Prevalence of anti-HAV antibodies in multitransfused patients with beta-thalassemia

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Abstract

AIM: To detect the prevalence of anti-HAV IgG antibodies in adult multitransfused beta-thalassemic patients.

METHODS: We studied 182 adult beta-thalassemic patients and 209 controls matched for age and sex from the same geographic area, at the same time. Anti-HAV IgG antibodies, viral markers of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection were evaluated.

RESULTS: Anti-HAV IgG antibodies were detected more frequently in thalassemic patients (133/182; 73.1%) than in healthy controls (38/209; 18.2%, P < 0.0005). When we retrospectively evaluated the prevalence of anti-HAV IgG antibodies in 176/182 (96.7%) thalassemic patients, whose medical history was available for the previous ten years, it was found that 83 (47.2%) of them were continuously anti-HAV IgG positive, 16 (9.1%) acquired anti-HAV IgG antibody during the previous ten years, 49 (27.8%) presented anti-HAV positivity intermittently and 28 (15.9%) were anti-HAV negative continuously.

CONCLUSION: Multitransfused adult beta-thalassemic patients present higher frequency of anti-HAV IgG antibodies than normal population of the same geographic area. This difference is difficult to explain, but it can be attributed to the higher vulnerability of thalassemics to HAV infection and to passive transfer of anti-HAV antibodies by blood transfusions.

Key words: Hepatitis A virus; Anti-HAV antibodies; Beta-thalassemia; Multiple transfusions; Hepatitis C virus

INTRODUCTION

Hepatitis A virus (HAV) hepatitis is usually spread by the fecal-oral route. Seroprevalence rates in the USA, Western Europe and in several Mediterranean countries have been falling during the past few decades. In some countries no more than 10% of the adult population has evidence of previous infection[1]. In Greece, the lack of epidemics of HAV since early 1980s and the improvement of socioeconomic and hygienic conditions over the last decade seems to have contributed to the decline of the prevalence of anti-HAV antibodies[2,3]. Dalekos et al tested 1984 healthy individuals of Greek nationality for anti-HAV antibodies and found a prevalence of 39.8% in males and 33.2% in females[4]. According to the National Centre for Surveillance and Intervention of Greece, the median annual prevalence of acute hepatitis A in Greece for the time 1998-2003 was 1.88 cases/100 000 of population, which is comparable to the hepatitis A prevalence of the Western Europe[5]. Parenteral transmission is extremely rare, but can follow transfusion of blood from a donor who is in the incubation period of the disease[6,7]. The relatively short duration of viremia in acute hepatitis A, together with the moderate titer of HAV viral load in the blood[8], diminishes the likelihood of transfusing a unit of blood infectious for HAV. The potential cotransfusion of HAV-specific antibodies to the recipient of multiple
blood units and the rising seroprevalence to HAV with age further diminish the risk of post-transfusion hepatitis A[9].

The aim of this study was to detect the prevalence of anti-HAV IgG antibodies in adult multi-transfused beta-thalassaemic patients and to compare this with the prevalence in healthy subjects of the same age and geographic area.

MATERIALS AND METHODS

Patients
We studied 182 adult multi-transfused patients from West Peloponnese (88 males, 94 females, mean age 31.6 ± 9.4 years, range 17-66 years) suffering from beta-thalassemia major (n = 136) or intermedia (n = 46). These patients were in follow up at the Thalassemia Center of the University Hospital of Patras. They were receiving regular transfusions of two units of packed red cells at about 3 wk intervals in order to maintain the haemoglobin level above 10 g/dL. The mean age at first blood transfusion was 2 ± 1.9 years, the mean duration of transfusion therapy was 26.6 ± 8.5 years and the mean number of transfusions received up until the time of the present study was 931 ± 482. Seventy-two patients (39.6%) had undergone splenectomy in the past. None of the patients had a history of intravenous drug use or chronic alcohol abuse. There were no reported cases of HAV hepatitis among the studied patients. In addition, none of the patients had received hepatitis A vaccination in the past. Serum used in the study was obtained just before a scheduled transfusion of packed red blood cells.

The control group was made up of 209 normal subjects from West Peloponnese, matched for age and sex (103 males, 106 females, mean age 31.2 ± 8.5 years, range 17-58 years) from the volunteer blood donor program of our Hospital. Each individual had indicated the absence of significant illness. Physical examination, normal liver function test results and absence of hepatitis B surface antigen (HBsAg) and anti-HCV antibodies in their serum, excluded liver diseases in the control group. None of the healthy subjects had a history of blood transfusion or hepatitis A vaccination in the past.

