

## Prevalence of hepatitis and its correlation with serum ferritin and aminotransferase levels among thalassemia major patients in Indonesia

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### Abstract

**Background** Thalassemia major patients who undergo routine transfusion have an increased risk of acquiring transfusion-transmitted infections (TTI), including hepatitis B and C. These diseases have serious implications and may affect the serum ferritin and aminotransferase levels of thalassemia major patients.

**Objectives** To identify the prevalence of hepatitis B and/or C infections among thalassemia major patients and to evaluate its correlation with serum ferritin and aminotransferase levels.

**Methods** This was across-sectional study conducted at the Thalassemia Center of Dr. Cipto Mangunkusumo Hospital in Jakarta, Indonesia. The subjects were screened for hepatitis B and C infections, and their serum ferritin and aminotransferase levels were also measured.

**Results** In total, 621 subjects were included in the study, among which 5 subjects tested positive for hepatitis B surface antigen (HBsAg) (0.8%), 111 subjects tested positive for anti-HCV (17.8%), and 5 subjects tested positive for both HBsAg and anti-HCV (0.8%). The subjects who tested positive for hepatitis B, hepatitis C, or both showed significantly higher values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum ferritin compared to their negative counterparts. Moreover, serum ferritin showed a positive, moderate correlation with both AST and ALT.

**Conclusion** This study shows a significant association between hepatitis and serum ferritin as well as aminotransferase levels. Early detection and early management of hepatitis B and C infections is warranted to minimize the occurrence of liver damage in thalassemia major patients. [Paediatr Indones. 2017;57:176-80; doi: <http://dx.doi.org/10.14238/pi57.4.2017.176-80> ].

**Keywords:** thalassemia major; hepatitis; serum ferritin; AST; ALT

Thalassemia is a genetic, blood disorder that requires multiple blood transfusions. Many patients show prolonged survival rates following routine transfusion, but blood transfusions cause iron accumulation in various tissues. One of the organs most prone to iron overload is the liver. Moreover, thalassemia patients are at risk of transfusion-transmitted infections (TTI), including hepatitis B and C. In addition to hepatic iron overload, hepatitis infection further damages liver cells.<sup>1</sup>

The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection vary among countries. Chronic HBV and HCV infection was estimated to be 240 million and 130-170 million people, respectively, worldwide.<sup>2</sup> The *World Health Organization* (WHO) also noted that HBV is highly endemic to countries in Southeast Asia, including Indonesia.<sup>3</sup> Although the mortality rate due to liver disease in thalassemia patients is low (2.7-4.1%), it should not be considered trivial. Liver cirrhosis

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in thalassemia has been linked to HCV and iron overload.<sup>4,5</sup> Among thalassemia patients worldwide, 0.3-5.7% are hepatitis B surface antigen (HBsAg) positive and 4.4-85.4% are anti-HCV positive.<sup>6</sup>

Indonesia is a developing country, with a majority of the population belonging to the low and middle socioeconomic demographic. Hence, the treatment of hepatitis using antivirals (ribavirin or interferon) is generally considered expensive and to a certain extent, unaffordable. Therefore, blood screening was proposed to prevent TTI. The *Indonesian Ministry of Health* has issued policies regarding blood services and stated that blood should only be collected from voluntary, non-remunerated blood donors, and not for commercial purposes. All blood donors are screened for TTI, including HBV, HCV, human immunodeficiency virus (HIV), and syphilis. Approximately 2.9% of blood products are disposed of due to positive TTI screening results every year.<sup>7</sup>

This study was aimed to describe the prevalence of HBV and HCV infection in thalassemia major patients by antibody detection, as well as to assess for correlations between hepatitis infection and liver transaminase enzyme as well as serum ferritin levels.

