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Distribution of serum lipids and lipoproteins in patients with beta thalassaemia major; an epidemiological study in young adults from Greece

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Abstract

Background: Beta-thalassaemia major (b-TM) has been defined as a combination of chronic hemolytic anemia, iron storage disease and myocarditis, and it has been associated with premature death especially due to heart failure. To the best of our knowledge the status of blood lipids in these patients has rarely been investigated. Thus, we assessed the levels of lipids and lipoproteins in a sample of cardiovascular disease free adult men and women with b-TM.

Methods: During 2003 we enrolled 192 consecutive patients with b-TM that visited our Institution for routine examinations. The Institution is considered the major reference center for b-TM in Greece. Of the 192 patients, 88 were men (25 ± 6 years old) and 104 women (26 ± 6 years old). Fasting blood lipid levels were measured in all participants.

Results: Data analysis revealed that 4% of men and 2% of women had total serum cholesterol levels > 200 mg/dl, and 11% of men and 17% of women had triglyceride levels > 150 mg/dl. In addition, mean HDL cholesterol levels were 32 ± 11 mg/dl in men and 38 ± 10 mg/dl in women, lipoprotein-a levels were 8.3 ± 9 mg/dl in men and 8.8 ± 9 mg/dl in women, apolipoprotein-A1 levels were 111 ± 17 mg/dl in men and 123 ± 29 mg/dl in women, and apolipoprotein-B levels were 60 ± 20 mg/dl in men and 59 ± 14 mg/dl in women. Total-to-HDL cholesterol ratios were 3.7 ± 1.2 and 3.8 ± 1.5 in men and women, respectively.

Conclusions: The majority of the patients had blood lipid levels (by the exception of HDL-cholesterol) within the normal range, and consequently the prevalence of lipid and lipoprotein abnormalities was much lower as compared to the general population of the same age. Interestingly, is that the total – to HDL cholesterol ratio was high in our patients, and may underline the importance of this index for the prognosis of future cardiac events in these patients.

Introduction

Beta thalassaemia major (b-TM) is a very serious blood condition since individuals with it are unable to make enough healthy red blood cells and depend on blood transfusions all their life. However, quality and duration of life of transfusion-dependent thalassaemic patients has been transformed over the last few years, with their life expectancy increasing well into the third decade and beyond, with a good quality of life. Nevertheless, cardiac symptoms and premature death from cardiac causes are still major problems since in the absence of effective iron chelation therapy, many patients develop evidence of iron-induced myocardial damage with cardiac failure, cardiac arrhythmia, sudden death, or a distressing lingering death from progressive congestive cardiac failure [1-4].

During the past years many scientific evidences have raised the adverse effect of abnormal blood lipid levels, like total cholesterol and other lipids and lipoproteins, on atherosclerotic disease [5-7]. At this point it should be mentioned that the relationships between blood lipids and atherosclerosis might be influenced by several other lifestyle-related factors, like glucose intolerance; blood pressure levels, dietary habits and smoking habits [8].

To the best of our knowledge data regarding the distribution of blood lipids levels among patients with b-TM are lacking. Therefore, we investigated the distribution of total-, HDL-, LDL-, cholesterol, triglycerides, apolipoprotein-A1 and B and lipoprotein – (a) levels, in a sample of patients with b-TM, in Greece.

