Serum levels of lipids and lipoproteins in Syrian patients with β–thalassemia major

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ABSTRACT

Objective: Evaluation serum lipids, lipoprotein (a), apolipoprotein A1, apolipoprotein B and total antioxidant status (TAS) in syrian patients with β-thalassemia major.

Methods: This study was carried out at Damascus University (Biochemical Laboratories of Medicine and Pharmacy Colleges), Syria between May 2002 and April 2003. This study included 30 patients with β-thalassemia major, aged between 1.5 and 16-years. All patients had undergone regular blood transfusions and iron chelation therapy (through thalassemia center, Damascus, Syria); also 30 control subjects matched for age were studied. Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, apolipoprotein A1 (apo A1), apolipoprotein B (apo B), lipoprotein (a) [Lp(a)] and total antioxidant status (TAS) were determined. Blood samples were withdrawn after at least 12-hours of patients'fasting and before the blood transfusion.

Results: β-thalassemia major patients had significantly lower total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and lower density lipoprotein (LDL-C) compared with control (P<0.0001, P<0.0001, P<0.003). While serum triglyceride (TG) and lipoprotein (a) [Lp(a)] levels were higher in β-thalassemia patients than in controls (P<0.0001). The reduction was significant (P<0.0001), in apolipoprotein A1 (apo A1) but not significant (P=0.537) in apo B serum levels, in patients compared to control subjects. Total antioxidant status (TAS) values were lower in β-thalassemia major patients than in controls.

Conclusion: The results might suggest that β-thalassemia may represent an interesting metabolic model: anemia, an activated macrophage system and defective liver function seem interrelated to the final serum lipoprotein pattern. This suggests that antioxidants counteract lipid peroxidation processes and have a protective effect against oxidative damage of red cells of β-thalassemia patients.

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Patient with beta thalassemia (β-thalassemia) major are at risk of an iron overloading in various organs, which is through repeated blood transfusion and increased iron absorption from the gastrointestinal tract.[1] Iron overload may particularly cause injury to the heart liver and endocrine glands. Iron-induced liver injury is often characterized by the development of fibrosis and eventually, cirrhosis.[2] In β-thalassemia major, liver damage accounts for the low total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) serum levels.[3] Moreover, it is known that severe chronic liver disease is characterized both by low total and LDL-cholesterol level[4] and by decrease in HDL-cholesterol.[5] The LDL-lowering effect of β-thalassemia major may be related to 1. The mild erythroid hyperplasia, which would increase the LDL removal by the bone marrow, and 2. The chronic activation of the monocyte-macrophage system, causing an increased secretion of some cytokines (interleukin-1, interleukin-6, and tumor necrosis factor-α) Known to affect to hepatic secretion and the receptor mediated removal of

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apolipoprotein B-containing lipoproteins.[6] Lipoprotein (a) [Lp(a)] is a LDL-like particle, which has been associated with increased cardiovascular risk.[7] Its characteristic constitutive proteins are apolipoprotein B and apolipoprotein (a) [apo (a)] joined by disulfide bonds. The metabolic pathways of Lp(a) are still incompletely understood. Studies in liver transplanted patients suggest that it is directly synthesized and secreted by the liver.[8] It is well documented that disturbances of oxidant –antioxidant balance occur disease.[9] Also, lipid peroxidation is enhanced in patients with β-thalassemia.[10]

To our knowledge no data are available on lipids serum concentrations in syrian patients with β-thalassemia major. This lack of information prompted us to determine the level of lipids from patients with β-thalassemia major in comparison to controls, to evaluate the lipid profile and its significance in syrian patients with β-thalassemia major.

