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ORIGINAL ARTICLE

Serum fasting lipid profile in children and adolescents with β-thalassaemia major in southern Pakistan

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Abstract

Objective: Serum fasting lipid profile has been studied in various clinical spectrum of Beta (β)-thalassaemia syndrome. Premature cardiac impairment in thalassaemia major appears primarily due to iron accumulation and oxidative injury; however it might be a sequel of abnormal lipoprotein concentrations. The rational of this study is to analyse the serum fasting lipid profile in cardiovascular disease free β-thalassaemia major (β-TM) patients. Relationships with age, gender, haematological parameters, liver enzymes and serum ferritin were observed. *Method:* Fasting serum lipid levels, liver function test (LFT), complete blood count (CBC) and serum ferritin were measured in 36 patients with homozygous β-TM from March 2012 to March 2014. Patients were stratified into two groups, age ≤15 and >15 years, to determine the possible lipid profile distinction in relation to age. *Results*: 17 were males and 19 were females, with median age of 12.0 years. The mean total cholesterol (TC) and triglyceride (TG) were 5.01±1.32 and 8.36±5.28 mmol/L respectively. High TG was detected in 36.1%, while high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) were markedly low, 0.98±0.51 and 2.35±1.22 mmol/L respectively. No statistically significant difference was noted between the two age groups. The median TC to HDL ratio (TC:HDL) was elevated, 5.7 (4.0). We established significant correlation of total bilirubin with TC (r=-0.4), HDL(r=-0.5) and LDL (r=-0.4) (P<0.05). *Conclusion:* Dyslipidaemia in β-TM patients is irrespective of age and gender including low HDL and high TC:HDL, whilst high TC:HDL may contribute as a significant risk marker for future cardiac events in these patients.

Keywords: β-thalassaemia major, triglyceride, HDL-cholesterol, LDL-cholesterol

INTRODUCTION

Beta (β) thalassaemia syndrome is an autosomal recessive heterogeneous disorder, as a result of inadequate beta globin chain synthesis.[1,2] It is a common genetic disorder with substantial morbidity and mortality globally,[1,2] with high prevalence in the Indian subcontinent, Middle East, central Asia and Mediterranean countries.[1,2] The estimated carrier rate of β-thalassaemia in the Pakistani population is 5-7%[2] and as anticipated, annually 5000-9000 newborns are being added to the existing disease pool of β-thalassaemia major (β-TM) in Pakistan.[2,3]

Blood transfusion and iron chelation are the mainstay of treatment, which have considerably increased the life expectancy and significantly improved the quality of life of these patients.[3] With the increasing average age and promptly managed siderotic complications various non-siderotic complications are being increasingly recognised.

Recent literature shows strong evidence that children with β-TM are at risk of developing subclinical atherosclerosis.[4] Studies have also revealed strong affiliation of dyslipidaemia with premature atherosclerosis as an emerging complication in these patients.[5,6] Subclinical atherosclerosis begins early in life and may evolve into coronary heart disease later. β-thalassaemic patients are subject to peroxidative tissue injury due to free radicals and low density lipoprotein cholesterol (LDL).[6,7] It has been postulated and identified that circulating LDL exerts enormous oxidative stress[8] on these patients.

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An abnormal lipid profile in thalassaemic patients has been reported in various studies, but its pathophysiology is still not defined.[9] This dyslipidaemia includes high triglyceride (TG), low total cholesterol (TC), low high density lipoprotein cholesterol (HDL) and high TC to HDL ratio (TC:HDL) in young thalassaemic patients.[6,7,10] Pro-atherogenic lipid profile (low HDL and high TC:HDL) in young thalassaemic patients highlights its importance as a prognostic factor for cardiac risk stratification. The underlying postulated mechanisms of hypocholesterolaemia include decreased production secondary to liver injury, increased consumption due to amplified erythropoiesis and an augmented uptake of cholesterol by histiocytes.[7,10]

Therefore we investigated the distribution of serum lipoprotein concentration in the samples of patients with β-TM. We also evaluated the relationship between these biochemical parameters with maternal characteristics (age, gender), haematological parameters, serum ferritin and liver function test (LFT).

MATERIALS AND METHODS

This is a cross-sectional study, in which 36 patients with β-TM were enrolled. The patients were registered from March 2012 to March 2014. Informed consent was obtained from patients aged ≥18 years and from parents/guardians for patients <18 years of age.

Patients were selected by non-probability sampling technique during the study duration. Demographic data including age, gender and medical history were recorded. All registered patients were diagnosed based on clinical history, physical examination findings and laboratory tests including complete blood count (CBC) and haemoglobin (Hb) electrophoresis. All patients were on regular blood transfusion and iron chelation therapy as per requirement. Patients on lipid lowering medications, having diabetes mellitus, hypothyroidism, renal failure and history of hereditary hyperlipidaemia were excluded. None of the patients had a cardiovascular event prior to the study. All the blood parameters tested were collected at one point of time from each patient.

