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جامعة عين شمس

تحت إشراف

د/ أميرة عبد المنعم عدلي

أستاذ طب الأطفال

جامعة عين شمس

د/ إيمان عبد الرحمن إسماعيل

إستشارى الباثولوجيا الأكلينكية

جامعة عين شمس

د/ نيرة هزاع خليل الشريف

أستاذ مساعد طب الأطفال

جامعة عين شمس

كلية الطب - جامعة عين شمس

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Ischemia modified albumin as a marker of oxidative stress in β-thalassemia Patients: relation to lipid peroxidation, iron overload and vascular dysfunction

Thesis

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By

Sara Hamed Elmetwally Shameia
M.B., B.Ch., Ain Shams University (2008)

Supervised By

Dr. Amira Abd ElMoneam Adly
Professor of Pediatrics
Faculty of Medicine-Ain Shams University
Dr. Eman Abdel Rahman Ismail
Consultant of Clinical Pathology
Faculty of Medicine- Ain Shams University
Dr. Nayera Hazaa Khalil ElSherif
Assistant Professor of Pediatrics
Faculty of Medicine-Ain Shams University

Faculty of Medicine - Ain Shams University
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INTRODUCTION

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production ***(Higgs et al., 2001)***. A mutation of β-globin gene leads to a defective β chain production. This defect leads to an imbalance in a/β globin synthesis causing ineffective erythropoiesis, chronic hemolytic anaemia and iron overload ***(Taher et al., 2011).***

The most common type arises as a result of decreased synthesis or total absence of the beta chain of hemoglobin. Consequently, excess free alpha chain accumulates and precipitates within erythrocytes leading to shortened life span and ineffective erythropoiesis ***(Olivier, 1999)***.

Furthermore, patients with β-thalassemia major require continuous blood transfusion which leads to iron overload with subsequent organ and tissue damage. The status of iron overload and iron-induced oxidative stress has been repeatedly investigated in patients with thalassemia major ***(Koren et al., 2010).***

Patients with β-thalassemia are under significant iron driven oxidative stress. Many studies reported increased blood levels of the redox active fractions of non-transferrin bound iron (NTBI) and labile plasma iron (LPI) in patients with β-thalassemia ***(Chakraborty et al., 2001; Cabantchik et al., 2005).*** It has also been demonstrated that such patients

experience decreased antioxidant capacity and increased products of peroxidative damage ***(Kassab-Chekiret al., 2003).***

β-Thalassemia (β-thal), especially β-thalassemia major (β-TM), is reported to be related to reactive oxygen species (ROS) and enhanced oxidation status. It is reflected by increased malondialdehyde (MDA), by membrane lipid peroxidation ***(Chris et al., 2014)*** which could be enhanced in thalassaemia patients because it may be dependent on the amount of circulating erythroid precursors and peripheral blood erythrocytes that have a high density of unpaired α-haemoglobin chains which are unstable and prone to denaturation and oxidation ***(Scott et al., 1993).***

Ischemia modified albumin (IMA) is an altered type of serum albumin that forms under conditions of oxidative stress ***(Awadallah et al., 2012)***. The N-terminus residues of human serum albumin tend to bind with transition metals such as cobalt, copper and nickel ***(Bar-or et al., 2001).*** Alterations in this region of albumin hinder its binding capacity to such elements. ROS resulting from conditions such as ischemia, hypoxia, acidosis, free radicals, and free iron can decrease the ability of the N-terminus to bind with transition metals ***(Bar-or et al., 2001; Roy et al., 2006).*** Human serum albumin with a decreased binding capacity as a result of ischemic events is referred to as IMA ***(Bar-Or et al., 2000).*** It has been suggested that elevated levels of IMA may reflect a generalized rather

than organ- or tissue-specific state of oxidative stress ***(Borderie et al., 2004***).

IMA is currently used as an early marker for myocardial ischemia and acute coronary syndrome ***(Sbarouni et al., 2011).*** It is also increased in diabetes mellitus, hyperlipidemia, chronic renal disease, obesity, and others ***(Borderie et al., 2004; Kaefer et al., 2010).*** Recently, increased levels of IMA have been reported in β-thalassemia major patients ***(Awadallah et al., 2012)***; however, its full clinical relevance remains to be fully explored.

AIM OF THE WORK

The aim of this work was to measure the levels of IMA in β-thalassemia patients as a marker of oxidative stress, and to assess its relation to lipid peroxidation as well as the effect on vascular complications, subclinical atherosclerosis and the efficacy of iron chelation.

Chapter One
BETA THALASSEMIA

Background

Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis ***(Galanello and Origa, 2010).***

Mutations that completely inactivate the β-gene resulting in no β-globin production cause β^0 -thalassemia. Other mutations allow the production of some β- globin, and depending on the degree of quantitative reduction in the output of the β chains, are classified as β^+- or β^{++}- ("silent") thalassemia ***(Thein, 2005).***

This defect leads to an imbalance in a/β globin synthesis causing ineffective erythropoiesis, chronic haemolytic anaemia and iron overload ***(Taher et al., 2011).***

Epidemiology

The thalassemias are the most common genetic disorder on a worldwide basis ***(Borgna-Pignatti et al., 2005).*** They are most common in the Mediterranean, the equatorial, or near equatorial regions of Africa and Asia ***(Weatherall, 2010).***

In Egypt, beta (β) thalassemia major represents a major public health problem. The carrier rate varies between 5.5 and

≥ 9%; it was estimated that 1000/1.5 million per year live births have β-thalassemia ***(El-Beshlawy et al., 2007),*** the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia ***(Weatherall et al., 2010)*** **(Figure 1**).