Methods. Thirty patients with β-thalassemia major were studied, age 1.5-16-years (mean, 8.80 ± 4.05 years): 18 females (mean age, 9.25 ± 4.37 years) and 12 males (mean age, 8.13 ± 3.58 years). Another 30 control subjects matched for sex and age were studied too, age 3.5 to 14 years (mean, 9.05 ± 2.70): 16 females (mean age, 9.22 ± 2.23 years) and 14 males (mean age, 8.86 ± 3.24 years). This study was conducted over an 11-month period (May 2002 through to April 2003), and it was carried out in the Biochemical Laboratories of the Medicine and Pharmacy Colleges, at Damascus University, Syria. According to age, most subjects of both control and patient groups were into the 2 age groups: 6-10 years old (18 subjects for controls and 13 subjects for patients), and 11-15 years old (9 subjects for controls and 10 subjects for patients). All thalassemic patients were attending thalassemia center (Ministry of Health, Damascus, Syria) for receiving frequent blood transfusion in order to maintain there hemoglobin concentration above 9 g/dl, and were under regular chelation therapy with desferrioxamine. Blood samples were taken after an overnight fast (12-14-hours.) and serum was obtained by centrifugation. Concentrations of glucose, TC and triglycerides (TG) were determined enzymatically by using commercial analytical kits form BioMerieux (RCS Lyon, France). The HDL-C was measured after precipitation of other lipoproteins by addition of phosphotungstic acid in the presence of magnesium ions, using kit from BioMerieux (RCS Lyon, France). The levels of LDL-C were estimated by calculation using the formula of Friedewald and Levy[11] LDL-cholesterol=(TC)-(TG/5)-(HDL cholesterol). Apolipoprotein A1 and B (Apo A1 and Apo B) were measured by turbidimetric immunoassay. These assays were performed in agglutination with the anti-Apo A1 or anti-Apo B antibodies in the reagent using a commercially available kits from Human (Wiesbaden, Germany). Serum lipoprotein (a) [Lp (a)] was determined quantitatively by latex-enhanced turbidimetric test, using the kit from Human (Wiesbaden, Germany). Total antioxidant status (TAS) was evaluated by using a commercial analytical kit from Randox (Antrim, United kingdom). All results are expressed as means ± standard deviation (SD). Comparison between controls and thalassemic patients were performed by using Student's t-test. Relationships were considered significant if the corresponding *P* value was lower than 0.05.

Results. Serum lipid levels in the control subjects and in the β-thalassemic patients are shown in **Table 1**. Serum total cholesterol, HDL-C and LDL-C levels were significantly lower in-patients with β-thalassemia major (119.10 ± 37.72, 28.37 ± 11.93 and 70.57 ± 36.71) than the levels in the control group (163.00 ± 9.38, $p<0.0001$, 35.37 ± 2.91, $p=0.003$ and 114.64 ± 9.71, $p<0.0001$). Triglycerides level was higher in β-thalassemic patients (97.30 ± 31.85) than in controls (63.13 ± 9.06, $p<0.0001$). Lipoprotein a [Lp(a)], apolipoprotein A1 (Apo A1), apolipoprotein B (Apo B) and total antioxidant status levels in serum of patients and control subjects are shown in **Table 2**. Beta-thalassemic patients showed a reduction in Apo A1 and Apo B serum levels compared to normal subjects (-23.9%, $p<0.0001$, and –3.9%, $p=0.537$); TAS values were slightly but significantly lower in β-thalassemia patients (1.20±0.61 mmol/L) than in controls (1.44 ± 0.15 mmol/L) ($p< 0.0001$). Lipoprotein (a) serum levels were higher in patient (30.97 ± 7.79 mg/dl) than control subjects (1.25 ± 0.81, $p<0.0001$), but they were into the normal values of used kit (up to 30 mg/dl). Serum fasting glucose levels were into normal values in both thalassemic and control groups (86.53 ± 11.75 and 83.60 ± 9.53). These findings were confirmed in the group as a whole and in females and males separately. Triglycerides, LDL-C, HDL-C, Apo A1 and Apo B were significantly lower and TG higher in females and males of homozygous β–thalassemic patients than in controls (**Tables 3 & 4**). Difference of lipid levels was not significant ($P>0.05$) between the 2 age groups (6-10 and 11-15-years-old) of controls or patients. But, that difference was significant by comparison lipid levels of controls and patients in the same age group ($P<0.05$). A strong positive correlation was found between serum LDL-C and TC (r=0.93; $p<0.0001$) (**Figure 1**) in thalassemia patients. No correlation existed between either Lp(a) or serum Apo B and the TC of

Table 1 - The mean (±SD) lipid profile in patients with β-thalassemia major and control subjects.

