

# Hypocholesterolemia in children and adolescents with $\beta$ -thalassemia intermedia

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**Objectives:** To conduct a prospective study to evaluate the lipid profile in children and adolescents with  $\beta$ -thalassemia intermedia and major, and to examine the contribution of different factors to hypocholesterolemia observed in these patients.

**Study design:** Demographic, clinical, and laboratory data were prospectively obtained from patients with  $\beta$ -thalassemia intermedia (n = 9) and major (n = 47). Lipid profiles were also determined in a control group of healthy children (n = 18). Lipid values of  $\beta$ -thalassemics and controls were compared and the relationships between lipid levels and different covariates were determined.

**Results:**  $\beta$ -thalassemia intermedia patients had significantly lower total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) compared with  $\beta$ -thalassemia major and controls ( $P < .001$ ). With regression analysis, serum lipid levels (TC, HDL-C, and triglycerides) correlated with diagnosis ( $\beta$ -thalassemia major or intermedia) but not with age, sex, hemoglobin, or ferritin. LDL-C was influenced by both diagnosis and ferritin levels.

**Conclusions:** Children and adolescents with  $\beta$ -thalassemia intermedia have significantly lower cholesterol levels than patients with  $\beta$ -thalassemia major. This is related to their disorder and not influenced by age, sex, hemoglobin, or ferritin levels. In these patients, needless investigations for hypolipidemia should be avoided. (*J Pediatr* 2002;141:543-7)

$\beta$ -thalassemia intermedia is a clinically defined syndrome applied to  $\beta$ -thalassemia patients who present a milder clinical course than patients with  $\beta$ -thalassemia major. Although mild organ

involvement (bone abnormalities, splenomegaly, and iron overload) may be present, in contrast to thalassemia major patients, these patients are not dependent on regular blood transfusions.<sup>1</sup>

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Low plasma levels of total cholesterol (TC) with or without hypertriglyceridemia have been frequently described in a variety of hematologic disorders in which anemia is a prominent feature.<sup>2,3</sup> It is well established that  $\beta$ -thalassemia is associated with changes in plasma lipids and lipoproteins. In  $\beta$ -thalassemia major, low cholesterol levels caused by a significant reduction of both low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels have been consistently reported, whereas reports on triglycerides (TG) levels were discordant. TC and LDL-C seemed similarly decreased in  $\beta$ -thalassemia trait carriers compared with controls, whereas HDL-C and TG blood levels were usually not modified.<sup>4-12</sup>

HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
TC	Total cholesterol
TG	Triglyceride

Several patients with  $\beta$ -thalassemia intermedia referred to the Pediatric Gastroenterology and Nutrition Clinic for evaluation of very low TC levels prompted us to investigate the lipid profile of these patients and determine whether lipid levels in  $\beta$ -thalassemia intermedia are different from those observed in  $\beta$ -thalassemia major, and evaluate the factors correlated to the hypocholesterolemia observed in  $\beta$ -thalassemia.

## METHODS

### Patients

The study population included patients with  $\beta$ -thalassemia intermedia (n = 9) and  $\beta$ -thalassemia major (n = 47) followed at 3 major referral centers in

**Table I.** Demographic and clinical characteristics of thalassemia patients

	Thalassemia major (n = 47)	Thalassemia intermedia (n = 9)
Age (y) (range)	11 ± 4.2 (4-19)	10.3 ± 5.1 (4-20)
Sex (F/M)	26/21	5/4
Israeli Arabs/Jews	45/2	8/1
Splenectomy (n)	12/47	0/9
Hemoglobin (g/dL) <sup>o</sup>	8.6 ± 0.9	7.5 ± 1.2
Ferritin µg/L <sup>†</sup>	3201 ± 1786	215 ± 237
Transfusions/year	21 ± 4	None
Desferoxamine (mg/kg/day)	29.6 ± 11.6	None
ALT (U/L)	52 ± 48	38 ± 38
Albumin (g/L)	4.3 ± 0.3	4.5 ± 0.25
Folic acid (ng/mL)	7.1 ± 3.4	5.9 ± 1.8
Vitamin B <sub>12</sub> (pg/mL)	379 ± 155	386 ± 196

Mean ± SD. Normal ferritin values 25 to 370 µg/L.  
<sup>o</sup>P = .002.  
<sup>†</sup>P < .001.

