

# Association between hepatitis C infection and number of screened blood unit transfusions in thalassemic children

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## ABSTRACT

**Background** Donor blood screening test for antibody against hepatitis C virus (HCV) by third generation ELISA is widely used. However, there is still a window period during which a donor may already be infected despite a negative screening test.

**Objectives** To determine the prevalence of hepatitis C infection in thalassemic children who had received screened donor blood and to seek the association between HCV infection and the number of blood unit transfusions received.

**Methods** This was an analytic cross-sectional study. Sixty-seven children who had received third generation ELISA screened donor blood were examined for HCV antibody. The study was conducted in Hasan Sadikin General Hospital, Bandung, from January to March 2004. The prevalence of hepatitis C was presented in percentage. The association between HCV infection and sex, age, interval between transfusions, and the number of blood unit transfusions received was determined by univariate analysis and logistic regression analysis.

**Results** In univariate analysis, significant difference between HCV-infected and uninfected subjects was found in the mean age and mean number of blood units transfused ( $P < 0.001$ ). In logistic regression analysis, we found a significant association between the quantity of transfused blood with positive HCV antibody ( $P < 0.001$ ). The odds ratio for positive HCV antibody was 1.08 for each blood unit transfusion received (95%CI 1.02;1.14). The prevalence of hepatitis C in thalassemic children who received third generation ELISA screened blood was 22.4% (95%CI 12.4%;32.4%). This prevalence is lower than that in a previous study of thalassemic children receiving unscreened blood (50.8%).

**Conclusions** The prevalence of HCV infection in thalassemic children who had received screened donor blood is 22.4%. HCV infection is significantly associated with the number of screened blood unit transfusions [**Paediatr Indones 2005;45:182-186**].

**Keywords:** hepatitis C, thalassemic children, screened donor blood, transfusion, ELISA

Children with thalassemia are at high risk for hepatitis C virus (HCV) infection. Multiple transfusions by donor blood containing the virus may cause HCV infection in patients with thalassemia.<sup>1-2</sup> Before routine HCV screening, the prevalence of positive HCV antibody in thalassemic patients requiring repeated transfusions in various countries ranged between 16.7 - 72.3%.<sup>3-4</sup> In Indonesia, the prevalence of hepatitis C infection in thalassemic children had been reported to be 31.37%<sup>5</sup> and 50.8%<sup>6</sup>. The risk of hepatitis C infection among patients receiving multiple transfusions increases with each additional unit transfused.<sup>3,5,7</sup> Nowadays, third generation ELISA is widely used as a serologic examination to detect HCV antibody in donor blood. Seroconversion takes place 2-3 weeks after exposure to HCV. The sensitivity and specificity of this screening test are 97% and 99%, respectively.<sup>8-10</sup> Using this test, approximately 50-70% of patients will test positive for HCV antibody at the onset of the disease.<sup>11</sup> However,

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there is still a window period during which a donor can be infected, yet screen negative.<sup>12</sup> The positive predictive value of this test is only 50-61% in patients who are at low risk for HCV infection.<sup>13</sup> Currently, hepatitis C polymerase chain reaction (PCR) is considered the most sensitive test.<sup>5,10</sup> With this test, more than 95% of patients with hepatitis C infection will test positive for HCV ribonucleic acid (HCV RNA).<sup>11</sup> With PCR, the window period is reduced to approximately 12 days.<sup>12</sup> However, financial constraints preclude wide usage of this test in Indonesia.

Generally, the prevention of HCV infection can be accomplished with screening of donor blood and high-risk groups. HCV donor blood screening might decrease the prevalence of HCV infection by as much as 50-80%.<sup>14</sup> The Ministry of Health of the Republic of Indonesia has issued a regulation concerning the prevention of disease transmission by the route of transfusion.<sup>15</sup> The Indonesian Red Cross in Bandung has started routine screenings for HCV antibody in donor blood using third generation ELISA since 1999.

