

## A Preliminary Report on Prevalence of Antibody Response to GBV-C, E2 Protein in Iranian Blood Donors and Multitransfused Patients

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### Abstract

**Background:** To analyze serologic markers of Hepatitis G virus (GBV-c) infection in Iranian blood donors and two major groups of multitransfused patients, hemophiliacs and thalassemics.

**Methods:** Nine hundred and five serum samples collected from the volunteer blood donors and two patient groups under the study were tested for the presence of antibodies to the GBV-C antigen (anti E2) by an ELISA assay. Those found positive for anti-E2 were also tested for possible exposure to HCV by detecting anti-HCV in their sera. Levels of ALT were also tested to evaluate impact on liver function.

**Results:** Approximately 8.6% of the volunteer blood donors were found positive for anti-E2. The prevalence rate in hemophiliacs was 41.4% and in thalassemia patients was 33.4%, which was significantly ( $P < 0.05$ ) higher than donors. However, the prevalence rate among the two high risk groups was not statistically significant.

**Conclusion:** A large number of the healthy blood donors in Iran have been exposed to the GBV-C. The significantly higher levels seen in the multitransfused patients can be regarded as an important route of transmission. It seems that no evidence of liver damage in individuals exposed confirming that GBV-C is not a hepatitis virus.

**Key words:** Hepatitis G virus, Blood donors, Thalassemia, Hemophilia, Iran

### Introduction

Despite systematically testing of blood donors for the presence of serologic markers of viral infection by HIV, hepatitis B and C (HBV, HCV) in most countries, there is still a low residual risk of post transfusion hepatitis (1). In about 10 to 15 percent of these cases none of the known hepatotropic viruses can be implicated (2).

A novel RNA virus termed GB virus type C (GBV-C) belonging to Flaviviridae was described in 1995 as a possible agent (3-5), however, two years later, despite a large number of investigations, the clinical significance of the GBV-C infection remained largely unresolved. Cases of acute or chronic hepatitis (3-5), fulminate hepatitis (6, 7) and aplastic anemia (8, 9), have been

linked to GBV-C/HGV but the role of the virus in those pathologies has been strongly questioned (10).

However it is difficult to establish whether this recently discovered virus is definitely an "orphan" virus. Indeed, GBV-C/HGV would not be the first virus for which its pathogenicity took a while to be defined, the B19 parvovirus was discovered in 1975, but its pathogenicity was described six years later (11, 12). Therefore, GBV-C/HGV should be taken under investigation, even if infection by this virus appears completely asymptomatic in the affected individuals. Earlier studies had reported that the majority of affected individuals develop an antibody to the E2 envelope protein of the virus (anti-E2), which in fact

is a neutralizing antibody, detectable after the loss of GBV/C-RNA (13, 14).

The present study, therefore, was designed to use anti-E2 as a marker of true level of past exposure in order to obtain an insight on the epidemiology of this infection in Iranian volunteer blood donors. Since recipients of blood products such as hemophilia and thalassemia patients have been shown to be at risk for GBV-C infection (15-17), a sample of these populations was also studied.

## Materials and Methods

Serum samples from 905 individuals were studied, in three groups as follows:

Group 1: Blood donors; 514 samples of volunteer blood donors who had given informed consent for the study and had gone through the routine physical examination and filled the appropriate questionnaire. All the individuals were Iranian living in the capital city of Tehran.

Group 2: Thalassemia patients; 251 samples from thalassemia major patients under treatment at a adult day-clinic in Tehran, who had received multiple transfusions of packed red cells, from Iranian blood donors with mean age of  $20.1 \pm 8.5$  yr.

Group 3: Hemophilia patients; 140 samples from hemophilia patients visiting a day-care clinic at the Imam Khomeini Hospital, Tehran, consisting of 120 hemophilia A and 20 type B with mean age of  $24.7 \pm 12.25$  yr. Unfortunately it was not possible to categorize the patients into groups who had used a particular plasma substitute, since they had all used single donor products as well as local and imported concentrates at some stage. Detection of serum anti-E2 was performed using an ELISA kit anti-HG env. From Roche, Diagnostics (GmbH) according to the manufacturer's instructions. Each positive sample was confirmed by the test procedure recommended by the manufacturer.

Anti-HCV was detected using Monolisa antiHCV kits from Sanofi-Pasteur (France) according to the manufacturer's instruction. All the positive samples were confirmed using RIBA as-

say, Deciscan HCV plus from Sanofi-Pasteur (France).

Estimation of ALT levels was carried out by an automated method using auto analyser Cobas Mira (Roche, Switzerland), and results expressed in IU/l. The upper limit of normal ALT level of laboratory was 50 IU/l.

Statistical analysis was conducted using chi-square test or Fisher's exact test. Differences were considered significant at  $P < 0.05$ .

