogenesis of hepatic steatosis. Future studies are needed to determine the role of HDV in hepatic steatosis.

In conclusion, there may be a number of possible explanations for the higher prevalence of HDV in female patients and patients living in urban areas, and this clearly demonstrates the urgent need for primary and secondary prevention of HBV infection. These findings also suggest that screening of women who have HBV infection with HDV-RNA could be an important and economically feasible public health strategy to improve the struggle against hepatitis delta infection in eastern Turkey. Additionally, we conclude that patients with delta hepatitis have a different HS pattern from patients with hepatitis B monoinfection.

Conflict of interest

The authors declare no conflict of interest.

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