Viral markers
Anti-HAV antibodies, IgG and IgM, were tested using standard commercially available enzyme immunoassays (HAVAB-M 2.0 and HAVAB 2.0, AxSYM, Abbott Laboratories, Wiesbaden, Germany) in 182 thalassemic patients and in 209 controls from 10 February 2005 to 10 June 2005. We also retrospectively evaluated the prevalence of anti-HAV IgM and anti-HAV IgG antibodies in 176/182 (96.7%) thalassemic patients whose medical history was available for the previous ten years (about 6-8 tests for each patient). Anti-HCV antibodies were tested by third generation ELISA (AxSYM-Abbott, Wiesbaden, Germany). HCV RNA was detected in anti-HCV positive sera by reverse transcriptase polymerase chain reaction (RT-PCR) (Hepatitis C virus test-version 3.0, Cobas Amplicor, Roche Diagnostics, Branchburg, NJ, USA).

Hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc) were determined using standard 3rd generation commercially available enzyme immunoassays [AxSYM HbsAg (V2), AxSYM CORE].

Statistical analysis
Values were expressed as prevalence rates or as the mean ± SD. Conventional chi-square and Fisher’s exact test were used to analyse qualitative differences. The differences between parametric data were evaluated with Student’s t-test. For non-parametric test values a Mann-Whitney test was used. P < 0.05 was considered significant. Statistical analysis was performed with SPSS 8.0 statistical software. The study was approved by the Ethical Committee of Patras University Hospital and informed consent to participate in the study was obtained from all patients and controls.

RESULTS

Prevalence of anti-HAV antibodies in the two groups
Table 1 shows the prevalence of anti-HAV antibodies in beta-thalassemic patients and controls. Anti-HAV IgM antibodies were not found in any patient of the two groups. Anti-HAV IgG antibodies were detected significantly more frequently in thalassemic patients (133/182; 73.1%) than in healthy controls (38/209; 18.2%, P < 0.0005). When we retrospectively evaluated anti-HAV IgM and anti-HAV IgG positivity over the past ten years in 176/182 (96.7%) thalassemic patients, we found that 83 (47.2%) of them presented anti-HAV IgG positivity continuously, 16 (9.1%) were initially anti-HAV IgG negative, but then they became persistently anti-HAV IgG positive, 49 (27.8%) presented anti-HAV IgG positivity intermittently (about two or three tests for anti-HAV negative, while the rest of them were found positive) and 28 (15.9%) were anti-HAV IgG negative continuously. Anti-HAV IgM antibodies were consistently negative in these previous tests. It must be emphasized that the mean numbers of anti-HAV IgG tests were not significantly different between the persistently positive and the intermittent group. When we considered the intermittently anti-HAV IgG positive thalassemics as anti-HAV IgG negative we found that the difference in anti-HAV IgG positivity between thalassemics and healthy subjects was still significant (99/176; 56.3% vs 38/209; 18.2%, P < 0.0005).

Table 1 General characteristics and prevalence of anti-HAV antibodies in beta-thalassemic patients and controls

| Characteristics | Thalassemic patients (n = 182) | Healthy donors (n = 209) | P value |
|-----------------|--------------------------------|-------------------------|---------|
| Age (yr)        | 31.6 ± 9.4                     | 31.2 ± 8.5              | 0.662   |
| Sex (M/F)       | 98/94                          | 103/106                 | 0.854   |
| AST (IU/L)      | 43.9 ± 29.1                    | 23.9 ± 6.9              | < 0.0005 |
| ALT (IU/L)      | 52.6 ± 37.8                    | 23.5 ± 10.6             | < 0.0005 |
| GGT (IU/L)      | 31.8 ± 26.6                    | 22.5 ± 14               | < 0.0005 |
| Anti-HAV IgM (+)| 0                              | 0                       |         |
| Anti-HAV IgG (+)| 133 (73.1%)                   | 38 (18.2%)              | < 0.0005 |

1Normal levels: Aspartate aminotransferase (AST) < 40 IU/L, alanine aminotransferase (ALT) < 40 IU/L, gamma-glutamyltranspeptidase (GGT) < 50 IU/L; 2Anti-HAV: Hepatitis A virus antibody.
Liver disease in thalassemic patients

Seventy-six thalassemic patients (41.2%) were anti-hepatitis C virus (anti-HCV) positive, while 44 (24.2%) of them were also HCV RNA positive. None of them was HBsAg positive. Anti-HBc antibody was found in 56/182 (30.8%) of them.