## Results

Among the 514 samples taken from healthy, volunteer blood donors, 44(8.6%) were positive for anti-E2. However, anti-E2 was positive in 84(33.4%) of the total thalassemia patients studied. The number of individuals found positive for anti-E2 in the hemophilia patients was 58 (41.4%). The results obtained are summarized in Table I. In order to obtain an insight to possibility of co-infection with HCV, the presence of anti-HCV was also tested in the individuals found to be positive for anti-E2. The number of individuals exposed to both viruses was 54 (93.1%) amongst hemophilia patients and 21(25%) in thalassemics. The levels of ALT in groups positive for anti-E2 and those found negative are compared in Table 2.

**Table 1:** Prevalence of anti-E2 in blood donors, thalassemia and hemophiliacs

Group	No.	Anti – E2 positive	
age group (yr)			
Healthy Blood donors	514	44 (8.6 %)	42+10.6
Thalassemics	251	84 (33.4 %)	20.1+ 8.5
Hemophiliacs	140	58(41.4%)	24.7+ 12.25
Total	905	186	

**Table 2:** ALT levels estimated in anti-E2 positive and negative individuals

ALT levels	Anti-E2 negative	Anti-E2 positive		Total
		Anti-HCV(+) n(%)	Anti-HCV(-) _n(%)	
Elevated > 50	234 (34.2)	33 (44)	38(35)	71(38)
Normal > 50	466(65.7)	42(56)	71(65.7)	113(61.7)
Total	709	75	108	183

## Discussion

The data presented in this study clearly indicates that 8.6% of the blood donor population in Iran has had previous exposure to GBV-C and that the prevalence in this carefully selected group is higher than what had been reported for anti-HCV in the same group (0.3%) (18).

Since at the time of this study, accurate GBV-C-RNA detection did not prove possible in Iran, it might be assumed that the prevalence of exposure, i.e. combination of GBV-C-RNA and anti-E2 assay, would be slightly higher (estimated at 2-4%).

The observed prevalence of anti-E2 reported (8.6%) compares well with those reported for European countries such as France 9.5%(17), Southern France 12%(19), Norway 7.3%(20), Germany 10,9% (21) and Canada 7.3%(22). However, the reported prevalence for some African and south American countries has been much higher (20.3 and 19.5%, respectively) (21).

However, the relatively high percentage of individuals exposed to GBV-C in the low- risk group namely, healthy volunteer blood donors indicates that parenteral transmission is probably not the exclusive route and alternative non- parenteral routes of transmission such as sexual transmission community acquisition must also be looked for. In fact, a recent report from Sweden finds sexual contact and medical procedures to be the main routes of transmission (23), another study reports that intrafamilial, vertical and horizontal transmission are efficient routes for this infection, in the

West (24). This is an interesting finding which calls on further investigation among a larger population of anti-E2 positive blood donors in Iran.

Transfusion is a well recognized route of transmission for GBV-C and this is well demonstrated in the significant ( $P < 0.05$ ) higher anti-E2 in thalassemia and hemophilia patients (33.4 and 41.4 respectively) compared to 8.6% in blood donors.

The 33.4% prevalence of anti-E2 amongst Iranian thalassemia patients compares well with 32% reported for Italian patients (25) and 27.3% reported from Taiwan (26). In addition the anti-E2 prevalence in hemophiliacs (41.4%) compares well with 32% reported for hemophiliacs in Italy (27), although slightly higher than report from Taiwan for this group (26).

The two multitransfused groups studied, thalassemia and hemophilia patients, are two major groups in Iran who are dependent on different blood products. Thalassemics are entirely dependent on local blood and red cell concentrates produced by the Iranian Blood Transfusion Organization whereas the hemophiliacs are dependent on both single donor, products from Iranian blood donors as well as imported coagulation concentrates.

However, the prevalence of anti-E2 amongst thalassemia and hemophilia patients is not significantly different, whereas the prevalence of anti-HCV in these two groups in Iran was found to be significantly different (15-27% and. 70% respectively) (28) and the rate of co-infection in the present study was also found to be significantly

different, 25% vs.93.1% ( $P < 0.05$ ). The high level of co-infection of GBV\_C with HCV often seen in patients with hemophilia seems not to affect the course of HCV infection or the response to interferon therapy (29). Indeed, in more recent reports it is also shown that co-infection with neither HBV nor HCV has no effect on disease severity but may accelerate progression of chronic liver disease (30). However, co-infection of HCV and GBV-C in HIV positive patients had no influence on the viral and cellular dynamics of these patients (31).

This significant difference between GBV-C and HCV in the multitransfused patients can be an appropriate indication for the low efficiency of GBV-C transmission via plasma derivatives, which has also been observed by others (17, 32) a low transmission rate was also observed in recipients of IVIg (33). Indeed, because of the frequency of GBV-C in blood donors, the starting plasma pools is bound to be infected, however, the putative presence of anti-E2 or other not yet recognized antibodies could have a protective effect, by neutralizing the infectious virus or by influencing the partitioning of infection particles during fractionation of the plasma pool.

The data presented also show that the pathogenicity of GBV-C is negligible, as far as liver damage is concerned, since there was no significant biochemical evidence of liver damage in individuals who were exposed to the virus and those never exposed. This finding is in accordance with previous report in high and low risk populations (10).

Finally, further study is needed to clarify the epidemiology and natural history of this infection in Iran.

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The authors declare that they have no conflict of Interests.

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