Methods

Population of the study

From January to December 2003 we enrolled all patients (i.e. 192) with b-TM who visited the "Saint Sophia" Pediatric Hospital for routine blood transfusion. The aforementioned Institute is the major reference center for b-TM in Greece. Of the 192 patients, 88 were males, aged 25 ± 6 years old (range 18–42) and 104 were females, aged 26 ± 6 years old (range 18–46). The number of enrolled participants is adequate to evaluate differences between the investigated parameters greater than 20%, achieving statistical power > 0.80 at < 0.05 probability level (P-value). Moreover, the selected sample can be considered as representative since there were only minor, insignificant, differences in sex and age distribution between the study population and the target population regarding the sex-age distribution. All patients were Caucasians, they were living in various regions of Greece and interviewed by trained personnel who used a standard questionnaire. Participants had hematologic evidence of b-TM, i.e. profound hypo chromic anemia, mean erythrocyte volume less than 75 fl, electrophoretic hemoglobin A_2 higher than 3.5% of total hemoglobin and both parents had beta thalassaemia. Moreover, presence of the disease was also evaluated by genetic analysis that performed in our Institution, which confirmed the absence or the reduced levels of alpha- or beta-chain synthesis in hemoglobin. Patients received red blood cell transfusions regularly, every 2 to 3 weeks, to maintain hemoglobin level 10 to 13 g/dl. All patients were under iron chelation therapy with deferoxamine, in a dose of 30 to 50 mg/kg given five to six rimes weekly subcutaneous. Chelation treatment was monitored by frequent estimation of feritine levels and urine excretion. All patients were without any evidence of heart failure at entry, as assessed according to the New York Heart Association (NYHA) classification classes I to IV [4], as well as by the recent guidelines of the European Society of Cardiology [9]. In addition, we did not include patients with other cardiovascular or systemic diseases, including rheumatic valve disease, chronic bronchitis and cirrhosis.

Blood lipids and lipoproteins evaluation

The blood samples were collected from the antecubital vein between 8 to 10 a.m., in a sitting position after 12 hours of fasting and avoiding of alcohol. The biochemical evaluation was carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. All biochemical examinations (serum total cholesterol, HDL-cholesterol and triglycerides) were measured using chromatographic enzymic method in a Technicon automatic analyser RA-1000 (Dade Behring, Marburg, Germany). HDL cholesterol was determined after precipitation of the Apolipoprotein B containing lipoproteins with dextran-magnesium-chloride. Lipoprotein (a) was measured by a latex enhanced turbidimetric immuno-assay. Serum for the measurement of these lipids was harvested immediately after admission. LDL cholesterol calculated using the Friedwald formula: {total cholesterol} - {HDL cholesterol} - 1/5 (triglycerides). Non-HDL cholesterol is the total cholesterol minus HDL cholesterol.

Hypercholesterolemia was defined as total serum cholesterol levels greater than 200 mg/dl or the use of lipid-lowering agents and diabetes mellitus as a blood glucose > 125 mg/dl or the use of antidiabetic medication.

An internal quality control was in place for assessing the validity of cholesterol, triglyceride and HDL methods. The intra and inter-assay coefficients of variation of cholesterol levels did not exceed 3%, triglycerides 2% and HDL 4%.

Demographic, clinical and lifestyle characteristics

The study's questionnaire also included demographic characteristics like age, gender, and residence of the participants. Information about smoking habits was collected using a standardized questionnaire developed for the

Study. Current smokers were defined as those who smoked at least one cigarette per day. Never smokers those who have never tried a cigarette in their life and former smokers were defined as those who had stopped smoking more than one year previously. For the multivariate statistical analyses cigarette smoking was quantified in pack-years (cigarette packs per day × years of smoking), adjusted for a nicotine content of 0.8 mg/cigarette.

Body mass index was calculated as weight (in kilograms) divided by standing height (in meters squared). Obesity was defined as body mass index > 29.9 Kg/m^2.

Arterial blood pressure was measured three times at the right arm (ELKA aneroid manometric sphygmometer, Von Schlieben Co, West Germany), at the end of the physical examination with subject in sitting position at least for 30 minutes. The systolic blood pressure level was determined by the first perception of sound (of tapping quality). The diastolic blood pressure level was determined by phase V when the repetitive sounds become fully muffed (disappear). Changes in loudness were not considered. Patients whose average blood pressure levels were greater or equal to 140/90 mm Hg or were under antihypertensive medication were classified as hypertensives.

