Severity of Hypocholesterolemia in β Thalassemia Intermedia Genotypes: A Communal Finding in Adults Suffering from β Thalassemia

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Abstract

Background: β Thalassemia Intermedia (βTI), known for its association with systemic implications and subsequent complications, requires active management of the disease with increased awareness about the outcomes. Increased erythropoietic activity, iron overload, and serum ferritin, with severe alterations in lipid profile predominantly hypocholesterolemia are all found in patients suffering from β Thalassemia Intermedia.

Objectives: This study aims to find and discuss the lipid profile alterations existing in between healthy individuals and β Thalassemia Intermedia patients with different genotypes.

***Methods*:** 140 subjects were included and divided into 2 groups according to selection criteria. Control group consisted of 70 healthy subjects from both genders of ages between 10 to 30 years. Case group consisted of 70 β Thalassemic Intermedia patients.

Results: Our study showed that severe hypocholesterolemia was observed in Thalassemia Intermedia patients as compared to control population with βºβº genotype having significantly decreased serum Total cholesterol and HDL

Conclusion: Our results conclude that patients with Thalassemia Intermedia suffer from altered lipid parameters particularly hypocholesterolemia.

Index Terms: β Thalassemia Intermedia; βTI, hypocholesterolemia, genotype, lipid profile.

I. INTRODUCTION

Thalassemias, a heterogeneous group of genetic disorder, results from diminished or decreased synthesis of beta or alpha chains of haemoglobin. The point mutations in beta-globin gene (β) cause β- thalassemia, whereas alpha- thalassemia (α) is caused by deletion of alpha globin gene that results in no or diminished production of alpha globin chains [1].

Out of these two, β- thalassemia is regarded as one of the most common blood disorders around the world and is in fact the most common monogenic inherited disease. A single or more than hundreds of mutations in associated genes might be considered as the cause of this disease which then leads to the instability of the unpaired globin chains resulting in intracellular precipitation. All of this is followed by haemolysis, early destruction of red blood cells (RBC), and an overall short life of circulatory RBCs. Heme and iron, which are formed as a result of haemoglobin breakdown, now act as catalysts to chemical reactions resulting in free radical generation inclusive or reactive oxygen species (ROS). Excess production of ROS almost always damages vital organs such as liver, heart, and endocrine system [2].

β Thalassemia is characterized as a type of hereditary anaemia that stems from the abnormalities associated with the beta-haemoglobin chain and as much as 200 different mutations have been recorded that affect the beta-globin gene, which are hence a cause of diverse and varying phenotypes and genotypes of the said disease [3].β Thalassemia is further characterized on the basis of its laboratory and clinical findings in which β-thalassemia minor, a usually asymptomatic mild anaemia, is a heterozygous state whereas compound heterozygosity and homozygosity account for β thalassemia major and β-thalassemia intermedia, a more severe form of anaemia [4]. Transfusion dependence distinguishes the two because β thalassemia major requires transfusions on a regular basis whereas it's not the same for intermedia [5].

The Thalassemia Intermedia is a clinically intermediate type that lie between severely diseased thalassemia major and asymptomatic thalassemia minor types [6].

Genotypically Thalassemia Intermedia inherit two β thalassemic alleles. The amount of synthesis of β globin chain

determines the severity in addition to further presence of secondary modifiers like polymorphism [7].

Lipid abnormalities have been associated with β thalassemia types and with other haematological disorders but the pathogenesis and exact aetiology of these findings are still unclear. Proposed mechanisms suggest that plasma dilution, erythropoiesis at an accelerated rate results in increased uptake of cholesterol, iron over load leading to impaired liver function, disturbances in hormonal system along with other causes might cause these changes [8].

The above mentioned findings make it important to understand and work towards finding a better association between lipid abnormalities and variants of β thalassemia which in this case is Thalassemia Intermedia. All of the results would help create better management modules for this disease so that patients suffering from blood disorders are better cared for.

