

Hepatitis B and C virus infections in patients with hemophilia in Zahedan, southeast Iran

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ABSTRACT

Objective: To determine the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in patients with hemophilia in Zahedan, Iran.

Methods: From March 2003 to January 2006, we evaluated 81 hemophiliac patients in Zahedan Hemophilia Center, southeast Iran, for hepatitis C virus antibody (HCV-Ab) and hepatitis B surface antigen (HBsAg), and evaluated the prevalence of HBV/HCV co-infection.

Results: The seroprevalence of HCV was 29.6%, and the HBsAg was positive in 4.9%. Four cases had HCV and HBV co-infection. All of the infected patients were unknowingly treated with contaminated plasma products before the middle of 1996.

Conclusion: All hemophiliacs, especially patients who have been treated with unheated clotting factor concentrates, should be evaluated for HCV and HBV infections.

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Patients with hemophilia and other coagulopathies treated with multiple blood transfusions and unheated clotting factor concentrates, including factors I, VIII, and IX have a high risk of acquiring hepatitis C, hepatitis B, and other viral infections.^{1,2} Although today, most cases of hepatitis C virus (HCV) infection are seen in injection

drug users (IDUs), it is the cause of most cases of post-transfusion hepatitis in some developed and undeveloped countries.^{3,4} In a report from the USA, the main cause of hepatitis transmitted by blood products was hepatitis C⁵ and in another report, anti-HCV was positive in 60-90% of hemophiliacs receiving commercial clotting factor concentrates.⁶ Screening blood donors for antibodies to hepatitis C virus (anti-HCV) and hepatitis B surface antigen (HBsAg) is effective in protecting against post-transfusion hepatitis.⁷ In southeast Iran, antibodies to hepatitis C have been documented in 2.1% of healthy blood donors, and 3.4% of the general population are infected with hepatitis B virus (HBV) (chronic carriers).^{8,9} Since 1996, new techniques have been used for treatment of hemophiliacs in Iran. Therefore, there is a high risk of acquiring viral infections in patients who were treated before 1996. The prevalence of HCV, HBV infections, and co-infection of HCV/HBV in hemophiliacs in Zahedan, southeast Iran is unknown. Therefore, our aim is to test all hemophiliac patients for antibodies to HCV and HBsAg, and define the prevalence of co-infection.

Methods. In a cross-sectional descriptive study, all patients who registered to Zahedan Hemophilia Society, southeast Iran, were evaluated for hepatitis C virus antibody (HCV-Ab) and HBsAg from March 2003 to January 2006. The laboratory tests were carried out in the blood transfusion organization. The sera were separated and tested for HCV specific antibodies using a commercial third generation anti-HCV enzyme immunoassay (EIA) kit (Ortho HCV 3.0, Amsterdam). All sera giving positive or intermediate EIA results were retested by a commercial HCV Western blot confirmatory test (Genelabs kit, Germany). Both EIA and the Western blot were performed according to the manufacturer's instructions. A serological tests for HBsAg was carried out using the enzyme-linked immunosorbent assay test kit (ELISA) (Diasorin Bio Medica, Saluggia, Italy).

Statistical analysis was performed using the Statistical Package for Social Sciences, version 11. The data were analyzed by Chi-square test, and $p < 0.05$ was considered significant.

Results. Eighty-one hemophilia patients (68 male, 13 female; mean age 12 years; age range 3-19 years) were enrolled in the study. Out of 68 males with hemophilia, 24 cases (29.6%) were positive for HCV-Ab. All the female with hemophilia were antibody-negative for HCV infection. There was a significant difference between the gender and seropositivity for HCV-Ab ($p=0.007$). Zahedan is a city in the Sistan and Baluchistan province, a subtropical area in southeast Iran, where 2 races (Persian and Baluch) are living together. Among the hemophiliacs, 39 cases were from Persian, and 42 were from the Baluch races. The HCV-Ab was positive in 18 cases of Persian race, and 6 cases of Baluch race. There was also a significant difference between the race and seropositivity ($p=0.003$). Seropositivity for HCV-Ab correlated with the time of treatment (before or after 1996) with clotting factors ($p=0.005$) (Table 1). Among the 81 cases with hemophilia, 4 cases (2 male and 2 female) were positive for HBsAg (4.9%). The recent cases also have the antibody for HCV. There was no difference between gender and race, and having a positive test for HBsAg. All infected patients with HBV were treated before the year 1996.

Discussion. In the present study, antibodies to HCV were detected in 28.3% of hemophiliacs who had been treated with multiple blood transfusions and unheated clotting factor concentrates before the year 1996. This rate is higher than the infection rate in the total population and blood donors in this area. In southeast Iran, antibodies to hepatitis C have been documented in 2.1% of healthy blood donors, and 2.3% in the general population before the year 1996.^{8,9} In a similar study in 2006, antibodies to hepatitis C