Comparison of anti-HAV positive and anti-HAV negative thalassemic patients

When anti-HAV positive (n = 133) were compared to anti-HAV negative (n = 49) thalassemic patients (Table 2) there was no difference in age, sex or duration of transfusion therapy. Anti-HAV positive patients had received more transfusions than anti-HAV negative thalassemics (P = 0.02). We also found that anti-HAV (+) thalassemics had lower frequency of previous splenectomy, although this difference did not achieve statistical significance (35.3% vs 51%, P = 0.055). An unexpected finding was that anti-HAV (+) thalassemics presented lower mean serum levels of aminotransferases (AST, P < 0.001; ALT, P = 0.039) and albumin (P = 0.025) than anti-HAV (-) patients. No statistical difference was found in ferritin values between these two groups. Patients with antibodies to HAV were also more frequently positive for HCV markers although this difference was not statistically significant.

DISCUSSION

Liver disease is a leading cause of death in patients with transfusion-dependent thalassemia. Transfusion-associated hepatotropic infections, especially HCV infection, and hepatic siderosis can act either synergistically or independently in promoting chronic liver disease, and they may induce cellular damage through similar oxidative pathways. There are no recent data on HAV epidemiology of that group of patients, and blood transfusion is not considered a significant predisposing factor for HAV infection.

In this study, adult beta-thalassemic patients were found to have significantly higher frequency of anti-HAV IgG antibodies than healthy subjects matched for age and sex in the same geographic area. IgG anti-HAV alone indicates past infection; it persists for decades after acute HAV infection and reflects recovery and immunity to reinfection[1].

It was found that 47.2% of thalassemic patients were continuously anti-HAV (+) over the past ten years. The possibility that multitransfused patients with beta-thalassemia acquire anti-HAV antibodies with higher frequency than normal people of the same geographic area because of socioeconomic reasons is difficult to be supported. There are also no data demonstrating a higher susceptibility of patients with thalassemia for hepatitis A. Unfortunately, we did not study the prevalence of anti-HAV antibodies in family members of these thalassemia patients. Immune deficiency attributed to multiple transfusions and to iron overload could be a factor that could predispose thalassemics to HAV infection, but no data exist that immune deficiency per se predispose to HAV infection. However, 41.2% of thalassemics were anti-HCV positive and 24.2% of them were HCV-RNA positive. In addition, thalassemics with anti-HAV positivity presented higher percentage of anti-HCV and HCV-RNA positivity, although these differences were not statistical significant. Two studies from Italy have shown that patients with chronic liver disease present high seropositivity for anti-HAV[6,7].

Another interesting finding was that 9.1% of thalassemic patients acquired anti-HAV IgG antibody during the previous ten years. However, anti-HAV IgM antibodies were not found in these patients during this time, so it is difficult to prove acute infections. IgM anti-HAV in serum is positive from the onset of symptoms and usually remains positive for approximately 4 mo[8]. These tests were performed every 1-2 years and it is possible not to detect IgM anti-HAV antibodies in these patients. This is a high rate of HAV seroconversion even for countries of high endemicity. Greece is considered, according to studies of previous decade as a country of low endemicity for HAV infection, with some regions of intermediate endemicity. Transfusion-associated hepatitis A virus is possible, but it is a very rare event (one per million transfusions)[9-22], so there is no evidence to consider polytransfused patients as a risk group for parenteral hepatitis A infection. Due to the frequent stay of thalassemics in ambulant and hospital premises of the health care system, hospital borne hepatitis A infection should also be taken into consideration. However, the transmission from the medical care stuff to the patients is even more rare[23]. In general, hospital-borne hepatitis A disease does not seem a possible explanation of HAV infection in thalassemics.

Finally, 27.8% thalassemics presented anti-HAV positivity intermittently. In this case anti-HAV IgG is apparently not the expression of an actively acquired immunity, but might have been transferred passively.