II. MATERIALS AND METHODS

This cross sectional study was carried out during Jan 2020 to Jan 2021 in Department of Physiology, Basic Medical Sciences Institute (BMSI) Jinnah Postgraduate Medical Centre (JPMC), Karachi. This study was carried out with the principles of the Declaration of Helsinki and was approved by the Institutional Research Ethics Committee. (Approval no. No.F.1-2/BMSI-E.COMT/JPMC).

Sample size was calculated by open EPI website calculator with the reference study [9].

140 subjects were included and divided into 2 groups according to selection criteria. Control group consisted of 70 healthy subjects from both genders of ages between 10 and 30 years. Case Group consisted of 70 diagnosed β Thalassemic Intermedia patients between the ages of 10-30 years, from both genders i.e. male and female. The diagnosis criteria was on Hb Electrophoresis with elevated HbF level and age at first transfusion was more than 2 years along with absence of regular transfusion dependency [7]. The diabetic patients, hypothyroid/hyperthyroid patients, patients with renal failure, patients with hereditary hyperlipidemia, hereditary persistence of Hb, Sickle β thalassemia, HbE β thalassemia, Hypertension, Coronary Artery Disease (CAD) patients, and smokers were excluded from the study.

Case group was further divided into three genotypes of Thalassemia Intermedia. $\beta^+\beta^+$, $\beta^+\beta^o$ and $\beta^o\beta^o$. $\beta^+\beta^+$with moderate amount of β globin synthesis, $\beta^+\beta^o$ with small amount of synthesis and $\beta^o\beta^o$ with mutations resulting in no β globin chain synthesis.

After taking consent, the blood samples of subjects fulfilling the inclusion criteria were collected after an overnight fasting of 8-10 hours. About 8 ml of venous blood was collected from antecubital vain after all aseptic measures in control subjects and in cases of Thalassemia. 4 ml of blood was transferred to a centrifuged tube and allowed to clot at 37°centigrade. The clotted sample was centrifuged at 3000rpm for ten minutes, the serum was separated and stored (-70° centigrade). The remaining 4 ml blood was transferred into an anticoagulant tube for haematological parameters. 2 ml for Complete blood count and 2 ml for DNA extraction and polymerase chain reaction (PCR) genotyping.

CBC was estimated by Sysmex 21 Cat No.KX21 Kobe, Japan. Serum cholesterol was measured by enzymatic colorimetric method HDL by precipitant method, Triglycerides by Glycerol-3-Phosphate Oxidase Phenol Aminophenenzone method, using kit A10085, Merck, Germany. By using a tetra primer based allele specific PCR, genotyping of β Thalassemia was done by on β globin gene for previously known, common mutations reported in Pakistan.

III. STATISTICAL ANALYSIS

Statistical Software SPSS version 17 was used for data feeding and analysis. A descriptive statistical analysis of continuous variable was performed. Data of variables were age, sex, Hb, MCV, MCH, Ferritin, TG, TC, HDL, LDL, VLDL and TC/HDL. Parameters were presented as Mean ± Standard Deviation (SD).Statistical comparison was done by t test between two groups and ANOVA was used for multiple groups of genotypes.

IV. RESULTS

Table # 3.1 describes the comparison of biophysical parameters between control and case groups. A total number of 70 control subjects (39 males and 31 females) and 70 patients of Thalassemia Intermedia (31 males and 39 females) were recruited for the study. Mean age of control group was 20.47±1.9 years while mean age of βTI was 19.9±1.57. Mean duration of transfusion in βTI was 5.26±1.3 years.

Table# 3.2 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 ($p<0.001$).

Table# 3.3 represents the comparison of serum Lipid profiles values of βTI to control group. TG, TC, HDL, LDL, VLDL and TC/HDL mean values of Thalassemia Intermedia group showed significant ($p<0.001$) decrement than matched controls.

