Original Article

Seroprevalence of Human Immunodificiency Virus (HIV) and Hepatitis C Infection in Hemophilic Patients in Iran

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ABSTRACT

Background and Objective: **Although transfusion therapy has lead to great improvement in longevity for hemophiliacs, but there have been tragic setbacks especially from transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV), HIV was reported to increase the rate of HCV-related liver failure by 4.2 times. In this study, we aimed to determine the seroprevalence of HIV and HCV, the association of HCV with abnormal liver tests, impact of HIV on HCV-related abnormalities and the distribution of HCV genotypes in Iranian hemophiliacs.**

Patients and Methods: **In a cross-sectional study, we determined virological, clinical and epidemiological characteristics for HIV and HCV infection of 236 hemophiliacs attending our center. Data were analyzed using Chi-square test.**

Results: **Ten (4.7%) out of 211 patients tested were HIV seropositive and 145 (83.3%) were HCV seropositive. All tested positive HIV patients also had HCV. HCV seroprevalence was significantly higher in patients with hemophilia A and B as compared to other congenital coagulopathies and it was directly related to coagulation severity. HCV seroprevalence was lower in hemophiliacs with positive HBsAg ($p = 0.03$) but it did not differ by HBcAb or HBsAb results. HCV genotype 1a (48.5%) was predominant type and genotype 3a (33.3%) was also common. Frequency of abnormal aspartate aminotransferase and alanine aminotransferase liver enzymes was significantly higher in the HCV positive group ($p = 0.006$).**

Conclusion: **This study provides evidence that hepatitis c virus infection is a major problem for Iranian hemophiliacs and it has higher prevalence in hemophiliacs with higher age, more severe coagulopathies, abnormal alanine aminotransferase level, and human immunodeficiency virus co-infection.**

Key words: Hepatitis C, Hemophilia, Human Immunodificiency Virus

Received: 8 February 2008
Accepted: 17 March 2008
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Introduction

The 1940s in the history of hemophilia treatment was the transitional time from primitive, conventional attempts to control bleeding episodes to transfusion therapy as a major accomplishment (1;2). Even though the discovery of cryoprecipitate and factor concentrates and lyophilized concentrates lead to great improvement in both longevity and quality of life for persons with hemophilia, but at the same time a group of tragic setbacks were reported (2). These significant negative consequences were complications resulting from transmission of hepatitis B virus (HBV), hepatitis C virus (hepatitis c virus), and human immunodeficiency virus (human immunodeficiency virus). It was discovered that virtually all hemophilia patients exposed to non-heat–treated factor were hepatitis c virus positive, and over 50% of hemophilia patients in the United States had human immuonodeficiency virus seroconvert, and 5-10% of such patients became chronic carriers of hepatitis B (3).

In general, the natural history of hepatitis c virus reveals a slowly progressive disease leading to cirrhosis in approximately 20% of patients after 20 years. The key risk factors that increase the rate of progression of hepatitis c virus are age at infection>40 yrs, alcohol, male gender, obesity, high grade of liver inflammation, hepatic steatosis, immuno-suppression, smoking, and co-infection with human immunodeficiency virus and HBV (4-7).

Iranian Hemophilia Center was established as a main health care system for Iranian hemophilia population in 1965. This reference center provided a good access of hemophilia patients and their family for early diagnosis and treatment. The programs for control of HBV and hepatitis c virus infection in Iran was started in 1993 by routine HBV vaccination and followed by screening for hepatitis c virus in blood products in 1997 (1;4).

In this study, we aimed to determine the prevalence of viral infections, specifically hepatitis c virus, HBV, and human immunodeficiency virus, their contribution to liver tests, the impact of human immunodeficiency virus on hepatitis c virus infection, and the genotypes of hepatitis c virus in Iranian hemophiliacs.

Materials and Methods

In a cross-sectional and prospective study, we enrolled all patients with hemophilia attending the Iranian Hemophilia Center through the year 2003 to determine their virological, clinical and epidemiological characteristics for chronic viral infections. A complete virological, biochemical and epidemiological assessment was done for all hemophiliacs registered at the center. They were tested for anti-hepatitis c virus-ELISA-3, HbsAg, HbsAb, HbcAb and human immunodeficiency virus serology. Also, biochemical liver-related tests including CBC, platelet, liver enzymes (ALT, AST), PT, and albumin were taken. The patients with positive anti-hepatitis c virus were tested for hepatitis c virus-RNA in order to obtain serological confirmation. Genotype was also determined.

Collected clinical information included age, gender, type of hemophilia (A or B or other), severity of hemophilia (classified as severe if factor VIII or IX blood levels were less than 1%, moderate if they were between 1 and 5% and mild if they were greater than 5%). In this study, ALT status was classified normal if ALT levels were below 40 IU/l, defined as the upper limit of normal (ULN). Data were analyzed with Chi-square test.