## Methods

This descriptive, cross-sectional study initially evaluated 1,088 thalassemia major patients who received routine blood transfusions once every 2 – 4 weeks at the Thalassemia Center, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia in 2015. Among these patients, 621 subjects were screened for hepatitis markers and subsequently enrolled in this study. The information collected included subjects' identity, sex, type of thalassemia, age at diagnosis, and age at first transfusion. Nucleic acid testing (NAT) has only been routinely available in four big cities in Indonesia (Jakarta, Bandung, Surabaya, and Denpasar) since 2015, so the data was collected from children who received routine transfusions prior to NAT testing.

Subjects were tested for HBsAg and anti-HCV antibodies, by electrochemiluminescence immunoassays (ECLIA), using the *Cobas e601* analyzer (*Roche Diagnostics*). Serum HBV DNA and HCV RNA tests were not conducted in this study.

Serum ferritin level was also analyzed by ECLIA. Alanine and aspartate aminotransferase levels were measured by immunoassay. The ALT and AST levels were considered to be increased if higher than 40 U/L.<sup>8</sup>

The data was analyzed using SPSS, SPSS Inc., Chicago, IL and *GraphPad Prism 6* software. A P value of <0.05 was considered to indicate a significant relationship between variables. Spearman's test was used to evaluate the correlation between two numeric parameters, while the Mann-Whitney and Kruskal-Wallis tests were used to compare two or more categorical groups.

## Results

Among the 621 subjects, positive hepatitis markers were found in 121 (19.4%) patients. Positive blood specimens for HBsAg were detected in 5 (0.8%) subjects, anti-HCV in 111 (17.8%) subjects, and for both HBsAg and anti-HCV in 5 (0.8%) subjects.

A total of 121 subjects were studied, comprising of 65 male and 56 female subjects. The age range of subjects was 5-42 years. There were 61 subjects with  $\beta$ -thalassemia and 60 with  $\beta$ -thalassemia/HbE. The medians and ranges of AST, ALT, and serum ferritin levels were 61 (9-194) U/L, 55 (6-218) U/L, and 6,117 (1,395-16,636) U/L, respectively. Increased ALT was found in 81 (66.9%) subjects.

**Table 1** shows the characteristics of subjects among the patients positive for HbsAg, anti-HCV, and both. The AST, ALT, and serum ferritin levels were found significantly higher in the hepatitis-positive compared to hepatitis-negative subjects ( $P=0.02$ ,  $0.029$ , and  $< 0.01$ , respectively) (**Table 2**). **Figures 1** and **2** show a moderate correlation between aminotransferase and serum ferritin levels among the various groups of subjects positive for hepatitis.

## Discussion

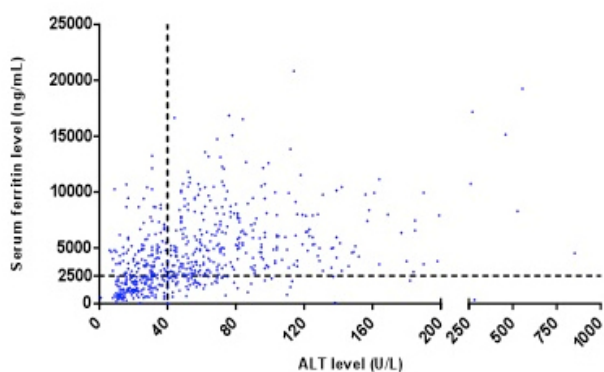
We found a higher prevalence of HCV than HBV in thalassemia patients, but past studies have reported varying prevalences of HCV compared to that of HBV in thalassemia patients.<sup>9-12</sup> Different assays may yield contrasting results. Purnamawati et al. found that

