EARLY MARKERS OF RENAL TUBULAR DYSFUTNCTION IN PATIENTS WITH β-THALASSEMIA MAJOR

A *thesis* submitted for fulfillment of master degree in Pediatrics

By

Moataz Khaled Abd El Wahab Badr

Supervisors

Dr. Mona Mohamed Hamdy Mahmoud

Ass. Professor of Pediatrics, Faculty of Medicine, Cairo University

Dr. Ahmed Mohamed Mahmoud Badr

Lecturer of Pediatrics, Faculty of Medicine, Cairo University .

Dr. Shahira Kamal Anis

Lecturer of Clinical Pathology Faculty of Medicine, Cairo University

Faculty of Medicine

Cairo University

2012

ACKNOWLEDGMENT

First and foremost, thanks to Allah, the most beneficial and merciful.

In few grateful words, I would like to express my greatest thanks to all my professor who helped me throughout this work.

I wish particularly to express my deepest gratitude and appreciation for unfailing support; valuable advice and generous help and patience rendered me by **Dr. Mona Mohamed Hamdy**, Ass.Professor of Pediatrics, CairoUniversity. She sacrificed a great deal of his precious time and effort guiding me throughout the preparation of this thesis.

Also, I want to thank **Dr. Ahmed Mohamed Badr,** Lecturer of Pediatrics, Cairo University and **Dr. Shahira Kamal Anis,** Lecturer of Clinical Pathology, Cairo University for thier great help and advice, And thier sincere care, untiring effort and thier great assistance during every step and every detail in this thesis.

Finally, I give all the thanks and grateful feelings and gratitude to my family for their unlimited support and help their offered me throughout my life.

Moataz Khaled Badr.
2012

CONTENTS

Abbreviations

ACE	Angiotensin converting enzyme.
ALT	Alanine Aminotransferase.
APP	American Academy Of Pediatrics.
AST	Aspartate Aminotransferase.
B-LCR	Beta- locus- control- region.
BM	Bone marrow.
BUN	Blood urea nitrogen.
Ca^{++}	Calcium.
CKD	Chronic kidney disease.
CS	Constant Spring .
Cys C	Cysteine C.
DFO	Desferrioxamine.
DM	Diabetes Mellitus.
DNA	Deoxy Ribonucleic Acid.
EPO	Erythropoietin
ERPF	Effective renal plasma flow.
ESRD	End stage renal disease.
FENa	Fractional execretion of sodium.
FSGS	Focal segmental glomerluosclrerosis.
GBM	Glomerular basement membrane.
GFR	Glomerular filtration rate.
GH-IGF-1	Growth hormone- Insulin like growth factor-1.
GVHD	Graft- versus- host- disease.

Hb	Hemoglobin.
HbF	Fetal Hemoglobin.
HCT	Hematocrit.
HCT	Hematopoietic cell transplantation.
HLA	Histocompatibilty.
HPLC	High performance liquid chromatography.
ICL670	Deferasirox.
IGF-1	Insulin like growth factor-1.
K^+	Potassium.
L1	Deferiprone
LDH	Lactate Dehydrogenase.
LMW	Low molecular weight.
MCH	Mean corpuscular hemoglobin.
MCV	Mean corpuscular volume
MDA	Malondialdehyde
TD- βTM	Transfusion dependent beta thalassemia major.
MHC	Major histiocompatibilty complex.
MRI	Magnetic resonance imaging.
Na^+	Sodium.
NAG	N-acetyl-beta D glucosaminidase.
N_P	Neuropilins.
P/C	Protein creatinie ratio.
PARADE	Proteinuria, Albuminuria, Risk, Assessment, Detection And Elimination.
PCR	Polymerase- Chain- Reaction.
PDGF	Platelet-derived growth factor.
RBC	Red blood cell.

RDW	Red cell distribution width.
RFLP	Restriction Fragment Length Polymorphism.
rHuEPO	Recombinant Human Erythropoietin.
SCAN	Sickle cell anemia-associated nephropathy.
SCD	Sickle cell disease.
SD	Slit diaphragm.
SD	Standard deviation.
sFlt-1	Soluble form of vascular endothelial growth factor receptor-1.
SPI	Selectivity of proteinuria index.
THP	Tamm-Horsfall protein.
TRP	Tubular reabsorption of phosphate.
UCB	Umbilical cord blood.
UCr	Urinary creatinine.
ULN	Upper limit of normal.
UMA	Microalbuminuria.
UPEP	Urine protein electropherisis.
URD	Unrelated donor.
VEGF A	Vascular endothelial growth factor A.
β_2MG	Beta$_2$ microglobulin
βTM	Beta Thalassemia Major.

