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Comparative study of oxidative status and some biochemical parameters in subjects suffered of intermediate and major Thalassemia

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***Abstract*---**In the β-thalassemia's, oxidative stress, resulting from chronic hemolysis, globin chain imbalance, iron overload and depleted antioxidant defenses, likely contributes to cell death, organ damage, anemia, hypoxia and inflammation. Between November 2020 and June 2021, the study assessed adults aged (25-35 years) attending to Hiwa Cancer Hospital/ Sulaimaniah Thalassemia and congenital blood disorders center/ Iraq: 60 patients with HbE β-thalassemia, 30 β-thalassemia major, 30 β-thalassemia intermediate and 30 control subjects. ferritin were measured as sources of oxidants; plasma antioxidants: Glutathione Reductase (GR) and Oxidative stress-induced growth inhibitor 2 (OSGIN2) , plasma D-dimer assessed thrombosis status and serum C-reactive protein assessed inflammation, the results showed increased in the levels of ferritin, plasma D-dimer and CRP in both β-thalassemia major and β-thalassemia intermediate (2789±789 ; 2657±725.8 ng/ml) , (175.4±54.02; 188.7±62.01 ng/ml) and (15.6±2.6; 13.3±3.1 mg/l) compared to control group (178.7±58.9 ng/ml) , (79.8±34.8 ng/ml) and (2.3± 0.7 mg/l) respectively. Whereas decreased in the levels of GR and OSGIN2 (38.4±12.7; 35±8.8 pg/ml) and (58±18.3; 78±20.5 mg/dl) compared to control group (96±17.3 pg/ml) and (156±65.6 mg/dl) respectively.

***Keywords*---**thalassemia, oxidative stress, d-dimer, glutathione reductase, OSGIN2.

Introduction

β-thalassemia (BT) is a genetic disease that affects thousands of people worldwide. It is characterized by anomalies in the synthesis of beta chains of hemoglobin (Hb) resulting in variable phenotypes, ranging from severe anemia to clinically asymptomatic individuals. This disease is classified as three different types: thalassemia major (BTM), intermediate (BTI) and minor (BTMi). BTM patients usually present severe anemia within the first two years of life and require regular blood transfusions. In contrast, BTI patients have moderate anemia and do not require regular blood transfusions, while patients presenting the BTMi phenotype are clinically asymptomatic and may have moderated anemia [1]. Despite being the most common monogenic diseases, the true disease burden of the inherited hemoglobin disorders is unknown. It is estimated that >7% of the world's population carry a hemoglobin variant resulting in 300,000–500,000 annual births with a serious hemoglobin disorder accounting for at least 3.4% of under-five deaths [2].

Oxidative stress is one of the leading factors that contribute to the early death of red blood cells, triggering them to undergo changes, such as denaturation of Hb and membrane lipids peroxidation, hence increasing inflammation and programmed cell death [3 ,4]. In hemolytic anemia's, the cellular environment becomes extremely prooxidant, culminating in increased reactive oxygen species (ROS) levels and consequent cell hemolysis, facilitating the diffusion of ROS to other cell types [5, 6]. It is widely recognized that major β-thalassemia patients have an increased risk of thrombosis. A study conducted by Michaeli J *et al.* reported 4% prevalence of thrombosis in thalassemia patients. Various cases have been reported, including neurological disorders that occur due to cerebral thrombosis[7]. C-reactive protein levels rise rapidly in response to infection or tissue inflammation [8,9]. Overall, many studies reported that the inflammation marker hsCRP was useful in thalassemia and has been shown to be positively associated with the risk of kidney and cardiovascular disease [10,11,12].

Materials and Methods

The study included 90 individuals from Hiwa Cancer Hospital/ Sulaimaniah Thalassemia and congenital blood disorders center/ Iraq: from February to July 2020, as 60 patients **:** HbE β-thalassemia, 30 β-thalassemia major, 30 β-thalassemia intermediate and 30 control subjects aged (25-35) years. Blood samples were collected from patients, and analysis was conducted to estimate the concentration of D-dimer , Ferritin , Glutathione Reductase, Oxidative stress-induced growth inhibitor 2 and C-reactive protein using Enzyme Linked Immunosorbent Assay (ELISA) Sunlong Biotech Company kits with the sandwich method [13]. Results were statistically analyzed using SPSS, with the data analyzed using a one-way contrast analysis of ANOVA followed by a multi-range Duncan test at a probability ($P<0.05$) to compare search groups.