Israel. The diagnoses of  $\beta$ -thalassemias intermedia and major were made based on the clinical, hematologic, and hemoglobin electrophoresis profiles and the results of  $\beta$ -globin chain synthesis.  $\beta$ -Thalassemia intermedia was diagnosed based on accepted criteria, including an abnormal blood smear, anemia, and splenomegaly more than expected for silent or  $\beta$ -thalassemia trait, together with older age at the time of diagnosis and satisfactory growth in the absence of regular transfusions, which distinguish these children from those with  $\beta$ -thalassemia major. Prospectively obtained data include age, sex, medical history (blood transfusions, amount of desferoxamine, and history of splenectomy), physical examination, dietary evaluation, and blood levels of hemoglobin, ferritin, albumin, folic acid, vitamin B<sub>12</sub>, liver enzymes and fasting lipid levels.

A group of healthy Israeli Arab children (n = 18) with a similar sex and age distribution as the  $\beta$ -thalassemia intermedia patients, were selected among patients with functional abdominal pain followed at the Pediatric Gastroenterology and Nutrition Unit at Rambam Medical Center, and their fasting serum lipid levels were determined. The ethic

committee approved the study protocol, and informed consent for this study was obtained for all study participants.

### Methods

Serum lipid profile was determined in all participants after an overnight fast of 12 hours. TC and TG were measured by enzymatic-colorimetric methods.<sup>13,14</sup> Direct measurement of HDL-C was done by using polyethylene glycol-modified enzymes as described previously.<sup>15</sup> LDL-C levels were calculated with the Friedewald equation, except when serum TG concentrations were >400 mg/dL.<sup>16</sup>

### Statistical Analysis

Statistical analysis was performed with SPSS 9.0 software (SPSS Inc, Chicago, Ill). Raw TG serum levels were skewed to the right, but the log transformation resulted in an acceptable normal distribution of values. Thus, log of serum TG values was used in the analyses. Mean lipid values in  $\beta$ -thalassemic and controls were compared by analysis of variance (after logarithmic transformation) and/or nonparametric analysis (Kruskal-Wallis test for highly skewed variables or small groups) when indicated. Pearson's correlations were used to determine the relationship be-

tween covariates. Multiple regression analysis was used to determine related variables controlled for covariates (including age, sex, hemoglobin, ferritin, and diagnosis).

## RESULTS

### Patients

The groups were homogenous with regard to age, sex, and ethnic origin (Table I). The average pretransfusion hemoglobin level and serum ferritin level were significantly higher in the  $\beta$ -thalassemia major group compared with the thalassemia intermedia group.  $\beta$ -Thalassemia major patients were frequently transfused and regularly chelated with desferoxamine. In the absence of symptoms and signs of severe anemia or growth failure, children with  $\beta$ -thalassemia intermedia were not transfused on a regular basis, in spite of their low hemoglobin levels, to avoid iron overload.

The first patient with  $\beta$ -thalassemia intermedia, brought to our attention because of hypocholesterolemia, was a 15-year-old boy. His TC and TG values were 46 mg/dL and 88 mg/dL, respectively; hemoglobin was 7.3 g/dL and ferritin was 250 mg/dL. He had no special complaints and except for pallor and mild splenomegaly, his physical examination was normal. His weight and height were in the 25th percentile. He had normal liver aminotransferases, vitamins A and E, normal vitamin B<sub>12</sub>, folic acid, zinc, and carotene serum levels. This patient underwent a thorough work-up in search of a cause for hypocholesterolemia that included evaluation for malnutrition, fat malabsorption, liver disorders, and hyperthyroidism without pathologic findings. In addition, an upper gastrointestinal endoscopy with biopsies from the duodenum before and after a fat meal test was done.<sup>17,18</sup> Small bowel biopsies were normal. There was no fat accumulation within enterocytes 5 hours after the fat load and a normal 2-fold increase of serum triglycerides was observed.

Subsequent patients with  $\beta$ -thalassemia intermedia and low cholesterol did not undergo endoscopy or a fat load test, which were judged not necessary.

### Serum Lipids

As seen in Table II,  $\beta$ -thalassemia intermedia patients had significantly lower TC, HDL-C, and LDL-C compared with the  $\beta$ -thalassemia major group and controls ( $P < .001$ ), whereas TG levels were higher than control levels but lower than those of  $\beta$ -thalassemia major patients.

**$\beta$ -THALASSEMIA MAJOR.** Within the  $\beta$ -thalassemia major group, patients with splenectomy were significantly older than patients without splenectomy ( $13 \pm 3$  vs  $10 \pm 4$ ,  $P < .03$ ), and their LDL-C levels were higher ( $58 \pm 20$  vs  $41 \pm 20$ ,  $P < .02$ ). TC, TG, and HDL-C, ferritin, and hemoglobin levels were not different in patients with or without splenectomy. Within the group as a whole, TC, HDL-C, and TG serum levels did not correlate with age, gender, hemoglobin or ferritin serum levels; however, there was a significant correlation between LDL-C and ferritin (Pearson's correlation).

