SERUM LIPID PROFILE ABNORMALITIES IN β THALASSEMIA MAJOR PATIENTS IN KARACHI.

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Abstract:

Objective: The aim of this study was to assess the lipid profile in beta thalassemia major patients and observe its correlation with age and gender.

Methods: In this cross sectional study, conducted during 2013, 70 βTM patients (38 males and 32 females) with mean age 19.92 who were undergoing periodic blood transfusions and desferrioxime as chelating agent were recruited. 70 controls of matched age and gender were also included in the study. Lipid profile was done by enzymatic colorimetric method on Micro lab 300 (Merck & Co Germany) and statistical comparison was done by using student's t test on SPSS: 17

Results: βTM patients showed significantly lowered blood cholesterol (105.5 ±19.2 mg/dl), LDL (44.23±19.4 mg/dl) and HDL(33±4.9 mg/dl) in comparison with controls (P value> 0.001) while TAG was level found to be increased(140.2±27 mg/dl) in comparison to normal healthy controls.

Conclusion: The mechanisms that may account for these findings are increased erythropoiesis and cholesterol consumption along with iron overload and oxidative stress in βTM patients. Awareness of these findings is helpful to avoid unnecessary evaluation and clinical consequences of impaired lipid profile in patients with β thalassemia major

Keywords: Beta thalassemia major (βTM), Lipid profile.

Introduction:

Thalassemia's are a heterogenous group of autosomal recessive hemoglobinopathies characterized by defects in genes producing alpha or beta globin chains (Mansi et al., 2008). Population migration and intermarriages between different ethnic groups have led to the prevalence of thalassemia in almost every country of the world and now it is considered to be the most common hemoglobinopathy (Kohne, 2011) About 1.5% of the global population (80 to 90 million people) are carriers of beta thalassemia (Vichinsky, 2005). β -thalassemia is also a major health problem in Pakistan. It is the most prevalent genetically transmitted blood disorder with a carrier rate of 5-8%; around 5000 children are diagnosed each year in the country (Khateeb et al., 2009)

Beta thalassemia is caused by mutation of beta globin gene on chromosome 11, resulting in reduced Hb in RBC's , decreased RBC production and anemia (Xu, SX et al., 2000).

Lipid abnormalities have been detected in different types of beta thalassemia (Amendola et al.,2007)

The defective β globin chain production may result in either a clinically silent thalassemia minor or a very severe transfusion dependent thalassemia major, while intermediate clinical manifestations are observed in patients of thalassemia Intermedia (Weatherall, 2010).

The reduced or absent beta globin chain leads to ineffective erythropoiesis and precipitation of excess alpha chains which cause hepatosplenomegaly, anemia and extra medullary hematopoiesis.(Thein,2005). Beta Thalassemia Major (βTM) or Cooley's anemia is the most common form in which subjects are homozygotes or compound heterozygotes (Melody et al., 2004). It is characterized by severe transfusion dependent anemia and entails risk of iron overload and multi organ dysfunction (Renzo & Rafaella, 2010).

Although an increasing number of patients are now treated with bone marrow transplantation, the majority of patients still depend on regular blood transfusions. These transfusions and chelation therapy have improved the span and quality of thalassemic patients' lives, but many suffer from clinical complications resulting from iron overload. A wide range of studies in the literature have documented multiple organ dysfunction along with many metabolic alterations, one of which is lipid abnormalities that have been frequently reported in thalassemic patients.

Socioeconomic and cultural factors in addition to lifestyle (particularly dietary habits) and genetic patterns of various ethnic groups have been reported to effect the lipid profile of beta thalassemia major patients (Hashemieh et.al. 2010). Therefore these factors may be different in Pakistani patients compared to other nations. However no earlier studies of lipid abnormalities in thalassemic patients of Pakistan have been reported in the literature.

The aim of this research is to estimate the lipid profile of β thalassemia major patients of Karachi and to compare the findings with healthy control participants and to investigate any correlation with age and to observe any difference in lipid profile on gender distribution.

