

Analysis of serum lipid profiles, metal ions and thyroid hormones levels abnormalities in β -thalassaemic children of Bangladesh

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Abstract

Objective: To assess the serum lipid profile of cardiovascular disease free male and female children with β -thalassaemia. Levels of zinc, copper and magnesium in the serum were also determined along with the Thyroid profile.

Methods: From January to December 2007, we enrolled 121 consecutive patients with β -thalassaemia that visited The Thalassaemia Center at Dhaka Shishu (Children) Hospital, Bangladesh every month for routine examinations. Fasting blood lipid levels were measured in all participants. Zinc, Copper and Magnesium levels in serums were determined. Thyroid function was also assessed by evaluating T3, T4 and TSH levels.

Results: Of the 121 patients, 65 were males (10.14 ± 3.91 years) and 56 were females (9.08 ± 4.32 years). Data analysis revealed that 2.0% males and 4.35% females had high total serum cholesterol, and 28.57% males and 21.74% females had high triglyceride levels. In addition, mean HDL-cholesterol levels were 21.14 ± 5.82 mg/dl in males and 21.17 ± 6.02 mg/dl in females; total-cholesterol to HDL-cholesterol ratios were 5.47 ± 1.66 and 5.96 ± 2.81 in males and females respectively. About 60% patients showed low serum level of Zn and Cu. Hypothyroidism was detected in 30% patients and 23% patients had abnormal experimental values of all the study parameters.

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 abnormalities was much lower as compared to the general population of the same age. Interestingly, the total-cholesterol 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. The serum Zn and Cu levels were low in most of the patients which may cause some metabolic abnormalities in future. Most of the patients also showed hypothyroidism indicating the presence of endocrine complications (JPMA 60:360; 2010).

Introduction

Beta-thalassaemia is a very serious blood disorder since individuals with it are unable to make enough healthy red blood cells and depend on blood transfusion throughout 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 and smoking habits.⁸

In recent years, several authors reported a high incidence of endocrine abnormalities in children, adolescents and young adults suffering from thalassaemia. However, the incidence of endocrinopathies varies among different series of the patients. This is multifactorial besides iron overload.²⁵ Trace metals deficiencies in patients with thalassaemia have been under debate. Trace minerals have shown to influence growth and hormones e.g. zinc deficiency is considered a causative factor in osteoporosis and endocrinopathies.

To the best of our knowledge, data regarding the distribution of blood lipid levels among patients with beta-thalassaemia are lacking. Therefore, we investigated the distribution of triglycerides, total-, LDL-, HDL-cholesterol in serum samples of patients with beta-thalassaemia in Bangladesh.

Patients and Methods

From January to December 2007, 121 consecutive patients with β -thalassaemia who visited The Thalassaemia Center at Dhaka Shishu (Children) Hospital, Bangladesh every month for routine examinations were enrolled. 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. Patients belonged to various regions from all over Bangladesh and interviewed by trained personnel (for some patients; especially for infants their parents were interviewed but the others were interviewed directly) who used a standard questionnaire. This questionnaire also had the option of their consent to analyze their serum sample. Participants had haematological evidence of beta-thalassaemia, i.e. profound hypochromic anaemia; mean erythrocyte volume less than 75 fl, electrophoretic haemoglobin A2 higher than 3.5% of total haemoglobin and both parents had beta-thalassaemia. Moreover, presence of the disease was also evaluated by genetic analysis, which confirmed the absence or the reduced levels of alpha- or beta-chain synthesis in haemoglobin. Patients received red blood cell transfusions regularly, every 2 to 3 weeks, to maintain haemoglobin 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 times weekly subcutaneously. Chelation treatment was monitored by frequent estimation of ferritin levels in serum and urine. All patients were without any evidence of heart failure at the time of 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 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 laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. All biochemical examinations (triglycerides, LDL-cholesterol, total-cholesterol, and HDL-cholesterol) were measured using chromatographic enzymatic method in a semiautomatic analyzer RA-50. Serum level of Zn, Cu and Mg were determined using the kits and procedures of ProDia International UAE. And final data was read using clinical chemistry analyzer RA-50 of Bayer Diagnostics, Ireland. Thyroid function was assessed by measuring serum level of T3, T4 and TSH using commercial quantitative ELISA kits and procedures

of ProDia International UAE by using ELISA reader 990 Win 6BV4 of GDV-Italy. Age of the patients was considered for determining the normal range of all the study parameters.

Continuous variables were analyzed as mean values \pm standard deviation.

Results

Of the 121 patients, 65 were males (10.14 ± 3.91 years) and 56 were females (9.08 ± 4.32 years).

The serum levels of haemoglobin of $6.23 \pm 1.32\%$ and ferritin of 3819 ± 3385.56 (ng/dl) for males of age 10.14 ± 3.91 years and the serum levels of haemoglobin of $6.25 \pm 0.86\%$ and ferritin of 2380.68 ± 1296.91 (ng/dl) for females of age 9.08 ± 4.32 years were the experimental values before transfusion of blood to the patients. The patients were subjected to blood transfusion every 15-21 days interval. All patients have undergone blood transfusion of on an average 144-336 times during their so far life cycle (irrespective of age).

