# THE PREVALENCE OF HEPATITIS C VIRUS ANTIBODIES IN THALASSEMIC PATIENTS IN THE SOUTH OF THAILAND

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**Abstract.** One hundred and one thalassemic patients, 37 with homozygous β-thalassemia, 60 with beta-thalassemia Hb E and 4 with hemoglobin H disease with Hb Constant Spring were studied. Twenty-four of 101 (23.8%) tested positive for antibody to hepatitis C virus (anti-HCV). Anti-HCV positivity among those with homozygous β-thalassemia was significantly higher than anti-HCV positivity among the β-thalassemic Hb E group. The number of blood transfusions received by anti-HCV positive thalassemic patients was significantly higher than that by anti-HCV negative thalassemic patients. Ninety per cent of anti- HCV positive thalassemic patients had persistently or intermittently raised SGPT levels.

## INTRODUCTION

The hepatitis C virus (HCV) was identified in 1989 (Choo *et al*, 1989) and is now recognized as the major etiologic agent of non A, non B hepatitis. About 50% of HCV infected patients develop chronic hepatitis and 20% of them go on to develop cirrhosis within 10 years (De Montalembert *et al*, 1995). Transfusion has been the principal means of HCV infection, the others being intravenous drug use, sexual contacts, and familial contacts (De Montalembert *et al*, 1995). The posttransfusional risk of HCV infection is directly dependent on the number of transfusions of blood products and on the prevalence of HCV in the blood donor population. Screening for anti-HCV positive blood donations drastically reduced the risk of transfusional HCV infection (De Montalembert *et al*, 1995). The prevalence of anti-HCV antibodies is high in hemophiliac (Troisi *et al*, 1993) and thalassemic patients (De Montalembert *et al*, 1995). The prevalence of anti-HCV antibodies in thalassemic patients varies geographically: 11.1% in India (Williams *et al*, 1992); 14% in Thailand (Poovorawan *et al*, 1991); 23.3% in the United Kingdom (Wonke *et al*, 1990); 34% in France (De Montalembert *et al*, 1992); 70% in Saudi Arabia (Al-Fawaz and Ramia, 1993); 72.3% in Italy (Rebulla *et al*, 1992) and 75% in Egypt (Khalifa *et al*, 1993). However, the serologic results of these studies were obtained with assays of different generations and are not fully comparable. The second generation enzyme-linked immunosorbent assay (ELISA) is more sensitive for the identification of HCV infection than the first generation ELISA (Lai *et al*, 1993).

In this report we evaluated the prevalence of HCV antibodies in multiple transfused thalassemic patients using the second generation ELISA.

## PATIENTS AND METHODS

One hundred and one transfusion-dependent thalassemic patients who attended the Pediatric Hematology Clinic, Songklanagarind Hospital, Faculty of Medicine, Prince of Songkla University, during the period of July 1993 to September 1995 were studied. They included 60 males and 41 females. Blood transfusion was given every 1

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week-3 months to keep the hematocrit value at 25% or above. Clinical data were kept for each patient and included information on height, weight, facial characteristics, jaundice, palpable liver and spleen below the costal margins and the amount of PRC transfusions that they received at each visit.

Fresh serum samples were obtained from each patient for HCV antibody testing and stored at -70°C until analysis. Serum samples of each patient was collected prior to PRC transfusion at each visit every 3 months for 1 year. We used second generation ELISA to detect the presence of HCV antibodies [Murex anti-HCV (Version III), Murex Diagnostics, Dartford, England]. The procedures were as stated by the manufacturer. In patients who had HCV antibodies, serum bilirubin and serum glutamic pyruvic transaminase (SGPT) were monitored every 3 months for 1 year. SGPT activities were considered abnormal if they were at least 1.5 times the normal value (0-40 IU/l).

Informed consent was obtained from a parent or legal guardian who accompanied the patient to our clinic.

**Statistical analysis**

Fisher's exact test and chi square test were used to determine the two-tailed statistical significance of differences between proportions in a two-by-two table. Mean values were compared using Student's *t*-test.