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Materials and Methods:

The research protocol was approved by the Basic Medical Sciences Institute (BMSI) Research Ethics Committee. All clinical investigations were conducted according to the principles expressed in the declaration of Helsinki. All the participants were explained the minimal risk procedure and were asked to complete a verbal and written informed consent.

This cross sectional comparative study was carried out in different thalassemia care centers in Karachi from Jan 2012 to Jan 2013

140 subjects of both sexes were included in our studies that were divided into two groups. Control group A comprised of normal healthy subjects and group B comprised of beta thalassemia major patients having no HbA, only HbF and HbA2 (HbF between 20 to 90 percent).

Patients having diabetes mellitus, hypothyroidism, hyperthyroidism, renal failure, hereditary hyperlipidemia, sickle / beta / HbE Beta Thalassemia, hypertension, CAD and smokers were excluded.

Following informed consent, venous blood samples were collected after 8 to 10 hours of overnight fasting. The samples were then centrifuged at 3000 RPM for ten minutes and supernatant was separated. Serum cholesterol was done by CHOD-PAP method. Serum triglyceride was done by GPO-PAP method. LDLc and VLDLc were done by Friedwal's formula.

A descriptive statistical analysis of continuous variables was performed using SPSS (version 17).

Biophysical and chemical parameters i.e. Hb, MCV, MCH, Ferritin, TC, HDL, LDL, VLDL & Total Cholesterol / HDL were calculated as mean ±standard deviation and comparison was done by student's t- test.

Spearmans, RHO coefficient of correlation (r) was used to correlate the levels of lipid profile with age. In all statistical analyses $p < 0.01$ was considered as significant.

Results:

Table # 1 describes the comparison of biophysical parameters between control and case groups. A total number of 70 control subjects (39 males and 31 females),70 patients of Thalassemia Major (40 males and 30 females) were recruited for the study. Mean age of control group was 20.47±1.9 years while mean age of βTM, was 19.9±1.17. Mean number of transfusion in Group D (βTM) patients was 20.49±0.69 years.

TABLE 1: COMPARISON OF BIOPHYSICAL PARAMETERS BETWEEN CONTROL AND CASE GROUPS.

	Control	Case Group
Parameters	Group A	Group B
	n=70	n=70
	Mean±S.D.	Mean±S.D.
Age (Years)	20.47±1.9	19.9±1.17
Gender		
M	39	40
F	31	30
Number of transfusion	-	20.49±0.69 years.

* Statistically significant as compared to control ($p< 0.001$)

Table # 2 shows Hemoglobin, MCV, MCH and serum Ferritin in control and case groups. Mean Hb was found to be significantly lower ($p<0.001$) in case group as compared to control. Similarly MCV and MCH were also found to be significantly low in case or thalassemic group as compared to controls ($p<0.001$). However in Group B (βTM patients) highly significant ($p<0.001$) increase in serum ferritin (3880±1670) was observed when compared to Group A i.e controls (43.63±1).

TABLE 2: COMPARISON OF BIOCHENICAL PARAMETERS BETWEEN CONTROL AND CASE GROUPS.

	Control	Case Group
Parameters	Group A	Group B
	n=70	n=70
	Mean±S.D.	Mean±S.D.
Hb (gm/dl)	14.1±1.9	7.5±1.5*
MCV (fl)	79.5±4.4	58.6±8.2
MCH (pg)	24.7±2.2	17.2±1.8
Ferritin (ng/ml)	43.63±1	3880±1670**

* Statistically significant as compared to control ($p< 0.05$)
** Statistically significant as compared to control ($p< 0.001$)

Table # 3 presents serum lipid profile of Group B (βTM) versus Group a (control) group. Mean values of TC, HDL -C, LDL-C, and TC/HDL ratio of βTM patients were found to be significantly decreased ($p<0.001$) when compared to healthy controls while TG and VLDL levels were significantly higher than control (Group A).

TABLE 3: SERUM LIPID PROFILE IN CONTROL AND THALASSEMIA MAJOR PATIENTS.