Figure (1): Geographical distribution of, β--thalassemia around the world. Black areas indicate the countries where thalassemia is prevalent ***(Weatherall and Clegg, 2001).***

Survival

During the past few decades, an impressive improvement in patients' survival has been noticed. In the mid-1960s, only 37% of patients in a small group of 41 patients with thalassemia major were alive at the age of 16 years ***(Engle et al., 1964).***

In contrast, a 95% survival was encountered among patients of similar age, 30 years later ***(Borgna-Pignatti et al., 1998).***

At the beginning of the new millennium, survival at the age of 35 years was 50% according to the UK thalassemia registryand 65% according to an Italian study whereas a 83% survival rate beyond 40 years was reported in 2004 ***(Modell et al., 2000; Davis et al., 2004; Borgna-Pignatti et al., 2005).***

A recent Iranian study revealed that the most common causes of death of thalassemic patients were cardiomyopathy (72.3%), and infections (17%) ***(Bazrgar et al., 2011).***

Modell and associates reported that in the UK, the main causes of death among beta-thalassemia patients before 1980 were anemia, infections, and cardiac hemosiderosis, with improvement in survival, likely due to appropriate intensification of Iron Chelation Therapy ***(Modell et al., 2008)***. ***Mokhtar et al. (2013)*** found that the main cause of death was infection (42.8%), followed by heart failure and liver cell failure.

Pathophysiology

Normal Hemoglobin structure

Hemoglobin is a polypeptide tetramer, globular in structure, and consisting of two pairs of unlike globin chains (i.e., α plus β, δ, or γ), which form a shell around a central

cavity containing four oxygen-binding heme groups each covalently linked to α globin chain. In healthy adults, 95% of the Hb is Hb A (α2β2) with small amounts (3.5%) of Hb A2 (α2δ2) and Hb F (α2γ2) present ***(Kutlar, 2007)*** **(Figure 2).**

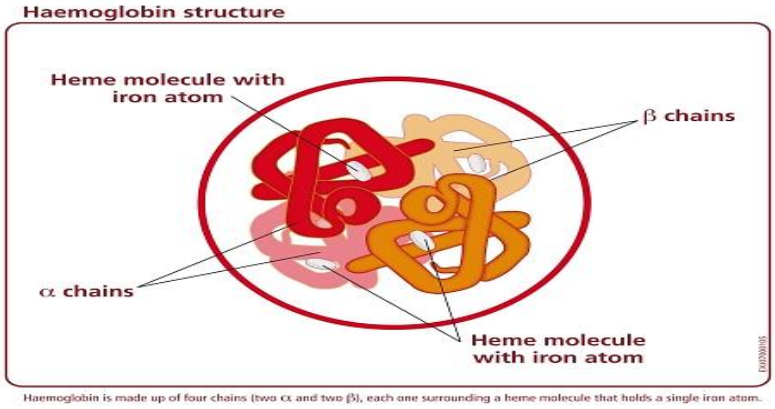

Figure (2): The structure of haemoglobin (2α chains and 2β chains). ***(http://164.109.71.105/Thalassaemia/General/Blood.html)***

β-thalassemia occurs when there is a quantitative reduction of β globin chains that are usually structurally normal ***(Weatherall and Clegg, 2001).***

In β thalassemia, the synthesis of normal α globin chains from the unaffected α globin genes continues as normal, resulting in the accumulation within the erythroid precursors of excess unmatched α globin. The free α globin chains are not able to form viable tetramers and instead precipitate in the red cell precursors in the bone marrow forming inclusion bodies. They are responsible for the extensive intramedullary destruction of the erythroid precursors and hence the ineffective

erythropoiesis that underlies all β thalassemias. Anemia in β thalassemia thus results from a combination of ineffective erythropoiesis, peripheral hemolysis, and an overall reduction in hemoglobin synthesis. The severity of disease in β thalassemia correlates well with the degree of imbalance between α and non-α globin chains and the size of the free α chain pool. Thus, factors that reduce the degree of chain imbalance and the magnitude of α chain excess in the red cell precursors will have an impact on the phenotype ***(Thein, 2005).***

β-Thalassemia Ineffective Erythropoiesis

1. Evidences for an Ineffective Erythropoiesis in β-Thalassemia

Dyserythropoiesis in β-thalassemic patients was suspected for a long time since it is largely recognized that many patients with an inadequate transfusional regimen have a dramatic expansion of the hematopoietic marrow and extramedullary hematopoiesis, which can lead to extensive bone deformity and/or bone marrow mass and splenomegaly**.** ferrokinetic studies done in the 70's, suggested that probably 60%–80% of erythroid progenitors were arrested in proliferation and/or underwent death ***(Pootrakul et al., 2000; Ribeil et al., 2013)***.