**$\beta$ -THALASSEMIA INTERMEDIA.** Within this group, no correlation existed between TC, HDL-C, LDL-C, and TG levels and age, sex, hemoglobin, or ferritin serum levels.

With the use of multiple regression analysis, we found that serum levels of TC, HDL-C, and TG were influenced only by diagnosis ( $\beta$ -thalassemia major or  $\beta$ -thalassemia intermedia) and not by age, sex, hemoglobin, or ferritin. LDL-C was influenced by both diagnosis and ferritin levels.

## DISCUSSION

The current study focuses on the observation that children and adolescents with  $\beta$ -thalassemia intermedia have significantly lower lipid blood levels than

**Table II.** Serum lipid and lipoprotein (mean  $\pm$  SD) levels of  $\beta$ -thalassemia patients and controls

Parameter	Thalassemia major (n = 47)	Thalassemia intermedia (n = 9)	Controls (n = 18)	P value*
TC (mg/dL)	106 $\pm$ 23	74 $\pm$ 24	155 $\pm$ 25	< .0001
TG (mg/dL)	154 $\pm$ 83	103 $\pm$ 41	73 $\pm$ 28	< .0001 <sup>†‡</sup>
HDL-C (mg/dL)	29 $\pm$ 8	18 $\pm$ 7	53 $\pm$ 13	< .0001
LDL-C (mg/dL)	45 $\pm$ 21	40 $\pm$ 23	87 $\pm$ 24	< .0001

\*Significant differences observed between all groups by using analysis of variance.  
<sup>†</sup>Test was performed on log TG.  
<sup>‡</sup>Kruskal-Wallis test.

patients with  $\beta$ -thalassemia major. Awareness to this finding may save unnecessary evaluation for malnutrition or hypolipidemia in patients with  $\beta$ -thalassemia intermedia who have lipid values in the abetalipoproteinemia range, as was done in our first patient.

The existence of hypocholesterolemia in all phenotypes of  $\beta$ -thalassemia has been reported since the beginning of the 20th century, and several studies showed that compared with healthy controls, cholesterol levels are lower in homo- and heterozygous  $\beta$ -thalassemia.<sup>4-12</sup> Our results are in agreement with previous studies demonstrating lower lipid levels in patients with  $\beta$ -thalassemia compared with nonthalassemic controls.

The general knowledge is that  $\beta$ -thalassemia major patients have the lowest cholesterol levels, whereas  $\beta$ -thalassemia trait carriers have less depressed levels. Thus, one would expect that intermediate lipid levels would be found in  $\beta$ -thalassemia intermedia. With one exception, however, previous studies that recorded cholesterol values in  $\beta$ -thalassemia intermedia, related lipid values only to healthy controls.<sup>19-21</sup> The one study to examine lipid levels in both  $\beta$ -thalassemia intermedia and  $\beta$ -thalassemia major patients found that TC and LDL-C levels were lower  $\beta$ -thalassemia intermedia compared with  $\beta$ -thalassemia major, whereas HDL-C was higher. Nevertheless no comments were made on this observation.<sup>11</sup>

Because the only differences between our  $\beta$ -thalassemic patients were their hemoglobin (lower in  $\beta$ -thalassemia intermedia) and ferritin levels (higher in  $\beta$ -thalassemia major), it was reasonable to presume that the distinction in cholesterol levels would be related to these variances. However, unexpectedly, the levels of all serum lipids except LDL-C (which was influenced by both group and ferritin) were independent of age, sex, hemoglobin, and ferritin levels either when examined in the  $\beta$ -thalassemia major and  $\beta$ -intermedia groups separately or in both  $\beta$ -thalassemic groups together. Actually, the only factor consistently correlated with the lipid levels was group affiliation (meaning, the disease itself). Taken together these observations raise again the question of the pathophysiology of hypocholesterolemia in  $\beta$ -thalassemia.

