Seroprevalence of HDV Infection in HBsAg Positive Population in Ismailia, Egypt

Nahed IM Gomaa, Lobna A Metwally, Nader Nemr, Soha Younis

Departments of Microbiology, Endemic and Infectious Disease Unit, Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Hepatitis D virus (HDV) is a defective RNA virus that needs hepatitis B surface antigen (HBsAg) from HBV for transmission. HDV can lead to fulminant hepatitis and the progression of chronic liver damage in patients with chronic hepatitis B. The aim of this study was to determine the seroprevalence of HDV among HBsAg positive individuals in Ismailia, Egypt. Serum samples were collected from 170 HBsAg positive healthy individuals from Suez Canal University blood bank over a one year period. All of them were seeking blood donation and found to be HBsAg positive during viral hepatitis screening workups which is routinely done prior to donation. Serum samples were screened for IgG antibodies to hepatitis delta virus (HDV) using enzyme-linked immunosorbent assay (ELISA) method. Anti-HDV antibodies were detected in 8 (4.7%) individuals aged from 29-43 years. Liver function tests showed that serum ALT and AST levels were elevated in the HBsAg/anti-HDV positive cases. It is concluded that the rate of HDV infection in Ismailia is high and further investigation is needed to validate the findings and raise awareness about the risk of dual HBV and HDV infection.
III is frequently associated with fulminant hepatitis (Casey et al., 1996).

Delta superinfection can transform asymptomatic or mild chronic hepatitis B infection to severe progressive chronic active hepatitis and cirrhosis and accelerate the course of chronic active hepatitis B. Therefore, in the present study, individuals volunteered for blood donation and found by routine screening to be HBsAg positive were investigated to assess the seroprevalence of HDV in Ismailia, Egypt and the safety of the unscreened blood supply in this area.

Materials and Methods

Serum samples

Serum samples were collected from 170 individuals who volunteered for blood donation and found to be HBsAg positive by routine screening. All were from Suez Canal University blood bank and collected over a one year period between June 2011 and June 2012. Informed consent was obtained from each donor in this study. Ethical approval of this investigation was obtained from the ethical committee of the Faculty of Medicine, Suez Canal University.

All participants completed a questionnaire including sociodemographic data including gender, age, occupation and their residence (urban vs. rural).

Liver enzymes assessment

The biochemical liver enzyme alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also assessed by standard laboratory tests.

Serological testing

The presence of HBsAg was determined by using the HBsAg enzyme-linked immunosorbent assay (ELISA) kit (Diasorin, Italy). The presence of IgG antibodies to HDV was determined by commercially available ELISA kit (DiaSorin, Italy) as described below. This method for qualitative anti-HDV determination is a simultaneous competitive assay. In principle, Anti-HDV present in the sample and labeled anti-HDV antibodies compete for a fixed quantity of HDVAg bound to the solid phase. The quantity of enzyme tracer bound to the solid phase and consequently the enzyme activity are inversely proportional to the anti-HDV concentration present in samples or controls. Enzyme activity is measured by adding a colourless chromogen/substrate solution. The enzyme action on chromogen/substrate produces a colour which is measured with a photometer (ETI-SYSTEM reader).

The procedure involves dispensing 50 µL negative control, positive control and samples into their respective wells and then adding 100 µL diluted enzyme tracer into all wells, then incubate for three hours ± 15 min at 37° ± 1°C. When incubation has been completed, the strip is rinsed and 100 µL chromogen/substrate solution is dispensed into all wells. After incubation for 30 ± 2 min at room temperature, away from intense light, 100 µL blocking reagent is added into all wells. The absorbance of specimens is measured with a photometer at 450/630 nm within one hour of adding the blocking reagent. The cut-off value is determined by adding the mean absorbance for the negative control values (NC) multiplied by 0.5 to the mean absorbance for the positive control values (PC) multiplied by 0.5. The presence or absence of anti-HDV is determined by comparing the absorbance of the unknown samples to that of the cut-off value. Unknown samples with absorbance values less than or equal to the cut-off value are considered reactive for anti-HDV (positive).