Results

The results showed a significant increase in concentration of D-dimer, Ferritin and CRP in patients , rather than the results showed decreased in the levels of

GR and OSGIN2 when compared to the control group at the probability (P<0.05), as shown in Table (1) as well as Figure (1,2,3,4 and 5).

Table 1
Concentrations of D-dimer, Ferritin, (GR), (OSGIN2) and (CRP) in β-thalassemia major and β-thalassemia intermediate patients, and control group

Groups	No.	Age (Year)	Parameters				
			D-dimer (ng/ml)	Ferritin (ng/ml)	GR (pg/ml)	OSGIN2 (mg/dl)	CRP (mg/l)
Control	30	25-35	79.8±34.8	178.7±58.9	96±17.3	156±65.6	2.3± 0.7
Patients major	30		175.4±54.02	2789±789	38.4±12.7	58±18.3	15.6±2.6
Patients Intermediate	30		188.7±62.01	2657±725.8	35±8.8	78±20.5	13.3±3.1
P value			0.001	0.001	0.000	0.001	0.000

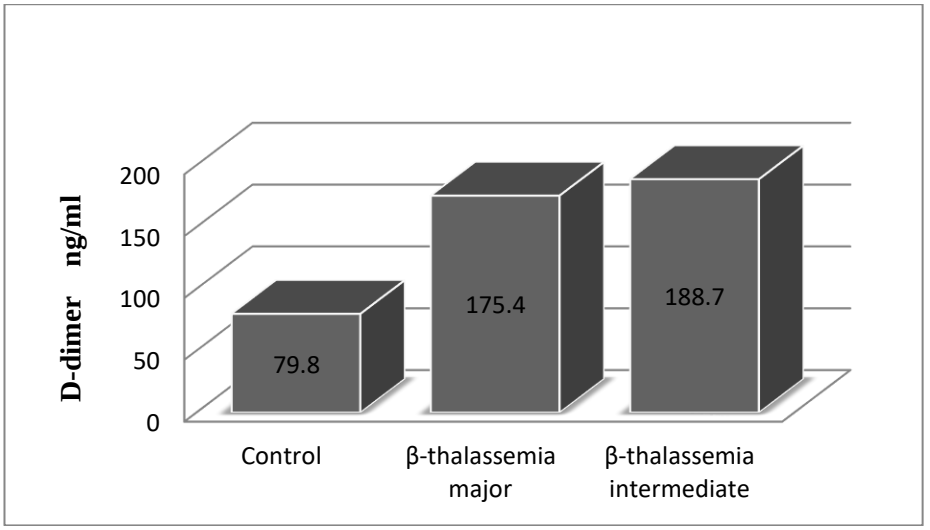

Fig. 1. Concentrations of D-dimer (ng/ml) in β-thalassemia major and β-thalassemia intermediate patients, and control group

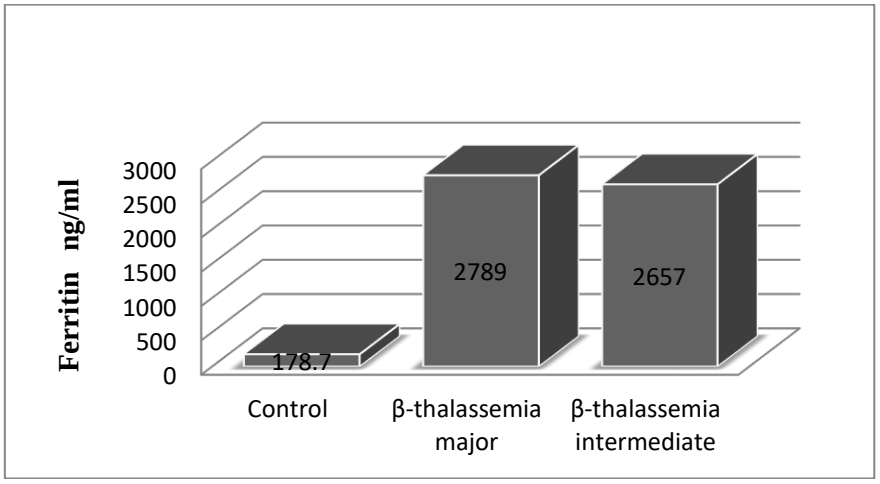

Fig. 2. Concentrations of Ferritin (ng/ml) in β-thalassemia major and β-thalassemia intermediate patients, and control group

