The association Between Serum Peroxiredoxin 2 and Iron Overload in Pediatric Patients with Beta Thalassemia: Single center study

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June 22, 2020

Abstract

Background: Oxidative stress is fundamental in initiating pathophysiological mechanisms leading to premature hemolysis resulting in iron overload in patients with beta thalassemia. Peroxiredoxin 2 (Prdx2) is one of the crucial cytoprotective and antioxidant systems that play a key role against oxidation. Our aim was to investigate serum Prdx2 levels in children with beta-thalassemia and to explore its possible relations with iron overload. Methods: The patients were divided into two groups: beta thalassemia major (BTM) group (n=40) and beta thalassemia intermedia (BTI) group (n=20). To compare serum Prdx2 and iron status parameters levels, a control group (n=20) was include in the study. Serum Prdx2 levels were determined by enzyme linked immunosorbent assay technique. Serum levels of iron and ferritin were measured using automated chemistry analyzer and electrochemiluminescence immunoassay respectively. Results: Serum Prdx2 concentrations in thalassemia major patients were significantly lower than those in thalassemia intermedia patients (P= 0.026); and Prdx2 concentrations in thalassemia intermedia patients were significantly lower than those in control group (P<0.001). In both thalassemia major and intermedia groups, serum Prdx2 concentration was positively correlated with serum iron (r = 0.558, P=0.002; r = 0.718, P=0.004, respectively) and ferritin levels (r = 0.422, P=0.007; r = 0.550, P=0.012, respectively). Conclusions: Our results demonstrate the positive association between Prdx2 and iron overload in thalassaemia patients. These findings may suggest unconventional therapeutic approach to control consequences of iron overload through modification of Prx2 activity.

Introduction

Beta-thalassemia (β-thalassemia) is a highly diverse group of inherited chronic anemia in which beta-globin chain synthesis is defective. Consequently, free unpaired alpha-globin chains are accumulated and precipitated, leading to erythrocytes destruction and ineffective erythropoiesis [1].

Beta thalassemia major (BTM) patients demand frequent packed red blood cells transfusion to maintain adequate hemoglobin levels and to reduce hepatosplenomegaly due to extramedullary erythropoiesis. Both chronic hemolysis and transfusional excess iron load cause exaggerated generation of free radicals and oxidative damage to biological macromolecules [2].

Both transfusion dependent and transfusion independent thalassemia patients are susceptible to iron overload [3]. This necessarily leads to considerable damage to many vital organs, such as the heart and liver [4]. There are no physiological mechanisms to eliminate excess iron and to prevent its deposition into end organs, hence it is very essential to remove it with a pharmacological tool and limit the serious clinical consequences of its overload [5].

Peroxiredoxins (Prdxs) are thiol-specific antioxidant proteins, which act as reactive oxygen species scavengers. In mammalian, they include six distinct isoforms (Prdx1–6) that are distributed over diverse cellular compartments [6]. Prdxs 1, 2, 3, 5 and 6 are found in the cytoplasm, Prdx 1 in the nucleus, Prdxs 3, 5 and 6 in mitochondria, and Prdx 5 in mitochondria and peroxisomes [7-9].

Peroxiredoxin 2 (Prdx2) is widely expressed in various tissues and is essential regulator of cell proliferation, differentiation and apoptosis by modifying the intracellular level of hydrogen peroxide (H_2O_2) level [10].

Erythrocytes are extremely exposed to oxidative stress due to plenty of heme iron and oxygen, which can generate reactive oxygen species (ROS). Under conditions of excessive oxidative stress, erythrocyte membrane damage may occur because of the reduced activity of membrane-bound enzymes, the suppression of transmembrane transport mechanisms, and the leakage of cellular constituents into circulation. Thus, erythrocytes have very efficient defense mechanisms against oxidative stress. Prdx2, the third most abundant protein in erythrocytes, is known to play a protective function against ROS–induced damage in these cells [11, 12]. In this study, we aimed to investigate serum peroxiredoxin 2 levels, as a marker of oxidative stress, in children with beta-thalassemia and to explore its possible relations with iron overload parameters.

Methods

Beta thalassemia patients included in this study were recruited from Hematology Unit, Department of Pediatric Hematology, Menoufia University. The patients were divided into two groups: β thalassemia major group (n=40, aged 2–18 years, 16 female and 24 male) and β thalassemia intermedia group (n=20, aged 3–16 years, 9 female and 11 male). All thalassemia major patients were on regular blood transfusion and received iron chelation therapy, oral deferasirox, according to their serum ferritin levels.

The control group (n=20, aged 2–18 years, 11 female and 9 male) included healthy volunteers. Complete blood count (CBC), peripheral blood smears and high-performance liquid chromatography (HPLC) were performed to ensure exclusion of thalassemia traits or any hemolytic anemia for the control.

This study was approved by the Ethics Committee of Menoufia University. Informed consent was taken from the guardians of healthy controls and thalassemia patients.

Blood sampling

Blood samples from patients were collected prior blood transfusion. Venous blood samples were collected in EDTA (for CBC, peripheral smear) and plain tubes (for iron profile and Peroxiredoxin 2) from patients and controls. Blood samples collected were sent immediately to Hematology Unit, Clinical Pathology Department. Blood samples for CBC and peripheral smear were analyzed within one hour after collection. Samples collected for iron profile and Peroxiredoxin 2 were left to clot, then centrifuged at 3000 rpm for 15 min; separated sera for estimation were kept frozen at –20 °C until analysis.