Thalassemic children appear to be at elevated risk for HCV infection due to multiple blood transfusions, despite screening of donor blood by third generation ELISA. This study was conducted to determine the prevalence of HCV infection in thalassemic children who had received screened donor blood, and to seek the association between hepatitis C infection and the number of blood units transfused in these children.

## Methods

This was an analytic, cross-sectional study involving 67 children with thalassemia, recruited consecutively. This study was conducted in the Thalassemia Outpatient Clinic, Department of Child Health, Hasan Sadikin General Hospital, Bandung from January to March 2004. The study was approved by the Medical Ethics Committee of the Medical School, Padjadjaran University.

The subjects had received transfusion of blood screened for HCV antibody by third generation ELISA in the period of January 1999 to November 2003. Anti-HCV was measured using UBI EIA 4.0. Laboratory examination was performed at the Indonesian Red Cross Laboratory, Bandung.

Patients were included in the study if all blood transfusions received had been screened for HCV antibody by third generation ELISA; medical records were complete with respect to age, sex, time of first transfusion, amount of transfused blood, and interval between transfusions; and parents agreed to enroll the child in the study by written informed consent. Exclusion criteria were history of surgery (minor or major); and hepatitis C already suffered prior to regular transfusions.

Subject characteristics, number of blood unit transfusions, interval between transfusions, family history of hepatitis C infection and desferal injection, and manifestations of hepatitis were recorded and tabulated. The prevalence of HCV infection was calculated and expressed in percentage. The association of HCV infection (positive HCV antibody) with age, sex, interval between transfusions, and the number of blood unit transfusions received was determined using univariate analysis and logistic regression analysis. Significance level was set at  $P < 0.05$ . Data analysis was done using SPSS version 10.0 (1999).

## Results

Sixty-seven thalassemic were included in this study. The characteristics of the subjects are shown in **Table 1**. There were 33 (49.3%) male and 34 (50.7%) female subjects, aged between 1 and 11 years old (mean age 3.5 years); 54 (80.6%) were under 5 years old. The amount of blood transfusions received ranged from 3

**TABLE 1.** SUBJECT CHARACTERISTICS

Characteristics	n	%
<b>1. Sex</b>		
- Male	33	
- Female	34	49.3
<b>2. Age (years)</b>		50.7
- < 5	54	
- ≥ 5	13	
<b>3. Interval between transfusions (weeks)</b>		79.4
- 2	1	19.1
- 3	13	
- 4	31	
- 5	15	1.5
- 6	4	19.4
- 8	2	46.3
- 20	1	22.4

to 76 units (mean 28.5 units). The interval between transfusions ranged between 2 to 20 weeks (mean 4.5 weeks). Most of the subjects (46 subjects; 68.7%) came from low socioeconomic class, 6 (9%) had health insurance, and 15 (22.3%) had no health insurance. Jaundice was found only in 1 subject (1.5%). None complained of nausea, vomiting, or right upper quadrant abdominal pain. None had history of hepatitis C infection in the family or had received parenteral medications such as desferal.

Of the 67 subjects, 15 (22.4%) had positive HCV antibody, while 52 (77.6%) tested negative for HCV antibody. Thus, the prevalence of hepatitis C infection in children with thalassemia who had received transfusions of donor blood screened for HCV antibody by third generation ELISA was 22.4% (95% CI 12.4%;32.4%).

**Table 2** shows results of univariate analysis comparing various variables in association with HCV infection. The mean age of HCV-infected subjects differed significantly from that of uninfected subjects ( $P < 0.001$ ). Significant difference was also found in the mean number of blood units transfused ( $P < 0.001$ ). No significant difference between infected and uninfected subjects was found in terms of sex and interval between transfusions.

In logistic regression analysis (**Table 3**), the number of blood units transfused was found to be significantly associated with HCV infection ( $P = 0.010$ ). The odds ratio for HCV infection was 1.08 for each blood unit transfused (95%CI 1.02;1.14). Age of  $\geq 4$  years was also significantly associated with positive HCV antibody, with odds ratio of 7.10 ( $P = 0.049$ , 95%CI 1.01;49.96). Interval between transfusions was not significantly associated with HCV infection.

