

Early Markers of Renal Affection In Patients with β Thalassemia Major

*Thesis submitted in partial fulfillment of the Master Degree
in Paediatrics*

By

Mohamed Mustafa Ahmed Attyah
(M.B, B.Ch)

Under Supervision Of

Prof. Dr. Mohsen Saleh El-Alfy

*Professor of Paediatrics
Faculty of Medicine
Ain Shams University*

Prof. Dr. Mona Mohammed Zakae

*Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University*

Dr. Samar Mohamed Farid

*Assistant. Professor of Paediatrics
Faculty of Medicine
Ain Shams University*

**Faculty of Medicine
Ain Shams University**

2012

دلائل الكشف المبكر لتأثر الكلى فى المرضى المصابين بأنيميا البحر الأبيض المتوسط

رسالة

توطئة للحصول على درجة الماجستير فى الأطفال

مقدمة من

الطبيب/محمد مصطفى أحمد عطية
بكالوريوس الطب والجراحة - جامعة الأزهر (٢٠٠١)

تحت إشراف

أ.د/ محسن صالح الألفي

أستاذ طب الأطفال
كلية الطب - جامعة عين شمس

أ.د/ منى محمد زكي

أستاذ الباثولوجيا الإكلينيكية
كلية الطب - جامعة عين شمس

د/سمر محمد فريد

أستاذ مساعد طب الأطفال
كلية الطب - جامعة عين شمس

كلية الطب
جامعة عين شمس

٢٠١٢

Summary

Beta thalassemia is the commonest type of thalassemia and usually produce severe anemia in their homozygous and compound heterozygous form. The use of regular and frequent blood transfusion in thalassemia has improved life span and quality of life of the patients, but it lead to chronic iron overload.

Severely affected patients are treated by blood transfusion every 3-4 weeks, which results in iron overload in various tissue including the liver heart and endocrine tissue. The kidneys are another site of iron accumulation in thalassemia. Unlike in the other organs, it is unclear whether kidney affection results solely from intravascular hemolysis, chronic transfusion or as a complication of iron chelation therapy.

Beta2-Microglobulin (beta2MG), an interesting and underutilized metabolite, can be used in assessing renal function, particularly in patients suspected of having renal tubulointerstitial disease.

The assay of urinary N-acetyl-beta-D-glucosaminidase (NAG) provides an early indication of tubular dysfunction resulting from renal disease or nephrotoxic damage. False positives are rare and its activity remains high during active disease or a sustained toxic insult but falls to normal levels on



Acknowledgement

*All Praise are to **Allah** and all thanks. He Has guided and enabled me by His mercy to fulfill this thesis, which I hope to be beneficial for people.*

*I would like to express my deepest gratitude and sincere appreciation to **Prof. Dr. Mohsen Saleh El Alfy** Professor of Pediatrics, Faculty of Medicine, Ain Shams University for his continuous encouragement, his kind support and appreciated suggestions that guided me to accomplish this work,*

*Special thanks are extended to **Prof. Dr. Mona Mohammed Zakae** Professor of Clinical Pathology Faculty of Medicine Ain Shams University for her constant encouragement and advice whenever needed.*

*I am also grateful to **Assistant, Prof. Dr. Samar Mohamed Farid** Assist, Prof, of Pediatrics, Faculty of Medicine, Ain Shams University for her sincere advise to bring up this work to the light.*

*Special thanks are extended to **Assistant, Prof. Dr. Amira Abdelmonaem Adly** Assist, Prof, of Pediatrics, Faculty of Medicine, Ain Shams University for her sincere advise to bring up this work to the light Who freely gave her time, effort and experience along with continuous guidance through out this work,*

Thanks for all staff of Pediatrics Department whose help and support are greatly appreciated. Also, I would like to express my warmest gratitude to my family.

Finally, I would like to convey my gratitude to my patients and their families and to every person who helped me while performing this work,

Mohamed Mustafa Ahmed



List of contents

List of Abbreviations	i
List of Tables	ii
List of Figures	v
Introduction and Aim of the work	1
Review of Literature	3
Subjects and Methods	58
Results	61
Discussion	86
Summary	95
Conclusion.....	98
Recommendations.....	99
References	100
Arabic Summary	--

List of Abbreviations

ALT	: Alanine transferase
AST	: Aspartate transferase
BM	: Bone marrow
BMI	: Body mass index
BUN	: Blood urea nitrogen
C HS4	: Chicken hypersensitive site-4 chicken insulator element
Creat	: Creatinine.
CT	: Computerized tomography.
DFO	: Desferrioxamine.
DFT	: Desferrithiocin.
DM	: Diabetes mellitus.
ELISA	: Enzyme linked immunosorbent assay
FS	: Frame-shifts.
GFR	: Glomerular filtration rate.
HBED	: Hydroxybenzyl-ethylenediamine-diaceticacid.
HbF	: Fetal hemoglobin.
HBV	: Hepatitis B virus.
HC V	: Hepatitis C virus .
HCT	: Haematocrit.
HGB	: Hemoglobin.
Ht	: Height.
K	: Potassium..
LCR	: Locus control region

List of Abbreviations (Cont.)

