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Full Length Research paper

Frequency of hepatitis B and hepatitis C infections and its association with development of factor VIII inhibitor in hemophiliacs in Hamadan Province of Iran

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The aim of the present study was to determine the frequency of hepatitis B and C infections; to analyze the markers of inflammation and liver function tests; and to assess the possible association between factor VIII inhibitor and hepatitis C and/or hepatitis B infections in hemophiliacs of Hamadan province of Iran. Subjects with confirmed diagnosis of hemophilia who agreed to participate were recruited in this case-series study. Hepatitis B and C serology, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and factor VIII levels, and factor VIII inhibitor titer were analyzed. Frequency of anti hepatitis C antibody and hepatitis B surface antigen were 59.1 and 5%, respectively. Factor VIII inhibitor was present in 47.8% of hemophilia A group, and its presence was neither associated with hepatitis B and hepatitis C infections nor with AST, ALT levels. Student's t-test showed a significant statistical association between duration of hemophilia and development of factor VIII inhibitor (p value = 0.038). Frequency of hepatitis B and C infections in hemophiliacs is significant. There was a significant association between duration of hemophilia and hepatitis C infection (p value <0.011). One suggestion is that current blood product screening methods for eliminating blood-borne viruses might not be effective enough. Further investigation is recommended.

Key words: Hepatitis B, hepatitis C, hemophilia, Iran.

INTRODUCTION

Hemophilia is an X-linked bleeding diathesis resulting from a deficiency of blood coagulation factor VIII (hemophilia A) or factor IX (hemophilia B). Clinically, the disease is characterized by frequent spontaneous bleeding episodes, mostly into joints or soft tissues. Bleeding can also occur into other critical closed spaces, such as the intracranial space or the retroperitoneal sp-ace, where it can be rapidly fatal. Hemophilia A occurs in about one in 5000 male births; hemophilia B is less common, occuring in about one in 30,000 births. Hemophilia is classified as mild, moderate, or severe based on circulating levels of clotting factor. Severe disease is defined as a diaease with plasma level < 1% of normal levels, moderate as 1 to 5%, and mild as >5% (High, 2001). Five to twenty percent of patients with hemophilia A and one to four percent of those with factor IX deficiency (hemophilia B) develop antibodies to the coagulation factors (High, 2001).

Currently screening the blood donors and inactivating enveloped viruses has essentially eliminated the danger of transmitting human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) by treatment concentrates to hemophiliacs. Despite the advances described above, approximately 60% of hemophilia treatment is still based on blood-derived products (High, 2001). Therefore, vigilance in maintaining the safety of the blood supply remains critical. Thousands of hemophiliacs worldwide were infected with HIV and/or HCV after receiving factor VIII and IX infusions from infected blood plasma in late 1970s through 1980s. Many of these patients have since died. Some were children or

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teenagers who lived the rest of their lives infected with HIV or hepatitis (Kasper et al., 1975). Despite precise provision of blood supply, there is a little risk of being infected with viral pathogens, such as hepatitis B and C and also HIV. In a study in Iran, 103 hemophiliacs and 103 controls were studied regarding hepatitis C. They found that 76.7% of patients and 0.97% of controls were anti-HCV positive (Kasper et al., 1975). This figure was higher than prevalence of anti-HCV Ab positivity among injection drug users (IDU) in a region of Iran in which 30% had anti-HCV Ab (Bouma and Starkenburg, 1974).

In this current article our aim was to determine the regional frequency of hepatitis B and C infections in hemophiliacs; as well as analyzing serum inflammatory markers to assess function of the liver and the possible association between development of factor VIII inhibitor and presence of hepatitis B or C infections.

MATERIALS AND METHODS

Subjects

All documented hemophiliacs in Western Province of Hamedan were announced by local hemophiliac support center and the study was explained. Through a counseling session, each participant was informed of the risk of post-transfusion infections and complications and necessity of vaccination against hepatitis B. All lab tests were free of charge.