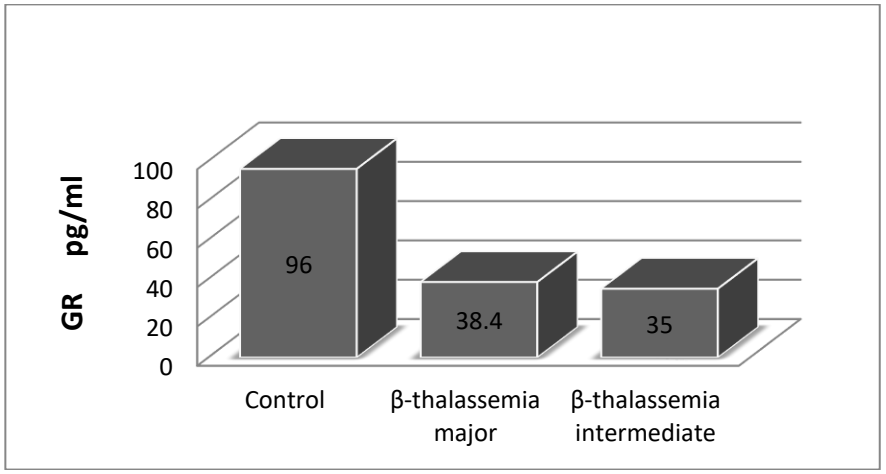

Fig. 3. Concentrations of GR (pg/ml) in β-thalassemia major and β-thalassemia intermediate patients, and control group

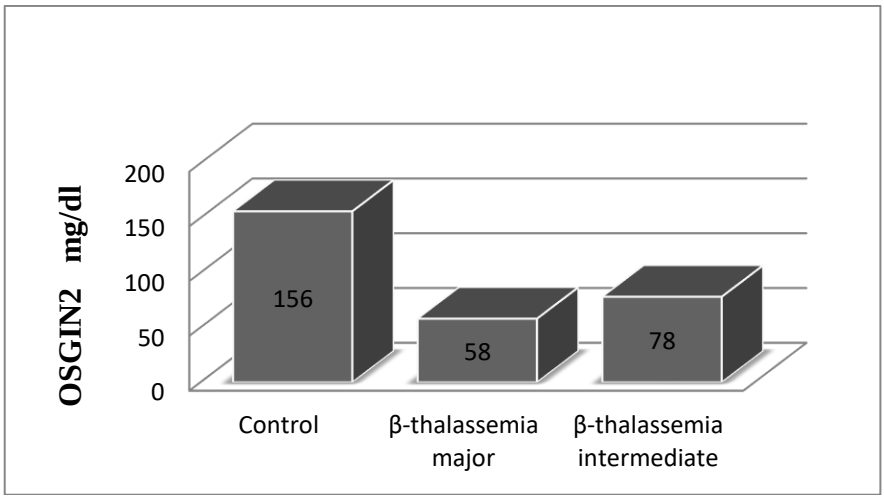

Fig.4. Concentrations of OSGIN2 (mg/dl) in β-thalassemia major and β-thalassemia intermediate patients, and control group

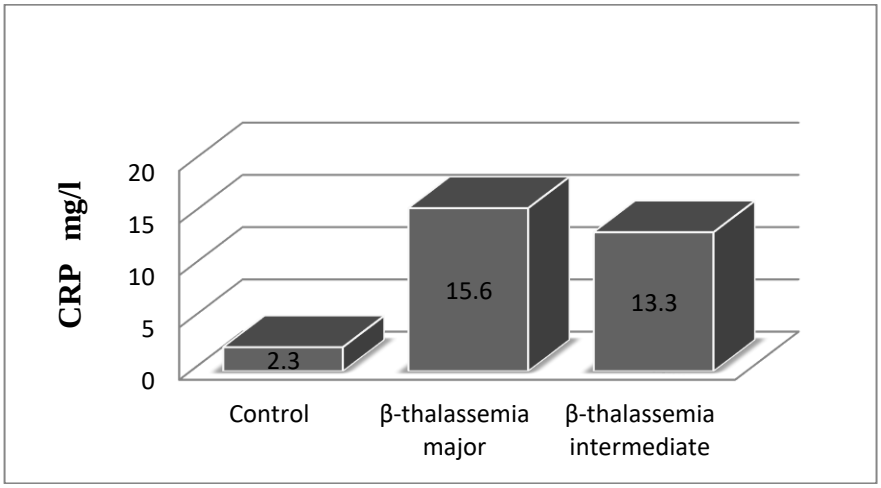

Fig. 5. Concentrations of CRP (mg/l) in β-thalassemia major and β-thalassemia intermediate patients, and control group

Discussion

This study revealed that D-dimer, ferritin, and CRP levels increased with a significant difference (P≤0.05) in the β-thalassemia major and β-thalassemia intermediate patients group rather than control; while the GR and OSGIN2 serum levels decreased with significant difference (P≤0.05). In this study have found that the patients with β-thalassemia had significant increase in serum ferritin level because multiple blood transfusions which believe life-saving therapy for β-thalassemia patients lead to iron overload and deposition of iron in tissue organ like liver caused hepatotoxicity and this results acceptable with the recent study [14]. The level of D-dimer was significantly increased in β-thalassemia major and β-thalassemia intermediate patients, compared with control group. Consistent with the research done by Hassan [15] in Egypt.

