

# Antibodies to hepatitis C virus in patients who have had multiple transfusions in Sri Lanka

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(Index words: Haemophiliacs, thalassaemics, anti-HCV enzyme immunoassay, HCV Western blot test)

## Abstract

**Objective** To determine the prevalence of antibodies to hepatitis C virus (HCV) in patients who have had multiple transfusions in Sri Lanka.

**Setting** University Medical Unit at the National Hospital, Colombo, the Cancer Institute, Maharagama, and the Lady Ridgeway Children's Hospital, Colombo.

**Patients** One to 5 ml of blood for serology was collected from 200 multiply transfused patients (those who have received five or more blood transfusions).

**Method** The sera were tested for HCV specific antibodies using a third generation anti-HCV enzyme immuno-assay (EIA) kit. All sera giving positive or intermediate EIA results were re-tested by a commercial HCV Western blot confirmatory test.

**Results** Of the 200 patients, 10 (5%) were repeatedly positive and confirmed by the Western blot. 33% (7/21) of haemophiliacs and 10% (3/31) of thalassaemics were positive for antibodies to HCV. Antibodies were not detected in other groups of multiply transfused patients (haemolytic disease, aplastic anemias, chronic renal failure, haematological and other malignancies)

Of the 200 patients, those who have had more than 80 blood transfusions had a significantly higher prevalence of antibodies to HCV. The frequency of HCV infection was also higher among those who had received factor concentrates.

**Conclusion** 33% of haemophiliacs and 10% of thalassaemics who have received multiple transfusions were infected with HCV. These findings warrant a larger study among blood donors, and justify screening and decontamination of blood and blood products given to haemophiliacs and thalassaemics in Sri Lanka.

## Introduction

Hepatitis C is the cause of most cases of post-transfusion hepatitis in developed countries (1). Screening blood donors for antibodies to hepatitis C virus (anti-HCV) is more accurate than screening for "surrogate markers" (antibody to hepatitis B core antigen and alanine transferase in serum), and more effective in protecting against post-transfusion non-A non-B hepatitis.

In Sri Lanka, antibodies to hepatitis C have been documented in 2.1 to 3% of patients with alcoholic cirrhosis (2,3).

Patients with hemophilia, thalassaemia and other coagulopathies treated with multiple blood transfusions and unheated clotting factor concentrates, including factors I, VIII and IX have a high risk approaching 100% of acquiring non-A non-B (NANB) hepatitis. The main cause of the NANB hepatitis transmitted by blood products is hepatitis C in the USA and other industrialised countries. Antibodies to HCV were positive in 60-90% of haemophiliacs receiving commercial clotting factor concentrates (4,5).

We do not know the prevalence of HCV infection in Sri Lanka. A study to determine the prevalence of HCV infection in Sri Lanka would be costly. We decided to test for antibodies to HCV in high risk groups (as recognised by published literature) as a preliminary investigation, to determine whether HCV infection in Sri Lanka is a problem that merits further study.

We sought to determine the proportion infected with hepatitis C among patients who had received multiple transfusions of blood products in Sri Lanka, and to identify the transfusion related high risk factors for hepatitis C in this group of patients.

## Methods

Over a period from July 1997 to Dec 1999, the proportion of patients with antibodies to HCV were determined in multiply transfused patients, defined as those who have received 5 or more transfusions of blood or blood products. Patients with other possible high risk factors such as haemodialysis, intravenous drug use, having multiple sex partners were excluded from the study.

One to 5 ml of clotted blood was collected from each of 200 multiply transfused patients attending the University Medical Unit at the National Hospital, Colombo, the Cancer Institute, Maharagama, and Lady Ridgeway Hospital, Colombo. Each patient's name, age, hospital, the reason for multiple transfusions, number of transfusions, types of transfusion, date of first transfusion and history of hepatitis were recorded. The study group consisted of patients with thalassaemia, haemophilia, haematological malignancies, solid tumours, chronic renal failure, haemolytic anaemia and patients with bone marrow dysfunction (including aplastic anaemia, myelofibrosis and myelodysplastic syndrome). The laboratory was kept 'blind' to patient details until the results were analysed.

The laboratory tests were carried out in the Department of Microbiology at the Faculty of Medical Sciences

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of the University of Sri Jayawardenepura. The sera were separated and stored at 4°C until they were tested for HCV specific antibodies using a commercial third generation anti-HCV enzyme immuno-assay (EIA) kit (Murex™). All sera giving positive or intermediate EIA results were re-tested by a commercial HCV Western blot confirmatory test (Murex™). Both EIA and the Western blot were performed according to the manufacturer's instructions. The same make of the commercial kit was used throughout the study. Statistical analysis was performed using SPSS version 6 and significance was assessed by the Chi-square test.

## Results

The 200 patients comprised 31 thalassaemics (15%), 21 haemophiliacs (11%), 38 with haematological malignancies (18%), 27 with solid tumours (14%), 30 with chronic renal failure (15%), 32 with haemolytic anaemia (16%) and 21 (11%) with aplastic anaemia, myelofibrosis or myelodysplastic syndrome. Anti-HCV was detected and confirmed by Western blot in 10 out of 200 patients (5%). The anti-HCV positivity was detected only among the thalassaemics (3/31; 10%) and haemophiliacs (7/21; 33%). Anti-HCV was detected in a significantly greater number of haemophiliacs than in thalassaemics ( $p < 0.05$ ).