Sixty six patients complied to participate and were enrolled into the study. Type of hemophilia (A or B) and duration of the disease were recorded in the data collection form.

Laboratory methods

Serum levels for SGOT, SGPT and antibodies to HBV and HCV were measured. Hepatitis B and C virus markers including HBsAg, HBe Ag , Anti- HBe , Anti HBc and HCV Ab were tested by a enzyme-linked immunosorbent assay .Serum HCV RNA level was detected by PCR (primers: 5' non-coding region; detection limit, 10^2-10^3 genome equivalent. Serum HBV DNA was measured by Cobas Amplicor method. Hepatitis C regarded positive if HCVRNA was positive. Hepatitis B regarded positive if serum was positive for HBsAg, anti-HBc and/or HBV-DNA. Factor VIII inhibitor titers were measured using the method described by Kasper and coworkers (Kasper et al., 1975). A titer of 1.0 Bethesda U/mL or more was considered positive. Assays for factor VIII inhibitors were performed using one-stage method (Ebrahim et al., 1997). Factor VIII recovery was measured before and 15 min after infusion of factor VIII (Alizadeh et al., 2005). All samples were analyzed at the coagulation laboratory of the Hamadan.

PCR method

Serum HBV DNA was measured by polymerase chain reaction using the Cobas Amplicor HBV monitor test kit (Roche Diagnostics, Indianapolis, Ind), an *in vitro* nucleic acid amplification test for quantifying HBV DNA. It has a certified lower limit of detection of 300 copies/mL. At this level of HBV DNA, the test has a 98.1% positivity rate and a 100% clinical specificity rate. The manufacturer's procedures for sample preparation, storage, and testing were followed. Whole blood was collected with a vacuum blood collection tube without an anticoagulant; the serum was separated by centrifugation at room temperature and was stored in a sterile tube at -70°C. This procedure was accomplished within 6 h of sample collection. Each sample ran for HBV DNA included a replicate of the negative, low-positive, and high-positive controls, and each run was determined to be valid.

Statistical analysis

Data were analyzed by SPSS software Ver.11.0 [SPSS Inc. Chicago, IL, USA], using Student's t-test and Chi square test. P value < 0.05 was regarded as significant. Diagrams were plotted using Microsoft Excel 2003 [Microsoft Corp. Redmond, WA, USA].

RESULTS

Of 66 hemophiliac patients, 46 subjects (69.7%) had type A and 20 subjects (30.3%) had type B hemophilia. 83.3% of patients were male. Mean age at the time of the study was 24.6 ± 15.01 years (23.4 ± 10.1 for hemophilia A and 28.2 ± 12.7 for hemophilia B) and mean age of diagnosis of hemophilia was 7.03 ± 8.49 years (4.6 ± 5.2 for hemophilia A and 9.8 ± 6.3 for hemophilia B). Mean age of subjects with hepatitis C infection was 29.17 ± 14.65 years at time of the study, compared to 17.78 ± 2.52 years for those not infected (p = 0.002). Eighteen patients were not aware of the exact date of diagnosis. Thirteen patients (19.7%) were previously vaccinated against hepatitis B virus. Table 1 summarizes age of patients at the time of diagnosis.