INTRODUCTION

Thalassemia is the most common type of genetic abnormality in the world ***(Ali D, et al, 2008).*** It was first described by Dr. Cooley in 1925 ***(Cooley T.B, et al, 1925).***

Thalassemia is more prevalent in the Mediterranean Region, North and West Africa, the Middle East and some other parts of the world ***(WeathheallDJ, et al, 1981).***

Thalassemia is a type of chronic, inherited, microcytic anemia that is characterized by defective hemoglobin synthesis and ineffective erythropoiesis. In all thalassemias, clinical features that result from anemia, transfusional, and absorptive iron overload are similar but vary in severity ***(Tunaci M, et al 1999).***

β -thalassemia is group of heterogeneous autosomal recessive disorders due to the absence or reduced synthesis of the β-globin chain ***(Cao A, et al 1994).***

Thalassemia major is characterized by grossly defective. synthesis of hemoglobin A, impaired red blood cell production and increased haemolysis of the defective RBC. Affected individuals are dependent on repeated blood transfusions ***(Barakat AY, et al, 1986).***

Shortened red cell lifespan and excess iron cause functional and physiological abnormalities in various organ systems in thalassaemia patients. β-thalassaemia patients have a high prevalence of renal tubular abnormalities. The severity correlated with the degree of anemia, being least severe in patients on hypertransfusion and iron chelation therapy, suggesting that the damage might be caused by the anaemia and increased oxidation induced by excess iron deposits ***(Sumboonnanonda A, et al 2003).***

In recent years, there have been few published studies demonstrating proteinuria, aminoaciduria, low urine osmolality, and excess secretion of the tubular damage markers, such as urinary β2 microglobulin and urinary N-acetyl-D-gloucosaminidase in patients with thalassemia ***(Valdislav Smolkin, et al 2008).***

Aim of the work

The aim of the study is early detection of renal tubular dysfunction in β-thalassemia major patients by estimation of early markers of renal tubular dysfunction as urinaty β_2 microglobulin.

THALASSEMIA

Thalassemia is one of the most common genetic disorders in Egypt **(Gaafar et al., *2006).*** About 100,000 Babies worldwide are born with severe forms of thalassemia each year. The Disease occurs most frequently in middle East, Southern Asian, Italian, Greek, and African ancestry ***(Eldor and Rachmilewitz, 2002).***

Historical background:

The first definitive descriptions of thalassemia were published independently in the United States and Italy in 1925. In the United States, Cooley, a pediatrician from Detroit, identified a group of children of Mediterranean origin with profound anemia, enlargement of the spleen and peculiar bone changes ***(Weatherall, 2004).***

The unusual name by which the disease is known today was given by Whipple when he was working as a pathologist in Rochester in 1932.Whipple decided on the name "thalassic anemia"-thalassa means sea in Greece and then shortened it to thalassemia. From early as the 1940s, it was clear that the term "thalassemia" is a geographical as well as literary misnomer ***(Weatherall, 2001).***

Definitions:

The thalassemia syndromes are the most common hereditary chronic hemolytic anemia due to impaired globin chain synthesis ***(Rund and Rachmilewitz, 2005).***

This impairment leads to deficient hemoglobin accumulation, resulting in hypochromic microcytic red cells, ineffective erythropoiesis and hemolytic anemia ***(Takeshita, 2006).***

Pathophysiology

The thalassemia syndromes were among the first genetic diseases to be understood at the molecular level. More than 200 β-globin and 30 alpha globin mutations have been identified; these mutations result in decreased or absent productions of one globin chain (alpha or beta) and a relative excess of the other. The resulting imbalance leads to unpaired globin chains which precipitate and cause premature death (apoptosis) of the red cell precursors within the marrow, termed ineffective erythropoiesis. Of the damaged but viable RBCs that are released from the bone marrow, many are removed by the spleen or hemolyzed directly in the circulation due to the hemoglobin precipitants. Combined RBCs destruction in the bone marrow, spleen and periphery causes anemia and, ultimately, an escalating cycle of pathology resulting in the clinical syndrome of severe thalassemia ***(Kearney et al., 2007).***

Damaged erythrocytes enter the spleen and are trapped in this low pH and low oxygen environment; subsequent splenomegaly exacerbates the trapping of cells and worsens the anemia. Anemia and poor tissue oxygenation stimulate increased kidney erythropoietin production that further drives marrow erythropoiesis, resulting in increased ineffective marrow activity and the classic bony deformities associated with poorly managed thalassemia major and severe thalassemia intermedia. Anemia in the severe thalassemia phenotypes necessitates multiple RBC transfusions and, over time, without proper chelation, results in transfusion-associated iron absorption and can result in iron overload, even in untransfused patients who have thalassemia intermedia ***(Kearney et al., 2007).***

It has long been recognized that the severity of ineffective erythropoiesis affects the degree of iron loading, but until the recent discovery of hepcidin and understanding its role in iron metabolism, iron deficiency and overload. Hepcidin initially was discovered due to its role in the etiology of anemia of chronic inflammation or chronic disease ***(Weinstein et al., 2002).***

Normal human hemoglobin

1. Function of hemoglobin:

Hemoglobin is essential for tissue to receive a constant supply of oxygen ***(Honig, 2000).***

2. **Genetics:**

The genes for the goblin chains occur in two clusters ε, δ, γ and β on chromosome 11 and ζ and α on chromosome 16. Two type of γ chain, Gγ and Aγ occur depending on whether there is a glycine or alanine amino acid at position 136 in polypeptide chain. The α chain gene is duplicated and both α genes (αl and α2) on each chromosome are active ***(Hoffbrand et al., 2001).***

3. Ontogency of human Hb:

Within the RBCs of an embryo, fetus, child and adult, six different hemoglobins may normally be detected-the embryonic hemoglobin Gower-1, Gower-2, and Portland, the fetal hemoglobin; HbF and the adult hemoglobins; HbA and A2.