## Discussion

The prevalence of hepatitis C infection in this study (22.4%) was lower than that found in studies conducted prior to the introduction of routine HCV screening of donor blood. In a study with similar design at the same center, Widjaja<sup>5</sup> measured HCV antibody in 61 children with thalassemia who received regular transfusion of unscreened blood. The prevalence of hepatitis C infection found in Widjaja's study was 50.8%. A more contemporaneous study in Malaysia by Jamal *et al* found a prevalence of HCV infection among thalassemic patients which is identical to that found in the present study.<sup>3</sup>

**TABLE 2.** UNIVARIATE ANALYSIS OF VARIOUS FACTORS IN ASSOCIATION WITH POSITIVE HCV ANTIBODY

Variable	VHC		P
	+	-	
<b>Sex</b>			
Male	7	26	0.820*
Female	8	26	
<b>Age (years)</b>			
Mean (SD)	4.5 (1.2)	3.2 (2.6)	<0.001**
<b>Interval between transfusions (weeks)</b>			
Mean (SD)	3.8 (1.0)	4.7 (2.4)	0.054**
<b>Number of units transfused</b>			
Mean (SD)	46.1 (17.5)	23.4 (12.9)	<0.001**

\*Chi-square test; \*\* Mann-Whitney test

**TABLE 3.** LOGISTIC REGRESSION ANALYSIS OF THE ASSOCIATION BETWEEN AGE, INTERVAL BETWEEN TRANSFUSIONS, AND NUMBER OF UNITS TRANSFUSED WITH POSITIVE HCV ANTIBODY

Variable	$\beta$	SE of $\beta$	P	OR (95%CI)
Age (<4; $\geq 4$ years)	1.961	0.995	0.049	7.10 (1.01-49.96)
Transfusion interval (<4; $\geq 4$ weeks)	-0.888	0.921	0.335	0.41 (0.07-2.50)
Amount of transfusion (units)	0.073	0.028	0.010	1.08 (1.02-1.14)

Accuracy: 82.09%

Previous studies have compared the prevalence of hepatitis C infection in post-operative patients who received blood transfusion before and after the application of routine screening for HCV. A study by Donahue *et al* found a decline in the prevalence of hepatitis C infection in post operative patients, from 3.8% to 0.6% after the application of routine screening with first generation ELISA.<sup>7</sup> However, transfusion-associated hepatitis C after donor blood screening is likely to remain a complication in transfusion therapy for at least two reasons: false negative screening tests performed in the window period and the presence of seronegative carriers.

The prevalence HCV infection did not differ significantly among males and females ( $P=0.820$ ). This confirmed results obtained by Widjaja<sup>5</sup> and Jamal *et al*.<sup>3</sup> This study found a significant association between age and positive HCV antibody. The mean age of infected individuals was 4.5 years. In Widjaja's study the mean age of HCV-infected subjects was 6.3 years,<sup>5</sup> while in the study of Lai *et al* it was 2.4 years.<sup>16</sup>

By logistic regression analysis, we found a significant association between the number of blood units transfused and positive HCV antibody ( $P=0.010$ ). The risk of HCV infection increased with each blood unit transfusion received ( $OR=1.08$  time for each blood unit). Such an association had also been shown in previous studies. Donohue *et al* showed that the patients who received more than 12 blood units had their risk for hepatitis C infection increased by 6-fold.<sup>7</sup> Widjaja found that patients receiving  $\geq 55$  blood units had 18.9 times higher risk for hepatitis C infection.<sup>17</sup> Saberi-Firozzi *et al* noted a 0.2% increase in the risk of acquiring hepatitis C infection after receiving 1 blood unit.<sup>4</sup>

We conclude that the prevalence of hepatitis C infection among thalassemic children receiving HCV-screened donor blood transfusions is 22.4%. This prevalence is lower than that in studies conducted prior to routine HCV screening of donor blood, suggesting that screening plays a role in lowering the rate of HCV transmission through blood transfusion. A significant association was found between the amount of blood units received with positive HCV antibody.

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