The mean values of the investigated blood lipids both in male and female are presented in Table-1. Data

Table 1: Lipid profiles of patients with beta-thalassaemia.

Parameters	Male		Female	
	Experimental Value	Standard Value	Experimental Value	Standard Value
Triglycerides (mg/dL)	106.07 ± 35.71	<130.98	104.74 ± 41.79	<135.40
LDL-cholesterol (mg/dL)	74.78 ± 10.27	<121.81	71.4 ± 34.92	<115.24
Total-cholesterol (mg/dL)	107.69 ± 14.35	<192.96	113.53 ± 32.76	<182.13
HDL-cholesterol (mg/dL)	21.14 ± 5.82	$?63.42$	21.17 ± 6.02	≥ 56.84
Total-cholesterol to HDL-cholesterol ratio	5.47 ± 1.66	<3.04	5.96 ± 2.81	<3.20

analysis revealed that 2.0% of males and 4.35% of females had high total serum cholesterol, and 28.57% of males and 21.74% of females had high triglyceride levels. In addition, mean HDL-cholesterol levels were 21.14 ± 5.82 mg/dl in males and 21.17 ± 6.02 mg/dl in females, total-cholesterol to HDL-cholesterol ratios were 5.47 ± 1.66 and 5.96 ± 2.81 in males and females respectively.

Estimation of serum metal ions is an important parameter to assess the clinical condition of patients. Serum Zn, Cu, and Mg level were assessed. All of these metals are important metabolic regulatory factors in different ways. The average value of each parameter is presented in Table-2 of either sex. Data indicates that

Table-2: Serum levels of Zinc, Copper and Magnesium.

Parameters	Male		Female	
	Experimental Value	Standard Value	Experimental Value	Standard Value
Serum Zinc (µg/dL)	56.73±5.87	63.8-110	57±6.68	63.8-110
Serum Copper (µg/dL)	65.54±4.59	70-140	69±4.69	80-155
Serum Magnesium (mg/dl)	1.91±0.02	1.9-2.5	1.90±0.02	1.9-2.5

Table-3: Serum levels of T3, T4 and TSH.

Parameters	Male		Female	
	Experimental Value	Standard Value	Experimental Value	Standard Value
T3 (ng/ml)	0.85±0.35	0.6-2.1	0.73±0.29	0.6-2.1
T4 (µg/dL)	5.23±3.48	4.7-12.5	4.765±4.13	4.7-12.5
TSH (mIU/L)	4.69±2.22	0.32-5.2	3.35±1.76	0.32-5.2

serum Zn and Cu levels were lower than the lower limit of standard value range but the Mg serum level was within the range (Table-2).

Hypothyroidism is associated with the abnormal production of thyroid hormone. Thus the assessment of serum T3, T4 and TSH could provide the important

information about the function of thyroid gland and the correlation with other disease(s). They were assessed and the results are shown in Table-3. Hypothyroidism was present in 28.57% of the male patients and 66.65% of the female patients. Patients showing abnormal serum level and suffering with hypothyroidism are summarized in Figure.

Discussion

In this study the serum lipid profiles in beta thalassaemic children of Bangladesh were evaluated. Majority of the participants had normal total-cholesterol levels; on the contrary a considerable proportion of the patients had very low HDL-cholesterol levels. Only 2.0% of males and 4.35% of females had high total serum cholesterol, and 28.57% of males and 21.74% of females had high triglyceride levels. A recent report from a study,¹⁰ which enrolled a representative and adequate sample of the general healthy population from Greece, suggested that roughly 25% patients had high total-cholesterol. Based on the previous report it could be speculated that patients with beta-thalassaemia have lower total-cholesterol levels as compared to healthy individuals of the same age. It is of interest that none of the male and female patients had LDL-cholesterol levels greater than 130 mg/dl. On the contrary, the other study¹⁰ reported that 17% men and 15% women had LDL-cholesterol levels above 130 mg/dl. A guideline suggests a cut off point of 150 mg/dl for defining elevated triglycerides levels.¹¹ It seems that patients with beta-thalassaemia are at low coronary risk as regards their triglycerides levels. When we focused our interest on HDL-cholesterol, we observed that thalassaemic patients had very low values. Comparing these findings with young adults from a study,¹⁰ it was 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 latter highlight the importance of total-cholesterol 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 total-cholesterol to HDL-cholesterol ratios were 5.47 ± 1.66 and 5.96 ± 2.81 in male and female respectively. About 25 % patients showed high TG level and about 58% patients showed low HDL-cholesterol level. Moreover, the average total-cholesterol to HDL-cholesterol ratios in male and female patients was above the threshold indicated by the guidelines (i.e. 3.5) for high-