The pathophysiology of hypocholesterolemia observed in  $\beta$ -thalassemia patients is obscure, and several mechanisms were proposed as explanation for the reported lipid abnormalities. Anemia, liver dysfunction, increased cholesterol consumption by the bone marrow in the process of increased but ineffective erythropoiesis, and an overrepresentative and overactive reticuloendothelial system were successively advanced as explanations of hypocholesterolemia in  $\beta$ -thalassemia.<sup>2,4,10</sup> The existence of a state of oxidative stress as reflected by the measurement of peroxidation products and depletion of lipid-soluble antioxidants, possibly related to the

presence of unpaired hemoglobin chains and abnormal iron homeostasis, has been demonstrated in  $\beta$ -thalassemia major patients. This is supported by recent *in vitro* studies in which oxidative interactions of unpaired hemoglobin chains with LDL ApoB and serve as triggers of oxidative modification of LDL-C.<sup>22,23</sup> Such oxidation processes could conceivably alter the metabolic behavior of both LDL-C and HDL-C and result in increased uptake of the 2 modified lipoproteins by already hyperplastic macrophage/monocytes system (scavenger pathway).<sup>20,21</sup>

The absence of any significant relationship between lipid levels (with the exception of LDL-C and ferritin in thalassemia major), hemoglobin, and ferritin levels, implies that absence of transfusions (iron overload) in  $\beta$ -thalassemia intermedia was not protective against hypocholesterolemia and, consequently, that an accelerated erythropoiesis is significantly implicated in hypocholesterolemia even more than iron oxidative stress. An additional protective factor, present only in  $\beta$ -thalassemia major patients was chelation therapy with desferrioxamine because this treatment was shown to inhibit hemoglobin-induced LDL-C oxidation independent of its iron-chelating property.<sup>23</sup>

The observation of correlation between LDL-C levels and ferritin in  $\beta$ -thalassemia major is in agreement with studies in the general population that demonstrates a significant positive correlation between ferritin and LDL-C blood levels.<sup>24,25</sup> In addition, in patients with  $\beta$ -thalassemia major, it may reflect the high susceptibility of this lipid fraction to the effects of extreme iron overload and peroxidation stress. It is possible that different mechanisms have dissimilar weight in the various  $\beta$ -thalassemia phenotypes; whereas in  $\beta$ -thalassemia major, severe iron overload and oxidative stress may be the dominant mechanisms implicated in hypocholesterolemia; in  $\beta$ -thalassemia intermedia where iron overload is negligible, accel-

erated erythropoiesis and enhanced cholesterol consumption may be more significant. In this study, there was a constant association with the group diagnosis. However, it is impossible to state whether the consistently lower cholesterol levels observed in  $\beta$ -thalassemia intermedia are secondary to the disturbances induced by the alterations in globin synthesis and erythropoiesis, or perhaps to an unknown linkage to a mechanism involved in the regulation of plasma cholesterol. We had few patients with thalassemia intermedia; however, this is certainly not an incidental observation and further investigation of the mechanisms involved in the pathophysiology of hypocholesterolemia in  $\beta$ -thalassemia of all phenotypes is warranted.

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### *50 Years Ago in The Journal of Pediatrics*

#### **POSTURAL AND RIGHTING RESPONSES IN CHILDREN**

*Silver AA. J Pediatr 1952;41:493-8*

Pathologic tonic neck reflexes and neck righting abnormalities may be observed in infants and children with severe central nervous system (CNS) impairment. Silver reported a technique to elicit remnants of these reflexes in older children (over 5 years of age) with learning disabilities and emotional disorders. With the child standing in an "arms extended" position with eyes closed, the subject's head is rotated passively to one side as far as possible without discomfort (stimulus), and both trunk rotation and arm movements (responses) are observed. Exaggerated responses to this procedure are correlated with CNS organicity, seizures, and psychologic problems such as schizophrenia, obsessive compulsive disorder, and phobias, reading disorders, and abnormalities on perceptual motor tests.

Objective data on the sensitivity and specificity for the results of this test with the author's large case experience at Bellevue Psychiatric Hospital are not reported in this paper. Neither has the maneuver survived as a component of any standardized or routine neurologic "soft sign" battery. However, since the child undergoing a neurodevelopmental examination is frequently placed in the "arms extended"/eyes closed position, this represents an easy and potentially informative addition to the pediatric neurologic examination.

With current emphasis on reading disorder as almost exclusively a phonologic processing (language-based) disorder, the neuromotor and perceptual correlates of dyslexia are often overlooked. This is similar to the minimal neurologic and minor dysmorphic features that frequently reflect the organic basis for the child's increased vulnerability to a variety of mental health problems. Delayed maturation of postural reflexes uncovered by Silver's head positioning stimulus may indicate a more generalized CNS maturational delay as contributing to both learning and emotional problems in children.

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