LDL	:	Low density lipoprotein
LV	:	Lentiviral
LV	:	Lentiviral
MRI	:	Magnetic resonance image
Na	:	Sodium
NAG	:	N-acetyl beta-D-glucosaminidase
NS	:	Nonsense
PIH	:	Pyridoxal isonicotinoyl hydrazone
PRBC	:	Packed red blood cells
RBCs	:	Red blood cells
RIA	:	Radio immune assay
S.TFR	:	Serum Transferrin Receptor
SIN	:	Self-inactivating
SPSS	:	Statistical program for social science
TLC	:	Total leucocytic count
Wt	:	Weight
β	:	Beta
β 2MG	:	Beta2 Microglobulin

List of tables

<i>Table</i>	<i>Title</i>	<i>Page</i>
1	Clinical and hematologic features of the principal forms of thalassemias	6
2	Pathophysiologic manifestations of CKD	30
3	Descriptive data of the studied thalassemic patients	61
4	Descriptive laboratory data among studied thalassemic patients	62
5	Comparison of clinical data between studied thalassemic patients and controls	63
6	Comparison of laboratory data between studied thalassemic patients and controls	66
7	Comparison between clinical data of the studied thalassemic patients according to type of chelation therapy	72
8	Comparison between laboratory data of the studied thalassemic patients according to type of chelation therapy	73
9	Comparison between clinical data of the studied thalassemic patients splenectomized and non splenectomized patients	74
10	Comparison between laboratory data of the studied thalassemic patients splenectomized and non splenectomized patients	75

List of tables (Cont.)

<i>Table</i>	<i>Title</i>	<i>Page</i>
11	Comparison between clinical data of the studied thalassemic patients non hepatitis and thalassemic patients with hepatitis.	76
12	Comparison between laboratory data of the studied thalassemic patients non hepatitis and thalassemic patients with hepatitis.	77
13	Urinary β 2 micro globulin correlations	78
14	Urinary NAG correlations	80
15	Comparison between clinical data of the studied thalassemic patients according to serum Ferritin	82
16	Comparison between laboratory data of the studied thalassemic patients according to serum Ferritin	83
17	Multiple regression analysis of β 2 micro globulin and other parameter	84
18	Multiple regression analysis of NAG and other parameter	85

List of Figures

<i>Figure</i>	<i>Title</i>	<i>Page</i>
1	The geographical distribution of the thalassemias and the more common, inherited structural haemoglobin abnormalities	4
2	Examples of mutations which produce β -thalassemia. FS= frame-shifts, NS= nonsense, SPL=spicing	8
3	Structure of haemoglobin tetramer	10
4	(a) The facial appearance of a child with β thalassemia major, (b) The skull x-ray in β thalassemia major	24
5	A flowchart showing an approach to the diagnosis of the thalassemia syndromes	21
6	Treatment and complication in β thalassemia	35
7	Comparison of Weight between thalassemic patients and controls	64
8	Comparison of height between thalassemic patients and controls	64
9	Comparison of BMI between thalassemic patients and controls	65
10	Median and inter quartil range of serum Ferritin (ng/ml)	67
11	Median and inter quartil range of β 2 micro globulin (mg/mL)	68

List of Figures (Cont.)

<i>Figure</i>	<i>Title</i>	<i>Page</i>
12	Median and inter quartil range of urinary NAG (IU/l)	68
13	Comparison of serum creatinine between thalassemic patients and controls	69
14	Comparison of creatinine clearance between thalassemic patients and controls	69
15	Comparison of AST between thalassemic patients and controls	70
16	Comparison of ALT between thalassemic patients and controls	70
17	Comparison of $\beta 2$ microglubulin between thalassemic patients and controls	71
18	Comparison of $\beta 2$ microglubulin/ceratinine ratio between thalassemic patients and controls	71
19	Correlation of TLC(x1000) with $\beta 2$ microglubulin	79
20	Correlation of creatinine(mg/dl) with Urinary NAG(IU/L)	81
21	Correlation of BUN(mg/dl) with Urinary NAG(IU/L)	81

Introduction

Beta thalassemia is the commonest type of thalassemia and usually produce severe anemia in their homozygous and compound heterozygous form. The use of regular and frequent blood transfusion in thalassemia has improved life span and quality of life of the patients, but it lead to chronic iron overload (*Chern et al., 2001*).

Severely affected patients are treated by blood transfusion every 3-4 weeks, which results in iron overload in various tissue including the liver heart and endocrine tissue. The kidneys are another site of iron accumulation in thalassemia. Unlike in the other organs, it is unclear whether kidney affection results solely from intravascular hemolysis, chronic transfusion or as a complication of iron chelation therapy (*Traez et al., 2007*).