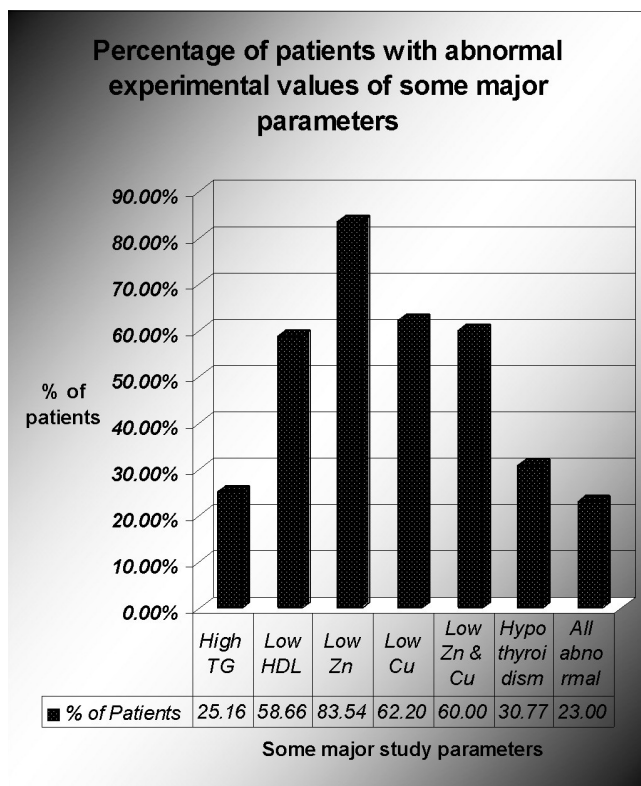


Figure: Percentage of patients with abnormal serum values of some major study parameters.

risk people.¹¹ If the previous rates are compared with the ones presented by another study¹⁰ (i.e. only 19% of men and 12% of women who had desirable total-cholesterol levels had low HDL-cholesterol levels), it could be suggested 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-cholesterol to HDL-cholesterol ratio thresholds (i.e. 3.5) identifies more high-risk subjects for treatment than other cholesterol threshold values alone. 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 LDL-cholesterol, total-cholesterol, and HDL-cholesterol were 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 triglyceride levels. Similar results were observed in the present study. In accordance to these findings Maioli et al.¹⁶ studying 70 individuals with beta-thalassaemia major from Italy found that these patients displayed significantly lower LDL-cholesterol, total-cholesterol, HDL-cholesterol, apolipoprotein A1, and B plasma levels and higher triglyceride concentration than controls. It appears therefore that many factors such as iron overload, liver injury, and hormonal disturbances affects lipid 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, it has been reported that some serum lipid polyunsaturated fatty acids were significantly decreased among patients with beta-thalassaemia major as compared to normal controls. However, it should be mentioned that contrary results from the previous reports have been reported in a recent study²⁰ 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, haemoglobin or ferritin 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. Zinc deficiency is considered as one of the main factors contributing to growth and puberty disorders in thalassaemic patients.²¹ About 60% patients showed both the low level of Zn and Cu. The zinc status of thalassaemic patients was previously reported by Arcasoy et al. and they showed that there was marked zinc deficiency in the presence of hyperzincuria.²² Kwan and colleagues²³ reported that only 3 of their 68 thalassaemic patients had zinc deficiency in their study population. Deficiencies of zinc and copper in patients with thalassaemia major have been under debate. Most of our patients showed low levels of serum zinc and copper but normal serum level of magnesium. It has been reported that there is high prevalence of deficiency of these two trace elements in Iranian general population.²⁴ The various metal ions such as zinc and copper are playing crucial role in cells especially in various enzymatic functions. Particularly Zn deficiency is relevant with depressed growth and diarrhea in child whereas copper is playing very important role in biological electron transport. Thus, their low levels in serum of beta-thalassaemic patients might be considered as metabolic abnormalities. Similarly thyroid hormones are essential to proper development, differentiation and metabolism of the cells and abnormal serum level of T3, T4 and TSH associated with beta-thalassaemia might link to endocrine complications. Finally it would speculate that the abnormal serum level of the parameters studied here can causes the several complications in beta-thalassaemia child patients which may ultimately lead to fatal consequences.

Conclusion

The present study revealed that the majority of males and females with beta-thalassaemia have their blood lipid levels within the normal range, and lower than the healthy individuals of the same age and population. An exception is that the very low HDL-cholesterol levels, which may underline the importance of total-cholesterol to HDL-cholesterol ratio as a prognostic factor for future cardiac events in this high-risk population. The deficiency of zinc, copper might lead some metabolic abnormalities. The prevalence of some endocrine complications among our thalassaemic

patients signifies the importance of our observations where we found abnormal levels of thyroid hormones. Thus, we prefer to conclude that the abnormalities found in the total-cholesterol to HDL-cholesterol ratio, thyroid hormone level and metal ions level in this study might be the underlying cause of the variety of complications that the thalassaemic patients develop.

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