Evaluation of lipid profile in patients with thalassemia

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ABSTRACT

Thalassemia can be defined as a condition in which a reduced rate of synthesis of one or more of the globin chains leads to imbalanced globin chain synthesis, defective hemoglobin production, and damage to the red cells or their precursors from the effects of the globin subunits that are produced in relative excess. While Lipid abnormalities, including low levels of all fractions of serum lipids, have been repeatedly reported in all phenotypes of B-thalassemia. Unexpectedly, the concentration of total cholesterol, HDL and LDL has been found in B-thalassemia intermedia patients even lower than in thalassemia major, without a clear explanation of pathophysiology of these findings. Accordingly Lipids are necessary to save life of cells because they are important sources of energy. A lipid profile is determination of the level of specific lipids in the blood. Two important lipids, cholesterol and triglycerides, are cycled in the blood by lipoprotein compounds. The lipoprotein particles are measured with a lipid profile. They are divided by their density into high-density lipoproteins , low-density lipoproteins , and very low density lipoproteins.

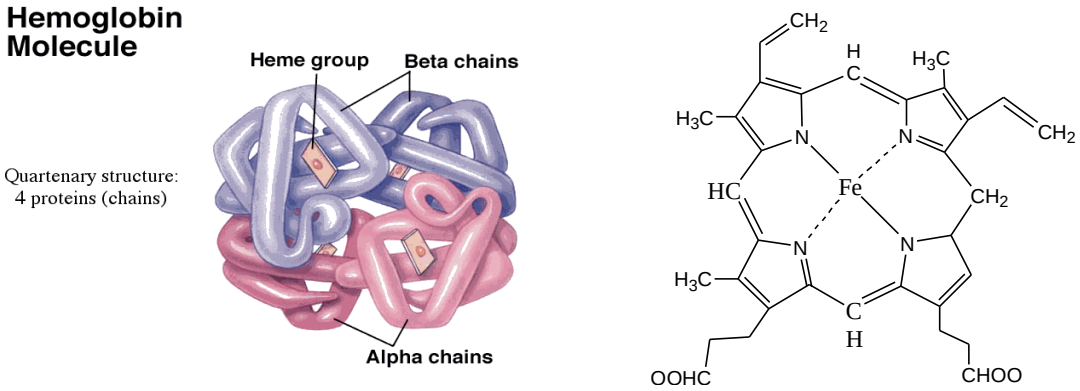
The present study is designed to investigate changes markers of thalassemia patients compared to the control group. Lipid profile examination has shown significant increase in TG levels and significant decrease in all cholesterol forms in patients.

Keywords: Thalassemia , lipid profile , patients

1.1. Hemoglobin structure.

Hemoglobin is a tetrameric globular protein which contains a hemi group of its four subunits, it's found exclusively in red blood cells, where its main function is to transport oxygen from the lungs to the capillaries of the tissues. Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains, two α chains and two β chains held together by noncovalent interaction. Heme is a complex of protoporphyrin and ferrous iron (Fe^{2+}). The iron is held in the center of the heme molecule by bonds to the four nitrogens of the porphyrin ring. The heme Fe^{2+} can form two additional bonds, one

on each side of the planar porphyrin ring. One of these positions is coordinated to the side chain of a histidine residue of the globin molecule, whereas the other position is available to bind oxygen [1].The red pigment of the erythrocyte is the conjugated protein hemoglobin. The normal concentration of hemoglobin in an adult is 14-16 g/dl of blood, all confined to the erythrocyte [2].The complete hemoglobin molecule has a molecular weight of around 67 KD. Hemoglobin is considered to be a complex protein which is composed of the protein (globin) and four heme groups as shown in (figure 1-1) [3].



Heme group

Figure 1-1: hemoglobin structure

1.2. Thalassemia

Thalassemia can be defined as a condition in which a reduced rate of synthesis of one or more of the globin chains leads to imbalanced globin-chain synthesis, defective hemoglobin production, and damage to the red cells or their precursors from the effects of the globin subunits that are produced in relative excess.

In 1925, Cooley and Lee first described a form of severe anemia that occurred early in life and was associated with splenomegaly and bone changes. In 1932, George H. Whipple and William L. Bradford published a comprehensive account of the pathologic findings in this disease. Whipple coined the phrase *thalassic anemia* and condensed it to *thalassemia*, from "the sea", (Greek words (thala) meaning sea and (emia) which stand for blood) because early patients were all of Mediterranean background. The true genetic character of the disorder became fully appreciated after 1940. The disease described by Cooley and Lee is the homozygous state of an autosomal gene for which the heterozygous state is associated with much milder hematologic changes. The severe homozygous condition became known as *thalassemia major*. The heterozygous states, thalassemia trait, were designated according to their severity as *thalassemia minor* or *minima*. Later, the term *thalassemia intermedia* was used to describe disorders that were milder than the major form but more severe than the traits [4].

2.1. Blood sampling

Five millimeters of venous blood were withdrawn by disposable syringes with stainless needles, divided into two portions. First portion was transferred to plain tube containing (ethylenediaminetetraacetic acid disodium) salt (EDTA) as anticoagulant for determination of hemoglobin and platelets, the second portion was transferred to plain polyethylene tube containing gel as a clot activator for serum separation.