Samples for serum fasting lipid profile (TC, TG, HDL, LDL) were taken after a 12 hour over night fast and were measured on Hitachi 912 using photometric assay by commercially provided kits from Roche Diagnostic according to manufacturer's instructions. Serum TC ranged between 5.55-12.22 mmol/L while hypocholesterolaemia was defined as < 5.55mmol/L. Hypertriglyceridaemia was defined as levels greater than 8.33 mmol/L. Normal values of LDL were considered up to 8.33 mmol/L and for HDL >1.94 mmol/L was considered as normal.

On the same Hitachi 912, LFT and serum ferritin were also measured using photometry and immunoturbidity methods, respectively. CBC was determined by Cell Dyne Ruby by impedance aperture method (Abbott, Diagnostic).

Patients were stratified into two groups, age ≤ 15 years and >15 years, to determine the possible lipid profile distinction in relation to age. The research protocol was approved by the Ethical and Research Committee Liaquat National Hospital prior to the study.

Statistical analyses were carried out using IBM statistics SPSS version 20. Results were reported as the median (IQR) and independent sample Mann–Whitney *U* test were used to compare study groups. Comparison of categorical data was carried by Spearman's rank correlation test. *P*-value of <0.05 was considered as statistically significant.

RESULTS

The study included 36 homozygous β-TM patients with median age of 12.0 (5-24) years, of whom 17 were males and 19 were females with a mean age of 15.5±5.9 and 10.1±4.5 years, respectively. The descriptive statistics and laboratory parameters are shown in Table 1.

Serum fasting lipid profiles along with reference ranges are illustrated in Table 2. Low level of TC was seen in 12 (33.3%) patients.

TABLE 1: Patients clinical and laboratory data

Parameters	Median (IQR)
Age (years)	12(9)
Hemoglobin (gm/dl)	6.9(3.6)
Hematocrit (%)	25(11)
Total Leucocyte Count	5.6(4.4)
Platelet Count	109(125)
Total bilirubin (μmol/L)	20.5(18.8)
Direct bilirubin(μmol/L)	7.6(5.1)
Indirect bilirubin(μmol/L)	11.9(15.3)
AST (ukat/L)	1.13(0.71)
ALT (ukat/L)	1.3 (1.22)
Serum ferritin	3850(5527)

TABLE 2: Fasting lipid profile of β-thalassaemia patients

Parameters	Reference ranges mmol/L	Median (IQR) mmol/L
Total lipid	25-55.5	26.6(6.8)
Cholesterol	5.55-12.22	5.0(1.6)
Triglyceride	3.88-8.33	7.44(4.1)
HDL	≥1.94	1.0(0.7)
LDL	≤8.33	2.19(1.7)
TC:HDL	<3.5	5.7(4.0)

High TG was detected in 13 (36.1%) patients. HDL and LDL were markedly low; 0.98±0.51 and 2.35±1.22 mmol/L respectively. The mean TC:HDL was significantly elevated, 5.7(4) (<3.5). The TC:HDL were also increased, irrespective of gender, which were 5.8(2.3) and 4.6(5.3) in males and females respectively (Table 3).

Data analysis in respect to gender distribution revealed that serum TG in females patients was higher than male patients, 8.1 (7.2) versus 5.7 (3.5). Overall 22.2% females and 13.8% males had high TG. No statistically significant difference was noted in two stratified age groups with respect to lipid profile (Table 4).

We established negative correlation of elevated total bilirubin with TC [r = -0.46, P=0.004], HDL [r= -0.44, P=0.001] and LDL [r= -0.44, P=0.006]. HDL correlated positively with LDL [r=0.5, P=0.000]. No significant correlations were noted between serum lipids and gender, haematological parameters, serum ferritin and liver enzymes.

DISCUSSION

Lipid abnormalities have been reported in β-TM, but its pathophysiology is still not entirely defined. In the present study, we report serum lipid levels of children and adolescents with

TABLE 3: Comparative analysis of serum lipid profile in relation to gender

Parameters	Male n= 17 median (IQR)	Females n= 19 median (IQR)	P- value
Total lipid	26.1(3.0)	27.7 (7.9)	0.2
Cholesterol	1.4 (2.3)	5.2 (1.1)	0.4
Serum triglyceride	5.7 (3.5)	8.1 (7.2)	0.1
HDL cholesterol	0.8 (0.5)	1.1(1.0)	0.4
LDL cholesterol	2.1 (1.3)	2.3 (1.8)	0.4
Cholesterol ratio	5.8 (2.3)	4.6 (5.3)	0.6

TABLE 4: Comparative analysis of serum lipid profile in relation to age

Parameters	Age ≤15 years n= 24 median (IQR)	Age >15 years n= 12 median (IQR)	P- value
Total lipid	27.6 (4.7)	25.7 (5.1)	0.05
Cholesterol	5.4 (1.2)	4.0 (0.7)	0.1
Serum triglyceride	7.7 (4.8)	5.7 (4.3)	0.1
HDL cholesterol	1.1 (0.9)	0.6 (0.4)	0.1
LDL cholesterol	2.4 (2.3)	2.0 (0.9)	0.5
Cholesterol ratio	4.7 (5)	6.0 (3.6)	0.7