Statistical analysis

Continuous variables are presented as mean values ± one standard deviation, while qualitative variables are presented as absolute and relative frequencies. The use of contingency tables and the calculation of chi-squared test tested associations between categorical variables. Comparisons between normally distributed continuous variables and categorical were performed by the calculation of Student's t-test as well as Analysis of Co-Variance, after testing for equality of variances (homoscedacity). In the case of asymmetric continuous variables the tested hypotheses were based on the calculations of the non-parametric test suggested by Kruskal and Wallis. Correlations between lipids levels and age, smoking habits (in pack-years) and body mass index were evaluated by the calculation of Pearson's correlation coefficient for the normally distributed variables and by the Spearman correlation coefficient for the skewed variables.

All reported *P*-values are based on two-sided tests and compared to a significance level of 5%. SPSS 11.0 software (SPSS Inc. 2002, Illinois, USA) was used for all the statistical calculations.

Results

Demographic and clinical characteristics of the patients are presented in Table 1. It is of particular interest the very low prevalence of hypertension, result of the low systolic and diastolic blood pressure levels observed in these patients. Additionally, mean body mass index was within normal range (i.e. < 25 kg/m^2); as result, obesity prevailed in less than 2% of the patients. On the other hand, a high proportion of men and women reported current smoking habits.

Blood lipids distribution

The mean values of the investigated blood lipids and lipoproteins both in men and women are presented in Table 2. Furthermore, Figure 1 illustrates the distribution of total, HDL cholesterol and triglycerides levels. Mean total cholesterol varied within normal values (< 200 mg/dl). In addition, none of the participants had cholesterol levels above 240 mg/dl, while only 4% of men and 2% of women had total serum cholesterol levels greater than 200 mg/dl. However, oppose results were observed regarding HDL cholesterol since 42% of men and 29% of women patients had very low HDL cholesterol levels (< 30 mg/dl). A group of people with particular interest is those who have normal total cholesterol, but low HDL cholesterol levels. In the our patients, 39% of men and 30% of women who had normal total cholesterol levels (i.e. < 200 mg/dl) had HDL cholesterol levels lower than 35 and 45 mg/dl, respectively. Mean triglycerides were also low, and 11% of men and 17% of women had triglyceride levels greater than 150 mg/dl. Finally, mean LDL cholesterol levels were also low and none of men and women had LDL cholesterol levels greater than 130 mg/dl.

Mean lipoprotein – (a) levels were considerably low both in men and women patients. Moreover, the distribution of lipoprotein – (a) was skewed to the right indicating that a very small proportion of the patients had high (> 15 mg/dl) lipoprotein – (a) values (Figure 2).

It is known that age is a factor that correlates well with blood lipid levels. In our study, age was positively and significantly associated with all blood lipids measurements in both men and women, by the exception of HDL-cholesterol levels. We expanded the previous findings by evaluating the association of the investigated blood lipids with age, after controlling for other potential confounders, like sex and smoking habits. We observed that a decade difference in age was associated with 7 mg/dl higher total cholesterol levels (95% confidence interval (CI) from 5 to 9 mg/dl, $p < 0.001$), 12 mg/dl higher triglycerides levels (95% CI from 10 to 14 mg/dl, $p < 0.001$), 7 mg/dl higher LDL cholesterol levels (95% CI from 5 to 9 mg/dl, $p < 0.001$), 0.9 mg/dl higher lipoprotein-(a) levels (95% CI from 0.4 to 1.4 mg/dl, $p < 0.001$), but only 2.5 mg/dl lower HDL cholesterol levels (95% CI from -1.5 to 6.5 mg/dl, $p = 0.31$).

