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REVIEW ARTICLE

CHRONIC LIVER DISEASES AND PARENTERALLY TRANSMITTED HEPATITIS VIRUSES

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Hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) are the leading cause of chronic liver diseases. The aims of the present study are to determine the etiological relationship of HBV and HCV in patients with chronic liver disease in North-Eastern Bulgaria and prevalence of dual and triple infections. A total of 434 patients were investigated for HBsAg, 402 of whom were also tested for anti-HCV. The HBsAg positive subjects were tested for anti-HDV and 32 of them also for HbeAg/anti-Hbe. Separated commercial ELISA kits were used. HBsAg was detected in 132 (30.4%); 10.6% were co-infected with HDV. Anti-HCV was detected in 15.4%. Five of 132 HbsAg positive patients (3.78%) were simultaneously HBV and HCV positive. Two patients out of 132 (1.52%) were positive to HBV, HCV and HDV. Our data indicate that HBV infection was the main cause of chronic liver diseases in North-Eastern Bulgaria, and 10.6% of the patients suffered from severe disease because of co-infection with HDV. HCV plays the same role in 15.4% of the cases. Recently, we observed dually infected (HBV and HCV) and triple infected (HBV, HCV, HDV) patients suffering from severe chronic liver diseases.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are a serious global health problem and are the leading causes of chronic liver diseases (1-3). HDV, a defective RNA virus that requires a helper function of HBV for packaging and transmission, plays an important role in the progression of chronic liver damage in patients chronically infected with HBV (4-6).

In the present study, an epidemiological survey to determine an etiological relationship of HBV, HCV and HDV in North/Eastern Bulgaria chronic liver disease patients were performed over a 3 year period. Patients, ranging from age 1 to 85 years (mean age 46.4±18.6), with clinically diagnosed chronic liver diseases (chronic persistent hepatitis, chronic active hepatitis, cirrhosis), were referred to the Laboratory of Clinical Microbiology and Virology, University Hospital St. Marina, Varna, from March 2002 to June 2005, by their gastroenterologist. For clinical purposes, the diagnosis was based primarily on serological data. The patients were divided into groups according to their age as follows: 1-15 yrs, 16-30 yrs, 31-45 yrs, 46-60 yrs, and over 60 years.

Single serum samples of 434 patients were tested for HBsAg and 402 of them also for anti-HCV. HBsAg positive subjects were investigated for anti-HDV and 32 of these for Hepatitis B e antigen (HbeAg) and antibody to Hepatitis B e antigen (anti-Hbe; HBeAb). HbsAg was determined using different commercial ELISA test kits, according to the manufacturer's recommendations: third generation ELISA (DIA PRO, Milano, Italy), ETI-MAK-4 (DiaSorin, Italy), biolelisa HBsAg (BIOKIT,

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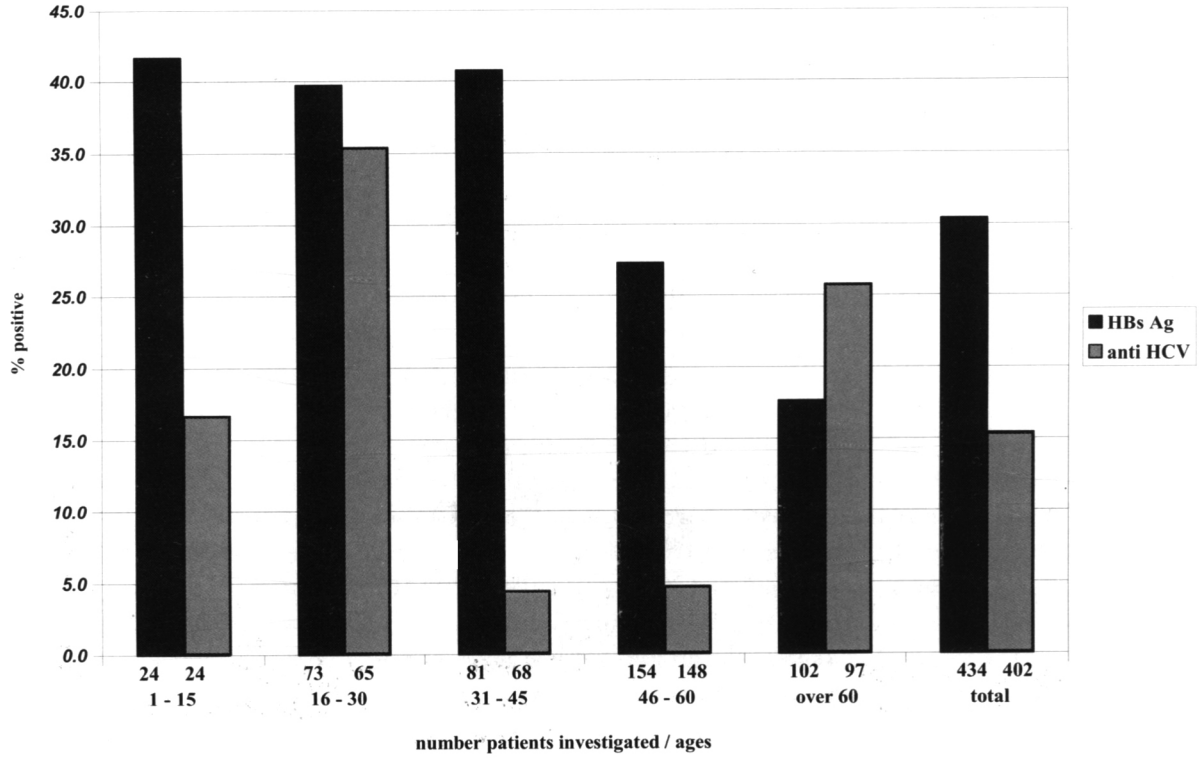