Hepatitis B and C markers in 66 studied subjects

Frequency of HBs Ag, HBs Ab, HBc Ab, HBe Ag, HBe Ab and HBV DNA were 6, 9.1, 39.4, 1.5, 56.1, and 15% respectively (6.5, 10.9, 43.5, 2., 63% and 15.2% for hemophilia A and 5, 5, 30, 0, 35 and 15% for hemophilia B, respectively). Anti-HCV Ab was positive in 39 (59.1%) (60.9%for hemophilia A and for 55% hemophilia B) and HCV RNA was positive in 28 (42.4%) (43.4% for hemophilia A and for 40% hemophilia B) subjects. HCV RNA $>10^5$ copies/mL was positive in 75.7% of Anti-HCV antibody positive patients. Hepatitis B and C co-infection detected in 4.5% of cases (6.5% for hemophilia A and for 0 hemophilia B). Factor VIII plasma level <1, 1 - 5%, and > 5% was detected in 60.5, 10.9 and 17.4% in hemophilia A patients respectively. Factor VIII inhibitor was positive in 47.8% of hemophilia A subjects, although not statistically associated with severity of hemophilia. Mean serum AST and ALT levels were 36.45 + 22.8 and 89.50 + 41.8 respectively (normal range 5-35 U/L). Mean AST and ALT serum levels in "factor VIII inhibitor positive" hemophiliac A subjects were 38.37 + 30.34 and 38.68 + 35.17, while in "factor VIII inhibitor negative" hemophiliac A subjects was 36.421+ 13.14 and 37.68+ 17.60 respectively. Figure 1 shows mean AST and ALT serum levels in patients positive for hepatitis B surface antigen, hepatitis C virus antibody, hepatitis B and C co-infection and in patients without hepatitis B and C infections.

Table 1. Age distribution of patients at the age of diagnosis of hemophilia*.

Age group (years)	Hemophilia A	Hemophilia B	Total
0-4	23 (65.7%)	3 (23.1%)	26 (54.2%)
5-9	5 (14.3%)	5 (38.5%)	10 (20.8%)
10-14	3 (8.6%)	1 (7.7%)	4 (8.3%)
15-19	2 (5.7%)	2 (15.4%)	4 (8.3%)
25-29	1 (2.9%)	1 (7.7%)	2 (4.2%)
30-33	1 (2.9%)	1 (7.7%)	2 (4.2%)
Total	35 (100%)	13 (100%)	48 (100%)

*P=0.16.

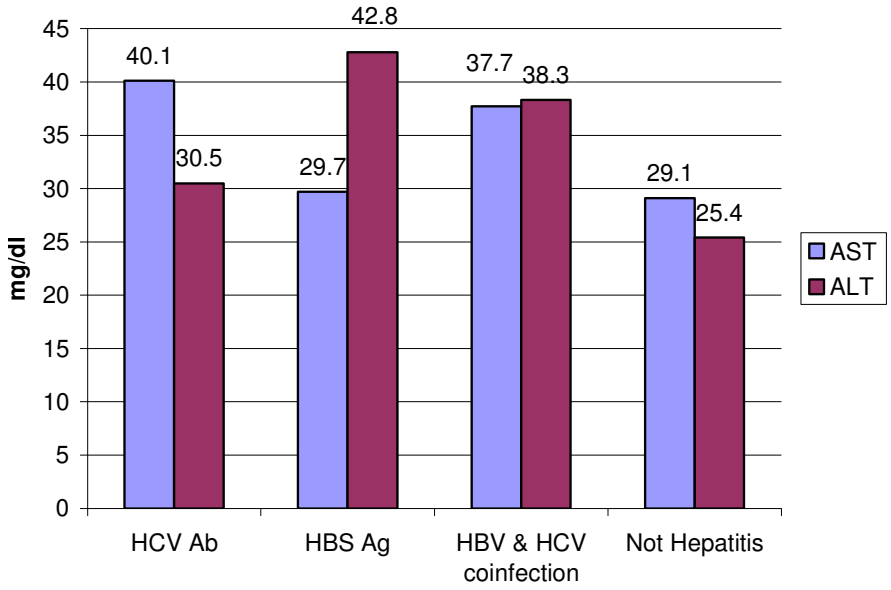

Figure 1. Mean serum level of AST and ALT in patients with hemophilia according to HBV and HCV infection.

There was no association between development of factor VIII inhibitor and mean AST / ALT serum levels and hepatitis B and C infections. However, there was a significant statistical correlation between development of factor VIII inhibitor and duration of hemophilia (Student's t-test, p value = 0.038). Duration of hemophilia was also associated with hepatitis C infection (p value = 0.011), but not with hepatitis B infection. Association between duration of hemophilia and hepatitis C infection in recent 5 and 10 years was significant by Chi square test (p values were 0.049 and 0.008 respectively). There was a statistically significant difference in mean serum AST level between hepatitis C infected and non-infected subjects (40.10 + 11.21 vs. 29.12 + 6.7 U/L, p = 0.036). Such a difference was not evident on subjects with hepatitis B infection.