The bone marrow of patients suffering from β-thalassemia contains five to six times the number of erythroid precursors observed in healthy controls, with increased basophilic and polychromatophilic erythroblasts and decreased orthochromatic erythroblasts ***(Centis et al., 2000).***

Moreover, it has been shown that β-thalassemic bone marrow erythroblasts contain electron-dense alpha-globin inclusion (aggregates) beginning at early polychromatophilic stages, which increase in size and frequency during subsequent maturation ***(Ribeil et al., 2013)***.

Expansion of very early erythroid precursors (proerythroblasts and earlier stages) and then ineffective erythropoiesis, which defines the suboptimal production of mature erythrocytes from a proliferating pool of immature erythroblasts are responsible for dyserythropoiesis in β-thalassemia patients. It is thus characterized by (1) accelerated erythroid differentiation, (2) maturation blockade at the polychromatophilic stage, and (3) death of erythroid precursors ***(Leecharoenkiat et al., 2011)*** **(Figure 3).**

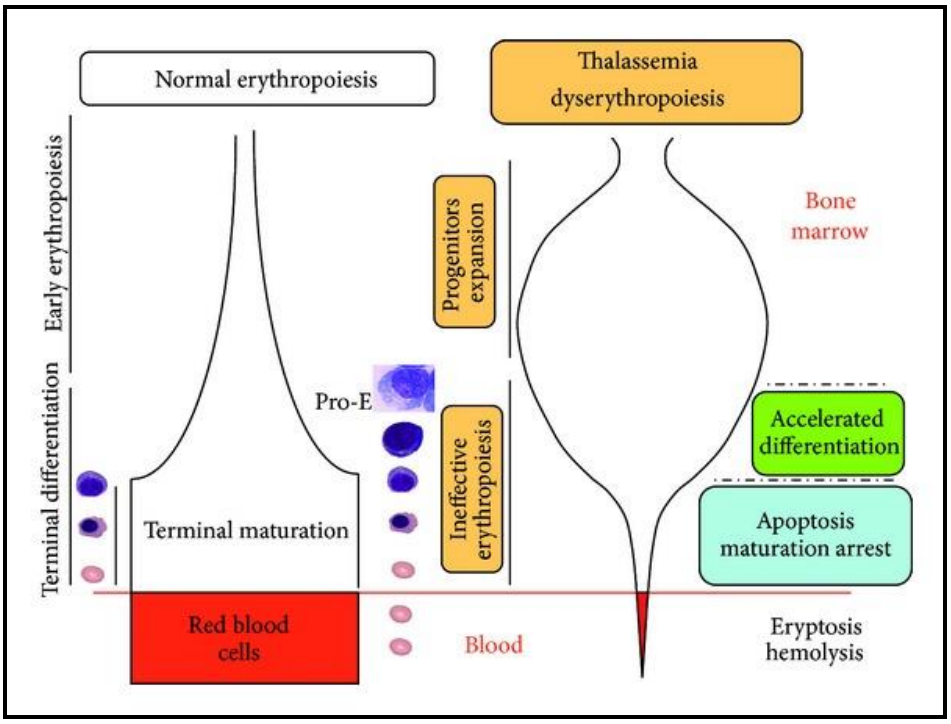

Figure (3): Difference between normal and β-thalassemia ineffective erythropoiesis ***(Ribeil et al., 2013).***

2. Enhanced Apoptosis Is a Key Feature of Ineffective Erythropoiesis in Human β-Thalassemia

In vitro findings corroborate the reduced cell expansion in β-TM erythroid cultures and enhanced apoptosis at the polychromatophilic stage of differentiation ***(Mathias et al., 2000).***

In spite of the markedly increased rate of apoptosis of β-thalassemic erythroid precursors, BM smears of these patients do not show high increased number of dying erythroblasts ***(Centis et al., 2000)***.

This paradox might be explained by increased phagocytosis of abnormal precursors erythroblasts expressing phosphatidyl serine by bone marrow macrophages whose number and activation are enhanced, respectively, by about 2-fold in TM ***(Kuypers and de Jong, 2004)***.

As a consequence, the delivery of thalassemic RBCs to the peripheral blood in the β-TM major patients is much reduced ***(Ribeil et al., 2013)***.

3. Apoptotic Pathways Involved in β-Thalassemia Ineffective Erythropoiesis

Studies of apoptotic death receptor pathways have shown that Fas and FasL are coexpressed early and at all stages of terminal differentiation. Both proteins are downregulated in bone marrow or spleen in proerythroblast and basophilic cells in β-thalassemic mice compared to control mice in vivo. This down regulation in Fas/FasL expression might be a marker of erythropoietic stress ***(Liu et al., 2006).***

Regarding the intrinsic apoptotic pathway, it was expected that it would have been also involved because it could be induced by cellular oxidant injury. Nevertheless, the involvement of this mitochondrial pathway has not been evidenced to date in β-thalassemia ***(Schrier et al., 2003).***