Group	n	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Triglycerides (mg/dl)
β-thalassemia major	30	119.10 ± 37.72	28.37 ± 11.93	70.57 ± 36.71	97.30 ± 31.85
Control	30	163.00 ± 9.38	35.37 ± 2.91	114.60 ± 9.71	63.13 ± 9.06
Significance		*P*<0.0001	*P*=0.003	*P*<0.0001	*P*<0.0001

HDL-C - high density lipoprotein cholesterol
LDL-C - low density lipoprotein cholesterol

Table 2 - Lipoprotein (a), Apo A1, Apo B, TAS (mean ±SD) serum concentration in patients with homozygous β-thalassemia and controls.

Group	Lp(a) (mg/dl)	Apo A1 (mg/dl)	Apo B (mg/dl)	TAS (mmol/dl)
β-thalassemia major	30.97 ± 7.79	109.70 ± 19.58	87.70 ± 22.36	1.20 ± 0.16
Control	1.25 ± 0.81	144.17 ± 21.92	91.27 ± 22.96	1.44 ± 0.15
Significance	*P*<0.0001	*P*<0.0001	*NS	*P*<0.0001

Lp (a) - lipoprotein (a), Apo A1 - Apolipoprotein A1, Apo B - Apolipoprotein
TAS - total antioxidant status, NS - non significant

patients. The serum Apo A1 of thalassemia patients showed a strong positive correlation with HDL-C (r=0.71, *p*<0.0001) (**Figure 2**). No correlation found between Apo B, TG or serum TC and Lp(a) concentrations of patients. Apo B: LDL-C ratio was higher in β–thalassemia major patients (1.371 ± 0.593 mmol/L) than in controls (1.005 ± 0.214 mmol/L, *P*<0.001) (data not shown).

Discussion. We observed low total serum cholesterol, low HDL-cholesterol and low LDL-cholesterol with elevation of triglycerides in β–thalassemia major patients, as compared to control subjects. Our results agree with previous findings with regard to the above altered serum lipid pattern[3,12-14] in patients with β–thalassemia major. This alteration is likely due to diminished hepatic biosynthesis as of anemia and iron overload, while a reduced extrahepatic lipolytic activity could account for the rise in circulating TG.[15] Lipoprotein (a) and Apo A1 levels were significantly lower, also Apo B was slightly lower but non-significant, in β–thalassemia major patients than in age and sex matched controls. These results are in agreement with those described previously[3,13,15] where patients with β–thalassemia major showed low Lp (a) and Apo A1 levels. It has been suggested that non-specific receptor uptake by activated monocytes or macrophages could represent a main route of Lp (a) clearance, and these cells have been identified in β-thalassemia.[3] Our finding of an increased Apo B: LDL-C ratio in β–thalassemia patients corroborates the hypothesis that iron-loading and the effects of repeated hemotransfusions, induce an hepatic acute-phase response, which could determine an LDL-class shift towards protein-rich, denser particles.[16] With regard to total antioxidants, we observed a clear decrease in thalassemic patients compared to control subjects. This is supported by previous studies[17,18] in which the plasma levels of antioxidants are greatly reduced in case of oxidative stress, which is a consequence of the disease process in β-thalassemia. This suggests that the use of antioxidants (like vitamin E, ascorbic acid and xanthin oxidase inhibitors) may have a protective effect improving red cell survival. Also, iron supplements and oxidative drugs should be avoided for the patients of β-thalassemia who receive a regularly blood transfusions. Using appropriate iron chelators (deferoxamine and deferiprone) will eliminate oxidative red cell damage in β-thalassemia.[9] The mechanism of inhibitory effects of these chelators on free radical processes is due to the oxidation of ferrous inside and ferrous ion-chelator complex. Antioxidant supplements have a protective effect against oxidative damage of

Table 3 - The mean (±SD) lipid-lipoprotein profile in female patients with β-thalassemia major (n=18) and female control subjects (n=16).