Fig. 1. *Patients with HBV and/or HCV infection.*

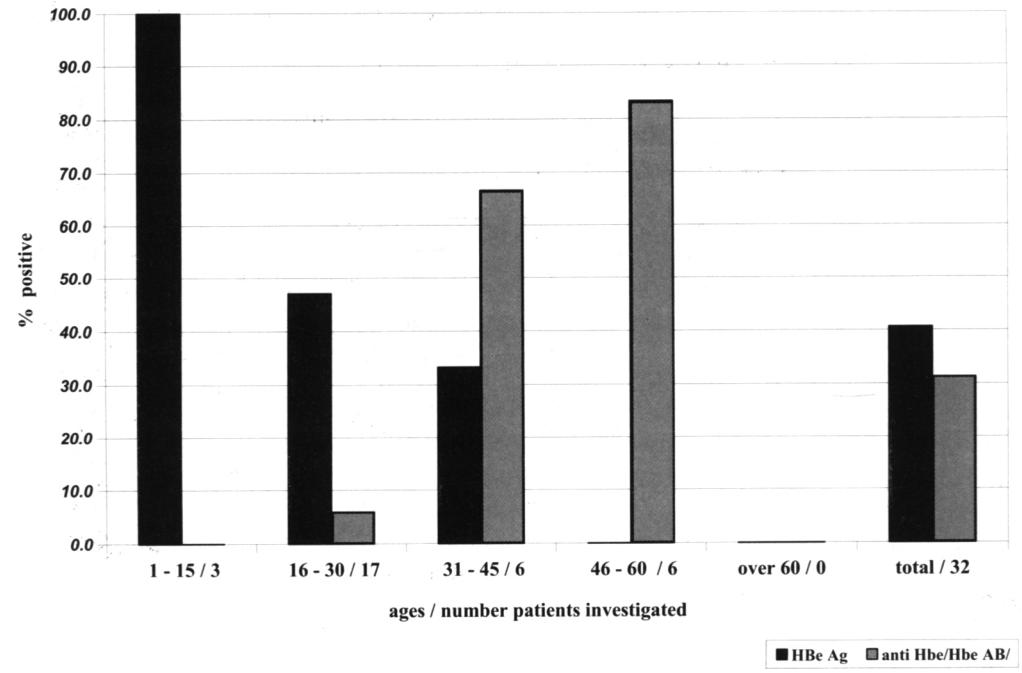

Fig. 2. *Patients with other HBV markers.*

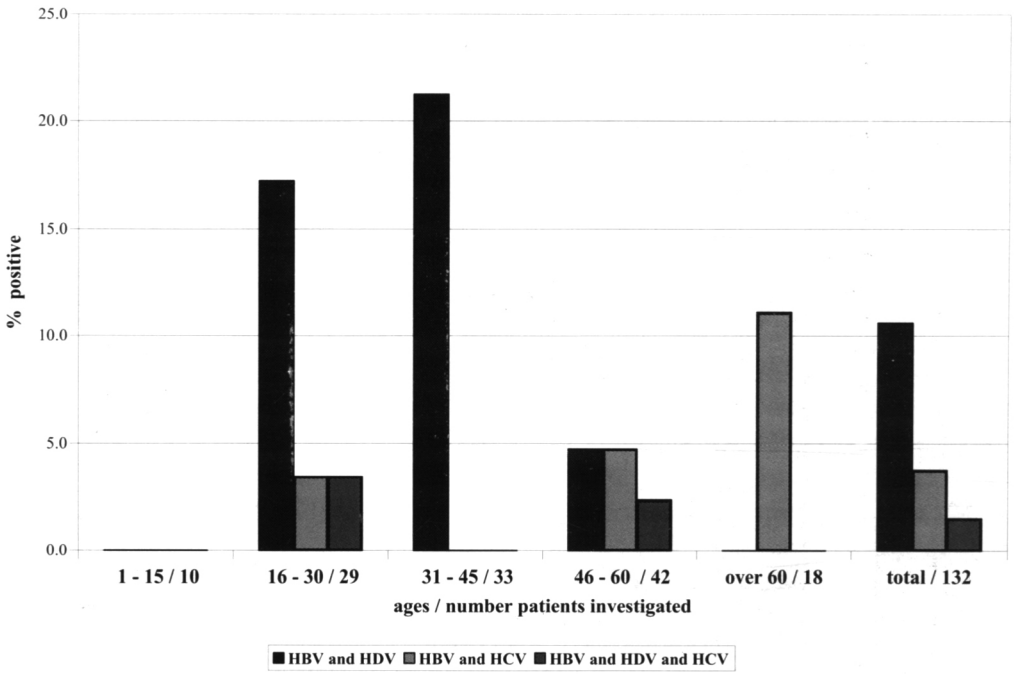

Fig. 3. *Patients with multiple infections.*

Barcelona, Spain), Hepatitis B surface antigen (ClinPro International, USA). HbeAg/anti-Hbe was also determined using different commercial ELISA test kits: HBeAg & Ab (DIA.PRO, Milano, Italy), ETI-EBK-2 (DiaSorin, Italy), Hepatitis Be Antigen Enzyme Immunoassay (ClinPro International, USA), and Hepatitis Be Antibody (ClinPro International, USA), according to the manufacturer's recommendations. Anti-HCV was determined using third generation Enzyme Immunoassay (DIA.PRO, Milano, Italy), ETI-AB-HCVK-3 (DiaSorin, Italy), Hepatitis C Virus Enzyme Immunoassay Test (ClinPro International, USA), bioELISA HCV (BIOKIT, Barcelona, Spain), in compliance with the manufacturer's recommendations. Total serum anti-HDV antibodies were detected, using HDV Ab Enzyme Immunoassay Test Kit (ClinPro International, USA), ETI-AB-DELTAK-2 (DiaSorin,.Italy), as per the manufacturer's recommendations.

Of 434 chronic liver disease patients investigated, HBsAg was detected in 132 (30.4%). HCV was the etiological agent in 15.4% of 402 of these (Fig.1). The data of other Bulgarian studies of patients with cirrhosis (7) shows that 38.5% were HBV positive and 22% anti-HCV positive. The statistically significant differences were proved only for the HBV prevalence ($p<0.05$). In a similar study by Chatterjee et al the prevalence of HCV infection was about 8% (8). The survey of distribution of these viruses, depending on the age, shows high prevalence of Hbs Ag in the groups up to 45 years of age (mean 41%). In Moscow, Russia (9), 77% of chronic hepatitis in children was found to be related with HBV infection. In our study there were only 24 children up to 15 years of age investigated, therefore it is difficult to compare our results with statistical data from these authors. The prevalence of anti-HCV (Fig.1) was higher in the patients 16-30 years old (35%) and in those over 60 years old (26%). Relative high anti-HCV prevalence in the older age may be explained by probable transmission of blood and blood products contaminated with HCV in the past. Surprisingly, in our present study, anti-HCV was detected with high prevalence in 16-30 years old patients (35%). High anti-HCV positivity in adolescents was also found in a study by Atanasova et al (10). These findings need further investigation and precise determination of risk factors for acquisition. About 0.4% to 26% of apparently healthy populations in different countries suffer from chronic HCV infection (3, 8, 11).

