

Relationship between elevated liver enzyme with iron overload and viral hepatitis in thalassemia major patients in Northern Iran

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ABSTRACT

الأهداف: تقييم العلاقة بين ارتفاع أنزيم الكبد مع زيادة حمل الحديد والتهاب الكبد الحموي لدى المرضى المصابين بالثلاسيميا الكبرى.

الطريقة: أجريت هذه الدراسة الوصفية العرضية على المرضى المصابين بالثلاسيميا بجناح مستشفى تونيكابون - مازنداران - إيران، خلال الفترة ما بين 20 أبريل 2006م وحتى 20 سبتمبر 2006م. تم دراسة المرضى وفقاً للعمر وأنزيم الكبد (LE) والأجسام المضادة (HCV) و (HBsAg)، والتشبع بالترانسفيرين (TSAT) ومدخلات التزويد بالدم (عدد تكرار ووحدات عملية نقل الدم). تم اعتبار عنصر ألانين أمينو ترانسفيريس مرتفعاً ≥ 40 U/L.

النتائج: تم تقييم حالة 65 مريضاً (يتراوح منتصف العمر ما بين 8.9 ± 19.51 عاماً، المدى من 4-5). كان هناك 11 مريضاً موجباً لمضادات HCV -16.9%. كان مصف فيريتين الرئيسي مرتفعاً بشكل ملحوظ لدى المرضى الذين لديهم $ALT \geq 40$ ($2553.08 \mu\text{g/L}$ versus $1783.7750 \mu\text{g/L}$) $p=0.012$. كان متوسط (ALT) مرتفعاً بشكل ملحوظ لدى المرضى ذوي المصابين TSAT $\geq 60\%$ (41.26 U/L versus 28.82 U/L) ($p=0.021$) كانت العلاقة بين $ALT \leq 40$ ومضادات HCV إيجابية بشكل ملحوظ. كان (ALT) الفعلي 60.91 U/L في مضادات HCV الموجبة و 39.29 U/L لدى السالبة ($p=0.001$). كان مصف عنصر الحديد الفعلي ومدخلات نقل الدم مرتفعاً بشكل ملحوظ لدى مرضى المضادات HCV الموجبة (234.0 versus 195.4815 ; $p=0.02$), (1693.6 versus 1036.29 , $p=0.014$)

الخلاصة: توصي العلاقة بين ارتفاع (ALT)، زيادة حمل عنصر الحديد، ومدخلات نقل الدم، العمر، وإيجابية مضادات (HCV) لدى المرضى المصابين بالثلاسيميا الكبرى لمستشفى تونيكابون، بإعادة تقييم نقل الدم وجرعات ديسفيرال والمعالجات بدلا عن نقل الدم لهؤلاء المرضى.

Objective: To determine the relationship between elevated liver enzymes with iron overload and viral hepatitis in thalassemic patients.

Methods: This descriptive cross-sectional study was carried out in the thalassemic ward of Tonekabon Hospital, Mazandaran, Northern Iran from 20 April to 20 September of 2006. Patients were studied with respect to age, liver enzymes, anti-hepatitis C

virus (anti-HCV) antibody, and hepatitis B surface antigen (HBsAg), transferrin saturation (TSAT), and blood transfusion index (multiplication of frequency and units of transfusion). Alanine aminotransferase (ALT) ≥ 40 U/L was considered elevated.

Result: Sixty-five patients were evaluated (median age 19.51 ± 8.9 years, range 4-54). Eleven patients were anti-HCV positive (16.9%). The mean serum ferritin was significantly higher in patients with ALT ≥ 40 ($2553.08 \mu\text{g/L}$ versus $1783.7750 \mu\text{g/L}$) ($p=0.012$). The mean ALT was significantly higher in patients with TSAT $\geq 60\%$ (41.26 U/L versus 28.82 U/L) ($p=0.021$). The relationship between ALT ≥ 40 and anti-HCV positivity was statistically significant. The mean ALT was 60.91 U/L in anti-HCV positive patients and 39.29 U/L in the negative group ($p=0.001$). The mean serum iron and transfusion index were significantly higher in anti-HCV positive versus negative patients (234.0 versus 195.4815 ; $p=0.02$), (1693.6 versus 1036.29 , $p=0.014$).

Conclusion: Close association between elevated ALT with iron overload, transfusion index, age, and anti-HCV positivity in thalassemic patients of Tonekabon is recommended to re-evaluate transfusion and Desferal doses and therapies other than blood transfusion.

