The Incidence of Hepatitis G Virus in Patients with Hematological Malignancies: The Relationship to the Number of Blood and Blood Products Transfusions

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ABSTRACT

The hepatitis G virus has been detected in patients with post-transfusion hepatitis. The precise transmission rate of the hepatitis G virus is not clear. This study aims to investigate the transmission rate of HGV and the relationship between the number of blood transfusions and the blood products used in multitransfused patients with hematological malignancies. Serum samples were obtained from 80 patients with hematologic malignancies hospitalized between January 1997 and December 1998 at Ibn'i Sina Hospital, University of Ankara. The patients were divided into three groups according to transfusion numbers. Group A received between 0 and 10 units of blood and blood products, Group B received 10-20 units, and Group C received more than 20 units. All patients received blood and blood products for a median of 6.8 Units/whole life. The hepatitis G virus was detected using the reverse transcription polymerase chain reaction. Of the eighty patients, four (5.0 %) were HBs-Ag positive, one (1.25%) was Anti-HCV positive, and one (1.25%) was HGV-RNA positive. Multiple blood transfusions may be an important risk factor for transmission-transmitted viral infections, but based upon the present experience, there is no significant relationship between the number of blood transfusions and blood products and the transmission rate of HGV infection in patients with hematological malignancies.

Key Words: Hepatitis G virus, Transfusion, Hematological malignancies, Blood-borne infection.

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INTRODUCTION

Hepatitis G virus (HGV) is a new human RNA virus that is mainly transmitted via blood and blood products[1,2]. The role of HGV in transfusion-associated infection and its relation to liver disease is not clear[2].

Previous studies reported that HGV prevalence in healthy blood donors ranged from 1.2 to 4.2 percent[3-5]. Although the precise transmission rate is unknown, an elevated prevalence of HGV-RNA has been described in subjects at risk for parenteral infections, such as blood and blood products recipients, intravenous drug abusers, and patients on hemodialysis[6-9].

Patients with hematological malignancies, such as acute or chronic leukemia require blood and blood products for sustaining life. Therefore, these patients are considered at a high risk for contracting transfusion-transmissible infections. For this reason, this study aims to investigate the prevalence of HGV infection in multitransfused patients with hematological malignancies in order to determine if there in a relationship between the incidence of infection in these patients and the number of times they have received blood and/or blood products.

PATIENTS and METHODS

Serum samples were obtained from 80 patients with hematological malignancies hospitalized between January 1997 and December 1998 at Ibn'i Sina Hospital, Department of Hematology and Oncology, University of Ankara. The underlying diseases included 34 cases of acute myeloblastic leukemia (AML), 21 cases of acute lymphoblastic leukemia (ALL), 5 cases of chronic myelocytic leukemia (CML), 6 cases of chronic lymphocytic leukemia (CLL) and 14 cases of lymphoma. The male to female ratio was 51/29. Their median age was 36.1 years (range 14-62 years).

All patients received blood and blood products for a median of 6.8 Units/whole life (range, 0-32 Units). Patients were divided into three groups according to transfusion frequency. Group A received between 0 and 10 units, Group B, 10-20 units, and Group C more than 20 units of blood and blood products. The characteristics of all patients are summarized in Table 1.

Control group blood donors were Turkish healthy volunteer donors.

Serological Tests

Hepatitis B surface antigen (HBs-Ag), hepatitis B surface antibody (Anti-HBs), hepatitis B e antigen (HBe-Ag), hepatitis B e antibody (Anti-HBe), hepatitis B core antibody (Anti-HBc-IgM, IgG), hepatitis delta antibody (Anti-D), hepatitis A virus antibody (Anti-HAV IgM, IgG), Cytomegalovirus (Anti-CMV IgM, IgG) antibody, Epstein Barr Virus antibody (Anti-EBV IgM, IgG) and the Human Immunodeficiency Virus antibody (Anti-HIV) were all assayed with a second ge-

Table 1. The demographic and clinical characteristics of all patients in study group

Characteristic		
Number of patients	80	
Age - Median (years)	36.1	
Range (years)	14-62	
Sex (Male/Female)	51/29	
Primary Diseases	**Patient Number**	**Male/Female**
AML	34	20/14
ALL	21	16/5
CML	5	3/2
CLL	6	3/3
Lymphoma	14	10/4

AML; Acute myeloblastic leukemia, ALL; Acute lymphoblastic leukemia,
CML; Chronic myelocytic leukemia, CLL; Chronic lymphocytic leukemia

neration enzyme-linked immunosorbent assay (ELISA; Abbott Laboraties, North Chicago, IL, USA). The hepatitis C virus antibody (Anti-HCV) was assayed using a second-generation ELISA assay (Abbott Laboraties, North Chicago, IL, USA). The serum HBV-DNA was tested by using the polymerase chain reaction (PCR) on serum samples of patients with HBs-Ag. The serum HCV-RNA was tested by using the PCR on serum samples from all of the patients with anti-HCV positive or abnormal liver function tests.

Liver Function Tests

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (AP) and bilirubin levels were measured on a 24 factor automated chemical analyzer using standard reagents in the Clinical Biochemical Department of Ibn'i Sina Hospital, Ankara-Turkey. The normal ranges are 0-40 IU/L for AST and ALT, 0-57 IU/L for GGT, 41-117 IU/L for AP, 90-230 IU/L for LDH, and 0-1.4 mg/dL for bilirubin. The tests were carried out at the same time as the viral serology. These tests were repeated when clinically indicated.