Methodology

Automated complete blood counts (CBC) were analyzed by Sysmex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan). Liver transaminases were measured using AU680 chemistry analyzer (Beckman Coulter Inc., Brea, California, USA). Iron and TIBC were done by Cobas Integra 400 plus analyzer (Roche Diagnostics, Mannheim, Germany) and serum ferritin levels were measured using Cobas e411 immunoassay analyzer (Roche Diagnostics). Serum peroxiredoxin 2 levels were determined by enzyme linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Inc., Houston, Texas, United States, Catalog No: E-EL-H1137) in accordance with manufacturer's protocol.

Statistical analysis

Results were statistically analyzed by SPSS statistical package version 23 (Armnok, NY: IBM Corp.). Student's t-test and Mann Whitney's test were used for comparison of quantitative variables between two groups. One-way analysis of variance (ANOVA) and Kruskal Wallis tests were used for comparison of quantitative variables between more than two groups. Spearman correlation was used for correlation analysis. Chi-square test (χ^2) was used to study association between qualitative variables. Two-sided P value less than 0.05 was considered statistically significant.

Results

The clinical and laboratory characteristics of the thalassemia major and intermedia patients are summarized in (table 1). There was no significant difference between both groups regarding TLC and platelets count (P>0.05). There was significant difference between both groups in terms of age at first diagnosis, interval between blood transfusions, pretransfusion Hb and ALT (P= 0.002; P<0.001; P=0.017; P=0.017 respectively).

Demographic and Biochemical parameters of thalassemia patient and control groups

There was no significant difference between ages or gender of the patients and controls. A statistically significant difference between thalassemia patients and control group was observed with respect to iron profile parameters and Prdx2 (P <0.001) (table 2).

Serum Prdx2 concentrations in thalassemia major patients (65.44 ± 8.17 ng/ml) were significantly lower than those in thalassemia intermedia patients (70.58 ± 9.44) (P= 0.026); and Prdx2 concentrations in thalassemia intermedia patients were significantly lower than those in control group (77.64 ± 7.13 ng/ml) (P<0.001) (Table 2) (Figure 1).

Correlation between Prdx2 and iron overload parameters:

Correlation analyses revealed a significantly positive correlation between serum Prdx2 concentration and iron level in both thalassemia major and intermedia groups (r = 0.558, P=0.002; r = 0.718, P=0.004, respectively) (table3). A positive correlation was observed between Prdx2 concentration and transferrin saturation in thalassemia intermedia group only (r = 0.564, P=0.001) (figure 2). A positive correlation between Prdx2 concentration and ferritin concentration was detected in both thalassemia major and intermedia groups (r = 0.422, P=0.007; r = 0.550, P=0.012, respectively) (figure 3).

Discussion

Beta-thalassemia is a typical model of stress erythropoiesis characterized by intense oxidative stress [13]. The control of the deleterious effects of oxidation during erythropoiesis is extremely substantial to ensure cell differentiation and maturation [14, 15]. Recently, the key role of peroxiredoxin-2 was reported in normal and pathologic erythropoiesis. Prdx2 is a potent antioxidant agent that can act as a molecular chaperone targeting free heme to control oxidative insults [16].

Prdx2 was reported as a key cyto-protector against iron overload induced either by iron administration or due to chronic hemolysis in a mouse model of β-thalassemia [17]. As there have been no satisfactory reports on the relation between human serum Prdx2 and iron status parameters levels in β-thalassemia; the present study aimed to investigate serum Prdx2 levels, as a marker of oxidative stress, in children with beta-thalassemia and to explore its possible relations with iron status.

Previous studies reported the upregulated gene expression of Prdx2 in β-thalassemic erythroid cells compared to the expression in controls both in mouse [18] and human cell culture models [19].

In contrast with results from mouse and in vitro models, no significant difference was demonstrated between mRNA levels of Prdx2 in reticulocytes of BTM patients and control. As regard BTI, there was reduced mRNA levels of Prdx2 in reticulocytes of patients in comparison to control, but with no differences in Prdx2 of erythrocytes at the protein level. In addition, the protein levels of Prdx2 were highly increased in erythrocytes of BTM when compared to BTI patients [20].

In this study, we revealed that serum Prdx2 concentrations in BTM and BTI patients were significantly lower than those in controls. These low Prdx2 levels indicated that thalassemia patients might require much more antioxidant levels to overcome oxidative stress caused by iron overload.

In contrast to our results, Prdx2 levels were analyzed in plasma samples with no difference was reported between thalassemia major patients and control group [21].

Additionally, we reported that serum Prdx2 concentrations in BTM were significantly lower than those in BTI patients. Consistent with our results, some studies have shown elevated state of oxidative stress in BTM

more than BTI, both in plasma and erythrocytes [22, 23].

In this study, we analyzed the correlation between serum Prdx2 and iron overload parameters. We detected significant positive correlation between serum Prdx2 concentration; and iron and ferritin levels in both thalassemia major and intermedia groups. A positive correlation was found between Prdx2 concentration and transferrin saturation in thalassemia intermedia group only. These findings could be explained by the increased oxidative stress due to iron overload in thalassemia patients.

Our study provides the first demonstration of the relation between serum Prdx2 and iron overload parameters in human model with beta thalassemia.

Further studies will be required to clarify Prx2 function and regulation in both normal and stress erythropoiesis. Also, estimation of Prx2 levels at different stage of erythroid maturation may be useful to identify the definite role of Prx2 in beta thalassemia erythropoiesis.

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Figures legends:

Figure 1: Serum peroxiredoxin 2 levels in the three studied groups

Figure 2: Correlation between serum peroxiredoxin 2 and transferrin saturation in thalassemia patients.

Figure 3: Correlation between serum peroxiredoxin 2 and ferritin in thalassemia patients.

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