Parameters	Group A	Group B
	n=70	n=70
	Mean±S.D.	Mean±S.D.
TG (mg / dl)	120.1 ± 2.4	140.3 ± 27.4
TC (mg / dl)	176.5 ± 2.7	105.5 ±19.23*
LDL (mg / dl)	111.2 ± 2.8	44.23 ±19.4*
HDL (mg / dl)	41.3± 6.4	33.2 ± 4.9*
VLDL (mg / dl)	24.02 ± 4.9	28.06 ± 5.4
TC / HDL	4.3 ± .98	3.22 ± .62*

* Statistically significant as compared to control ($p< 0.05$)

Table# 4 shows the gender distribution of lipid profile in Group B. We found no significant difference between male and female in Thalassemia Major group. (p<0.005).

TABLE 4: COMPARISON OF SERUM LIPID PROFILE BETWEEN MALE AND FEMALE IN THALASSEMIA MAJOR GROUP.

Parameters	Group B n=70 Mean±S.D.	
	Male	Female
TC (mg / dl)	103.55±20.08	107.84±18.21
TG (mg / dl)	142.86±28.33	137.31±26.37
HDL (mg / dl)	32.13±3.37	34.50±6.08
LDL (mg / dl)	42.84±20.16	45.88±18.95
VLDL (mg / dl)	28.57±5.66	27.46±5.27
TC / HDL	3.24±0.62	3.19±0.63

* Statistically significant as compared to control (P < 0.001)

Table # 5 reveals the correlation between age and lipid profile of normal control and thalassemia group. TG (r= 0.498), HDL (r=0.729), VLDL (r=0.498) were significantly negatively correlated (p = 000) with age in group A. Group B (βTM) revealed negative correlation with TG and VLDL though results were not significant. Thalassemia group B, i.e βTM showed negative correlation of TC/ HDL ratio (r=-0.044) depicting their protection against CAD with age but the results are not significant with p= 0.720. No significant correlation was observed between LDL and TC with case or control groups.

TABLE 5: CORRELATION BETWEEN AGE AND LIPID PROFILE IN CONTROL AND CASE GROUPS.

Parameters	Group A n=70 Mean±S.D.	Group B n=70 Mean±S.D.
TC (mg / dl)	0.164	0.131
TG (mg / dl)	-0.498*	-0.146
HDL (mg/dl)	-0.729*	0.192
LDL (mg/dl)	0.72	0.66
VLDL(mg/ dl)	0.498*	-0.146
TC / HDL	0.644*	-0.042

*Statistically significant as compared to control (P < 0.001)

Discussion:

In this study we investigated for the first time in Pakistan the lipid profile in Thalassemia Major Patients of Karachi. 140 subjects were enrolled in our study, which were then divided into one case group and a healthy control group. The case group was beta thalassemia major (βTM).

In the present study we observed low total serum cholesterol, low HDL & low LDL cholesterol in thalassemia major patients as compared to control subjects. Our results are in agreement with previous studies' findings with regard to above altered serum lipid pattern by Patne et al in India (2012) and Ferdaus et al. in Bangladesh (2010).

These lipid alterations in patients with βTM are likely due to diminished hepatic biosynthesis because of anemia and iron overload. The proposed mechanisms include increased erythropoietic activity resulting in increased cholesterol requirement, liver injury due to iron overload and macrophage system activation with cytokines as interleukin 1, 6 and Tumor Necrosis Factor α release (Shalev et al., 2007),

Moreover it is known that severe chronic liver disease which is a consequence of iron overload and hence oxidative stress is characterized both by low total and LDL cholesterol level and by decrease in HDL cholesterol. (Al -Quobaili et al., 2004)

The results reported by Ricchi et al.,(2009) also support the idea that main mechanism of hypocholesterolemia in βTM is severe iron overload and oxidative stress.

It has been documented that the circulating LDL molecule in beta thalassemia patients shows marked oxidative stress and is altered or modified. This modified LDL is then phagocytosed by macrophages which lead to the formation of foam cells, which infiltrate and deposit in the arterial walls and this is the initial step in the formation of the atherosclerotic plaque (Liveria et al., 1998). Also iron overload leads to the development of free radicals which further increase the oxidative stress and alteration of LDL (Omran, 2010).