β-TM in Pakistan. To the best of our knowledge, this is the first report from Pakistan. The results of the present study showed that the β-TM patients had lower serum TC, LDL and HDL, with raised serum TG and TC:HDL with respect to reference ranges; this is in agreement with the results reported from Turkey, Jordan, Iran, Algeria and Egypt.[5,7,9,11,12] The proposed contributory mechanisms include plasma dilution because of anaemia, accelerated erythropoiesis resulting in increased cholesterol uptake by macrophages and histiocytes of the reticuloendothelial system, liver damage because of iron overload, macrophage activation with cytokine release and hormonal disturbances.[10,13,14] However, the main speculated causative mechanisms of dyslipidaemia are severe iron overload and oxidative stress.[13,15]

In our study, serum TC was lower (33.3%) irrespective of age and gender. Decreased concentrations of cholesterol were observed in most published studies reported from Turkey, Italy and Thailand on thalassaemic patients.[5,16,17] In parallel to our findings, one prior study also demonstrated no correlation of serum cholesterol with advancing age.[18] Another investigator speculated that this dyslipidaemia is related to the underlying disorder and not influenced by age, gender, haemoglobin and ferritin levels.[7] In 1997, Maioli and colleagues in their study suggested that an increased uptake of LDL by macrophages and histiocytes of the reticuloendothelial system are the main determinants of low serum cholesterol in thalassaemics.[16] Amendola *et al* in 2007 suggested that the brisk bone marrow activity with enhanced cholesterol consumption could be the basis of low cholesterol levels.[19]

Although the serum TG did not demonstrate significance in some studies, it was established to be high in most studies,[16,20,21] concurring with our findings. Hypertriglyceridaemia was observed in 36.1% in our series of patients. Similarly, Shams *et al* reported high TG in 34.6% of Iranian thalassaemic patients.[18] One previous study also disclosed high serum TG in female thalassaemics compared to males, which also strengthens our findings. Elevated TG was also reported in Egyptian and Italian studies.[4,16] A reduced lipolytic activity could be the explanation for the increase in circulating TG in patients with β-TM. Triglyceride lipase enzymatic activities (both hepatic and extrahepatic) have been reported significantly lower in β-TM.[7]

In accordance with the preceding findings, we also found very low median LDL levels in our patients.[14] Papanastasiuo *et al* and Hartman *et al*, from Greece and Israel respectively, reported the mean LDL levels significantly lower in patients with β-thalassaemia.[21,22] It is of consideration that none of our patients had LDL levels greater than 6.9 mmol/L. We determined a significant correlation of total bilirubin with serum TC and LDL; similarly Arica *et al* ascertained hepatocellular damage as a cause of dyslipidaemia, however liver lipid peroxidation may be one causative factor.[23]

Virtually, all males and females had HDL levels below 1.6 mmol/L in the present study. The instant cleaning of HDL by activated macrophages was considered as one an underlying mechanism.[3] Our results were analogous to previous studies.[10,24] Low HDL could be considered as a predictive factor of cardiovascular risk stratification in patients with β-TM. Various studies established that the risk for ischaemic myocardial infarction is high with low HDL levels regardless of normal TC levels.[10,25] Thus, thalassaemic patients are at a much higher coronary risk owing to the low HDL levels.

The proatherogenic ratio, TC:HDL was significantly higher in our thalassaemic patients. This is in concurrence with Chrysohoouet al, from Greece. Furthermore the median TC:HDL of 5.7 in our thalassaemic patients was above the threshold of 3.5 stipulated by the ATP III guidelines for high-risk persons.[25] This ratio also predicts the risk of coronary heart disease regardless of the LDL and HDL levels.[10,25] TC:HDL would be helpful for the evaluation of atherosclerotic disease. Bersot *et al* also suggested that, based on high TC:HDL, subjects are classified as high-risk for coronary events and are candidate for life style modification and drug treatment.[26]

The limitations of the study need to be mentioned: Firstly is small sample size, the results of which could not be generalized to all patients and secondly its observational nature and lack of control group for comparison. Thus, we recommend that more future studies are needed for validation and confirmation of our findings and also to include a control group for comparison. Despite the limitations discussed, the strength of this study is that this is the first local study, which provides preliminary information about dyslipidaemia in β-TM in Pakistani thalassaemic patients and will provide the base for future prospective studies for management and preventive strategies in the local setting.

Conclusion

Our study revealed that deranged lipid profile is not uncommon in β-TM patients irrespective of age and gender. Considerable deficiency of HDL and significantly high TC:HDL could be predictive for future coronary events. We propose that lipid profile should be evaluated in these patients as low HDL and raised TC:HDL are important tools for coronary risk assessment. The exact mechanisms and clinical consequences of dyslipidaemia in β-TM should be further investigated in larger prospective studies to represent the Pakistan population.

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Conflict of interest

The authors report no potential conflict of interest

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