Table # 3.4 describes the gender distribution of lipid profile in βTI. Thalassemia Intermedia group also presented the same depiction i.e. no significant difference in mean values of TC, TG, HDL, LDL, VLDL and ratio of TC/HDL between male and female while high mean values of TC, HDL, LDL, TC/HDL were found in males.

Table # 3.5 reveals the correlation between age and lipid profile of normal control and βTI group. TG (r= 0.498), HDL (r=0.729), VLDL (r=0.498) were significantly negatively correlated (p = 000) with age in control group. Group C (βTI) revealed negative correlation with TG and VLDL though results were not significantly reported. Significant positive correlation was found between age and TC/HDL ratio (r= 0.644) of control group indicating increased risk of CAD with age however βTI group showed negative correlation of TC/HDL ratio (with age r=-0.042, r= 0.008 & r=-0.044 respectively) depicting their protection against CAD with age but the results are not

significant with p= 0.730, 0.949 and 0.720 respectively. No significant correlation was observed between LDL and TC with βTI or control group.

Table # 3.6 describes the comparison of mean lipid profile values among genotypes of Thalassemia Intermedia patients i.e. among $\beta^+\beta^+$, $\beta^+\beta^o$ and $\beta^o\beta^o$ through Anova. Serum TC and HDL showed significant difference. Serum TC and HDL-C levels were significantly lowered in $\beta^o\beta^o$ genotype than $\beta^+\beta^+$ and $\beta^+\beta^o$ genotypes.

Table 3.1
Comparison of Biophysical Parameters between Control and Case Groups

Parameters	Control	βTI group
	n=70 Mean±S.D.	n=70 Mean±S.D.
Age (Years)	20.47±1.9	19.9±1.57
Gender		
M	39	31
F	31	39
Duration of transfusion	-	5.2±1.3

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

TABLE 3.2
Comparison of Haematological and Biochemical Parameters between Control and Case Groups

Parameters	Control	βTI group
	n=70 Mean±S.D.	n=70 Mean±S.D.
Hb (gm/dl)	14.1±1.9	7.4±1.1*
MCV (fl)	79.5±4.4	54.2±13.2
MCH (pg)	24.7±2.2	15.3±12.6*
Ferritin (ng/ml)	43.63±1	1026±1215

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

TABLE 3.3
Serum Lipid Profile In Control and Thalassemia Intermedia Patients

Parameters	Control group n=70 Mean±S.D.	βTI group n=70 Mean±S.D.
TG (mg / dl)	120.1 ± 2.4	103.0 ± 25.2*
TC (mg / dl)	176.5 ± 2.7	89.0 ± 20.7**
LDL (mg / dl)	111.2 ± 2.8	34.6 ± 18.5**
HDL (mg / dl)	41.3± 6.4	33.7 ± 4.9*
VLDL (mg / dl)	24.02 ± 4.9	20.6 ± 5.05*
TC / HDL	4.3 ± .98	2.6 ± .5*

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

TABLE 3.4
Comparison of Serum Lipid Profile between Male and Female in Thalassemia Intermedia Group

Parameters	βTI group n=70 Mean±S.D.	
	Male	Female
TC (mg / dl)	88.38±19.51	89.64±21.91
TG (mg / dl)	108.00±22.13	99.12±27.13
HDL (mg / dl)	33.41±4.65	34.07±5.15
LDL (mg / dl)	33.36±16.95	35.73±19.89
VLDL (mg / dl)	21.60±4.42	19.82±5.42
TC / HDL	2.63±0.47	2.65±0.61

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

TABLE 3.5
Correlation between Age and Lipid Profile in Control and Case Groups

Parameters	Control group n=70 Mean±S.D.	βTI group n=70 Mean±S.D.
TC (mg / dl)	0.164	0.031
TG (mg / dl)	-0.498*	-0.169
HDL (mg/dl)	-0.729*	0.066
LDL (mg/dl)	0.72	-0.047
VLDL(mg/ dl)	-0.498*	-0.169
TC / HDL	0.644*	-0.008