Our findings are also in agreement with those of ladaa et al. (2013) and Arica et al. (2012) who showed that total choleterol and LDL cholesterol were lower in patients with β thalassemia major as compared to normal subjects.

In our study we found that serum TG was significantly higher in βTM patients compared to controls. Increased concentrations of TG have been observed in most published studies on lipid profiles of thalassemic patients (Al Quobaili et al., 2004).

In 2008, Mansi et al. reported not only a significant increase in TG level but they also found positive correlation between TG and Ferritin.

Triglyceride lipase activities (both hepatic and extra hepatic) have been found to be significantly lower in thalassemic patients (ladaa et al., 2013) and this could explain the increase in TG in our study as has been speculated by Christina et al.(2004) who suggested that decreased level of the enzymatic activities could play a role in determining not only the increased TG levels but also a de-

crease in HDLc. Thus when we focussed our interest on HDL cholesterol, we also observed that βTM patients had very low values as compared to our controls.

This increases the risk of CAD in patients with βTM as studies suggest that risk for myocardial infarction is high when HDL cholesterol is low (Brewer, 2003) .

However we found that the TC/HDL ratio of beta thalassemia major patients in our study decreased compared to normal controls which might contribute to the protection of these individuals from development of CAD.

Thus in thalassemia major patients the atherogenic process could be enhanced in part by increased iron stores and induced oxidative stress, and in part counterbalanced by the reduced level of cholesterol.(Ricchi et al.,I 2013).

In our study, lipid profile values in males of the control group were significantly higher with the exception of TC and LDL when compared to their age matched female counterparts. LDL and TC were more in the females but the difference was statistically insignificant. However within the same gender the thalassemia type showed no significant difference amongst them. Mansi et al. (2008) in their study on thalassemia minor patients observed that lipid profile of thalassemic patients is not influenced by gender. On the contrary TG was positively correlated with age in a study conducted by Papanastasiou et al. (cited by Omran 2010).

It is known that age is a factor that correlates well with blood lipid levels and in our study lipid profile levels of normal controls was significantly associated with age but in the thalessemic patients age had no correlation with lipid levels.

Al-Quobaili(2004) observed that a ten year difference in age in BTM patients was associated with 7mg/dl higher TC levels, 12mg/dl higher TG, 7mg/dl higher LDLc but only 2.5mg/dl lower HDLc level after every decade of life. Christina et al. (2004) also observed a positive correlation between age and TC, TG & LDL in BTM patients which was not observed in our study where the thalassemia group showed no such correlation with age. This difference could be due to age of the sample taken in by the above two studies whose subjects were older than 25 years of age while the mean age age of our subjects was less than 20 years of age. This fact has also been highlighted by the study of Hashemieh et al. (2011) who also observed no correlation when they studied younger subjects of less than 25 years of age.

Conclusion:

In conclusion, our study reveals that beta thalassemia patients have decreased levels of TC, LDL, HDL and increased value of triglycerides as compared to healthy individuals of same age and population.

Low levels of cholesterol in BTM patients depict their inability to balance the increased cholesterol consumption for RBC membrane formation. Cholesterol is also needed for steroid hormone synthesis and in many common clinical conditions such as infections and hypercoagulability. Hypocholesterolemia could thus impact negatively in conditions given above.

Even though risk for coronary events is increased in beta thalassemia, our study showed that TC / HDL of BTM patients were found to be lower than matched controls. Therefore this ratio cannot be used to identify risk for coronary events, and better methods to assess coronary risk are needed for beta thalassemia patients, for example, iron overload.This should be a motive for concern.

Further studies on larger scale are suggested to establish the relationship between complications of thalassemia and hypocholesterolemia. Addition of good food with cholesterol supplements may be recommended for the enhancement of their life expectancy.