Statistical Analysis

Data analysis was performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Qualitative data were analyzed using the χ2 test. Quantitative variables were also analyzed with the Student’s t test. A p value < 0.05 was considered significant.

Results

During a one year period of this study, volunteer blood donors presenting to Suez Canal University blood bank were tested for the presence of HBV as part of the routine clinical assessment. 170 subjects were found to HBsAg+, and rejected from donation. Their serum samples were screened for IgG antibodies to hepatitis delta virus using the enzyme-linked immunosorbent assay (ELISA) method.

All subjects were males and the average age was 35.6 ± 14.7 years. Of the 170 subjects, 117 lived in rural areas (68.6%) and 100 (58.8%) worked in non-governmental jobs. Anti-HDV antibodies were detected in 8 (4.7%) subjects with an average age of 36.9 ± 12.6. Anti-HDV antibodies in rural population
was higher than in urban population (6 versus 2 cases) and 5 cases had non-governmental jobs. No significant association was observed between HDV seropositivity and sociodemographic characteristics. A study of serum ALT in HBsAg/anti-HDV positive cases showed elevation in all of them as compared to anti HDV negative cases (55.6 ± 38.0 IU/L in dual infection versus 40.1 ± 26.0 in anti-HDV negative cases). As for AST level it was 41.9 ± 22.0 IU/L in dual infection compared to 32.9 ± 9.9 in anti HDV negative cases.

Discussion

Delta hepatitis affects 15-20 million individuals worldwide which makes it a major global health problem (Gupta et al., 2005). In this study, Sera of 170 HBsAg-positive blood donors were screened for antibodies to hepatitis delta antigen (anti-HDV). Anti-HDV was found in 8 (4.7%) of the cases.

A similar prevalence was observed in the study of Saudy et al., 2003, who screened 48 blood donors and found 2 cases to be positive for HDV antibodies (4.2%).

In the present study, subjects living in rural areas showed a higher prevalence of HDV antibodies than the urban population. This is consistent with the study of Mumtaz et al., 2005 from Pakistan.

Earlirer studies conducted in Egypt showed different results. In 1988, El Zayadi et al., reported the prevalence of delta infection among urban Egyptians sera of 44 HBsAg-positive chronic liver disease (CLD) patients and 48 asymptomatic HBsAg carriers. Anti-HDV was found in 21 (47.7%) of the patients compared with 4 (8.3%) of the asymptomatic carriers. Abdelfattah et al., 1991, carried out their work on 45 patients with chronic liver diseases, including 24 cases of liver cirrhosis and 21 cases of chronic hepatitis. The study showed that IgG anti-HDV was detected in 8.9% of cases with chronic liver diseases. On the other hand, HBsAg was detected in 53.3% of cases (54.2% of them with cirrhosis and 45.8% with chronic hepatitis) with no significant association between HBsAg positivity and type of hepatic illness. Moreover, IgG anti-HDV was positive in only 4.2% of HBsAg positive cases. Darwish et al., 1992, demonstrated the presence of HDV antibodies in 16.94% of patients with acute HBV, 23.53% in patients with chronic HBV and 21.9% among chronic HBsAg carriers (9 out of 41). In another study conducted on 45 Egyptian children, aged 2 to 15 years, with chronic hepatitis B, IgG anti-HDV was detected in only four children (8.9%) (Morcos et al., 2000). Zaki et al., 2010, detected twenty anti-delta positive samples out of the 100 cases studied (20%). The highest frequency of anti-HDV positivity was found among Schistosomal hepatic fibrosis patients (46.7%) followed by drug abusers (25%), hepatocellular carcinoma group (14.3%), and patients on maintenance haemodialysis (9.09%). None of the acute hepatitis B patients or chronic asymptomatic HBsAg carriers showed Delta antibodies.

In the rest of the Arab and non-Arab world the situation is quite different. Several studies have been conducted in Iran. A study conducted in West of Iran in 2010 showed a dramatic increase in positive HDV serology (17.3%) among HBV carriers compared with the study conducted in the same region almost a decade ago (Alizadeh et al., 2010). In a study carried out in Tehran in 1988, 2.5% of HBV carriers (n = 120) were reported to be HDV positive (Rezvan et al., 1990). In a more recent study in 2005, 5.8% of HBsAg positive individuals were found to be HDV infected in Golestan province (Gholamreza et al., 2007).