Table 1: Characteristics of the patients (% by gender)

	Men (n = 88)	Women (n = 104)	p
Age (years)	25 ± 6	26 ± 6	0.33
Current smoking, n (%)	34 (45%)	36 (34%)	0.08
Systolic blood pressure (mmHg)	120 ± 43	114 ± 15	0.03
Diastolic blood pressure (mmHg)	71 ± 10	74 ± 10	0.11
Hypertension, n (%)	0 (0%)	1 (1%)	-
Glucose (mg/dl)	104 ± 28	88 ± 16	0.06
Diabetes mellitus, n (%)	12 (15%)	17 (15%)	0.88
Body mass index (kg/m^2)	21.8 ± 2	22.2 ± 3	0.56
Obesity, n (%)	2 (2%)	0 (0%)	0.13
Family history of CHD, n (%)	8 (10%)	17 (17%)	0.16
Hematocrit (%)			
Feritine (ng/dl)	2411 ± 1784	2076 ± 1350	0.17
Hemoglobin (g/dl)	10.4 ± 1	10.6 ± 1	0.77
White blood cell (counts)	9959 ± 4999	9401 ± 4074	0.78

Table 2: Blood lipids distribution in men and women with b-TM

	Men (n = 88)	Women (n = 104)	P*
Total cholesterol (mg/dl)	114 ± 33	129 ± 30	0.018
Triglycerides (mg/dl)	113 ± 43	112 ± 44	0.98
HDL-cholesterol (mg/dl)	32 ± 11	37 ± 13	0.065
Total – HDL cholesterol ratio	3.7 ± 1.2	3.8 ± 1.5	0.75
LDL-cholesterol (mg/dl)	128 ± 38	122 ± 36	0.01
Apolipoprotein-A1 (mg/dl)	123 ± 28	111 ± 17	0.01
Apolipoprotein-B (mg/dl)	60 ± 20	59 ± 15	0.10
Lipoprotein (a) (mg/dl)	6.3 ± 8.8	8.3 ± 9.1	0.01

P value derived from the comparison between men vs. women after taking into account the effect of age, body mass index, and smoking habits.

Finally, none of the investigated blood lipids or lipoproteins was associated with feritine, hemoglobin levels or white blood cell counts (data not shown in text).

Discussion

In this work we evaluated the distribution of several blood lipids and lipoproteins in a sample of Greek adults with beta thalassaemia major. To our knowledge the distribution of blood lipids and lipoproteins among patients with this disorder are presented for the first time in the literature. We found that the majority of the participants had normal total cholesterol levels, on the contrary a considerable proportion of the patients had very low HDL cholesterol levels. In addition, LDL cholesterol, triglycerides, as well lipoprotein – (a) and apolipoproteins A1 and B were substantially low.

Epidemiology of blood lipids in patients with beta thalassaemia major

Only 4% of men and 2% of women had total cholesterol levels greater than 200 mg/dl. A recent report from the ATTICA study [10], which enrolled a representative and adequate sample of the general healthy population from Greece, suggest that roughly 25% of men and women with the same age of our patients (i.e. < 45 years old) had high total cholesterol. Based on the previous report it could be speculated that patients with beta thalassaemia major have lower total cholesterol levels as compared to healthy individuals of the same age. In accordance with the previous findings we also observed very low mean LDL cholesterol levels in thalassaemic patients. It is of interest that none of men and women patients had LDL cholesterol levels greater than 130 mg/dl. On the contrary, the ATTICA study [10] reported that 17% of men and 15% of women of the same age with our patients had LDL cholesterol levels above 130 mg/dl.

Mean triglycerides were also low in beta thalassaemic patients. Roughly one out of ten men and two out of ten women had triglyceride levels greater than 150 mg/dl. If we compare these figures with the ones reported by the ATTICA study investigators, i.e. that approximately one