## RESULTS

One hundred and one thalassemic patients, comprising 37 cases of homozygous β-thalassemia, 60 cases of β-thalassemia Hb E disease and 4 cases of Hemoglobin H with Hb Constant Spring (Hb CS), were studied. Age of the study patients was in the range 0.8-23.8 years with a mean of 7.65±4.8 years. The male to female ratio was 1.46. Twenty-four (23.8%) of the 101 patients were anti-HCV positive (Table 1). The ages of the anti-HCV positive patients were in the range 1.2-17.4 years with a mean of 9.69±4.86 years. The male to female ratio in anti-HCV positive patients was 0.86. Anti-HCV positivity among patients with homozygous β-thalassemia, β-thalassemia Hb E disease and Hb H disease with Hb CS were 32.4%, 15% and 75% respectively (Table 1). Anti-HCV

Table 1

Prevalence of HCV antibodies in multitransfused thalassemic patients in the south of Thailand.

| Thalassemic disease | Age(yr) | Sex | HCV antibodies | | Total |
|---|---|---|---|---|---|
| | X±SD | M :F | negative | positive | |
| 1. **Homozygous β- thalassemia** | 7.96±5.1 | 2.5:1 | 25[a] | 12[a] (32.4)[b] | 37[a] |
| No. of blood transfusion | | | | | |
| -range | | | 6-141 | 34-151 | 6-151 |
| -X±SD | | | 55.1±38.6 | 94.7±38 | 67.9±41.7 |
| 2. **β-thalassemia Hb E** | 7.21±4.6 | 1.2:1 | 51[a] | 9[a] (15%)[b] | 60[a] |
| No. of blood transfusion | | | | | |
| -range | | | 2-92 | 18-118 | 2-118 |
| -X±SD | | | 28.5±21.6 | 54.9±34.7 | 32.5±25.3 |
| 3. **Hb H with Hb CS** | 9.94±5.3 | 1:3 | 1[a] | 3[a] (75%)[b] | 4[a] |
| No. of blood transfusion | | | | | |
| -range | | | 2 | 19-51 | 2-51 |
| -X±SD | | | 2 | 28.5±21.4 | 26±20.7 |
| **All thalassemic patients** | 7.65±4.8 | 1:1.2 | 77[a] | 24[a] | 101[a] |
| No. of blood transfusion (X±SD) | | | 36.9±30.8 | 72.2±41.3 | 48.3±36.6 |

a = number of patients, Hb CS = Hb Constant spring
b = percentage of HCV Ab positive in each group of thalassemic patients
X±SD = nean±standard deviation

was significantly higher in those with homozygous β-thalassemia than in those with β-thalassemia Hb E disease ($p<0.05$). The number of blood transfusions was significantly higher in thalassemic patients who had anti-HCV than in those who did not have anti-HCV ($p<0.05$). This findings was true for each group of thalassemic patients (homozygous β-thalassemia and and β-thalassemia Hb E disease).

Nineteen of 21 thalassemic patients (90%) who had HCV antibodies had raised serum glutamic pyruvate transaminase (SGPT>60 IU/l) during the time of follow up after detection of HCV antibodies (Table 2 ). Among patients who had HCV Ab, the range of serum total bilirubin was 0.76-7.07 mg/dl (Table 2).

## DISCUSSION

The prevalence of anti-HCV antibodies (Ab) among thalassemic patients in this study was higher than in a previous study in Bangkok in Thailand (23.8% *vs* 14%) (Poovarawan *et al*, 1991). This difference result may be due to a difference in the two patient populations and time of study, or the technology: we used the second generation ELISA which more sensitive than that used in the previous study (Poovorawan *et al*, 1991). When comparing the populations, however, our group (mean age 9.69 years) was slightly younger than that in the previous report (mean age 10.6 years). The prevalence of seropositivity for anti-HCV antibodies in thalassemic patients had been shown to be corre-

Table 2

Serum bilirubin and SGPT in thalassemic patients who had HCV Ab.

