Arlyesterase activity of Paraoxonase-1 enzyme in Iraqi patients with β-thalassemia minor

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Abstract

Background: Arylesterase activity of Paraoxonase-1 (ARE-PON-1) exhibits an antioxidant role which protects lipoprotein from oxidation. It is known that ARE-PON-1 antioxidant activity associated with high density lipoprotein cholesterol (HDL-C) reduces the oxidative damage mediated by low density lipoprotein cholesterol (LDL-C). The present study was aimed to examine the level of serum ARE-PON1 in Iraqi patients with β-thalassemia minor and its relationship with lipid profile (total cholesterol (TC), HDL-C, very low density lipoprotein (VLDL-C), and LDL-C) and hematologic changes as a part of antioxidant system action. Methods: In the present study, the ARE-PON-1 activity was investigated in serum of patients with β-thalassemia minor. Results: It has been revealed that the ARE-PON-1 activity was significantly decreased (P<0.0001) in patients group compared to healthy group. This was associated with slight decrease in the lipid profile of patient group (HDL, LDL-C, very low density lipoprotein cholesterol (VLDL-C), and total cholesterol (TC), except of triglyceride (TG) which showed a significant decrease (P<0.0001) compared to healthy group). Also, hematologic changes were investigated and showed depleting in most of white blood cell (WBC) and red blood cell (RBC) components. Conclusion: Patients with β-thalassemia minor are suffering from weakness in the antioxidant defence system expressed by dropping in the ARE-PON1 level and lipid profile that stimulate oxidation of many cell particles such as HGB, promote EOS apoptosis and rise of CD risk development.

Keywords: Paraoxonase-1, arylesterase, β-thalassemia minor, lipid profile, WBC, RBC.


Introduction

β-thalassemias are an inherited disorder of blood haemoglobin disorder diagnosed by abnormality in the creation of β-chains of haemoglobin (HGB) causing different phenotypes and leading to chronic haemolytic anaemia [1]. In β-thalassemias, the abnormality in β-globin gene causes reduction (beta+) or complete lacking (beta0) in the synthesis of the normal β-globin chains [2, 3]. This leads to reducing the synthesis of HGB and accumulation of α-globin chains which are responsible for the pathology of the disorder [6].

The total annual incidence of β-thalassemia estimates that the symptomatic individuals at 1 of 100,000 through the world (about 1.5% of the world, i.e. 80-90 million persons are harbouring β-thalassemia) and in the European Union at 1 of 10,000 people. It is also reported that β-thalassemia is highly prevalent in the Mediterranean countries such as Cyprus 14%, Sardinia 10.3% and Greece 6.3% along with South America, north coast of Africa and Asian countries [2, 5].

Depending on clinical severity and genetic disorder, β-thalassemia can be categorised into three main groups; thalassemia major, thalassemia intermedia and thalassemia minor [2]. Thalassemia major is associated with severe anaemia and usually diagnosed in the first two years of life, necessitating regular red blood cell (RBC) transfusions as well as iron chelating treatment to prevent organs damage by iron overload [2, 6, 7]. Patients may need periodic blood transfusion, depending on iron overload development, and chelating therapy [2, 8].

In thalassemia minor form, patients, clinically, are considered asymptomatic; however, some individuals might have mild anaemia. This form of anaemia can be controlled without blood transfusion or treatment. However, patients remain susceptible to iron overload [2, 9].
Moreover, β-thalassemia minor is usually accompanied with biochemical and rheological variations which can cause cardiovascular disease (CD)\(^{[5]}\). CD has a higher prevalence in patients with β-thalassemia and the potential mechanisms for this include rising in nitric oxide destruction, enhanced macrophage and platelet activation, and stimulation of low density lipoprotein cholesterol (LDL-C) oxidation. Another possibility is that reduction in HGB levels in anaemia that is reported as independent risk factor for CD\(^{[3]}\).

Iron overload that observed in β-thalassemia patients, which is due to increase intestinal absorption of iron, impaired erythrocyte hemolysis and transfusion of erythrocyte, has a key role in stimulation of oxidative stress (OS). OS in the presence of free iron leads to cell deterioration and causes oxidative damage in main organs, particularly in the CD system. These complications start in the early stage of thalassemia and initiate severe CDs in future\(^{[10, 11]}\).