Fig. 2 shows the prevalence of various HBV serological markers among 32 HbsAg positive

patients stratified by age. HBeAg and HBeAb were detected in 40.6% and 31.3% respectively. Most of the patients up to 30 years of age were HbeAg positive. HBeAb were detected more frequently in patients older than 30 years. The detection of HBeAg in HBsAg positive patients is considered as evidence of active viral replication (12). Our data confirmed the current understanding that the age of acquiring of HBV infection affects the course of the disease. In children and adolescents who acquire the infection, there is a long immune tolerance phase with HBeAg positivity. For those, who acquired infection during adolescence or adulthood, the disease progresses directly to the immune clearance phase with seroconversion from HBeAg to HBeAb (13-14). It was observed, as in other studies (15), that severe exacerbations occur with equal frequency in patients who are HBeAg positive and in those with HBeAb.

Over the past few years more complete information about etiological relationship of HBV and HCV with chronic liver diseases has been gathered. Very little data is available regarding coinfections/superinfections with HDV in our region and the interactions between these hepatotropic viruses. Fourteen of 132 (10.6%) HbsAg positive patients were co-infected with HDV, most of them between 16–45 years of age (Fig. 3). The data of another Bulgarian study (7) shows that 6.1% of patients with cirrhosis were co-infected with HDV. Prevalence of HDV in HbsAg carriers varies from 1% to 5% in USA, Western Europe and Asia (16), but is high in those with repeated exposure, such as intravenous drug abusers (20% to 30%) (17). In patients with HDV infection, it has been proved that HDV suppresses HBV. More than 70% of patients superinfected with HDV become chronic carriers of HDV and are at high risk of developing chronic active hepatitis and cirrhosis, and the majority of these die from liver disease (4, 18). Of the patients in this study, only 3 (21%) had laboratory data for active HBV replication (presence of HBeAg in serum).

Five of 132 HbsAg positive patients (3.8%) were simultaneously HBV and HCV positive (Fig. 3) and only 1 of these (20%) was positive for HbeAb. Co-infection with both viruses may occur, because of shared routes of infection. In patients with chronic hepatitis B, the rates of HCV coinfection vary from 9% to 30%, depending on the geographical region (19). Other Bulgarian studies show that 6.6% of the patients with cirrhosis and 4% of chronic HCV infection patients were co-infected with HBV (7, 20). There are no statistical differences when comparing our data with these results. Most of the patients in our study were over 45 years of age. An Italian study shows that dual infections are more common in patients over 50 years of age (21). The exact number of patients infected with both HCV and HBV is unknown, because no large-scale studies have been performed, and also there is a well-described phenomenon of "serologically silent" occult HBV infection (22). A total anti-HBc antibody was not investigated in the present study of chronic liver disease patients. But in our region, there are about 5% of randomly chosen patients with only anti-HBc positivity and HbsAg negative (23). Co-infected patients are often found to have evidence of both HBV and HCV infection, without a clear chronology of infection (12), as was also found in our patients. There are various immune profiles of dually infected patients, and HBV and HCV exert an alternative dominant replication (12, 18, 24-25). There is an inverse relationship between serum HBV replication and serum HCV replication and both viruses have the ability to induce seroconversion of the other (12, 25-26). In our study, among patients with dual infection, only 1 (20%) was Hbe Ag positive.

Two of 132 (1.5%) HbsAg positive patients were anti-HCV and also anti-HDV positive (Fig. 3). In patients with multiple hepatotropic viral infections, the reciprocal influence of each virus remains controversial (19, 25). In most cases HDV acts as the dominant virus (24-25). The interaction between hepatitis viruses in this study was not determined. We assess only HbeAg/HbeAb activity. Concerning liver injury, it has been suggested that patients with dual or triple infection suffer from more severe liver lesions with a higher prevalence of cirrhosis and are at an increased risk for progression to hepatocellular carcinoma (12, 24, 27). Patients with multiple infections should be carefully monitored, and virological assessment is necessary to determine which virus will emerge as a dominant virus, in order to select the most appropriate antiviral treatment.

In summary, the prevalence of HBV and HCV infections varies according to the geographical areas. Patients with chronic viral diseases constitute a reservoir of infected individuals who perpetuate

the infection from generation to generation. We found that nearly 1/3 of chronic liver diseases were related to HBV, and nearly 50% are related to parenterally transmitted hepatitis viruses. The routes and risk factors of acquisition of the infections involved were not identified. The study including a heterogeneity group of patients and all of the routs of infection were possible. The risk factors for acquisition need future investigation, especially in young people. Detection of HBV DNA, HCV RNA and HDV RNA is necessary to be carried out in future investigations. HBV vaccination applied in HCV positive individuals reduces the high risk of dual or triple infections. In Bulgaria, a national expanded program of immunization against HBV has already been started.

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