was detected in 3.1% of the general population.¹⁰ Our study showed that 29.6% of hemophilia patients had antibodies to HCV. This high rate of infection in hemophiliacs indicates that multiple blood transfusions and unheated clotting factor concentrates were 2 risk factors for occurrence of HCV infection in this group. At present, IDU is the most common risk factor for the occurrence of HCV infection in the world.¹¹ However, we observed an increased rate in IDUs in southeast Iran during the last decade.¹² Considering that hepatitis C is generally transmitted through IDU (80%), and intrafamilial transmission can occur,¹⁰⁻¹¹ therefore, the rate of infection can be higher than the past in the total population. At present, anti-HCV ELISA is the only diagnostic test in most clinical laboratories in the world. In Iran, it is the only screening test for detection of HCV infection in all blood donors.¹³ Hence, this infection can be a serious risk factor for multi-transfused patients as the anti-HCV ELISA test is not so valuable especially for early diagnosis, or for the past, or resolved cases,^{8,13} therefore, it is very important to use new techniques (such as the polymerase chain reaction or third generation antibody diagnostic test) for screening of blood donors. Although, these tests are not cost-beneficial in Iran and many other countries, using new viricidal techniques in blood products can reduce the rate of infection in our hemophilia patients. In the 1980s, the risk of transmitting viral contaminants in commercial factor VIII concentrates was well recognized. By the mid-1980s, most patients with severe hemophilia had been exposed to hepatitis A, B, and C viruses and human immunodeficiency virus (HIV).^{1,4-6} Fresh frozen plasma and cryoprecipitate are no longer used in hemophilia A and B because of the lack of safe viral elimination and concerns regarding volume overload. New viricidal techniques have been effective in eliminating the HIV transmissions and virtually eliminating hepatitis B and C exposures.^{1,5,6} Our results showed that transfusions of cryoprecipitate and factor

Table 1 - Frequency of hepatitis B surface antigen (HbsAg) and hepatitis C virus antibody (HCV-Ab) according to gender, race, and time of treatment.

| Variables | HbsAg | | HCV-Ab | |
|------------------|--------------|--------------|--------------|--------------|
| | Positive (n) | Negative (n) | Positive (n) | Negative (n) |
| <i>Gender</i> | | | | |
| Male | 2 | 66 | 24 | 44 |
| Female | 2 | 11 | 0 | 13 |
| <i>Race</i> | | | | |
| Persian | 2 | 37 | 18 | 21 |
| Baluch | 2 | 40 | 6 | 36 |
| <i>Treatment</i> | | | | |
| Before 1996 | 4 | 64 | 23 | 42 |
| After 1996 | 0 | 13 | 1 | 15 |

Seropositivity of HCV-Ab and HBs-Ag correlated with the time of treatment with the clotting factors (before and after 1996).

concentrates were significantly associated with HCV infection (28.3%). The results are in agreement with the results of other studies showing that multi transfused hemophilia patients have often been exposed to HCV and HBV.^{1,2,4,5-7,14-16} In the present study, all cases that had a positive test for HBsAg had been treated by unheated clotting factor concentrates before the year 1996. Since 1992, all children and many of the health care workers in Iran have received the vaccine against HBV and the incidence rate of HBV infection was decreased in the total population during the last years (from 5.4% in 1994, to 3.4% in 2006).⁸⁻¹⁰ Although it can be concluded that the use of new products and vaccination were 2 important factors for reduction of HBV infection in our patients, infections such as HCV are still a serious risk factor for multi-transfused patients. This is because HBsAg is the only test for screening of HBV infection in many of the blood transfusion centers in Iran and other developing countries, and many cases who are HBsAg negative but are infected with HBV, can be missed.¹³ On the other hand, a lot of people are not vaccinated and all of these factors can led to occurrence of infection. Today, approximately 3% of the world's population is chronically infected with HCV, and 5% with HBV.^{8,13} Therefore, the use of a new diagnostic test followed by urgent treatment are very important in prevention of severe complications.

Previous reports shows that in the USA, 88% of hemophiliacs were infected with HCV and two-thirds of these were co-infected with HIV.^{1,5} In this study with 16 years of active follow-up, there was an increased mortality rate in hemophilic patients who were infected with HCV. In 1994, Brenner et al¹⁷ showed that 83% of hemophiliacs who were treated with nonheat-treated factors were HBV seropositive. Chow and associates¹⁸ showed that out of 11 patients with hemophilia, 9 cases were HBsAg positive. One study showed that 26.7% of hemophilic patients were HBV seropositive, and only 2.3% were HBsAg positive. As in a previous study, as our present study, almost all of the infected hemophilia patients received nonheat-treated factors.¹⁹ Al-Kubaisy et al²⁰ showed that 0.66% of Iraqi hemophilia patients who are infected with HIV, were also infected with HCV. Viral inactivation techniques such as dry heating, pasteurization, solvent detergent treatment, and irradiation have improved the safety of these blood products.²¹ Today, these products offer greatly reduced risk for HIV, and hepatitis B and C transmission, but there is still some risk.^{21,22} In developing countries where the prevalence of HCV, HBV, and HIV is high and the use of new products is not a routine,²³ infections in hemophiliacs should be observed and we recommend hepatitis B and A vaccines for individuals with hemophilia, as they are at increased risk of developing hepatitis due to exposure to blood products.

With the results of this study, we conclude that all hemophiliacs especially patients who have been treated with unheated clotting factor concentrates, and those who live in the endemic area where there is no sensitive screening test for detection of HBV and HCV infection in blood donors, should be evaluated for HCV and HBV infections.

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