Evaluation of Paraoxonase1 Activities and Lipid Profiles Concentration in Sera of β-Thalassemia Major Patients

Ghufran Mohammed Hussein*

Abstract

Determination of serum paraoxonase1 activities and lipid profiles concentration and its action in the increasing the susceptibility for atherosclerosis development in βTM patients. The study included βTM patients in addition to apparently healthy control group. The study was conducted on 35 patients with βTM (22 males and 13 females), aged 5 to 15 years and the control group include 31 subjects (19 males and 12 females) those are apparently healthy, aged 6 to 17 years were used as control. Blood was taken from all subjects for determination of serum paraoxonase1 activities, lipid profile, iron and ferritin concentration. The results of present study show significant drop in serum paraoxonase and arylesterase activity, LDL-cholesterol, total cholesterol, HDL-cholesterol concentration and significant increase in serum iron, ferritin and triglycerides concentration in βTM group in comparism with control (healthy) group. In conclusion, a drop-in serum PON1 activities and raise serum ferritin and iron concentration are increase the susceptibility for development of atherosclerosis in βTM patients.

Keyword: β-thalassemia major; Paraoxonase-1; Lipid profiles and Atherosclerosis

*Corresponding Author: ghufran.mohammed@qu.edu.iq
Department of Clinical Laboratory Science, Collage of Pharmacy, Al-Qadisiyah University
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Introduction

Thalassemias is an inherited disease that resulted from inadequate or missing in of alpha or beta globin chains synthesis in hemoglobin [1]. β-thalassaemia major (βTM) is a hereditary severe anemia resulted from severely impaired synthesis of β-globulin chain [2,3]. The βTM is allied with hemolysis, impaired erythropoeisis, jaundice, bone marrow damage and cardiomegaly. The excess of iron is deposited in several organs such as heart, reticuloendothelial system, and endocrine glands lead to damage to these organs [4,5]. In patients with βTM, the iron overload is responsible for reactive oxygen species formation for example hydroxyl radical, that

promote oxidative stress by Fenton reaction [6,7]. The increasing in free radical formation lead to oxidation of LDL (low density lipoprotein) [8]. The lipids (oxidized), such as LDL are the main reason of inflammation and are responsible for the beginning and/or proliferation of several inflammatory diseases including atherosclerosis [9]. Paraoxonase (PON) is a group of three enzymes named as paraoxonase1 (PON1), paraoxonase2 (PON2) and paraoxonase3 (PON3) [10]. In human, serum PON1 enzyme is bound to HDL (high-density lipoprotein) and it have paraoxonase/ arylesterase activity that give the action to the HDL as antioxidant [11,12]. The liver is the main site of PON1 synthesis and then secreted into plasma [13]. The antioxidant enzymes prevent oxidation of biomolecules, one of these antioxidant enzymes is PON1 which linked with HDL to avert of LDL oxidation. Consequently, the PON1 perform an important protecting function from atherosclerosis through decreasing serum lipoproteins oxidation [14]. Also, a number of studies showed that oxidative stress is responsible for decreasing the serum PON1 activities and down regulation the expression of serum PON1 [15,16]. The goal of the study to estimate paraoxonase1 activities and lipid profiles concentration and its action in an increasing the susceptibility for atherosclerosis development in βTM patients.

Materials and Methods

The study built-in βTM patients and healthy control group. The study was conducted on 35 patients with βTM (22 males and 13 females), aged 5 to 15 years, who attended in the thalassemia center at Babylon Maternity and Pediatric Hospital in Babylon province. All cases were diagnosed by specialist pediatric physicians depending on the family history, clinical features and established by Hb-electrophoresis, blood film and serum ferritin. The control group include 31 subjects (19 males and 12 females) those are apparently healthy, aged 6 to 17 years were used as control.

Keeping out criteria for subjects were having hypothyroidism, hyperthyroidism, hereditary hyperlipidemia and renal failure.