The prevalence of HDV infection among the HBsAg+ population was 24.4%, 16.6%, 4%, 2.2%, 1.6%, and 1.5%, in Bangladesh (Zaki et al., 2003), Pakistan (Mumtaz et al., 2005), Mexico (Munoz Espinosa & Ibarra;
HDV antibodies in Egyptian blood donors

Salas, 1997), Taiwan (Chen et al., 1992), Spain (de Miguel et al., 1994) and Yugoslavia (Delic' et al., 1993), respectively. In a study conducted in Mongolia in 2005, the seroprevalence of anti HDV among the screened 403 blood donors was 10.2% irrespective of their HBsAg status. Their ages ranged from 20- 49 years (Tsatsralt et al., 2005), as was observed in our study.

Other studies conducted in the Middle East region have reported different results. A study by Njoh & Zimmo, 1998, in Saudi Arabia found that 13.6% of HBV carriers (n = 81) were also positive for anti-HDV. In Lebanon, the prevalence of delta antibody among HBsAg-positive Lebanese patients and blood donors is ~1% (three patients: one blood donor and two with chronic hepatitis) (Ramia et al., 2007). In asymptomatic carriers of HBsAg from Jordan, Kuwait, and Turkey the seroprevalence rate was reported as 2%, 31%, 5.2%, respectively (Alavian & Alavian, 2005).

Some countries have showed a decrease in the prevalence of HDV infection. This decrease in HDV prevalence internationally may be due to worldwide HBV vaccination and treatment. The tendency in HDV decline has been observed in Italy (Jacobson et al., 1985), India (Chakraborty et al., 2005) and Turkey (Deertekin et al., 2006). In Taiwan, Huo et al., 1997, reported a decrease in HDV infection from 23.7% in 1983 to 4.2% in 1995. Whether in Egypt we have witnessed this worldwide decline tendency cannot be judged due to paucity of studies conducted in this field.

Although HDV requires the help from HBV and thus HDV infection is necessarily associated with HBV infection (Sureau et al., 1993), in a study conducted in Mongolia, 2005, HDV RNA was detected in 26% of the investigated donors which were HBsAg negative and anti-HDV-positive (Tsatsralt et al., 2005). In another study done in Egypt, Abdelfattah et al., 1991 found that 14.3% of HBsAg negative cases were positive for IgG anti-HDV. Moreover in the study of Darwish et al., 1992 out of the twelve chronic HB cases with delta infection, four cases were negative for HBsAg (33.33%). This phenomenon seems to be due to replicative suppression by HDV. The large delta antigen of HDV, which was proven in vitro to inhibit DNA-dependent RNA polymerase II in the host (Lo et al., 1998; Modahl & Lai, 2000) may be involved in this suppressive effect.

Our study has some limitations. For example, the sample size is small as only 170 HBsAg positive sera samples were included and this cannot be representative for the population of Egypt. Although of this limitation, still the results are important as this is the first study from Ismailia on the subject where a considerable prevalence rate of HDV-HBV co-infection has been observed. Other points that should have been addressed in our study is that only HBs Ag positive cases were screened for anti-HDV and as reported earlier, anti-HDV can be found in HBsAg negative sera. Evaluation of the anti-HDV status in HBsAg-negative donors would help in the screening of blood.

Due to paucity in sera, HDV RNA was not analyzed in the positive cases in this study to confirm HDV infection.

All our cases were males; probably they are the common population in Egypt who donate blood. Some studies have found a gender association with HDV seropositivity (Seifi & Ghannad, 2010; Gholamreza et al., 2007) which couldn't be assessed in the present study.

In conclusion, safe supply of blood is essential for patients requiring transfusion. Therefore, further efforts to improve the screening tests and to include screening of delta markers, in addition to hepatitis B viral markers would help to reduce the risks of hepatitis viruses infections via blood
transfusion. Proper vaccination and health education programs should be implemented to prevent this HBV/HDV coinfection.

Acknowledgment
The authors would like to thank all the staff working in Suez Canal University blood bank for their help in sample collection.

References