References:

1. Al-Quobaili FA, AbouAsali IE. Serum levels of lipids and lipoproteins in Syrian patients with beta-thalassemia major. Saudi Med J 2004;25(7):871-5.
2. Amendola G, Danise P, Todisco N, D'Urzo G, Di Palma A, Di Concilio R. Lipid profile in beta-thalassemia intermedia patients: correlation with erythroid bone marrow activity. Int J Lab Hematol 2007;29(3):172-6
3. Arica V., Arica S., Özer C., Çevik M. (2012): Serum Lipid Values in Children with Beta Thalassemia Major. Pediatrics and Therapeutics 2:130.
4. Brewer Jr HB. New features of the National Cholesterol Education Program Adult Treatment Panel III Lipid lowering guidelines. Clin Cardiol 2003; 26:19-24.
5. Christina C, Demosthenes B, Christos P. Distribution of serum lipids and lipoproteins in patients with beta thalassemia major; an epidemiological study in young adults from Greece. Lipids in Health and Disease 3: 328-334, 2004
6. Ferdaus MZ, Hasan AKMM and Shekhar HU. Analysis of serum lipid profiles, metal ions and thyroid hormones levels abnormalities in β-thalassaemic children of Bangladesh. JPMA 2010; 60(5):360-364.
7. Hashemieh,M,Javadzadeh,M,Shirkavand,A, Sheibani,K. Lipid profile in minor thalassemic patients:a historical cohort study.Bangladesh Med Res Counc Bull 2011;37:24-27
8. Iadaa NFN, Al-Kotobe, F and Sorhat AR. Estimation of lipid profile in beta thalassemia major in Tikrit City. Tikrit Med J 2013; 19(2):185-192.
9. Khateeb B, Moatter T, Shanghil AM, et al. Genetic diversity of beta-thalassemia mutations in Pakistani population. J Pak Med Assoc. 2009; 50 (9):293-6
10. Kohne E. Hemoglobinopathies Clinical Manifestation, Diagnosis and Treatment. DtschArztebleInt 2011; 108 (31-32) 532-40
11. Livrea MA, Tesoriere L, Maggio A et al. Oxidative modification of low density lipoprotein and atherogenetic risk in beta thalassemia. Blood 1998; 92 : 3936-3942.

12. Mansi K.M., Aburjai TA. Lipid Profile in Jordanian Children with β-thalassemia Major. International Journal of Hematology and Oncology 2 2008; 18:93-98.
13. Melody J, Eric A, Ellis J, Alan R. Complications of s-thalassemia major in North America. Blood 104: 34-39, 2004
14. Omran DG. Lipid profile in children with beta thalassemia major in Babylon. J Basby Pure 5 (18):20101899-1901.
15. Papanastasiou DA, Siorokou T, Haliotis FA. Beta-thalassaemia and factors affecting the metabolism of lipids and lipoproteins. Haematologia (Budap) 1996; 27(3):143-53.
16. PatneAB, Hisalkar PJ, Gaikwad SB. Lipid abnormalities in patients of beta thalassemia major. Intern J Pharm and BiolSci 2012; 2:106-112.
17. Renzo Galanello and Rafaella Origa. BetaThalassemia.Orphanaet Journal of Rare Disease. 2010; 5:11
18. Ricchi P, Ammirabile M and Magglo A. Hypocholesterolaemia in thalassemia – pathogenesis, implications and clinical effects. EurHaematol 2013; 20-23.
19. Ricchi P, Ammirabile M, Spasiano A, Costantini S, Di Matola T, Cinque P, et al. Hypocholesterolemia in adult patients with thalassemia: a link with the severity of genotype in thalassemia intermedia patients. Eur J Haematol. 2009; 82(3):219-22.
20. Thein SL. Pathophysiology of {beta} Thalassemia—A Guide to Molecular Therapies. Hematology Am SocHematolEduc Program. 2005:31-37.
21. Vichinsky E. Changing Patterns of Thalassemia Worldwide. New YorkAcademy of Sciences 2005; 1054: 18.24.
22. Weatherall D.J. Thalassemia as a global health problem: recent progress toward its control in the developing countries. Ann New YorkAcadSci 2010; 1202: 17-23.
23. Xu XS, Glazer PM, Wang G. (2000) Activation of human gamma-globin gene expression via triplex forming oligonucleotide (TFO)-directed mutations in the gamma-globin gene 5' flanking region. Gene. 242:219-228.