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Thalassemia is a hereditary anemia resulting from defects in hemoglobin production.¹ Beta-thalassemia, which is caused by a decrease in the production of beta-globin chains, affects multiple organs and is associated with considerable morbidity and mortality.² The anemia that is associated with thalassemia may be severe and is accompanied by ineffective erythropoiesis, with bone expansion and extramedullary hematopoiesis in the liver, spleen, and other sites, such as paravertebral masses. Transfusion therapy, which is the mainstay of treatment, allows for normal growth and development and suppresses ineffective erythropoiesis. Iron overload results both from transfusional hemosiderosis and excess gastrointestinal iron absorption. Iron deposition in the heart, liver, and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. The most serious result of iron overload is life-threatening cardiotoxicity, for which chelation therapy is required.³ Approximately 73% of those born with thalassemia can potentially develop iron overload and toxicity from transfusions and/or increased iron absorption. In particular, beta-thalassemia major has a high mortality rate if regular red blood cell transfusions are carried out, but no chelation therapy is available.⁴ The most common and indirect methods for estimating body iron status are serum ferritin and transferrin iron saturation. Usually, serum ferritin levels >2.5 mg/l, transferrin iron saturation >100% and liver iron concentration >7 mg iron per gram liver dry weight suggest the presence of toxic levels of iron in the tissues and the need for the use of a more aggressive chelation therapy regime.⁵ Iron-chelation therapy is largely responsible for doubling the life expectancy of patients with thalassemia major.⁶⁻⁸ Adequate iron chelation is expensive. Therefore, alternatives to blood transfusion and chelation (such as reactivation of HbF, antioxidant agents, stem cell transplantation, gene therapy and other molecular approaches) would be valuable to increase the safety and decrease the costs of thalassemia treatment and to provide effective treatment for patients who do not have access to blood transfusions.^{9,10} Transfusion-transmitted infections (TTI) continue to be a major challenge for blood transfusion organizations across the world. The problem is more serious in the developing countries with lower economic means. Studies demonstrated that hepatitis C virus (HCV) is the most prevalent TTI and remains a major health problem for these patients.¹¹ Hepatitis C virus is a major cause of chronic hepatitis in patients with thalassemia major and is associated with raised aspartate aminotransferase (AST) activity and serum ferritin concentration compared with patients seronegative for anti-HCV.¹² Improved survival of patients with thalassemia has given new importance to adult complications such

as endocrinopathies and hepatitis that have a major impact on the quality of life.¹³ The aim of this study was to determine the relationship between elevated liver enzyme with serum iron status and HCV infection in thalassemic patients.

Methods. In a descriptive cross sectional study, 65 patients with thalassemia major ranging in age from 4-54 years and mean age \pm SD of 19.51 \pm 8.9 years and who were recruited regularly to the thalassemic ward of Tonekabon Hospital, Iran from April to September of 2006 were enrolled. Patients with irregular transfusion and in compliance were excluded. The patients were given transfusions of red cells as needed to raise their hemoglobin level from 8-9 gram per deciliter to 12-14 gram per deciliter. They underwent blood transfusion minimum one and maximum 3 times a week. Each unit of blood transfused was considered to contain 4 mmol (225 mg) of iron. This study included only patients for whom there was reliable information on the use of blood products. At each patient visit, a detailed clinical and laboratory evaluation was taken. All the patients selected for the study, after being fully briefed, gave their informed consent. The project was approved by the Ethics Committee of the Faculty of Medicine, Iran University of Medical Sciences.

Serum iron status was assessed in blood samples obtained from each patient 10 days after blood transfusion to measure the serum iron, serum ferritin and transferrin saturation. Transferrin saturation was calculated as follows: (serum iron/total iron body capacity (TIBC)) x 100 and values were expressed as percentage (%). The transferrin saturation was considered elevated at values \geq 60%. Alanine aminotransferase (ALT, U/L) was determined in serum samples. For comparisons, we considered values of ALT as an index based upon 40 U/L, classifying patients as normal (<40 U/L) or as elevated ALT (\geq 40 UL). Anti-HCV antibody and HBsAg were detected in serum samples by a third-generation enzyme-linked immunosorbent assay. We considered transfusion index expressed as transfusion frequency times the number of red blood cell units transfused in the month. Mean ALT was compared in patients with transferrin saturation \geq 60% to patients with transferrin saturation <60%, and in anti-HCV positive versus anti-HCV negative patients. Also, mean serum iron, serum ferritin, and transferrin saturation was compared with anti-HCV positives and negatives. The K2 test, the t-test, and odds ratio (OR) with 95% confidence intervals were used when appropriate. A *p*-value of 0.01 was considered to indicate statistical significance. The Statistical Package for Social Sciences 14.0 was used for data analysis.

Results. Of 65 patients, 25 (38.5%) had an elevated ALT. Approximately 27.7% had a transferrin saturation of 30-60 and 72.3% had a transferrin saturation of ≥ 60 , and all of patients had a high normal serum ferritin. None of them was HBsAg positive, but 11 (16.9%) patients were anti-HCV positive. In comparison between normal and elevated ALT patients based on iron status parameters and anti-HCV positivity; the mean serum ferritin in elevated ALT (2553.08 $\mu\text{g/L}$) was significantly higher than with normal ALT (1783.775 $\mu\text{g/L}$) [$T_{(63)} = -2.596$; $p=0.012$]. Mean ALT in patients with transferrin saturation ≥ 60 was significantly higher than patients with transferrin saturation 30-60. [$T_{(47)} = 2.37$; $p=0.021$] (Table 1). Nine (81.8%) of elevated ALT patients but only 2 (18.2%) of normal ALT patients were anti-HCV positive therefore we found a significant relationship between elevated ALT and anti-HCV positivity [$\chi^2_{(1)} = 10.86$; $p=0.001$] (Table 1). In comparison, between anti-HCV positive and negative patients; mean age, serum iron, and transfusion index in positive patients were significantly higher than those in negative patients ($p=0.025$, $p=0.02$ and $p=0.014$) (Table 2).