### Relationship of HCV status with number of blood transfusions

The 10 anti-HCV positive patients had received a sig-

nificantly greater number of transfusions of blood or blood products than the negative patients. Median 323 vs. 65,  $P < 0.001$  (Chi square test). The median number of transfusions received was significantly higher ( $p < 0.02$  by Chi square test) among the haemophiliacs (100 transfusions) and the thalassaemics (108 transfusions) compared to the patients with haemolytic anaemia (66 transfusions) and other groups of patients (7 to 15 transfusions).

Of 151 patients who have had more than 80 transfusions, 10 were positive for anti-HCV antibodies, whereas none of the 49 who had less than 80 blood transfusions were positive.

### Relationship of the HCV status with the type of transfusion

Table 1 shows the frequency of transfusion of blood and blood products in each patient category. The transfusions received by the 21 haemophiliacs in this study group consisted of cryoprecipitate with or without factor concentrates, and whole blood transfusions. The patients with haematological malignancies received platelets in addition to whole blood transfusions and other patient categories including thalassaemics received only whole blood.

Patients who had received cryoprecipitate and factor concentrates were at a significantly higher risk ( $p < 0.001$ ) of being positive for anti-HCV than those who had received only whole blood transfusions or platelets (Table 1).

**Table 1. Comparison of the major types of blood products received by different groups of multiply transfused patients with the HCV status (transfusions of cryoprecipitate and factor concentrates were confined to the haemophiliacs)**

Underlying condition for multiple transfusions	Type of blood product							
	Cryoprecipitate with or without factor concentrates		Blood only		Blood+platelets		Total	
	Total number of patients	Number positive for HCV	Total number of patients	Number positive for HCV	Total number of patients	Number positive for HCV	Total number of patients	Number positive for HCV
	number	Number of positive for HCV	number	Number of positive for HCV	number	Number of positive for HCV	number	Number of positive for HCV
Haematological malignancies	0	-	13	0	25	0	28	0
Solid tumors	0	-	27	0	0	-	27	0
Bone marrow dysfunction	0	-	3	0	18	0	21	0
Chronic renal failure	0	-	30	0	0	-	30	0
Haemolytic anaemia	0	-	32	0	0	-	32	0
Haemophilia	21	7	0	-	0	-	21	7
Thalassaemia	0	-	31	3	0	-	31	3
<b>Total</b>	<b>21</b>	<b>7</b>	<b>136</b>	<b>3</b>	<b>43</b>	<b>0</b>	<b>200</b>	<b>10</b>

## Discussion

Antibodies to HCV were detected in 10% of thalassaemics and 33% of haemophiliacs in this study indicating that certain categories of patients receiving multiple transfusions of blood and blood products in Sri Lanka carry a greater risk of HCV infection. Antibodies to HCV were detected significantly more frequently in patients who have had more than 80 transfusions; and thalassaemics and haemophiliacs had received more than a median of 100 blood transfusions each when compared to the other categories.

The prevalence of antibodies to HCV among 246 thalassaemics in Sri Lanka has been reported as 3.25% (6), which contrasts with the figure of 10% in the present study. Technical reasons are unlikely to account for this difference since both studies had used third generation commercial EIA for anti-HCV detection. However, the 31 patients with thalassaemia in our study were from the Lady Ridgeway Hospital (LRH) and the National Hospital, Sri Lanka, whereas in that study (9), 24 patients were from the LRH and 222 patients were from the Kurunegala General Hospital. It would be interesting to find out whether the age and geographical differences could account for this difference in prevalence of HCV positivity among thalassaemics. Haemophilia patients are known to be at a greater risk of hepatitis C infection in many countries, including Japan (13-84%) (7), Turkey (24%) (8), Singapore

(60%) (9) and Poland (80%) (10). The prevalence of antibodies to HCV among thalassaemics in India varies from 25% (11) to 36% (12). (Table 2).

In the present study, transfusions of cryoprecipitate and factor concentrates were significantly associated with HCV infection. Haemophiliacs exclusively had received these blood products. This may account for the significantly greater proportion of haemophiliacs infected with hepatitis C virus than the thalassaemics. The pooling of blood collected from a number of donors in the preparation of cryoprecipitate and factor concentrates is likely to increase the chances of carrying the hepatitis C virus.

The prevalence of antibodies to HCV among 1748 blood donors in Sri Lanka is 0.7% tested by a second-generation anti-HCV EIA (13). Our results warrant a large-scale study of anti-HCV in blood donors and a cost benefit analysis, to assess the need for routine screening of all blood donors in Sri Lanka.

Haemophiliacs often receive factor concentrates that increase the risk of transmission of HCV. Viral inactivation techniques such as dry heating, pasteurisation, solvent detergent treatment and irradiation have improved the safety of these blood products (14,15,16). Our results justify examination of the feasibility of providing HCV screened and decontaminated blood and blood products, at least initially to the haemophiliacs and thalassaemics in Sri Lanka.

**Table 2. Results of studies on antibody to HCV in patients who have had multiple transfusions. (One should bear in mind the varying sensitivities and specificities of the different HCV antibody tests used in these studies when comparing the data)**

Country/City	Multiple Transfusions	Haemophilia	Thalassaemia	Reference
Turkey	4%	24%		17,8
Poland		80%		10
Japan		13-14%		7
Wellington	3%	89%		18
UK		59%		19
France		80%		22
Singapore		60%		9
Calcutta		25%	14.3%	20
Mumbai	36.4%			21
Lucknow			23 - 35.9%	12
Sri Lanka			3.25%	6
Sri Lanka	5%	33%	10.3%	Present study

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