DISCUSSION

This study found that Frequency of anti-hepatitis C antibody and hepatitis B surface antigen were 59.1 and 5% respectively. Factor VIII inhibitor was present in 47.8% of hemophilia A group, and its presence was neither associated with hepatitis B and hepatitis C infections nor with AST, ALT levels. There was a significant association between duration of hemophilia and development of factor VIII inhibitor.

In a study by Ebrahim et al. (1997) on 103 hemophiliacs and 103 controls, 76.7% of patients and 0.97% of controls were anti-HCV positive (Kasper et al., 1975). According to the study, significant correlation was found between the prevalence of anti-HCV antibody and serum AST and ALT levels, patient's age and the amount of blood products transfused. In the present study, we found a significant prevalence of hepatitis B and C infections in hemophiliacs (5 and 59.1% respectively). The lower prevalence of hepatitis C might be related to different time frames of the two studies and also the endemicity of hepatitis C in the provinces studied by Ebrahim (1997).

In a study on general population in Hamedan Iran, fre-

frequency of hepatitis B surface antigen (HBs Ag) was 2.3% (Mohammad et al., 2002). The authors showed that history of imprisonment and surgery was a great risk factor for acquisition of hepatitis B. Here we found HBs Ag in 5% of hemophiliacs in the same geographical region. This difference may be caused by repetitive hospitalizations or contamination of blood products, although more studies are required for confirmation. In 2000, Ghavanini et al. (2000) studied on 7897 voluntary blood donors in Shiraz, Iran. The prevalence of HBs Ag and anti-HCV Ab was 1.07 and 0.59% respectively (Ghavanini and Sabri, 2000). Although, blood donors are usually self-regarded as healthy and so should not be regarded as a sample of the general population, the difference in prevalence of hepatitis B and C between this group and hemophiliacs is substantial. However, hospitalization by itself and recurrent use of IV drugs and other high-risk habits should be accounted in hemophiliacs. Association of hemophilia and hepatitis C infection warrants more scrutiny in terms of controlling safety of blood products, considering that mean duration of hemophilia in our study was recent 16.1 years.

Mean age of hemophiliacs with hepatitis C infection also deserves further discussion. It was 29.17 + 14.65 versus 17.78 + 2.52 years for hemophiliacs not infected with hepatitis C. This means that new cases of HCV infection have declined over the recent years. Association between hepatitis C infection and duration of hemophilia in recent 5 and 10 years was still significant. This study regarded frequency of viral hepatitis B, C infections irrespective of type, name and number of product used. In this study only 19.7% of hemophiliacs were vaccinated against hepatitis B; we recommend vaccination against hepatitis B for all hemophiliacs and their families. Factor VIII inhibitor detected in 61.1% of our hemophilia A patients, and was much higher than 5-20% that mentioned in literature (High, 2001). Its presence was not associated with severity of hemophilia. In this study, the effect of viral hepatitis B, C on production of factor VIII inhibitor was investigated, and no significant association between viral hepatitis B, C and factor VIII inhibitor was determined. Unusual case of acquired serious factor VIII deficiency due to the development of human factor VIII inhibitor in a female patient 2 months after having had an acute episode of hepatitis C was reported (Dentale et al., 1998). Other studies on hemophiliacs have mentioned hepatitis C infection accompanied by HIV. Treatment of HCV is increasingly important in HIV-infected patients due to the improved HIV-associated morbidity and mortality and also frequency with which HCV occurs in patients with HIV-HCV confections (Centers for Disease Control and Prevention, 1998). Epidemiology, disease course, and management of HCV are different in HIV-HCV co infected individuals compared with HCV-monoinfected individuals. Most HIV- and HCV-infected individuals do not develop symptoms until late in the course of their disease (Centers for Disease Control and Prevention, 1998). During primary HIV infection the HIV RNA level increases rapidly as virion production greatly out-paces virion clearance. During the first 6 months after seroconversion, the immune system is able to gain partial control over viral replication, and the level of HIV RNA in plasma is decreased. During the asymptomatic phase of the infection, a steady state is achieved in which virion production equals virion clearance, presumably by the immune system. However, a progressive destruction of CD4+ cells eventually results in profound immunodeficiency (Centers for Disease Control and Prevention, 1998). The immune response plays an important role in HCV pathogenesis (Talal et al., 2002). A broad and strong anti-HCV specific CD4 + immune response is an important determinant of recovery during the acute phase of HCV (12). Both CD 4+ and CD 8+ responses to HCV structural proteins (core, E1, and E2) are important determinants of a successful outcome to therapy. Through the destruction of CD 4+ cells with reactivity for HCV, HIV may have a deleterious effect on immune responses in coinfected patients, which may be one of the reasons why higher CD 4+ T-cell counts and lower HCV viremia have been associated with improved responsiveness to interferon (Cerny and Chisari, 1999). Recent reports have demonstrated that the rate of hepatic fibrosis is accelerated in HIV-HCV coinfected individuals (Gerlan et al., 1999). An estimated 60 to 90% of persons with hemophilia and 50-60% of injection drug users who have HIV are co-infected with hepatitis C (Centers for Disease Control and Prevention, 1998).