Results

In total, 236 patients completed the registration. In this population, 73% had hemophilia A, 10% hemophilia B, and 17% other types of congenital coagulopathies like platelet disorders and factor V deficiency. Most of the patients were in the third decade of life. The mean ± SD of their age was 26.6 ± 12.1 years (Table 1).

On virological examination, more than 80% of patients were anti-hepatitis c virus (ELISA) positive. The frequency of hepatitis c virus-RNA was 73%. Forty four percent of patients with positive HBcAb revealed contact with hepatitis B virus. In this population, only 3% of patients were chronic carrier for hepatitis B virus. About 80% of patients had HBsAb in their blood and showed natural or vaccine immunity to HBV. Only less than 5% of patients were human immuonodeficiency virus seroconverted.

No child under the age of 10 was anti-hepatitis c virus, HBsAg, or anti-human immunodeficiency virus (ELISA) positive, in contrast the frequency of these

Tabel-1:Demography of 236 Patients With Hemophilia

	Hemophilia A	Hemophilia B	Others*	Total
Gender				
Female	2	1	18	21 (8.9%)
Male	169	24	22	215 (91.1%)
Total	171 (72.5%)	25 (10.6%)	40 (16.9%)	236 (100%)
Age				
Range (Yr)	4.0-60	6.0-69	9.0-58	4.0-69
Mean±SD	26.1±11.1	32.5±15.9	25.5±12.5	26.6±12.1
0-9	2	2	1	5
10.0-19	47	2	16	65
20-29	70	9	13	92
30-39	29	6	4	39
40-49	15	2	2	19
50-59	7	1	4	12
60 and over	1	3	0	4
Severity				
Severe	128 (74.9%)	16 (64.0%)	(-)	144 (73.5%)
Moderate	24 (14.0%)	3 (12.0%)	(-)	27 (13.8%)
Mild	19 (11.1%)	6 (24.0%)	(-)	25 (12.8%)

*** Others= Platelate disorders, Factor V deficiency**

infections among older subjects were higher. The age distribution of positive anti-hepatitis c virus and HBcAb differs statistically among the hemophiliacs.

There was no prominent difference in anti-hepatitis c virus (ELISA) positivity between male and female hemophiliacs. The anti-hepatitis c virus (ELISA) positivity significantly increased in hemophilia A and B as compared to other congenital coagulopathies (platelate disorders and factor V deficiency).

We did anti-hepatitis c virus (ELISA) test in 8 patients with positive human immunodeficiency virus serology. All of them were anti-hepatitis c virus positive (100%). This relationship due to low number of patients in this group was not statistically significant. There was a lower frequency of anti-hepatitis c virus (ELISA) positive test in hemophilia patients with HBsAg positivity. The anti-hepatitis c virus test (ELISA) was positive in 2 out of 5 HBsAg positive patients with hemophilia in contrast to 134 out of 160 HBsAg negative hemophilics. ($p = 0.03$). We did not find any significant difference in anti-hepatitis c virus (ELISA) positivity by HBcAb (contact to HBV) or HBsAb (immune to HBV) positive and negative.

Hepatitis c virus RNA test by reverse transcriptase-polymerase chain reaction (RT-PCR) was positive in 80.2% of anti-hepatitis c virus (ELISA) positive hemophilia patients. It means that viremia was found out in 80% of anti-hepatitis c virus (ELISA) positive patients. Non-viremic infection (liver cell or mononuclear cell sequestration), transient absence (or fall) of viremia, recovered infection, viremia below limit of assay, and false positive (or non-specific) ELISA results are possible explanations for the rest of them.

In a study based on the results of PCR positivity, hepatitis c virus genotyping was carried out using genotype specific probes and the dot blot hybridization assay. Genotype 1a (48.5%) was predominant type, and genotype 3a (33.3%) was common. Four patients were found infected with type 1b (12.1%), 1 patient with type 2b (3%), and one patient with type 4b (3%). The double infection with two hepatitis c virus types was not tried in this study.

In registration of laboratory findings in 236 hemophiliacs, frequency of abnormal AST liver enzyme was significantly higher in anti-hepatitis c

virus (ELISA) positive group. (p = 0.015). There was no difference in frequency of abnormal AST between human immunodeficiency virus positive and negative groups. The frequency of abnormal ALT liver enzyme was significantly higher in anti-hepatitis c virus (ELISA) positive group. (p = 0.006). There was also no difference in frequency of abnormal ALT between human immunodeficiency virus positive and negative groups.

Patients with hepatitis c virus and human immunodeficiency virus seropositive status had a higher ratio of prolonged prothrombin time in respect of hepatitis c virus or human immunodeficiency virus negative patients (p = non-significant). Patients with factor V deficiency were excluded in this analysis. But patients with anti-hepatitis c virus (ELISA) and human immunodeficiency virus positive tests had a higher frequency of platelet lower than 150000/ µl. In analysis of serum albumin results among hemophilic patients, there was only a significant difference between human immunodeficiency virus positive and negative groups (p = 0.008). The human immunodeficiency virus seropositive patients had a lower serum albumin level.