Modified LDL-C resulting from oxidative stress initiates a process that leads to atherogenesis-related vascular variations. Modified LDL-C is localised in monocytes-derived macrophages causing the creation of foam cells that are infiltrated and stored in the arterial wall\(^{[12]}\). These processes are considered the starting steps to promote atherosclerotic plaque. LDL-C particles are undergoing oxidation inside the vessel wall by different types of processes such as myeloperoxidase and lipoxygenase pathways. The particles of oxidised LDL-C activate the production and release of some factors such as adhesion molecules and monocytes chemotactic protein-1 which contribute to the transporting of inflammatory cells to arterial wall\(^{[12]}\). High density lipoprotein cholesterol (HDL-C) displays a defence action to circumvent this inflammatory cycle through protecting LDL-C from oxidation. This process may include paraoxonase-1 (PON1) that abolishes inflammatory cells migration to arterial wall\(^{[12]}\).

Human PON1 is an esterase calcium-dependent glycoprotein, belongs to PON family (PON1, PON2, PON3)\(^{[13]}\). It is mainly synthesized in the liver and then secreted in blood stream where the PON1 is associated with HDL. PON1 is also distributed in the endothelial layer of several tissues, such as kidneys and small intestines\(^{[12, 14, 15]}\). It has been found that PON1 exhibits activity of arylesterase (ARE) (aromatic carboxylic acid esters hydrolysis), lactonase (lactones hydrolysis), and paraoxonase (organophosphatase hydrolysis)\(^{[15-17]}\). These activities are described as one of PON1 physiological functions as well as the antioxidant activity which is considered another biological role of PON1\(^{[18, 19]}\). It was also demonstrated that more than 95% of the PON1 in serum exhibits ARE activity (ARE-PON1) as in vitro investigated by controlling phenylacetate hydrolysis\(^{[16]}\). Antioxidant activity appears when PON1 is structurally binding to HDL-C. This activity is expressed through inhibiting the oxidation process of lipoprotein, especially LDL-C, to lipid peroxidation. It has also been reported that the deficiency in PON1 is correlated to increase of LDL-C oxidation susceptibility and progress of atherosclerosis\(^{[20, 21]}\).

The present study was aimed to examine the level of serum ARE-PON1 in Iraqi patients with β-thalassemia minor and its relationship with lipid profile (total cholesterol (TC), HDL-C, very low density lipoprotein (VLDL-C), and LDL-C) and hematologic changes as a part of antioxidant system action.

**Subjects and Methods**

This study was performed on 15 subjects with β-thalassemia minor (with an age range of 20-50 years) who were attending Mohammed Baqer Al-Hakim Hospital, and 14 gender- and age-matched healthy individuals. Blood samples were collected in plane tubes (free from anticoagulant materials) left for 15 minutes at room temperature before centrifugation at 3500rpm for 10 minutes. Sera were alliquoted into new tubes and ARE-PON1 activity and lipid parameters were determined immediately. Blood samples for Complete Blood Count (CBC) were collected using a 3- or 5-ML K3 EDTA tube on all samples. The CBC was run immediately after blood drawing. Remaining sera were stored at -20°C for further measurements.

Lipid profile analysis (TG, TC, and HDL-C) was determined using commercially available assay kits (cromatest®) with spectrophotometer (PD-303 APEL, Japan). HDL-C was determined after selective precipitation of LDL-C and VLDL-C by phosphotungstic acid/MgCl\(_2\) using cromatest® kit. Non-HDL-C concentration was calculated by subtracting HDL-C values from TC values. LDL-C concentration was calculated using Friedewald’s equation. VLDL-C concentration was estimated through dividing TG by 5.

ARE-PON1 activity was determined using phenylacetate substrate. An assay included use of tris- HCl buffer (100mM) pH 8.0, CaCl\(_2\) (2mM) and phenyl acetate (4mM) solution. This solution should prepared fresh daily. In this assay the rate of phenylacetate hydrolysis was determined spectrophotometrically using kinetic mode through monitoring the increase in the generation rate of phenol at 270nm and 25°C for 2 minutes. The enzyme activity was determined from the molar absorptivity coefficient of phenol (1310 M\(^{-1}\)cm\(^{-1}\)). ARE-PON1 activity was expressed as U/L serum, where one unit of enzyme activity was defined as 1µmol of phenol generated/minutes under the above conditions.

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Serum total protein (TP) assay: TP concentration was determined using Biuret method. Serial dilutions of Bovine serum albumin (BSA) were prepared from 10mg/mL of BSA stock solution which was used as a standard for protein determination. Each of standards and samples (1mL) were mixed with Biuret reagent (2mL), and allowed to stand for 15 minutes at room temperature. The absorbance was read at 550nm.