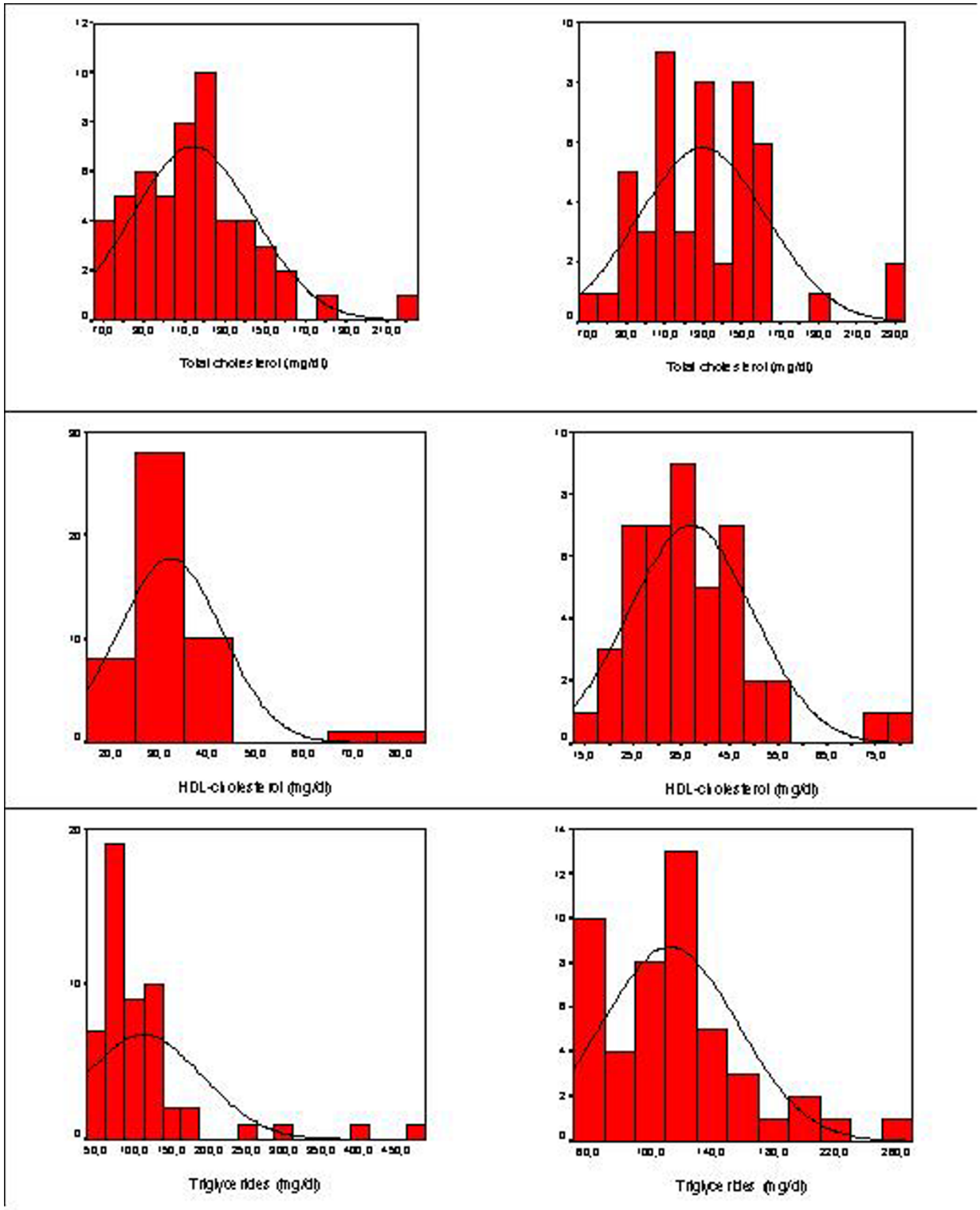

Figure 1
Distribution of selected blood lipids in men (left column) and women (right column) with beta thalassaemia major