As in our study, hepatitis C is frequent in hemophiliacs, its occurrence is at young age and its occurrence is associated with alterations of serum AST level. Liver biopsy in these patients is hazardous but routine screening for viral transmission and early detection, treatment and surveillance for chronic liver disease is mandatory. In the past 10 to 15 years, advances in screening of blood donors, laboratory testing of donated blood, and techniques to inactivate viruses in blood and blood products have remarkably increased the safety of blood products used to treat hemophilia. Although treatment-related infection with the HIV or most of the hepatitis viruses is a thing of the past, these measures do not completely avoid viruses such as hepatitis A and parvovirus. There is a great deal of concern about Creutzfeldt-Jakob disease (CJD), a rare transmissible nervous system disease that is inevitably fatal, being transmitted through transfusion. Counseling and HIV antibody testing have been recommended for persons at risk for infection including hemophilia patients, intravenous drug abusers, and persons who have had sexual contact with members of these groups (Gerlan et al., 1999). Routine counseling and antibody testing have not been recommended for blood transfusion recipients, because in general, their risk for infection is extremely low. However, as illustrated by a report (Mauss et al., 1998), some multiple transfused persons may be at a higher risk for HIV infection. In addition, some persons

with transfusion-associated HIV infection have transmitted the virus to their sexual partners and their infant children. In the present study, all patients were negative for HIV.

To ensure absolute safety from transfusion-transmitted viruses and other agents, hemophilia may now be treated with factor VIII, which has been produced through biotechnology. When inhibitors are present in large amounts, the patient may require very high and expensive quantities of transfused clotting factors to stem bleeding, and in some instances, even that may not be effective. More research is needed to investigate the role of genetic factors in risk of inhibitor development, design of specific immunosuppressive treatments to block inhibittor formation, or to neutralize inhibitors, and gene therapy.

Conclusion

Hepatitis B and C infections have a high occurrence rate in hemophiliacs, which could be related to recurrent hospitalization of hemophiliacs. Until new treatment modalities are established for hemophiliacs, several protective measurements should be considered. Namely employing more effective virus elimination processes for preparing blood products; educating hemophiliacs on transmission routes of hepatitis infections; routine screening of the patients and their family members for viral hepatitis B and vaccination against it; and surveillance of this population for liver diseases to commence early treatment for viral hepatitis.

The authors suggest further studies on pain management with avoidance of narcotics abuse, and social support and educating hemophiliacs and their family members on transmission of infections by blood products.

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