Beta 2-Microglobulin (beta 2M), an interesting and underutilized metabolite, can be used in assessing renal function, particularly in patients suspected of having renal tubulointerstitial disease (*Bethea and Forman, 1990*).

The assay of urinary N-acetyl-beta-D-glucosaminidase (NAG) provides an early indication of tubular dysfunction resulting from renal disease or nephrotoxic damage. False positive are rare and its activity remains high during active disease or a sustained toxic insult but falls to normal levels on recovery or removal of the toxin. Urinary NAG activity can be used in conjunction with other tests to assess disease activity and prognosis (*Price, 1992*).

Aim of the Work

Our study designed to evaluate the prevalence of renal tubular dysfunction among patients with transfusion dependent β -thalassemia major- in the period from January 2009 till June 2011 recruited from the Hematology and Oncology clinic, children's hospital, Ain Shams University.

Thalassemia Syndrome

Definition:

Thalassemia is one of the most common single gene disorders and is widely distributed in the Mediterranean region (*Barragan et al., 2006*). Thalassemia syndromes are a heterogeneous group of inherited anemia's characterized by defects in the synthesis of one or more of the globin chain subunits of the hemoglobin tetramer (*Forget, 2000*).

The thalassemia syndromes are the most common hereditary chronic hemolytic anemia due to impaired globin chain synthesis (*Cighetti, 2002*).

The clinical syndromes associated with thalassemia arise from the combined consequences of inadequate hemoglobin accumulation and unbalanced accumulation of globin subunits. The former causes hypochromia and microcytosis, the latter leads to ineffective erythropoiesis and hemolytic anemia (*Schwartz et al., 1995*).

Types:

The severity of the imbalance of globin chain generates the different thalassemia phenotypes (*Scott et al., 1993*).

This disease represents the homozygous state of a partially autosomal dominant gene for which the heterozygous state is associated with much milder hematological changes. The severe homozygous condition is known as thalassemia major, where the heterozygous states were designated according to their severity thalassemia minor or minim. Later, the term thalassemia intermedia was used to describe disorders that are milder than the minor form, but more severe than the traits (*Weatherall, 1995*).

Thalassemia minor is an asymptomatic disorder associated with prominent abnormalities of erythrocyte morphology but with little or no anemia (*Lukens, 1993*).

Prevalence and Geographic Distribution:

Thalassemia is considered the most common genetic disorder worldwide, about 3% of the world's population (150 million people) carry β -thalassemia genes and in Southeast Asia 5-10% of the population carry genes for α -thalassemia (*Honig, 2004*).

According to ethnic group, α -thalassemia trait is more prevalent in South East Asia, affect 2.7% of American black newborn and is less common in the Mediterranean region.

β -thalassemia >5% in certain area of Italy, Greece, Sardinia, India and 8% in American black (*Williams, 2001*).

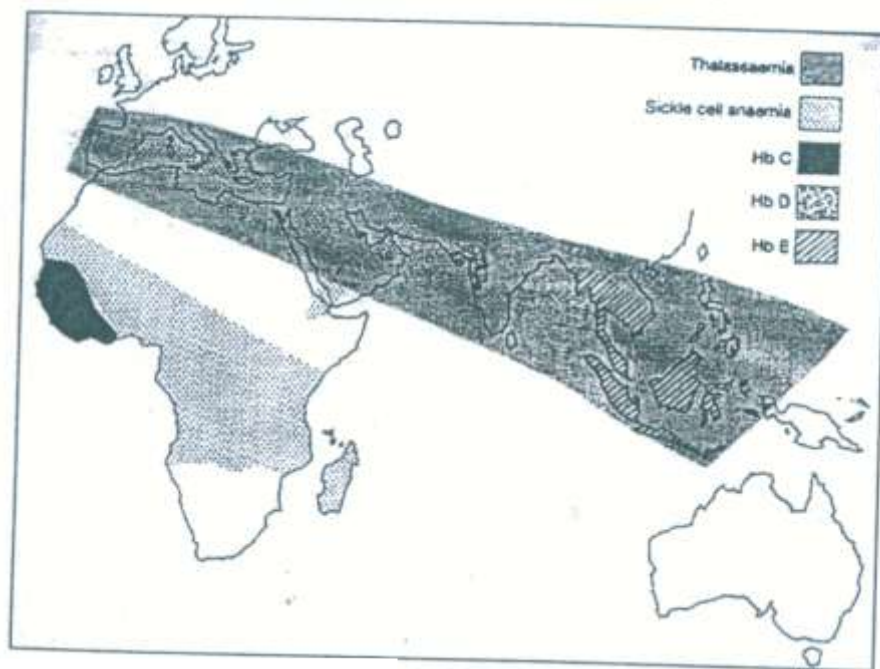


Fig. (1): The geographical distribution of the thalassemias and the more common, inherited structural hemoglobin abnormalities (*Hoffbrand et al., 2001*).