Table 1 - Relationship between undesirable alanine aminotransferase (ALT) with ferritin, transferrin saturation, and anti-hepatitis C virus (anti-HCV).

Parameters	ALT		P-value
	Normal n=40	Elevated n=25	
Ferritin			0.012
Number	39	26	
Mean	1783.7750	2553.0800	
SD	10.86228	1262.72298	
Transferrin saturation			0.021
30-60 (%)	13 (72.2)	5 (24.8)	
≥ 60 (%)	27 (57.4)	20 (42.6)	
Anti-HCV			0.001
Positive	2 (18.2)	9 (81.8)	
Negative	38 (71.4)	16 (29.6)	

Discussion. We investigated the relationship between elevated liver enzyme (ALT) and iron overload and viral hepatitis in thalassemia major patients; however, a bigger sample size may detect more reliable association. We found that 27.7% of patients had TS of 30-60 and 72.3% of them had TS of ≥ 60 and all patients had a high normal serum ferritin. Silva et al¹⁴ reported serum iron was elevated in 28%, FS in 27%, and transferrin saturation in 12.5% of patients. In our study, the mean serum ferritin in elevated ALT patients was significantly higher than those with normal ALT. Mean ALT in patients with transferrin saturation ≥ 60 was significantly higher than those patients with transferrin saturation 30-60. At multivariate analysis, Parti et al¹⁵ showed that necroinflammation was related to the increased serum aminotransferases and higher iron stores including serum ferritin ($p<0.05$). Significant fibrosis and its progression is mostly influenced by iron overload. Ruhl and Everhart's¹⁶ study suggested that abnormal ALT levels ($p<0.05$) included higher transferrin saturation and iron. Worwood et al¹⁷ showed a high correlation between serum ferritin concentration and ALT activity. In our study, we found a significant relationship between elevated ALT and anti-HCV positivity. Maier et al¹⁸ showed that the most common causes of elevated amino transferase levels are chronic hepatitis B and C. Wu et al¹⁹ study suggested that increased ALT's levels occurred more frequently in hepatitis C positive thalassemia major patients. Chang et al²⁰ showed that the prevalence of raised ALT and AST (≥ 45 IU/liter) in the HCV-positive group was more significant than in the negative group. Wang et al²¹ showed a strong positive correlation between the prevalence of an elevated ALT level and anti-HCV positivity. Wang et al²² showed that subjects with elevated ALT levels were more likely to be seropositive for anti-HCV. The serum level of alanine aminotransferase was found to be significantly altered between the 2 groups of HCV-infected and non-infected thalassemic patients in Chakravarti and Verma's²³ study. In our study, mean age, serum iron, and

Table 2 - Relationship between anti-hepatitis C virus (anti-HCV) positivity with age, serum iron, and transfusion index.

Parameters	Anti-HCV		P-value
	Positive	Negative	
Age (n)	11	54	0.025
Mean \pm SD	28.27 \pm 9.634	17.42 \pm 7.718	
Serum iron (n)	11	54	0.02
Mean \pm SD	234.0000 \pm 49.48939	195.4815 \pm 48.69068	
Transfusion index (n)	11	54	0.014
Mean \pm SD	1693.60 \pm 716.357	1036.29 \pm 7558.237	

transfusion index in anti-HCV positive patients (28.27 years, 234.00µg/dl and 1693.60) were significantly higher than the negative group. Wanachiwanawin et al²⁴ showed that there was no significant relationship between the presence of anti-HCV antibodies and the number and frequency of blood transfusions. The findings of Ocak et al²⁵ indicated that patients who were anti-HCV positive had a significantly higher mean number of blood transfusions and peak serum alanine transaminase level than anti-HCV-negative patients. Gattoni et al²⁶ showed that patients with elevated serum iron markers have more chronic hepatitis. The prevalence of hepatitis viruses and raised ALT levels are found to be significantly associated with the increasing age and number of blood units transfused to them that was shown in Jaiswal's et al²⁷ study. Mirmomen et al²⁸ in a univariate analysis showed that beta-thalassemia major ($p=0.01$), older age ($p=0.001$), longer transfusion duration ($p=0.000$), HBsAg seropositivity ($p=0.03$), and higher serum ferritin level ($p=0.002$) were significantly associated with a higher prevalence of HCV.

It is concluded that HCV is a major cause of chronic hepatitis in patients with thalassaemia major and is associated with raised ALT activity, serum iron concentration, and transfusion index compared with patients seronegative for anti-HCV. The findings suggest that both HCV and iron overload are the main causes of abnormal liver function in patients with thalassemia. The treatment of both problems, if coexisting in patients with thalassemia, is required to prevent progression to chronic liver disease. We recommended to reevaluate transfusion and desferal doses and therapies other than blood transfusion in these patients.

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Related topics

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