Discussion

It should be noted that Iranian Hemophilia Center started to diagnose and treat hemophilic population (1965) before inactivation of blood products for transfusion-transmitted viral infections (8). In this center, hemophiliacs were treated with imported lyophilized concentrates and locally produced cryoprecipitate and fresh frozen plasma without viral inactivation treatment before 1997. This could lead to early and ready exposure of this population to contaminated clotting factor products. The high prevalence of hepatitis c virus infection observed in this hemophilic population (80%) indicates that hepatitis C largely contributes to the morbidity of persons with hemophilia in Iran. This high prevalence is comparable to developed countries (8-10) with a good access of patients to products and the widespread use of clotting factor concentrates in replacement therapy before the era of inactivated products.

Similar to the general hemophilic population, hemophilia A was the predominant type (73%). The risk of infection with each of these viruses is directly related to the severity of the hemophilia. The predominance of severe hemophilia was expected in a population infected by hepatitis c virus with such a high exposure. Individuals with more severe disease bleed more frequently and require more clotting factor, thus increasing their risk of blood-borne infections. This shows that the likelihood of infection increases with bleeding frequency and amount of factor used, indicating that these are the key risk factors of interest.

Another important problem is multiple infections, especially the combination of hepatitis C and human immunodeficiency virus. As expected, given their similar routes of transmission, such co-infection is common among persons with hemophilia (9;11-13). We detected a high proportion of past exposure to HBV (44%), with 3% of positive HBsAg, and important frequency of co-infection with human immunodeficiency virus (5%). This shows higher exposure of this population to transfusion-transmitted viral infections as compared to Iranian general population. We registered lower prevalence of human immunodeficiency virus infection in our hemophilic population as compared to developed countries (13;14). Based on our anecdotal observations in our center, lower survival of human immunodeficiency virus positive patients could affect this lower number in our registry. However, co-infection especially with human immunodeficiency virus is growing in importance because these patients are at a higher risk of progression to chronic liver disease than those infected with hepatitis c virus alone (2;14;15). This is illustrated by our surveillance data, which indicated that among human immunodeficiency virus infected subjects, 100% were hepatitis c virus positive and these patients had a lower serum albumin level and a higher ratio of prolonged prothrombin time.

Children who were born after 1993 have no evidence of hepatitis c virus, HBV, and human immunodeficiency virus infection. Others have observed this decline in risk as well (16-18). Although the occurrence of new infections with HBV, hepatitis c virus, and human immuonodeficiency virus has substantially decreased in recent years, chronic liver disease by these viruses continues to be of significant concern to patients with hemophilia. The impact of human immunodeficiency virus infection has been devastating and despite recent advances in treatment, it is likely to be felt for some time into the future.

The observation that most anti-hepatitis c virus-positive hemophiliacs (57%) did not show abnormal ALT levels at the time of study was interesting, demonstrating the lack of a relationship between the degree of abnormality of serum transaminase levels

and seropositivity for hepatitis c virus. Also, in this regard absence of a history of alcoholism in our patients needs to be considered.

The association between hepatitis c virus viremia and raised ALT levels ($p = 0.006$) was also reported in hemophiliac populations by other authors (2;19). This information can be useful for clinicians who treat positive anti-hepatitis c virus hemophiliacs but do not have any molecular method (i.e. PCR) available to determine the persistence of hepatitis c virus infection. Raised ALT can be a clue of hepatitis c virus viremia, an important piece of information for hemophiliacs, for whom there has been an obvious reluctance to perform liver biopsies due to the risk of bleeding complications (20-22). The protective effect of chronic HBV infection (positive HBsAg) found in this study ($p = 0.03$) has also been described by other authors (23-25). It has been attributed to viral interference between HBV and hepatitis c virus, with the mechanism still not elucidated.

The distribution of hepatitis c virus genotypes in the studied population did not significantly differ from the published data on hemophilic and non-hemophilic populations in Iran, where genotype 1a infection is predominant (26-28). Actually, the distribution of hepatitis c virus genotype is similar with England, European countries and USA, where usually reflects the origin of the blood donors used in the manufacture of imported pooled factor VIII and IX concentrates (29;30). In Iran, the hepatitis c virus genotype distribution would be more a reflex of indigenous and some intermixing of strains with strains from other parts of the world (23).

Conclusion

In summary, this study provides evidence that hepatitis c virus infection is a major problem for Iranian hemophiliacs and it has a higher prevalence in patients with higher age, more severe coagulopathies, abnormal ALT level, and uman immunodeficiency virus co-infection. Transmission of viruses via blood products has been a significant source of morbidity for persons with hemophilia. Fortunately, a number of effective strategies including viral inactivation procedures have been implemented in order to prevent such transmission in the future. The most recent innovation in hemophilia treatment has been the introduction of recombinant factor therapy, which holds great promise for safety as well as efficacy.

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