CBC measurements: CBC was performed using Five-Part Differential method according to Lab Protocol for NHANES 2003–2004 data with Beckman Coulter MAXM. Beckman Coulter method for counting and sizing used to derive CBC parameters. An automatic mixing and diluting device was used for sample treatment and a single beam spectrophotometer for haemoglobinometry. Three simultaneous measurements were used for analysis and classification of white blood cells (WBC) including individual cell volume (V), high frequency conductivity (C), and laser light scatter (S) which is known as VCS technology. The scattergram for the cells was plotted upon measurements of these three parameters.

Statistical analysis

Results are expressed as mean value ± standard deviation. Statistical analysis was performed using $t$-test by Graph Pad Prism software to estimate the differences between $\beta$-thalassemia patients group and healthy persons group. Variable distribution was determined using the Holm-Sidak method, where $P<0.05$ was accepted as statistical significance. Correlations were applied for ARE-PON1 with significant variable parameters to study the association of ARE-PON1 with these parameters.

Results and Discussion

$\beta$-thalassemia minor is widely distributed in the world that can develop to CD. OS stimulated by $\beta$-thalassemia is mainly contributed in CD. This study investigated the changes in the ARE-PON1 activity and TP along with lipid profile as part of defence mechanisms to neutralize the OS effects in $\beta$-thalassemia minor patients. WBCs and red blood cells (RBCs) changes were also examined to introduce a complete study that links between antioxidant system and haematology in case of $\beta$-thalassemia minor.

The results showed that the activity of ARE-PON1 enzyme has been significantly decreased in patients with $\beta$-thalassemia minor compared to healthy group ($P<0.0001$). However, serum TP level remained nearly unchanged between these two groups.

This was accompanied with a slight decrease in the concentration of HDL-C, LDL-C, VLDL and TC in patients group compared to healthy group (Table 1).

Table (1) ARE-PON1 activity, TP concentration and lipid profile levels in patients with $\beta$-thalassemia minor compared to healthy individuals

<table>
<thead>
<tr>
<th>ARE-PON1 (U/L)</th>
<th>TP (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>Non-HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>VLDL-C (mg/dL)</th>
<th>TG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20.80±3.74</td>
<td>7.77±0.1</td>
<td>179.87±69.9</td>
<td>37.24±18.4</td>
<td>139.51±58.4</td>
<td>118.52±52.2</td>
<td>20.81±14.15</td>
</tr>
<tr>
<td>Patient</td>
<td>0.87±0.7</td>
<td>7.70±0.0</td>
<td>153.66±32.0</td>
<td>33.89±11.8</td>
<td>125.61±34.3</td>
<td>106.41±22.3</td>
<td>12.02±3.05</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.1</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

concentration was dramatically declined in patients group compared to healthy group. It has also been found that there was a positive correlation of TG with ARE-PON1 (Figure 1).

ARE activity is strongly related with the circulating quantity of PON1 protein [23]. Therefore, the dropping in ARE activity in $\beta$-thalassemia may reflect the disturbance in serum and blood cells oxidant/antioxidant balance that leads to OS. It has been reported that ARE-PON1is involved in the antioxidant system through preventing LDL oxidation [23]. Hence, it can be hypothesized that the variation in ARE-PON1 level is associated with diseases in which OS is a major part in their pathogenesis such as breast cancer [24] and brain cancer [25]. In $\beta$-thalassemia minor the decrease in ARE-
PON1 activity may also be considered as a marker for prediction of increased CD and atherosclerosis incidence as has been reported by many studies [26-28].

Our outcomes agreed with [29] who reported that the ARE-PON1 activity was markedly decreased in patients with β-thalassemia minor which contributed to developing CD risk factor as the ARE-PON1 activity contributes to preventing LDL modified oxidation. Furthermore, the vast majority of research that studied the ARE-PON1 activity in diseases associated with OS suggested that there was a substantial dropping in enzyme activity compared to healthy persons suggesting the strong relationship between OS and ARE-PON1 activity [30-36].

Moreover, our results showed a decrease in lipid profile that was in parallel with ARE-PON1 level. Several studies showed the strong correlation of HDL level with ARE-PON1 activity [12, 21, 33]. Decrease in HDL associated with ARE-PON1 contributes to the weakness of antioxidant system where the ARE-PON1 has been found to be responsible for HDL-C antioxidant activity. Consequently, this leads to an increase in LDL-C oxidation, accumulation of lipid peroxide and OS occurrence. Furthermore, dropping in LDL-C concentration in β-thalassemia minor might be due to an increase of its bone marrow uptake to supplement with cholesterol. This is necessary for increasing inflammatory cytokines production and rising of erythroid progenitor cell proliferation which decreases the hepatic secretion and surge of LDL catabolism.