From all subjects, 6 mL of blood was drawn by vein puncture after an overnight fast and the blood was separated into two portions, the first portion contain 4 mL of blood sited in plain tube. This portion for determination of serum serum paraoxonase1 (paraoxonase and arylesterase) activities, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, ferritin and iron concentration). A second portion (two milliliters) placed in EDTA containing tube for determination of serum hemoglobin

concentration and hemoglobin-electrophoresis analysis. The activity of serum paraoxonase was measured by utilizing the paraoxon (as a substrate) and because of the generation of 4-nitrophenol, its activity increased at 412 nm absorbance. The activity of serum paraoxonase was determined at room temperature by addition of the serum (50 µL) to Tris-HCl buffer (1 mL) (100 mM at PH=8) including $CaCl_2$ (2 Mm) with paraoxon (5 mM). The generation velocity of 4-nitrophenol was measured at 412 nm. The 17 100 M^{-1} cm^{-1} (molar extinction coefficient) was used for calculating the activity of enzyme. The activity of serum paraoxonase was uttered in international units per 1 litre of sera (U/L) [17].

The activity of serum arylesterase was measured by utilizing the substrate (phenylacetate). The dilution of serum was four hundred times within Tris-HCl buffer (100 mM at pH=8). The reaction mixture includes phenylacetate (2.0 mM) and $CaCl_2$ (2.0 mM) within Tris-HCl buffer (100 mM at PH=8). Initial hydrolysis velocity was measured through subsequent the raise in concentration of phenol at 37 °C and 270 nm. The activity of serum arylesterase was uttered in international units per 1 litre of sera (U/L) [18].

Serum total cholesterol, triglycerides, and HDL-cholesterol concentrations are measured by Biolabo SA (France) kit. Friedewald equation was used for measurement of serum LDL-cholesterol concentration [19]. Serum iron concentration are determined by Human (Germany) kit. Serum ferritin concentration is determined by creative diagnostics (USA) ELISA kit. Statistical analyses were done by SPSS.

Results

The demographic and laboratory characteristics of βTM and the control (healthy) group as revealed in table (1-1). The results of present study show a significant decrease in serum paraoxonase and arylesterase activity in βTM group in comparism with control (healthy) group as revealed in table (1-2). Also, this study show the serum LDL-cholesterol, total cholesterol, HDL-cholesterol concentration were significantly decreased and significant increase in the serum triglycerides concentration in βTM group in comparism with control (healthy) group as revealed in table (1-3).

Table (1-1):

General characteristics of βTM and control (healthy) group

Character	Group	Mean ± SD	P value
Number	Control	31	
	βTM	35	
Sex (Male/Female)	Control	19/12	
	βTM	22/13	
Age (year)	Control	11.25 ± 3.50	P > 0.05
	βTM	10.71 ± 3.08	
Hemoglobin g/L	Control	12.19 ± 1.57	P < 0.001
	βTM	6.82 ± 1.33	
Iron (µg/dL)	Control	105.09 ± 29.67	P < 0.001
	βTM	161.97 ± 36.38	
Ferritin (µg/L)	Control	63.83 ± 21.62	P < 0.001
	βTM	2942.71 ± 1512.25	

Table (1-2):

Mean serum paraoxonase1 activities in βTM and healthy control group

Parameter	Group	Mean ± SD	P-value
Paraoxonase (U/L)	Control	123.22 ± 38.29	P < 0.001
	βTM	85.34 ± 26.31	
Arylesterase (U/L)	Control	68.18 ± 9.32	P < 0.001
	βTM	52.74 ± 6.45	

Table (1-3):

Mean serum lipid profiles concentration in βTM and healthy control group

Parameter	Group	Mean ± SD	P-value
Triglycerides (mg/dL)	Control	101.9 ± 32.50	P < 0.001
	βTM	143.90 ± 40.97	
Total Cholesterol (mg/dL)	Control	167.06 ± 27.60	P < 0.001
	βTM	119.22 ± 28.06	
LDL-Cholesterol (mg/dL)	Control	99.51 ± 28.53	P < 0.001
	βTM	64.57 ± 19.58	
HDL-Cholesterol (mg/dL)	Control	48.87 ± 8.33	P < 0.01
	βTM	30.28 ± 4.52	