| Patient No. | Age/Sex (yr) | SGPT (IU/l) Range | SGPT (IU/l) X±SD | Total bilirubin (mg/dl) Range | Total bilirubin (mg/dl) X±SD | Thalassemia disease |
|---|---|---|---|---|---|---|
| 1 | 1.2/F | 58-67 | 62.5±6.4 | 1.9-2.16 | 2.03±0.18 | Thal major[b] |
| 2 | 3.8/F | 54-124 | 84.1±23.1 | 3-3.96 | 3.35±0.46 | Thal major[b] |
| 3 | 5.4/F | 69-156 | 124±31.9 | 1.18-3.29 | 1.95±0.68 | Thal major,S[a] |
| 4 | 5.8/M | 7-129 | 74.9±41.1 | 0.76-1.05 | 0.88±0.13 | Thal major,S[a] |
| 5 | 6.1/F | 114-200 | 159.5±29.4 | 1.36-2.55 | 2.04±0.56 | Thal major,S[a] |
| 6 | 6.9/M | 63-103 | 85.8±41.8 | 1.7-3.06 | 2.33±0.56 | Thal major,S[a] |
| 7 | 7.3/M | 79-147 | 119.67± 35.9 | 1.88-2.8 | 2.43± 0.49 | Thal major,S[a] |
| 8 | 8.8/M | 71-197 | 139.3±57.4 | 1.3-3.76 | 2.54±1.04 | Thal major,S[a] |
| 9 | 9.2/M | 85-92 | 88.5±4.9 | 1.56-2.91 | 2.24±0.96 | Thal major,S[a] |
| 10 | 13.7/F | 126-184 | 156.6±37.9 | 2.25-2.9 | 2.54±0.32 | Thal major,S[a] |
| 11 | 16.3/M | 48-53 | 50.5±3.5 | 6.44-7.07 | 6.76±0.45 | Thal major,S[a] |
| 12 | 3.3/F | 49-87 | 64±13.9 | 1.5-2.86 | 2.16±0.5 | β-Thal Hb E |
| 13 | 6.6/M | 56-94 | 75±26.9 | 2.04-2.52 | 2.28±0.34 | β-Thal Hb E,S[a] |
| 14 | 7.5/M | 88-174 | 126.2±32.9 | 2.89-4.02 | 3.49±0.46 | β-Thal Hb E |
| 15 | 11.6/F | 13-142 | 77.5±91.2 | 2.46-4.14 | 3.3±0.84 | β-Thal Hb E,S[a] |
| 16 | 13.9/M | 79-210 | 118.6±47.2 | 1.9-3.59 | 2.79±0.66 | β-Thal Hb E,S[a] |
| 17 | 14/F | 32-115 | 87.8±31.3 | 0.78-2.74 | 2.52±0.88 | β-Thal Hb E |
| 18 | 14.8/F | 27-80 | 53.5±37.5 | 4.07-4.51 | 4.29±0.31 | β-Thal Hb E |
| 19 | 17.4/M | 34-36 | 35±1.4 | 1.93-2.11 | 2.02±0.13 | β-Thal Hb E |
| 20 | 2.5/F | 65-173 | 116±54.3 | 2.49-4.31 | 3.12±1.03 | Hb H c Hb CS |
| 21 | 14.3/F | 84-174 | 125±63.6 | 4.35-5.64 | 5.0±0.91 | Hb H c Hb CS |

[a] S = splenectomy,
[b] Thal major = homozygous β-thalassemia
X±SD = mean±standard deviation

lated with transfusional age and therefore the number of transfusions received (Borzini *et al*, 1991; Al-Fawaz and Ramia 1993). However, other studies have shown no correlation (Resti *et al*, 1991; Wonke *et al*, 1990). Our study showed that HCV Ab positive thalassemic patients received a higher number of blood transfusions than patients who were HCV Ab negative.

Higher frequency of receiving blood transfusion in homozygous β- thalassemia patients compared with β-thalassemia Hb E patients may explain the difference in exposure rate to HCV found among the two groups. A higher exposure rate to HCV in patients with Hb H disease with Hb CS was observed but the number of patients studied was very low.

The post transfusion risk of HCV infection depends on the prevalence of HCV in the blood donor population. Patients who received anti-HCV positive blood products are 20 times more likely to have HCV infection than patients of anti-HCV negative blood (Van Der Poel *et al*, 1990). The prevalence of anti-HCV antibodies in normal blood donors in the United Kingdom was 0.5-1% while the prevalence of anti-HCV seropositivity among thalassemic patients was 12.2% (Wonke *et al*, 1990). In Italy, the prevalence of anti-HCV antibodies positive donors was 0.68% and the prevalence of anti-HCV Ab among thalassemic patients was 55% (Borzini *et al*, 1991). In India, the prevalence of HCV antibodies positive blood donors was 1.6% and the prevalence of HCV antibodies positive among thalassemic patients was 11.1% (Williams *et al*, 1992). The prevalence of HCV antibody positive Thai donors in previous studies was 1.48%, the prevalence of HCV antibody positive thalassemic patients was 14% (Poovorawan *et al*, 1991). The prevalence of anti-HCV Ab positive blood donors at our blood bank was 1.2%, the prevalence of anti-HCV Ab positives among thalassemic patients was 23.8%. The difference in prevalence of anti-HCV Ab among these studies may depend partly on difference in the the method used in detection the antibodies, especially that between first and second generation ELISA.