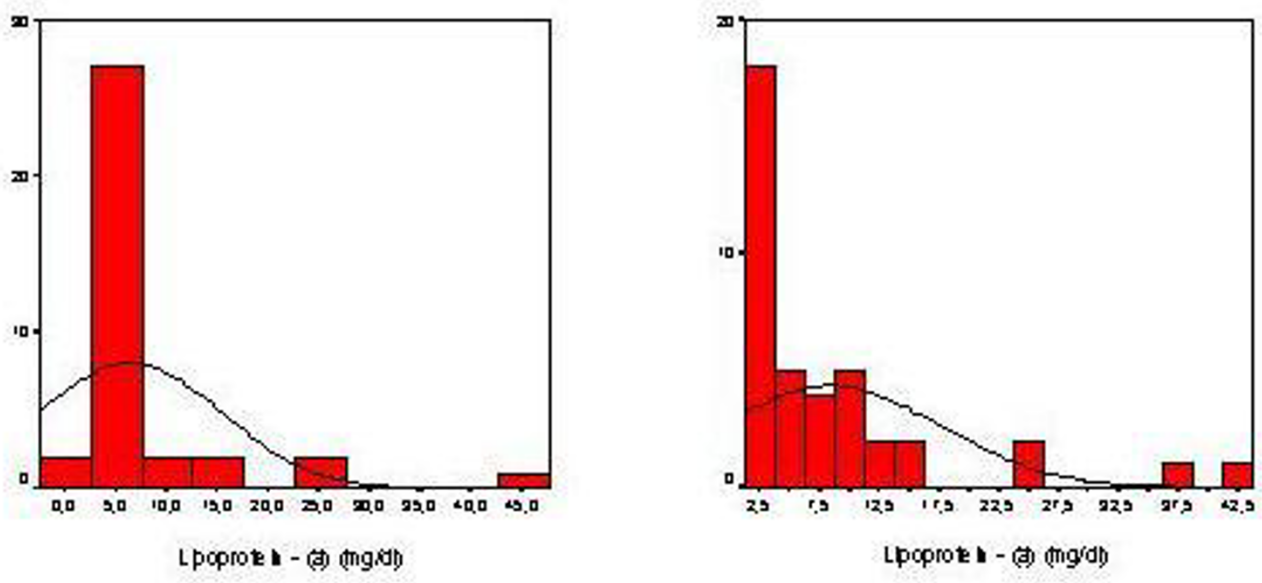

Figure 2
Distribution of lipoprotein – (a) in men (left column) and women (right column) with beta thalassaemia major

third of men and only 13% of women had triglycerides levels higher than 150 mg/dl, we may assume that thalassaemic men had substantially lower triglyceride levels, while thalassaemic women have similar prevalence of high triglyceride as compared to normal individuals. The ATP III suggests a cut off point of 150 mg/dl for defining elevated triglycerides levels [11]. It seems that patients with beta thalassaemia major are at low coronary risk as regards their triglycerides levels.

On the contrary, when we focused our interest on HDL cholesterol we observed that thalassaemic patients had very low values. Particularly, 42% of men and 29% of women had HDL cholesterol levels below 30 mg/dl. Comparing these findings with young adults from the ATTICA study [10] we observed that the rates of low HDL cholesterol levels among our patients were substantially higher. Studies suggest that even for those with normal levels of total cholesterol, risk for myocardial infarction is high when HDL cholesterol is low [11,12]. The later may highlight the importance of total-to-HDL cholesterol ratio for the evaluation of blood lipids and the prevention of atherosclerotic disease. It has also been reported that the total cholesterol-to-HDL cholesterol ratio predicts coronary heart disease risk regardless of the absolute LDL- and HDL-cholesterol [12]. We observed that 39% of men and 30% of women patients who had total cholesterol levels below 200 mg/dl had HDL-cholesterol lower than 35 and 45 mg/dl, respectively. Moreover, the average total-to-HDL cholesterol ratios in men and women patients were above the threshold indicated by the ATP III guidelines (i.e. 3.5) for high-risk people [11]. If we compare the previous rates with the ones presented by the ATTICA study (i.e. only 19% of men and 12% of women who had desirable total cholesterol levels had low HDL cholesterol levels), we could suggest that thalassaemic patients are at much higher coronary risk than their matched controls, because of the low HDL cholesterol production, even if they are within normal values of total cholesterol. Bersot et al [12] suggested that in populations at risk for coronary heart disease caused by low HDL cholesterol, qualification of subjects for treatment based on the total – to – HDL cholesterol ratio thresholds (i.e. 3.5) identifies more high-risk subjects for treatment than other cholesterol threshold values alone.