**Table 1.** Demographic comparison for subjects with positive HBsAg results, positive anti-HCV results, and both

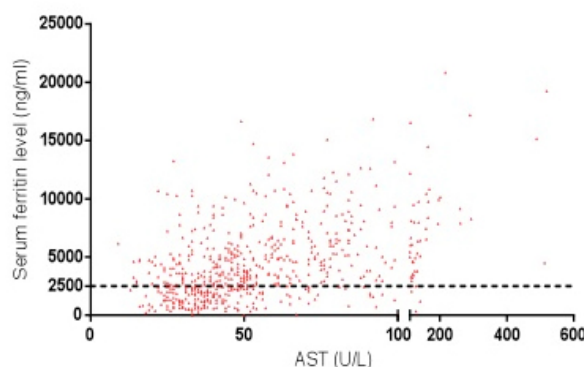
Characteristics	Positive HBsAg (n=5)	Positive anti-HCV (n=111)	Positive HBsAg + anti-HCV (n=5)
Mean age (SD), years	14.4 (6.6)	24.0 (6.3)	22.0 (7.1)
Gender, n			
Males	4	58	3
Females	1	53	2
β-thalassemia subjects, n	2	55	4
β-thalassemia/HbE subjects, n	3	56	1
Mean age at first diagnosis of thalassemia, years	7.0	5.3	3.0
Mean age at first transfusion, n			

**Table 2.** Comparison of AST, ALT, and serum ferritin levels between hepatitis-positive and hepatitis-negative subjects

Variables	Positive HBsAg (n=5)	Positive anti-HCV (n=111)	Positive HBsAg + anti-HCV (n=5)
Median AST (range), U/L	61 (9-194)	48 (14-5190)	0.02
Median ALT (range), U/L	55 (6-128)	46 (1-853)	0.029
Median serum ferritin (range), ng/mL	6,177 (1,395-16,636)	3,324 (3-20,831)	<0.0001



**Figure 1.** Correlation between ALT and serum ferritin level ( $r=0.53$ ,  $P<0.0001$ )



**Figure 2.** Correlation between AST and serum ferritin level ( $r=0.46$ ,  $P<0.0001$ )

the prevalence of hepatitis C among subjects with thalassemia was 6.7% (6/90). They used the Entebbe dipstick anti-HCV test. Among the six subjects with hepatitis C, five had increased ALT levels ( $>40$  U/L), and four had increased transferrin saturation.<sup>9</sup>

A previous study in thalassemia patients identified positive anti-HCV in 49.5% of subjects and positive HbsAg in 3.2% of subjects, using a commercial ELISA kit (*version-1*, China).<sup>10</sup> Also, Wanachiwanawin et al. found that among thalassemia patients, 2% had positive HBsAg results and 20.2% had positive anti-HCV results. The HBsAg was evaluated using passive hemagglutination assay kits (MyCell, Tokyo, Japan), and anti-HCV by second generation enzyme immunoassay (*EIA II*, Tokyo,

Japan).<sup>11</sup> In addition, Vidja et al. found that 2% of thalassemia patients had positive HBsAg results, and 2% had positive anti-HCV results, both of which were detected with the ELISA method.<sup>12</sup> Further assay development is needed to distinguish a reactive result from a weak antigen/antibody concentration.

Electro-chemiluminescence immunoassay was shown to have weak sensitivity and specificity for cut-off indexes (COI) between 1.0 and 4.0, which would, therefore, require confirmatory testing. However, for COIs between 4.0 and 10.0, the confirmatory testing showed the same positive results.<sup>13</sup> Besides the assay kit, the implementation of screening on donated blood is another factor that may account for differences in HBV and HCV infection rates. In the US, hepatitis

C screening has been done since 1992, while in Iran since 1996. It should also be noted that NAT screening began in Indonesia in 2014, but a majority of our patients had received transfusions long before this period. The provision of free NAT testing for patients has only recently been conducted (since 2015).