Regarding haematology, size and types of WBCs as well as RBCs were also determined in this study. Our results indicated that the major changes in WBCs counts were in eosinophils (EOS) and basophiles (BASO). The former were dramatically decreased in patients with β-thalassemia minor compared to healthy group; whereas basophiles (BASO) were significantly increased in patients group compared to healthy group (Table 2).

### Table (2) WBCs counts including neutrophil (NEU), lymphocyte (LYM), monocyte (MONO), EOS and BASO in patients with β-thalassemia minor and healthy persons

<table>
<thead>
<tr>
<th></th>
<th>WBC (10^9/μl)</th>
<th>NEU (%)</th>
<th>LYM (%)</th>
<th>MONO (%)</th>
<th>EOS (%)</th>
<th>BASO (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.33±1.73</td>
<td>4.56±1.52</td>
<td>2.52±0.58</td>
<td>0.54±0.12</td>
<td>0.23±0.10</td>
<td>0.05±0.03</td>
<td>0.9</td>
</tr>
<tr>
<td>Patient</td>
<td>7.29±3.43</td>
<td>3.86±2.15</td>
<td>2.83±1.33</td>
<td>0.41±0.32</td>
<td>0.08±0.07</td>
<td>0.10±0.07</td>
<td>&lt;0.0001 &lt;0.05</td>
</tr>
</tbody>
</table>

It was reported that eosinophils are involved in cell apoptosis and this is associated with oxidant/antioxidant status. [37] stated that the oxygen-dependent mechanisms play important role in the regulation of eosinophils survival/apoptosis. They found that the eosinophils apoptosis is stimulated by cellular oxidative metabolisms and repressed by antioxidants. Therefore, we speculated that the reduction in ARE-PON1 activity might have stimulated EOS apoptosis and led to EOS cell death.

Interestingly, we found that there was a negative correlation between ARE-PON1 activity and EOS (Figure 1). We supposed that the cell defence mechanisms are stimulated by the oxidants to maintain the oxidant/antioxidant balance. Simultaneously, EOSs undergo apoptosis mediated by rising of cellular oxidative metabolisms.

### Table (3) RBCs indices including HGB, HCT, MCV, MCH, MCHC, RDW and PLT in patients with β-thalassemia minor and healthy persons

<table>
<thead>
<tr>
<th></th>
<th>RBC (10^9/μl)</th>
<th>HGB (g/dL)</th>
<th>HCT (%)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>RDW (%)</th>
<th>PLT (10^9/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.27±0.64</td>
<td>14.36±1.37</td>
<td>43.52±4.0</td>
<td>87.24±4.3</td>
<td>29.26±1.9</td>
<td>27.66±2.8</td>
<td>12.42±1.16</td>
<td>289±75.4</td>
</tr>
<tr>
<td>Patient</td>
<td>4.81±0.88</td>
<td>9.31±3.13</td>
<td>33.46±6.2</td>
<td>69.47±4.7</td>
<td>19.35±3.3</td>
<td>27.69±2.8</td>
<td>16.46±2.04</td>
<td>186±75.4</td>
</tr>
</tbody>
</table>

Haematological analysis also showed that patients with β-thalassemia minor underwent from markedly decreased in each of haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red cells distribution width (RDW)and mean platelets volume (PLT) compared to healthy persons (Table 3)
It was also observed that there were positive correlations between ARE-PON1 activity with each of HGB, HCT, MCV and MCH. However, ARE-PON1 activity has a negative correlation with RDW and PLT (Figure 1).

The depletion in ARE-PON1 enzyme gives an indication of increase in oxidants levels and this might lead to enhancing oxidation of RBC components such as HGB. As a result of this process, the RBC loses its function and becomes inefficient to oxygen transfer. The reticuloendothelial system removes these abnormal RBCs and this leads to decrease the circulating RBCs quantity and HGB concentration which induces haemolytic anaemia.

**Conclusion**

We can conclude that patients with \( \beta \)-thalassemia minor suffering from weakness in the antioxidant defence system expressed by dropping in the ARE-PON1 level and lipid profile that stimulate oxidation of many cell particles such as HGB promotion of EOS apoptosis and rise of CD risk development.

**Ethical Clearance**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**

The authors declare that they have no conflict of interest

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