The electrophoretic immobilites of hemoglobins vary with their chemical structures ***(Cohen et al., 2004).***

a) Embryonic hemoglobins:

The blood of early human embryos contains two slowly migrating hemoglobins, Gower-1 and Gower-2, and Hb Portland, which has HbF, like mobility. Hb Gower-1 has the structure ζ2, ε2 and Gower-2, α2, ε2. Hb Portland has the structure ζ2, γ2. In embryos of 4-8 weeks gestation, the Gower hemoglobins predominate, but by the 3rd month they have disappeared ***(Stamatayannopoulos et al., 2001).***

b) Fetal Hb:

HbF contains γ polypeptide chains in place of the β-chain HbA. After the 8th gestational week, HbF is the predominant hemoglobin, during the 3rd trimester, a gradual decline occurs, so decreases rapidly postnatally, and by 6-12 months of age only a trace is present ***(Nelson, 2004).***

c) Adult Hb:

Some Hb (α2, β2) can be detected in even the smallest embryo. Accordingly, it is possible as early as 16-20 weeks gestation to make a prenatal diagnosis of major β-chain hemoglobinopathies, such as thalassemia major ***(Inati et* al., *2006).***

Table (1): The composition of embryonic, fetal and adult Hb

Embryonic	Gower 1 Gower 2 Portland	ζ2 ε2 α2 ε2 ζ2 γ2
Fetal	Hemoglobin F	α2 γ2
Adult	Hemoglobin Al Hemoglobin A2	α2 β2 α2 δ2

(Ohls and *Christensen, 2008)*

Alteration of the hemoglobins by disease:

HbF levels may be influenced by various factors, because the HbF level is elevated during the 1st year of life, knowledge of its normal decline is important. In persons heterozygous for β-thalassemia (β-thalassemia trait), postpartum decrease of Hb F is delayed: about 50% of such persons have elevated levels of HbF (>2.0%) in later life. In homozygous thalassemia (Cooley's anemia) and in hereditary persistence of HbF, large amounts of HbF characteristically are found. In patients with major β-chain hemoglobinopathies (Hb SS, Sc), HbF usually is increased, particularly during childhood. Preterm infants treated with human recombinant erythropoietin (EPO) had increased in level of HbF production during active erythropoiesis.Moderate elevations of HbF may occur in many disease accompanied by hematologic stress, such as hemolytic anemias, leukemia, and aplastic anemia, because of a minor population of RBCs that contains increased amounts of HbF. Tetramers of γ chains (γ4 or Hb barts) or β chains (β4 or HbH) may be found in α-thalassemia syndromes ***(Ohls and Christensen, 2008).***

The normal adult level of HbA2 (2.0-3.4%) is seldom altered. Levels of HbA2>3.4 are found in most persons with the β-thalassemia trait and in those with megaloblastic anemias secondary to vitamin B12 and folic acid deficiency.Decreased HbA2 levels are found in those with iron deficiency anemia and α-thalassemia ***(Ohls and Christensen, 2008***)

Classification of Thalassemia:

A large number of thalassemic syndromes are currently known; each involves decreased production of one globin chain or more, which form the different Hbs normally found in RBCs. The most important types in clinical practice are those that affect α or β chain synthesis ***(Nathan, 2005).***

α- Thalassemia:

Two α thalassemia phenotypes are recognized; one is characterized by thalassemia minor in the homozygous state (deletion of 2α-genes) and the other is marked by no clinical or hematologic abnormality in the heterozygous state (deletion of one α-gene). The former phenotype has been referred to as α thalassemia 1 and the latter has been labeled α thalassemia 2 ***(Weatherall, 2001).***

It is now recognized that the α thalassemia determinants are associated with complete absence and the α thalassemia 2 phenotypes with only a reduction in α-globin syntheses. Accordingly, these two major α thalassemia variants are now designated α° thalassemia and α+ thalassemia. The α°thalassemia result from deletions that involve both α-globin genes.

Some of the α+ thalassemia result from deletions involving only one of the two α-globin genes (-α) and others from non deletion mutations that limit α-gene expression.

Interactions of the mutations causing deficient α-globin synthesis produce a spectrum of phenotypes that can be grouped into four clinical syndromes.In each syndrome, the severity of the symptoms correlates closely with the deficiency of α-globin chains relative to β-chains ***(Weatherall, 2001).***