Since starting screening for anti-HCV Ab positive blood products in our blood bank in June 1994, we have not detected any new patient with anti-HCV Ab.

Thalassemic patients who had HCV Ab had high levels of serum alanine aminotransferase activities within the range of 15% (Al Fawaz and Ramia, 1993) to 32% of the patients (Borzini *et al*, 1991). But the data from Wonke *et al* (1990) showed that 16 of 17 anti-HCV positive thalassemic patients had had persistently or intermittently raised plasma alanine aminotransferase activities. Our data also show that 19 of the 21 (90%) anti-HCV positive thalassemic patients had had intermittently or persistently raised serum glutamic pyruvate transaminase (SGPT).

## REFERENCES

Al-Fawaz I, Ramia S. Decline in hepatitis B infection in sickle cell anemia and β thalassemia major. *Arch Dis Child* 1993; 69: 594-6.

Borzini P, Cazzaniga G, Vecchi L, Prevalence of anti-hepatitis C virus seroconversion in polytransfused thalassemic patients. *Vox Sang* 1991; 60: 188.

Choo QL, Kuo G, Weiner AJ, *et al*. Isolation of a cDNA derived from a blood-borne non-A non-B viral hepatitis genome. *Science* 1989; 244: 359-61.

De Montalembert M, Girot R, Mattlinger B, Lefrere JJ. Transfusion-dependent thalassemia: viral complications (epidemiology and follow up). *Semin Hematol* 1995; 32: 280-7.

De Montalembert M, Costagliola D, Lefrere JJ, *et al*. Prevalence of markers for human immunodeficiency virus type 1, cytomegalovirus, and hepatitis B and C in multiply transfused thalassemia patients. *Transfusion* 1992; 32: 509-12.

Khalifa AS, Mitchell BS, Watts DM, *et al*. Prevalence of hepatitis C viral antibody in transfused and non transfused Egyptian children. *Am J Trop Med Hyg* 1993; 49: 316-21.

Lai ME, De Virgillis S, Angiolu F, *et al*. Evaluation of antibodies to hepatitis C virus in a long-term prospective study of posttransfusion hepatitis among thalassemic children: comparison between first and second generation asssy. *J Pediatr Gastroenterol Nutr* 1993; 16: 458-464.

Poovorawan Y, Pongpunlert W, Chumdermpadetsuk S, *et al*. The prevalence of hepatitis C virus antibodies among high risk groups in Thailand. *Southeast Asian J Trop Med Public Health* 1991; 22: 133-4.

Rebulla P, Mozzi F, Coutinho G, *et al*. Antibody to hepatitis C virus in 1305 Italian multiply transfused thalassemics: a comparison of first and second generation tests. *Transf Med* 1992; 2: 69-70.

Resti M, Azzari C, Rossi ME, *et al.* Prevalence of hepatitis C virus antibody in beta-thalassemic polytransfused children in a long term follow up. *Vox Sang* 1991; 60: 246-7.

Troisi CL, Hollinger FB, Hoots WK, *et al.* A multicenter study of viral hepatitis in a United States hemophilic population. *Blood* 1993; 81: 412-8.

Van Der Poel CL, Reesink HW, Schaasberg W, *et al.* Infectivity of blood seropositive for hepatitis C virus antibodies. *Lancet* 1990; 335: 558-60.

Williams TN, Wonke B, Donohue SM. A study of hepatitis B and C prevalence and liver function in multiply transfused thalassemic and their parents. *Indian Paediatr* 1992; 29: 1119-24.

Wonke B, Hoffbrand AV, Brown D, Dusheiko G. Antibody to hepatitis C virus in multiply transfused patients with thalassemia major. *J Clin Pathol* 1990; 43: 638-40.