Group	TC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	Apo A1 (mg/dl)	Apo B (mg/dl)	Apo TG (mg/dl)
β-thalassemia major	127.39 ± 45.15	84.06 ± 40.92	24.50 ± 11.73	106.39 ± 19.16	91.67 ± 18.05	88.39 ± 32.74
Control	160.25 ± 9.06	112.19 ± 9.80	35.69 ± 2.25	145.63 ± 19.62	86.50 ± 24.40	61.38 ± 10.44
Significance	*P*<0.016	*P*=0.015	*P*=0.003	*P*<0.0001	NS	*P*=0.011

TC - triglycerides, HDL - high-density lipoprotein, LDL - low-density lipoprotein, APO - apolipoprotein, NS - not significant

Table 4 - The mean (±SD) lipid-lipoprotein profile in male patients with β-thalassemia major (n=12) and male control subjects (n=14).

Group	TC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	Apo A1 (mg/dl)	Apo B (mg/dl)	Apo TG (mg/dl)
β-thalassemia major	106.67 ± 17.89	50.33 ± 14.87	34.17 ± 10.07	114.67 ± 19.95	81.75 ± 27.40	110.67 ± 26.33
Control	166.14 ± 9.05	117.36 ± 9.18	35.00 ± 3.35	142.50 ± 24.95	96.71 ± 20.72	65.14 ± 7.03
Significance	*P*<0.0001	*P*<0.0001	NS	*P*=0.024	NS**	*P*<0.0001

TC - triglycerides, HDL - high-density lipoprotein, LDL - low-density lipoprotein, APO - apolipoprotein, NS - not significant

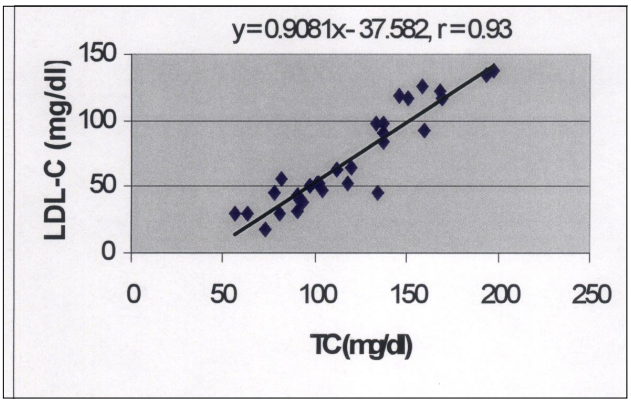

Figure 1 - Correlation between serum low-density lipoprotein-C and total cholesterol in patients with β-thalassemia major.

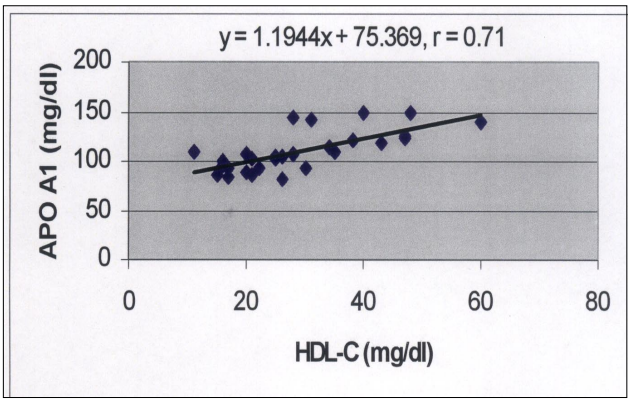

Figure 2 - Correlation between serum apolipoprotein Al and high-density-C in patients with β-thalassemia major.

red cell membrane of β-thalassemia patients. It is assumed a promising result when antioxidants and iron chelating drugs are administered simultaneously to thalassemic patients, but drug interactions have to be considered.

In this study, we have had the chance to ascertain the altered lipoprotein pattern in syrian β-thalassemia major patients. We found, as it well established that β−thalassemia has a major impact on serum lipids and lipoproteins, and it may represent an interesting metabolic model: anemia, an activated macrophage system and defective liver function seem variably interrelated to the lipoprotein pattern. The oxidative denaturation of the abnormal red blood cells in thalassemia could be a source of excess oxygen free radicals. Such oxidation process could alter the metabolic behavior of LDL and HDL and result in increased uptake of the two modified lipoproteins by macrophages. Blood transfusion and iron overload would affect the oxidative status LDL in β-thalassemia major patients. It is suggested that iron chelators and antioxidant supplements improve antioxidant/oxidant balance, LDL particles and red blood cells in β−thalassemia patients.

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