Hepatitis G Virus Reverse Transcription Polymerase Chain Reaction

RNA Extraction: The serum HGV RNA was extracted by using the acid-guanidinium-phenol-chloroform method. In this method, 150 µL plasma was mixed with 400 µL of the guanidinium buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate), 0.5% sarcosyl, 1% 2-mercaptoethanol), then 5 µL of transfer RNA was added. This solution was extracted with phenol/chloroform. After precipitation with isopropanol, the dried pellet was re-suspended in 10 µL of RNA'se block (930 µL RNA'se free water, 20 mM 20 µL dithiothreitol T). The obtained RNA was stored at -80°C until use[10].

RT-PCR: The RNA pellet was reverse transcribed to complementary cDNA using a random hexanucleotide mix and 10 units of AMV RT in a final volume of 20 µL. This mixture was incubated in a thermal cycler at 42°C for 1 hour.

HGV RT-PCR: Reverse transcription was performed in a buffer consisting of 20 U M-MuLV-RT, 100 µM mixture of PCR nucleotide, 50 nm random hexamer and 1xRT buffer at 42°C for 30 minutes incubation. A program including fortyfive cycles: 94°C for 45 seconds, 55°C for 60 seconds and 72°C for 60 seconds following an initial heating at 94°C for 5 minutes and 7 minutes extension at 72°C at the end of reaction was used for PCR. Two pairs of primers, derived from the 5'NCR and NS5a regions of HGV-RNA, and two biotin labelled probes complementer the amplified region were used. The optic density of the color reaction was measured with the digoxigenin detection system' for the evaluation of PCR products (Boehringer Mannheim, Germany).

Statistical analysis: All eligible patients were included in the analysis. Data was analyzed by Kruskal-Wallis 1-Way Anova, Wilcoxon Matched-Pairs Signed-Ranks and Chi-Square tests.

RESULTS

There were no significant differences in age, gender, and the time of illness among the three different groups ($p > 0.05$). Of the eighty patients with hematological malignancies, four (5.0%) had HBs-Ag positivity, one (1.25%) had Anti-HCV positivity and one (1.25%) had HGV-RNA positivity. HBV-DNA by PCR was negative in all patients with HBsAg. The anti-Delta antibody was not detected in HBs-Ag positive patients the HCV-RNA was negative by PCR in the patient who was anti-HCV positive. The patients was HBs-Ag and Anti-HCV positivity had normal liver injury tests. All 80 patients with hematological malignancies were seronegative for anti-HAV IgM, CMV-IgM antibody, EBV-IgM antibody, and anti-HIV. The results are shown in Table 2.

One patient with HGV-RNA positivity had normal liver injury tests, HBs-Ag, Anti-HCV and other virologic tests which were negative during the HGV-RNA positivity. Because patient's liver injury and cholestatic enzyme levels were normal, a liver biopsy was not performed. No abnormal liver injury tests were observed during his follow-up. The patient is currently alive and HGV-RNA positive.

DISCUSSION

HGV or the GB virus C (GBV-C) have recently been isolated in patients with acute, chronic or post-transfusion hepatitis[10-14]. It is now absolutely clear that HGV and its variant, GBV-C, are prevalent agents with

a high carrier rate in the volunteer donor population[8,11,13]. Although there has been a rapid increase in the epidemiologic and molecular knowledge of about this virus, its clinical relevance remains largely unresolved. The most important unresolved issue is whether HGV plays a role in the origin of virus-induced hepatitis.

Patients with hematological malignancies are exposed to multiple units of blood and blood products which certainly represent a major route for transfusion-transmitted infections such as hepatitis (HCV, HBV, HGV) and human immunodeficiency viruses[3,6,8,15]. In the present study, four patients (5%) with hematological malignancies were seropositive for HBs-Ag and one patient (1.25%) was seropositive for Anti-HCV. Three patients with HBs-Ag positivity had received more than 20 units of blood and blood products, and the remaining patient with HBs-Ag positive had received 14 units of blood and blood products. One patient with HCV positivity had received more than 20 units of blood and blood products. Although HBV and HCV are the causative agents of transfusion-transmitted infections, there are no significant differences in the incidences of these viruses between multiply transfused-patients with hematological malignancies and the volunteer group ($p > 0.05$).

Recently, some reports have suggested that a high prevalence of HGV infection is seen among intravenous drug users, thalassemic patients, hemophiliacs, and patients on maintenance haemodialysis[6-9,16-18]. In this study, HGV-RNA by RT-PCR was detected in one patient (1.25%) who had received more than 20 units of blood and blood products. HGV did not influence the liver injury tests and the cholestatic enzyme patterns in this patient.

In conclusion, although multiple blood transfusions are the most important risk factor for transfusion-related viral infections, this study showed that there is no significant relationship between the number of blood transfusions and blood products and the transmission rate of HGV infection in patients with hematologic malignancies. These findings may be the result of strict blood screening for HCV and HBV, and we suggest that strict blood screening for HCV and HBV may also be enough to prevent HGV transmission.

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Table 2. The viral serology of all patients with hematological malignancies

Groups n= patients number	HBs-Ag	Anti-HCV	HGV-RNA	Co-infection
Group A	Not	Not	Not	Not
(0-10) n= 34	Detected	Detected	Detected	Detected
Group B	1	Not	Not	Not
(10-20) n= 18	(Lymphoma)	Detected	Detected	Detected
Group C	3 (11%)	1 (3.6%)	1 (3.6%)	Not
(> 20) n= 28	(2 AML,1 CLL)	(AML)	(ALL)	Detected
Volunteers*	7.16%	1.15%	3.33%	-
	5017/70094	235/20430	2/60	

AML; Acute myeloblastic leukemia, ALL; Acute lymphoblastic leukemia, CLL; Chronic lymphocytic leukemia
* These data have been taken from the studies numbered 19-21

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