Oxidative stress plays a major role in pathophysiology of β-thalassemia, although it is not the primary etiology of disease. The cell oxidative status depends on the equilibrium between oxidants and anti-oxidants. The reactive oxygen species (ROS) are oxidants produced mainly as byproducts of cellular respiration, while reduced glutathione is an example of anti-oxidants. A balance between oxidants and anti-oxidants is crucial for normal physiology. ROS are utilized from the cells as regulators in many physiological processes, including proliferation and differentiation of the erythroid precursors. Oxidative stress ensues in many pathological processes when the balance between oxidants and anti-oxidants is broken, as it occurs in β-thalassemia. Excess ROS cause cytotoxicity by binding to cell components such as DNA, proteins, and membrane lipids [16].

Thalassemic patients undergo chronic oxidative stress and prooxidant pools, evidenced by high levels of serum iron, ferritin, and no transferrin bound iron [17,18].Many studies have reported on increased levels of oxidative products of biomolecules including malondialdehyde (MDA), protein carbonyl, and 8-hydroxyguanine in patients with thalassemia [17, 19, 20]. Reduced glutathione is one of the most important intracellular and extracellular antioxidants, synthesized from its amino acid constituents (cysteine, glutamic acid, and lysine) by two sequential enzymes, γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthetase. The rate limiting step in GSH synthesis is the formation of γ- glutamylcysteine, catalyzed by γ-GCS [21]. Glutathione serves as an antioxidant by direct radical scavenging or participating in enzymatic reactions. Consequently, GSH is oxidized to GSSG, which can be converted back to GSH in a reaction catalyzed by NADPH-dependent GR.

The intracellular GSH level depends on levels of GSH synthesis, efflux, utilization, and recycling. In our study, (GR) and (OSGIN2) figures (3 and 4) did show decreased with significant differences among thalassemic patients compared with control group. Therefore, the status of the redox environment in thalassemia is mainly regulated by the rate of GSH export and GSH synthesis. Oxidative stress-induced GSH efflux through membrane transporters in the families of cystic fibrosis trans membrane conductance regulator (CFTR) and multidrug resistance associated protein (MRP)were reported in β-thalassemia/HbE erythrocytes [22]. CRP was used as a biomarker of various inflammatory conditions. The results of current study showed an increased CRP level in β-thalassemic patients than in control groups as in figure (4) which are in accordance with the results of similar studies. A research done in Pakistan by [23] concluded that CRP can serve as a biomarker of inflammatory conditions, the progression of cardiovascular diseases and as indicator of morbidity and mortality. High C-reactive proteins in these

Patients indicate ongoing iron overload toxicity related damage in these patients. Lynch, 2012[24], has found that interestingly there was a trend towards increasing C-reactive protein levels in beta thal/HbE postsplenec patients with higher platelet count, although no correlation was observed. Besides the inflammatory process, platelet and/or factor(s) that control(s) thrombopoiesis seem(s) to play a role in the high serum C-reactive protein levels in the studied population. C-reactive proteins and cytokines have been used by a number of workers as a biomarker of inflammation in thalassaemia patients as well for another disease as pyogenic infection including pneumonia, infective pulmonary

exacerbation in cystic fibrosis, diabetes, hepatitis and as marker for the development of cardiovascular diseases which is a major morbid complication of thalassaemia . Kanavaki et al., 2009[25] they found that all endothelial adhesion molecules and CRP were significantly increased in β-thalassemia intermedia patients ($p<0.001$) and not influenced by treatment. These results agree with the study published earlier where CRP levels were found elevated in β-thalassemia patients compared to healthy individuals, especially in splenectomized patients.

Conclusion

Alteration in iron homeostasis is associated with oxidative stress and inflammation. In the β-thalassemia's, oxidative stress, resulting from chronic hemolysis, globin chain imbalance, iron overload and depleted antioxidant defenses, likely contributes to cell death, organ damage, anemia, hypoxia and inflammation. Studies of oxidative stress to improve outcomes in patients with thalassemia should consider individual patient variation in oxidative status both between and within the thalassemia patients.

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