Table 2 Clinical characteristics of anti-HAV positive and anti-HAV negative beta-thalassemic patients

|                        | Anti-HAV (+)     | Anti-HAV (-)    | P value  |
|------------------------|-----------------|----------------|---------|
| (n = 133)              | (n = 49)        |                |         |
| Age (yr)               | 31.5 ± 9.6      | 31.8 ± 8.7     | 0.844   |
| Sex (M/F)              | 62/71           | 26/23          | 0.440   |
| Duration of transfusion therapy (y) | 26.5 ± 8.1      | 27.1 ± 9.6     | 0.683   |
| Number of transfusions | 989 ± 441       | 772 ± 556      | 0.020   |
| Spleenectomy           | 47 (35.3%)      | 25 (51%)       | 0.055   |
| AST (IU/L)†            | 38.6 ± 24.2     | 58.3 ± 36.1    | 0.001   |
| ALT (IU/L)             | 49.2 ± 36.2     | 62.5 ± 40.8    | 0.039   |
| GGT (IU/L)             | 29.6 ± 26.0     | 39.1 ± 27.3    | 0.057   |
| Albumin (g/dL)         | 4.7 ± 0.45      | 4.47 ± 0.57    | 0.025   |
| Globulin (g/dL)        | 3.03 ± 0.85     | 3.26 ± 0.79    | 0.122   |
| Ferritin (median, ng/mL) | 1515            | 1815           | 0.590   |
| Anti-HCV (+)           | 61 (45.9%)      | 15 (30.6%)     | 0.064   |
| HCV RNA (+)            | 36 (27.1%)      | 8 (16.3%)      | 0.133   |

†Normal levels: Aspartate aminotransferase (AST) < 40 IU/L, alanine aminotransferase (ALT) < 40 IU/L, gamma-glutamyltranspeptidase (GGT) < 50 IU/L. Anti-HAV: Hepatitis A virus antibody; anti-HCV: Hepatitis C virus antibody.
by means of transfusions. It is well known that packed red cells contain leukocytes, microaggregates, plasma containing proteins and immunoglobulin. The level of protection that might be conferred by these antibodies is unknown. This finding is similar to that in substituted haemophiliacs where the detection of anti-HAV antibodies is also a frequent finding. In haemophiliacs, the high concentration of antibodies in plasma preparations of multiple donors and the frequency of their administration facilitate the passive transmission of antibodies. It can be supported that some of the continuously anti-HAV (+) sera in the other thalassemic patients could be also attributed to the passive transfer of anti-HAV antibodies. We think that it is not very possible, as many tests for anti-HAV antibodies just before a transfusion and for the previous ten years were found persistently positive.

Previous studies have shown lower prevalence of anti-HAV antibodies in multiply transfused thalassemic patients. However, in these studies the mean age of thalassemic patients was much lower than that of our population and it is known that seroprevalence rates of HAV are increasing with age.

The lower mean levels of AST in anti-HAV IgG positive thalassemics, is difficult to explain. Ferritin values were found comparable between the two groups. Body mass index (BMI) and alcohol consumption were not different between these two groups. Moreover, no statistical difference was found in anti-HCV or HCV RNA positivity between anti-HAV positive and anti-HAV negative thalassemics that could explain this difference between the two groups.

There are some important limitations in this study. Volunteer blood donors are selected subjects, not comparable with general population. In cases where passive transfer of anti-HAV antibodies was suspected, the frozen aliquots of the donor’s plasma were not tested for the presence of anti-HAV antibodies. Additional studies may be needed to confirm our findings.

In accordance to the findings of this study, we suggest that thalassemic patients present higher prevalence of anti-HAV IgG antibodies than matched healthy subjects of the same geographic area. This difference is difficult to explain, but it can be attributed to the higher vulnerability of thalassemics to HAV infection and to passive transfer of anti-HAV antibodies by blood transfusions.

**Comments**

**Background**

In Greece, the lack of epidemics of hepatitis A virus (HAV) since early 1980s and the improvement of socioeconomic and hygienic conditions over the last decade seems to have contributed to the decline of prevalence of anti-HAV antibodies. However, we observed that the majority of our beta-thalassemia patients were anti-HAV positive.

**Innovations and breakthroughs**

Previous studies have shown lower prevalence of anti-HAV antibodies in multiply transfused thalassemic patients. However, in these studies the mean age of thalassemic patients was much lower than that of our population and it is known that seroprevalence rates of HAV are increasing with age.

**Applications**

In order to reduce further the incidence of liver infections in polytransfused thalassemic patients, we recommend an active immunization for HAV.

**Terminology**

Beta-thalassemia, also known as Cooley’s anemia, is a chronic recessively inherited hemoglobinopathy, characterized by severe hemolysis.

**Peer review**

The authors reported that transfused adult beta-thalassemic patients present higher frequency of anti-HAV IgG antibodies than normal population of the same geographic area. This result is interesting. The strength of this study is surely the dimension of the population and the long history of the patients (10 years).

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