The second portion was centrifuged at 4000 rpm for 10 minutes, and the serum was separated immediately to four labeled eppendroft tubes.

The serum was used to determination of Lipid profile.

2.2. Determination of some biochemical parameters in serum of thalassemia control

2.2.1.Determination of lipid profile

2.2.1.1.Determination of Cholesterol concentration

Principle

The determination of cholesterol is based on enzymatic hydrolysis and oxidation. The formation of quinoneimine from hydrogen peroxide, (that released from the enzymatic hydrolysis), and 4-aminoantipyrine in the presence of phenol and peroxidase [66-67].

Cholesterol-ester + H_2O —Cholesterolesterase→ Cholesterol + Fatty acid

Cholesterol + O_2 —Cholesteroloxidase→ Cholestene-3-one + H_2O_2

$2H_2O_2$ + phenol + 4-Aminoantipyrine —peroxidase→ Quinoneimine + H_2O

Reagents:

Contents	Concentration of solutions
Reagent	
4-Aminoantipyrine	0.30 mmol/L
Phenol	6 mmol/L
Peroxidase	≥ 0.5 U/mL
Cholesterol esterase	≥ 0.15 U/mL
Cholesterol oxidase	≥ 0.1 U/mL
Pipes Buffer	80 mmol/L; pH 6.8
Standard	5.17 mmol/L (200 mg/dl)

Procedure

The following steps were followed

Reagent	Reagent blank	Standard	Sample
D. W.	10 µL	———	———
Standard	———	10 µL	———
Sample	———	———	10 µL
Reagent	1.0 mL	1.0 mL	1.0 mL

The tubes are mixed well and incubated for 10 minutes at 25 ºC, then the absorbance was read against the reagent blank at 500 nm.

Calculations

Cholesterol concentration (mg/dL) = $A_{sample} / A_{standard} \times 200$

2.2.1.2. Determination of serum Triglyceride

Principle

The determination of triglycerides is based on enzymatic hydrolysis by lipase. The colored compound is a quinoneimine that formed by the react of hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol. The last reaction catalyzed by peroxidase [68].

Triglycerides + H_2O —lipase→ Glycerol + Fatty acids

Glycerol + ATP —Glycerol kinase→ Glycerol -3-phosphate + ADP

Glycerol -3-phosphate + O_2 —GPO→ dihydroxyacetonephosphate + H_2O_2

$2H_2O_2$ + 4-chlorophenol + 4-Aminophenazone —peroxidase→ Quinoneimine + HCl + $4H_2O$

Reagents

Contents	Concentration of solutions
Buffer	
Pipes Buffer	40 mmol/L; pH 7.6
4-chloro-phenol	5.5 mmol/L

Magnesium-ions	17.5 mmol/L
Enzymatic reagent	
4-aminophenazone	0.5 mmol/L
ATP	1.0 mmol/L
Lipases	≥ 150 U/mL
Glycerol-kinase	≥ 0.4 U/mL
Glycerol-3-phosphate oxidase	≥ 1.5 U/mL
Peroxidase	≥ 0.5 U/mL
Standard	2.29 mmol/L (200 mg/dl)

Working reagent was prepared by quantitative transfer of enzyme vial content to buffer container.

Procedure

The following steps were followed

Reagent	Reagent blank	Standard	Sample
D. W.	10 µL	———	———
Standard	———	10 µL	———
Sample	———	———	10 µL
W. R.	1.0 mL	1.0 mL	1.0 mL

The tubes are mixed well and incubated for 10 minutes at 25 ºC, and then the absorbance was read at 500 nm against the reagent blank.

Calculations

Triglyceride concentration (mg/dL) = $A_{sample} / A_{standard} \times 200$

2.2.1.3.Determination of High density lipoprotein Cholesterol concentration

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After separating, the high density lipoprotein cholesterol (HDL) remains in the in the supernatant and can be determined according to cholesterol colorimetric determination method.

Reagents

Contents	Concentration of solutions
Precipitating reagent	
Phosphotungstic Acid	0.55 mmol/L
Magnesium Chloride	25 mmol/L

Procedure

The following steps were followed

A. Precipitation

Reagent	Sample
Precipitating reagent	500 µL
Sample	200 µL

The tube had been mixed well and incubated for 10 minutes at 25 ºC. Then it's centrifuged for 10 minutes at 4000 rpm and Separated off the clear supernatant.

B. HDL assay

Reagent	Reagent blank	Standard	Sample
D. W.	100 µL	———	———
Standard	———	100 µL	———

Sample	———	———	100 µL
Reagent*	1.0 mL	1.0 mL	1.0 mL

** The same reagent that used in cholesterol determination.*

The tubes are mixed well and incubated for 10 minutes at 25 ºC, and then the absorbance was read at 500 nm for standard and sample against the reagent blank.