Discussion

This study shows significant raise in serum iron and ferritin concentration and significant decrease in serum hemoglobin concentration. The main causes of iron overload are ineffective erythropoiesis and blood transfusion. The patients with βTM suffer from anemia as a result of ineffective erythropoiesis [20,21]. The excess of iron can produce reactive oxygen species and encourage oxidation of cells and the excess of iron is build up in several organs for example heart, liver, pancreas and endothelial tissue [22,23]. The reactive oxygen species can promote oxidation of protein and lipid. Oxidation of lipoproteins such as LDL that is lead to formation of oxidized LDL in endothelial layer of vessels [24]. Modified or oxidized LDL is scavenged by macrophages through the scavenger receptors that present in cell surface, that is leads to formation of foam cell. The deposition and infiltration of foam cells in the wall of the artery are represent the beginning step in the atherometous formation [25].

There are several mechanisms by which the antioxidants reduce atherogenesis and improve vascular function [26]. Many enzyme systems provide the defense from the oxidants (reactive oxygen species) and responsible for distraction the oxidized protein and lipids. One of these antioxidants enzyme is PON1 (paraoxonase/arylesterase activities) [27]. Paraoxonase-1 hydrolyses organophosphates, phenyl acetate, aromatic esters, lipid peroxidation products, and

prevent the damaging effect of oxidized LDL. Therefore, PON1 prevents the atherosclerosis acceleration [28].

This study shows the serum paraoxonase and arylesterase activity were significantly decreased in βTM group in comparism with healthy control group, the current results are in consistence with the result of Rageb M. [29] and Asif M. [30] studies wherein the activity of both paraoxonase and arylesterase were decreased in βTM group in comparism with healthy control group. A number of studies have obtained that paraoxonase1 activities avert the oxidation of HDL and LDL. In oxidized LDL, the active lipids can have obliterated by paraoxonase 1, also paraoxonase 1 prevent the initiation of inflammatory responses in the cells of wall of the artery. The LDL oxidation is documented as a beginning step in the atherosclerosis development, that resulting in LDL uptake by the scavenger receptor of macrophage and therefore formation of foam cells [31,32]. Also this study show the serum LDL-cholesterol, total cholesterol, HDL-cholesterol concentration were significantly decreased while there are a significant increase of serum triglycerides concentration in βTM group in comparism with healthy control group, the current results are in consistence with Arıca1 V. [33] and Cece H. [34] studies wherein the serum HDL-cholesterol, LDL-cholesterol, total cholesterol, concentration were established to be considerably lesser in βTM patients than control group, whereas the serum triglycerides concentration were considerably increased in βTM group in comparism with healthy control group. Thalassemia syndromes have lowering effect on LDL-cholesterol concentration. The increasing cellular LDL uptake via bone marrow to supply cholesterol to accelerate the proliferation of erythroid cells and increased in an inflammatory cytokines production which decrease the hepatic secretion and accelerate the LDL-cholesterol catabolism, all these reasons have been suggested to be the main causes for decreasing LDL-cholesterol concentration in βTM patients [35].

Inconclusion; the drop-in serum PON1 activities and raise serum ferritin and iron concentration are increase the susceptibility for development of atherosclerosis in β-thalassemia major patients.

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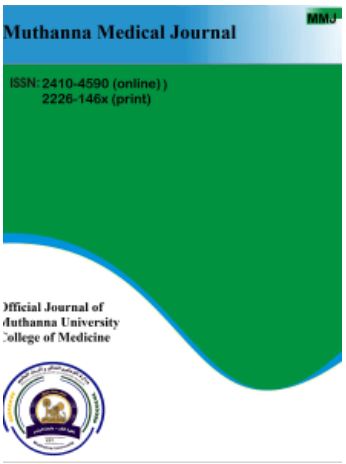

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Postal Mail
Muthanna Medical School
Samawah
Tel: +964 (782) 542-5669
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