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

TABLE 3.5
Comparison of mean lipid profile values among genotypes of Thalassemia Intermedia patients

Parameters	B^+B^+ (n=31) Mean ±SD	B^+B° (n=22) Mean ±SD	$B^\circ+ B^\circ$ (n=17) Mean ±SD	P value
TC (mg/dl)	87±3.55	80 ±2.06	75±1.57	0.001*
TG (mg/dl)	108±0.53	107±1.25	106±0.5	0.425
HDL (mg/dl)	35±1.5	33±1.87	29±1.75	0.001*
LDL (mg/dl)	39±0.05	38±1.02	37±0.85	0.366
VLDL (mg/dl)	22±1.45	20±2.50	20±1.45	0.680
TC/HDL	2.68±1.5	2.55±1.58	2.42±0.85	0.725

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

V. DISCUSSION

Thalassemia Intermedia patients from our study showed decreased levels of all the lipid profile parameters. Our findings are aligned with the results of Amendola et al that showed lower levels of concentration of total cholesterol and high and low density lipoprotein cholesterol in β Thalassemia Intermedia patients, typically lower than in thalassemia major patients. [10] Biochemical and clinical observations quite severely pointed towards erythropoietic marrow expansion in patients suffering from β thalassemia Intermedia as found in the study conducted, as a result of which accelerated erythropoiesis leads to decreased lipid profile levels [11]. However, it was also noticed that β Thalassemia Intermedia patients can survive on occasional transfusions rather than continued and regular transfusions except in the cases of pregnancy, surgery, or any other dangerous disease as explained by [12].

Oxidative stress, found in βTI patients was associated with Hypocholesterolemia directing in one way or another the increased cholesterol consumption linked with RBC formation [13].

Our findings were in line with Lai et al [14] and Hartman et al [15] where the findings matched with that of our studies concerning the lowered lipid profile particularly the triglycerides (TG).

Lower levels of high density lipoprotein cholesterol as found out in our study were also reported by another study [16].

A significant decrease in TC/HDL ratio in the β Thalassemia Intermedia group was found out in our study as compared to the control group, this lowered ration is considered and associated with increased risk for cardiovascular diseases and might be a cause of high morbidity for patients suffering from βTI [17].

Our study presented us with significantly lower levels of low density lipoproteins (LDL), Very low density lipoproteins (VLDL), Total Cholesterol (TC), and TC/HDL ratio, which was clearly in line with the study conducted in southern Iran[18]. This also presented us with the fact that lipid profile alterations were more severe in βTI patients than any other thalassemia group easily showing the severity of Hypocholesterolemia, among all other thalassemia forms too

Our study reveals that Genotype βºβº is inflicted with severely low levels all lipid profile parameters. Another study also found that this genotype is more involved in complications and dyslipidemia with endocrinopathies [19]. Another study reported that Hypolipidemia decreases binding capacity of Lipopolysaccharides, resulting in ligation of the CD14 complex and mononuclear system activation. This can further induce oxidative stress in these patients [20].

The detrimental role of hypocholesterolemia can increases susceptibility to infections, complications even endocrinopathies especially of lipid derived hormones leading to hypogonadism, growth and puberty in thalassemia Intermedia patients [21]. Similar complications due to hypocholesterolemia were found in [22].

VI. CONCLUSION

Our study revealed the severity of Hypocholesterolemia in β Thalassemia Intermedia as compared to control group with others marked alterations in lipid parameters making these patients protective to cardiovascular changes and subsequent atherogenic activity. Cholesterol supplements are recommended for the management but therapies and management for βTI patients should be aimed at keeping the Hb level normal, prevent hypoxia, oxidative stress and other potential endocrine complications. However, aggressive hypolipidimic management should be avoided and other factors for cardiovascular risk should be evaluated at regular intervals. Hence, increased clinical awareness and checks need to be established on such patients especially with βºβº genotype in helping improve their quality of life.

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