Calculations

HDL concentration (mg/dL) = $A_{sample} / A_{standard} \times 200$

3.1. Lipids levels in serum of TM, TI and control groups

Lipids are necessary to save life of cells because they are important sources of energy. A lipid profile is determination of the level of specific lipids in the blood.

Two important lipids, cholesterol and triglycerides, are cycled in the blood by lipoprotein compounds. The lipoprotein particles are measured with a lipid profile. They are divided by their density into high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) [63].

Table (3-1) and figure (3-1) show the result of lipid profile of two groups of thalassemia patients (TM and TI) and control group. Cholesterol and LDL levels were lower in TI patients when compared with TM patients, and the last was lower than control group, these parameters were significantly decreased.

The level of TG was higher in TM patients than TI, and in TI was higher than control group, it was significantly increased.

Hypocholesterolemia in all phenotype of thalassemia patients has been reported since the beginning of the20th century [69-70]. Our result agree with the study of Hartman *et al*., who explained that TI patients showed significantly lower TC, HDL-C and LDL-C compared with TM, and TM lower than control group. No clear explanation was proposed for the reported lipid abnormalities in TI patients; accelerated erythropoiesis and enhanced cholesterol consumption were suggested as the more acceptable mechanism implicated in the hypocholesterolemia of TI patients [71].

Hypertriglyceridemia in thalassemia patients that's obtained in our results is in agreement with Al-Quobaili *et al*. and Ragab *et al*. [72,73] they suggested that anemia places the thalassemic patients at risk for decreased extrahepatic lipolytic activity, resulting in high serum triglycerides.

Table (3-1): Serum lipids levels of TM, TI and control

Variables	Statistics	Major	Intermedia	Control
T. G.	Mean± SD (mg/dl)	171.56±69.714	162.32±72.357	92.84±39.009
	ANOVA	**<0.001**		
Cholesterol	Mean± SD (mg/dl)	144.37±57.621	126.97±37.84	185.32±40.963
	ANOVA	**<0.001**		
H.D.L.	Mean± SD (mg/dl)	14.941±6.6979	14.780±5.6494	33.330±9.3026
	ANOVA	**<0.001**		
L.D.L.	Mean± SD (mg/dl)	95.114±52.7646	79.726±34.6976	133.4187±36.8666
	ANOVA	**<0.001**		

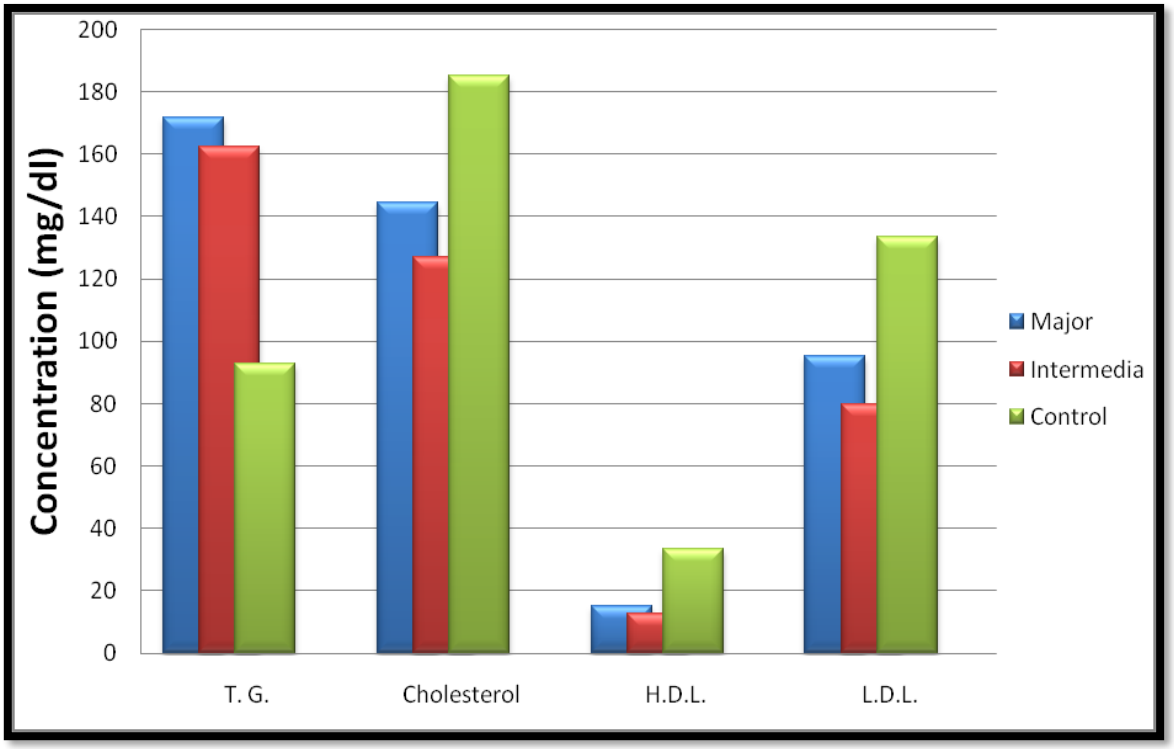


Figure (3-1): Serum lipids levels of TM, TI and control groups

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