Measurement of serum Klotho and its correlation with bone mineral density in patients withβ-Thalassemia Major

Thesis

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قياس كلوثو بروتين فى الدم و علاقته بكثافة المعادن في العظام لدى مرضى أنيميا البحر المتوسط

رسالة

توطئة للحصول علي درجة الماجيستير فى أمراض الدم الاكلينيكية

مقدمة من

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Contents

List of Abbreviations

ADHR	:Autosomal dominant hypophosphatemic rickets
BMD	:Bone mineral density
CKD	:Chronic kidney disease
DXA	:Dual energy X-ray absorptiometry
FGF23	:Fibroblast growth factor 23
FGFR1	:Fibroblast growth factor receptor 1
GH	:Growth hormone
Hb	:Hemoglobin
HCV	:Hepatitis c virus
HIV	:Human immunodeficiency virus (AIDS)
IGF-1	:Insulin like Growth Factor
MnSOD	:Manganese superoxide dismutase
PTH	:Parathyroid hormone
TRPV5	:Transient receptor potential cation channel subfamily member 5
β-TI	:Beta thalassemia intermedia
β-TM	:Beta thalassemia major

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Abstract:

The aim of this study was to measure plasma levels of the secreted protein Klotho in β-thalassemia major patient and the existence of correlations between the protein level and osteoporosis and fragility fractures. Also, we compared the level of the protein in patients and in healthy controls.50 patients with β-thalassemia major and 30 healthy volunteers were enrolled. Klotho level in plasma was measured by mean of an ELISA test. CBC, renal functions, liver functions, viral markers (HBs Ag, HCV Ab), calcium, phosphorus and serum ferritin level were measured by standard clinical techniques. DEXA was used to measure bone mineral density (BMD) at the lumbar spine (L2–L4) and femoral neck. We found that the Klotho protein concentration was lower in the blood of patients with β-thalassemia major than in healthy controls. Also, the klotho concentration was lower in patients with osteoporosis or osteopenia than those with normal BMD. Also, lower in patients with history of fragility fractures.

Key words:

Klotho, Osteoporosis, fragility fractures.

Introduction

β- thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent β-globin synthesis resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals ***(Borgna-Pignatti et al., 2011)***.

Complications of the disease include heart problems which are responsible for 70% of deaths ***(Borgna-Pignatti et al., 2004)***, Liver diseases due to transfusional iron overload and transmitted viral infections, endocrine problems as hypogonadism in over 50% of patients, diabetes and hypothyroidism ***(Costin et al., 1979), (Borgna-Pignatti et al.1985).***

Fragility fractures are common in these patients (5). Osteoporosis affects approximately 51% of the patients with another 45% suffering from osteopenias ***(Origa et al., 2005)***. The reduced bone mineral density and susceptibility to fractures has been attributed, in addition to hyperactivity of the bone marrow due to endocrine dysfunction, iron overload, chelation therapy, Vitamin D deficiency, and lack of exercise ***(Jensen et al., 1998).***

In recent years, researchers have focused the attention on the Klotho gene which encodes a protein that is expressed in the kidney, parathyroid glands, and in the choroid plexus. The gene was identified for the first time in mouse knock-out mutants for the gene. These animals

develop an ‘aging’ phenotype which includes, reduced bone density, growth retardation, hypogonadism and vascular calcification ***(Kuro-o et al., 2010)***.

The secreted protein is present in blood, urine and cerebrospinal fluid ***(Imura et al., 2004)***. it is involved in calcium reabsorption in the kidney in distal tubules the loss of calcium in the kidney may lead to an increment in calcium absorption from the bone ***(Imura et al., 2007)***.

Researchers hypothesized a possible involvement of Klotho in the appearance of clinical complication in b thalassemia major due to similarity between b thalassemia major and knock-out mouse phenotype.

Aim of the work

The aim of this study is to compare Klotho plasma levels in β-Thalassemia major patients with healthy controls and to correlate between the protein level and osteoporosis.

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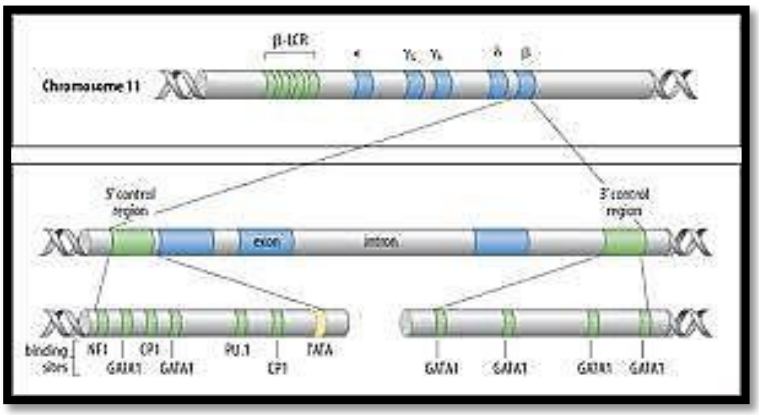
Thalassemia

The term thalassemia is derived from the Greek, Thalassa (sea) and haima (blood). Beta-thalassemia includes three main forms: Thalassemia Major, variably referred to as "Cooley's Anemia" and "Mediterranean Anemia", Thalassemia Intermedia and Thalassemia Minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia". Apart from the rare dominant forms, subjects with thalassemia major are homozygotes or compound heterozygotes for $beta^0$ or $beta^+$ genes, subjects with thalassemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassemia minor are mostly heterozygotes.

The β-globin gene is located in the short arm of chromosome 11 in a region containing also the δ gene, the embryonic ε gene, the fetal G gamma and A gamma genes, and the pseudogene β1***(Fritsch et al.,1980)***

The five functional globin genes are arranged in the order of their developmental expression. The β-globin genes are subject to a very complex regulatory mechanism, acting at the level of single genes as well as of the entire β cluster**,** β-Thalassemia mutations result in either a complete absence of β-globin chains (β0-thalassemia) or in a largely variable reduction of β-globin synthesis (β+-thalassemia). More than 200 different mutations producing β-thalassemia have been so far described; the large majority are point

mutations in functionally important sequences of the β-globin gene, while in contrast to α-thalassemia, gene deletion is a rare cause of β-thalassemia ***(Fullerton et al., 1994).***



β-thalassemia phenotypes are variable, ranging from the severe transfusion dependent thalassemia major to the mild form of thalassemia intermedia. Thalassemia intermedia does not require transfusion or only sporadic or intermittent transfusions. Thalassemia minor indicates the heterozygous state, which is usually completely asymptomatic. Thalassemia minima was used in the Italian literature to indicate a carrier in whom no hematologic symptoms. Sometimes the term of thalassemia minima was used to indicate the condition of silent carrier ***(Danjou et al., 2011).***