A SSESSMENT OF BLEEDING TENDENCY & PLATELET ACTIVATION IN THALASSEMIC CHILDREN

Thesis

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By

Hayam Mohamed Abd El-All

M.B.B.CH.

Cairo University 1996

Supervisors

Dr. Eman Abdel Raouf

Assistant Professor of Pediatrics

Pediatrics Hospital – Cairo University

Dr. Sherif Nasseh Amin

Professor of Clinical Pathology

Cairo University

Dr. Magy Samir Abdel Wahab

Lecturer of Pediatrics

Pediatrics Hospital – Cairo University

Faculty of Medicine

Cairo University

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Content

List of tables

List o figure

List of abbreviations

TI	Thalassemia intermedia
TM	Thalassemia major
L 1	Deferriprone
C.B.C	Complete blood count
F.B.C	Full blood count
M.C.V	Mean corpuscular volume
R.B.C	Red blood cells
R.D.W	Red cell distribution width
A.D.P	Adenosine 5- diphosphate
PC	Prothrombin concentration
PT	Prothrombin time
PTT	Activated partial thromboplastin time
ALT	Alanin transaminase
AST	Aspartate transaminase
CD_{62p}	Cluster designation 62 p-selectin
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
W.B.C	White blood cells
S. Ferritin	Serum ferritin
TAX	Thromboxane A2
PF_3	Platelet factor 3
PF_4	Platelet factor 4
VWF	Von Willebrand factor
TG	Thromboglobulin
ICAM-1	Intracellular adhesion molecule- 1
VCAM-1	Vascular cell adhesion molecule- 1
AT III	Antithrombin III
HC II	Heparin cofactor II
ELAM - 1	E – selectin
ACDD	Antibody- dependent cell cytotoxicity
TAT	Thrombin antithrombin III

Abstract

Standard of care for thalassemic patients have improved in recent years, resulting in almost doubling of the average life expectancy. As a consequence, additional previously unscribed complications are new being recognized.

In particular, profound hemostatic changes have been observed in patients with thalassemia major or thalassemia intermedia. These hemostatic changes may be in the form of thrombotic or hemorrhagic events. To study hemorrhagic events we assessed platelet aggregation to ADP and Ristocetin in thalassemic children presenting with epistaxis and compare the results to group of normal children in the same age a sex group presented to E.N.T. clinic by recurrent epistaxis. Control group had no bleeding tendency due to haematological condition.

Both cases and control were subjected to complete clinical examination, Full Blood Count (FBC.), liver enzymes (AST, ALT), PC,PT,PTT and serum ferritin. In addition, platelet aggregation to ADP and ristocetin were assessed for both cases and control too. The levels of plalelet aggregation to ADP and ristoctein found to be statistically lower in thalassemic children as compared to control.

The results were positively correlated with ALT, which indicate that liver status is contributing factor in bleeding tendency in thalassemic cases.

In conclusion, bleeding tendency in thalassemic patients can be attributed to a defect in platelet aggregation namely platelet hypoaggregation. However, hepatic dysfunction associated with the disease can be a contributing factor as well.

Thalassemic children with bleeding tendency should receive vitamin K supplementation to compensate for hepatic synthesis defect.

On the other hand to study hypercoagulable status we assessed CD62p as a marker of in vivo platelet activation which in turn indicate hypercoagulability state in thalassemic children.

To compare the results with normal children attending out patient clinic of new children hospital, Cairo University both cases and control were subjected to complete clinical examination, Full Blood Count (FBC), liver enzymes (AST,ALT), PC, PT, PTT. And flow cytometric study for CD_{62P} as a marker of in vivo platelet activation were assessed for both cases and control.

Patients result found to be statistically insignificant as compared to control.

In spite of that detection of membrane P-selectin by flow cytometry is accepted as a sensitive marker of platelet activation, it is not specific for platelets. So, CD62p can not be considered as a reliable marker for assessing platelet activation due to many complexities associated with its estimation.

Key words:

Platelet - Thalassemia

Aim of the Work:

To assess the hemostatic defect underlying epistaxis in thalassemic children and to compare results with matched controls having espistaxis as the presenting symptom but not due to a hematological disorder.

To assess platelet activation using CD62P as a marker of hypercoaguability in steady state thalassemic children and compare the results with matched controls.