Hepatitis infection tends to induce iron accumulation in the liver. Therefore, we would expect increased iron profile levels such as serum ferritin, serum iron, and transferrin saturation.<sup>14,15</sup> The hypothesis for this mechanism is that the virus inside liver cells accumulates the iron for its replication, and the immune status of the host response to viral infection may be modified.<sup>16</sup>

Our low numbers of hepatitis B-positive patients may not represent a true low, as our facility can only currently conduct serologic examinations, and it is costly to evaluate viral load and genotype. As such, HBV DNA testing could not be conducted on a routine basis. This limitation also highlights the importance of predicting the true burden of HBV infection. Several studies found the presence of occult hepatitis B (OHB) infections in thalassemia patients. Studies in Egypt and India, for instance, found 32.5-32.8% occult HBV infections in children with thalassemia.<sup>17,18</sup> Moreover, Shaker *et al.* found OHB in all HCV-infected subjects.<sup>17</sup> Occult hepatitis B is defined as the detection of HBV DNA in patients with serial negative results for HbsAg, with or without hepatitis B antibodies. The diagnosis of OHB requires an assay with high sensitivity and specificity that can detect a low limit of < 10 IU/mL of HBV DNA.<sup>19</sup>

The increase in aminotransferase enzymes may reflect the occurrence of hepatocellular injury. For instance, ALT maintains its highest concentration in the liver, while AST may be found in other organs besides the liver, such as the heart, the kidney, as well as skeletal muscles. Therefore, ALT is more specific as a marker for hepatocellular injury compared to AST. We found a significant correlation between aminotransferase and serum ferritin in patients with hepatitis. An increase in ALT levels could be due to iron overload, alcohol use, or hepatitis virus infection. History of alcohol consumption was not evaluated in these subjects. However, we assumed that a large percentage of subjects were not alcohol abusers since it is restricted by law in Indonesia. Ideally, hepatitis B vaccinations should be provided for all thalassemia

patients, to prevent hepatitis B virus infection, which can further contribute to liver failure. As shown in **Table 2**, the median levels of aminotransferase and serum ferritin in hepatitis-positive subjects were higher than those of negative subjects. Wanachiwanawin *et al.* reported that subjects with positive anti-HCV results generally had higher levels of ALT and AST compared to those with negative anti-HCV results.<sup>11</sup> Other factors such as use of iron chelation and frequency of transfusion may also affect the serum ferritin and aminotransferase level.

However, the presence of HCV does not alter the effects of iron overload on liver function. Triantos *et al.* found that the survival of thalassemia patients was not associated with the presence of hepatocellular carcinoma and other liver diseases. Instead, an association was found between the former and cardiac failure, as well as non-adherence to chelation treatment.<sup>20</sup> Liver MRI is recommended for those patients to more specifically evaluate iron overload. Azarkeivan *et al.* found a moderate correlation between serum ferritin level and relaxation time of liver MRI T2 ( $r = -0.535$ ).<sup>21</sup> Hepatitis B antibody testing was not conducted, nor was hepatitis B vaccination status known in our subjects.

With regards to iron chelation therapy, a study in Indonesia found that a higher dose of deferiprone is required to treat thalassemia major patients with consequent hepatitis B and/or C infections as opposed to hepatitis-negative patients.<sup>22</sup> This, in turn, also highlights the importance of hepatitis detection in thalassemia patients.

In summary, this study demonstrates the current prevalence and clinical significance of HBV and HCV infection among thalassemia patients in Indonesia. The subjects who tested positive for HBV and HCV tended to have higher serum ferritin, AST, and ALT levels than those without infection. Therefore, early and aggressive management should be considered to prevent further liver damage in thalassemia patients with concomitant hepatitis. In the future, now that NAT screening in Indonesia has been routinely practiced since 2015, we hope that the incidence of hepatitis B and C, as well as other infections such as HIV, in thalassemia patients can be drastically decreased.

## Conflict of Interest

None declared.