It has been reported that the distribution of lipoprotein – (a) levels in the general population are different from the bell-shaped curve of serum cholesterol [14,15]. Figure 1 expands the previous suggestion among patients with beta thalassaemia major. Moreover, the mean lipoprotein – (a) values in our sample were approximately 6 mg/dl among men and 8 mg/dl among women. These figures were much lower than those reported by the ATTICA study investigators in healthy people of the same age [10]. However, none of our patients had lipoprotein – (a) levels higher than 30 mg/dl, while on the contrary, approximately, 10% of men and women from the ATTICA study

had high lipoprotein – (a) levels. The gender difference that observed in the general Greek population seems to hold in our patients, too.

Papanastasiou et al [15] studied a total of 104 patients with major and minor beta thalassaemia and compared them with 112 healthy controls. The investigators reported that total cholesterol; HDL and LDL-cholesterol was significantly decreased, while triglycerides were significantly increased in the thalassaemic patients compared to the control subjects. They also found a positive correlation between age and triglycerides levels. By the exception of triglycerides we also observed similar results regarding blood lipids levels among our patients and the healthy controls from the general population of Greece (based on the ATTICA study). In accordance to these findings Maioli et al [16] studying 70 individuals with beta thalassaemia major from Italy found that these patients disclosed significantly lower total-cholesterol, LDL-cholesterol, HDL-cholesterol, apolipoprotein A1, and B plasma levels and higher triglyceride concentration controls people. It appears, therefore, that many factors such as iron overload, liver injury, and hormonal disturbances affects lipids pattern among patients with major form of beta-thalassaemia. Moreover, Maioli et al [17,18] in previous reports suggested that accelerated erythropoiesis and increased uptake of LDL by macrophages and histiocytes of the reticuloendothelial system are the main determinants of low plasma cholesterol levels in beta thalassaemia major. In addition, Giardini et al [19] observed that total serum phospholipids, their fractions and cholesterol were significantly lower among patients with thalassaemia major. These changes were referred to hepatic damage and to severe anaemia, respectively. Furthermore, some serum lipid polyunsaturated fatty acids were significantly decreased among patients with beta thalassaemia major as compared to normal controls. Since these alterations are a sign of lipid oxidation, the causes of this phenomenon are discussed. These differences on blood lipids and lipoprotein levels could also attribute to the adherence of a healthier lifestyle by people with beta thalassaemia, which could include consumption of healthy foods since childhood. However, it should be mentioned that oppose results from the previous reports have been reported in a recent study [20] which suggested that adolescents with beta-thalassaemia minor have significantly lower cholesterol levels than patients with beta thalassaemia major. The investigators suggested that this has been related to their disorder and not influenced by age, sex, hemoglobin, or feritine levels. In these patients, needless investigations for hypolipidemia should be avoided.

At this point it should be noted that the extrapolation of our findings into other populations with beta thalassaemia major may be under scrutiny, since thalassaemia is genetically oriented and various expressions of the related polymorphisms may be involved in the distribution of blood lipids and lipoprotein levels.

Conclusion

The present study revealed that men and women with beta thalassaemia major have their blood lipid and lipoprotein levels within the normal range, and lower than the healthy individuals of the same age and population. An exception is the observed very low HDL cholesterol levels, which may underline the importance of total-to-HDL cholesterol ratio as a prognostic factor for future cardiac events in this high-risk population.

Authors' contributions

CC: design of the study, drafted the manuscript, DP: design of the study, data analysis and interpretation of the findings, CP, JB: drafted the manuscript, CK: design of the study, MK, JL: field investigator, drafted the manuscript

All authors read and approved the final manuscript.

Competing interests

None declared.

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