## References

1. US Public Health Service. Updated US Public Health Service guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. *MMWR Recomm Rep.* 2001;50:1-52.
2. Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol.* 2007;13:2436-41.
3. Okada S, Taketa K, Ishikawa T, Koji T, Swe T, Win N, et al. High prevalence of hepatitis C in patients with Thalassemia and patients with liver diseases in Myanmar. *Acta Med Okayama.* 2000;54:137-8.
4. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and Complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica.* 2004;89:1187-93.
5. Ladis V, Chouliaras G, Berdoukas V, Chatziliami A, Fragodimitri C, Karabatsos F, et al. Survival in a large cohort of Greek patients with transfusion-dependent beta thalassaemia and mortality ratios compared to the general population. *Eur J Haematol.* 2011;86:332-8.
6. Riaz H, Riaz T, Ullah F, Aziz S, Khan MU, Pervaiz R, et al. Assessment of the seroprevalence of viral hepatitis B, Viral hepatitis C, and HIV in multitransfused thalassemia major patients in Karachi, Pakistan. *Trop Doct.* 2011;41:23-5.
7. Soedarmono YS. Donor issues in Indonesia: a developing country in South East Asia. *Biologicals.* 2010;38:436.
8. Jaiswal SP, Chitnis DS, Jain AK, Inamdar S, Porwal A, Jain SC. Prevalence of hepatitis viruses among multi transfused hemogenous thalassemia patients. *Hepatol Res.* 2001;19:247-53.
9. Purnamawati SP, Kartoyo P, Chair I, Bisanto J, Oswari H. Profile of alanine aminotransferase and hepatic iron accumulation in thalassaemic patients with or without anti-hepatitis C virus. *Paediatr Indones.* 2004;44:85-9.
10. Din G, Malik S, Ali I, Ahmed S, Dasti JI. Prevalence of hepatitis C virus infection among thalassemia patients: A perspective from a multi-ethnic population of Pakistan. *Asian Pac J Trop Med.* 2014;7:S127-33.
11. Wanachiwanawin W, Luengrojanakul P, Sirangkapracha P, Leowattana W, Fucharoen S. Prevalence and Clinical significance of hepatitis C virus infection in Thai patients with thalassemia. *Int J Hematol.* 2003;78:37-48.
12. Vidja PJ, Vachhani JH, Sheikh SS, Santwani PM. Blood transfusion transmitted infections in multiple blood Transfused patients of beta thalassemia. *Indian J Hematol Blood Transfus.* 2011;27:65-9.
13. Shao H, Li Y, Xu WZ, Zhou X. Increased need for testing to confirm initial weakly reactive results for hepatitis B virus surface antigen. *Lab Med.* 2012;43:15-7.
14. Bonkovsky HL, Banner BF, Rothman AL. Iron and chronic viral hepatitis. *Hepatology.* 1997;25:759-68.
15. Bassett SE, Di Bisceglie AM, Bacon BR, Sharp RM, Govindarajan S, Hubbard GB, et al. Effects of iron loading On pathogenecity in hepatitis C virus-infected chimpanzees. *Hepatology.* 1999;29:1884-92.
16. Shan Y, Lambrecht RW, Bonkovsky HL. Association of hepatitis C virus infection with serum iron status: analysis of data from the Third National Health and Nutrition Examination Survey. *Clin Infect Dis.* 2005;40:834-41.
17. Shaker O, Ahmed A, Abdel SI, El Ahl H, Shousha W, Doss W. Occult hepatitis B in Egyptian thalassaemic children. *J Infect Dev Ctries.* 2012;6:340-6.
18. Singh H, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, et al. High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. *Vox Sang.* 2003;84:292-9.
19. Alavian SM. Occult hepatitis B in thalassemia: a need for further study. *J Infect Dev Ctries.* 2012;6:650-1.
20. Triantos C, Kourakli A, Kalafateli M, Giannakopoulou D, Koukias N, Thomopoulos K, et al. Hepatitis C in patients with b-thalassemia major: a single-centre experience. *Ann Hematol.* 2013;92:739-46.
21. Azarkeivan A, Hashemieh M, Akhlaghpour S, Shirkavand A, Yaseri M, Sheibani K. Relation between serum ferritin and liver and heart MRI T2\* in beta thalassemia major patients. *East Mediterr Health J.* 2013;19:727-32.
22. Ananta Y, Wahidiyat PA, Oswari H. Deferiprone effectiveness in thalassemia major children with or without hepatitis B or C virus infection: a non-randomized study. *Paediatr Indones.* 2010;50:105-12.