Thalassemia

Introduction and Prevalence

The term thalasemia is derived from a Greek term, which means (the sea) Mediterranean in blood. ***(Hoffman, 1995).***

It was first described as a clinical entity in 1925 by Thomas Cooley, pediatrician, as a syndrome among children of Italian descent characterized by profound anemia, splenomegally and bony deformities**.** ***(Cooley and Lee, 1925).*** In Egypt, β-thalassemia was first described in 1944 as an erythroblastic anaemia with bony changes, possibly Cooley's type ***(Diwany, 1944).***

Thalassemic syndromes are heterogenous group of inherited anaemias characterized by defect in the synthesis of the globin chain subunits of the hemoglobin tetramer (Fig 1 A, B). Clinical syndromes associated with thalassemia arise from inadequate hemoglobin accumulation and unbalanced accumulation of globin subunits. The former leads to hypochromia and microcytosis, and the latter leads to ineffective erythropoiesis and hemolytic anaemia.***(Hoffman , 1995.)***

Prevalence

Thalassemia is considered the most common genetic disorder worldwide, about 3% of the world s population carry β-thalassemia gene. ***(Lukens, 1993; Bernard, 2000)***Estimates of gene frequencies range from 3 to 10% in some areas. ***(Weatherall et al., 1994).***

Thalassemia are the most common in the Mediterranean basin and equatorial or near equatorial region of Asia and Africa. The thalassemia belt extend along the shores of the Mediterranean and throughout the

Arabian peninsula, turkey, Iran, India and southeastern Asia, especially Thailand, Cambodia and southern China. Gene frequencies in these regions range from 2.5 to 15%. (***Hoffman, 1995).***

Worldwide, about 60.000 children with thalassemia major are born annually and about 150 million people carry β- thalassemia genes (***Cao et al., 2002)***.

In Egypt, Hematology Clinic Study Group at Cairo University Children Hospital reported a carrier state varying between 6 and 10%. In the Hematology clinic, thalassemia represented 20% of all cases visiting the clinic (7640 in 1997) and 80%of the chronic hemolytic anemia patients. Moreover, 1000 children affected with thalassemia are expected out of 1.5 million live births per year ***(El_Beshlawy et al., 2003).***

Thalassemia are quantitative disorders. The primary lesion lies in the amount of globin produced. Thalassemic syndromes are classified according to globin chain whose synthesis is adversely affected. Thus, α globin synthesis is reduced in α- thalassemia and β globin synthesis is reduced in β_thalassemia. β- thalassemia is sub classified according to whether synthesis of the affected globin chain is totally absent e.g. β^{o} or only partially reduced e.g. β^{+}. (***Adams JG III., 1990).***

Fig. (1A) : Structure of hemoglobin tetramer, University of Arizona 2002

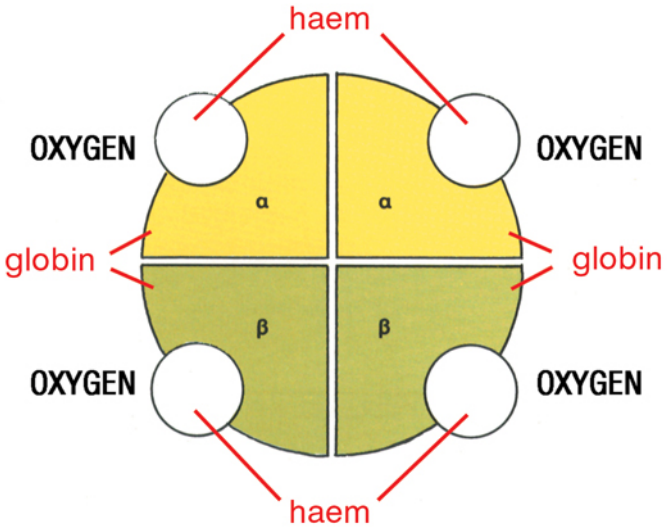

Fig. (1B) : Structure of hemoglobin tetramer, T.I.F.

Molecular pathology & Classification:

The thalassemia syndromes were the first to be characterized at the level of underlying DNA mutations, and remain the most thoroughly understood human inherited disease at the molecular level. The small size of the globin genes, the fact that they were among the first human genes to be isolated and characterized at the DNA sequence level, and the high frequency of thalassemia genes in many human populations have led to a better understanding of molecular epidemiology of thalassemia syndromes ***(Edwards et al., 1990).***

The production of human hemoglobin is controlled by two gene clusters; the alpha globin genes are located on chromosome 16, while the non-alpha genes are on chromosome 11 (Fig. 2). different forms of thalassemia usually arise from genetic mutations. About 150 various mutations have been described in β-thalassemia and related disorders ***(Cao et al., 1997)***. Deletions involving two or more genes are found in rare cases ***(Wratherall et al., 1999)***.

The majority of β-thalassemia are due to point mutations affecting one or few bases. Mutations not only affect amino acid coding regions of β-globin exons, but also sites surrounding the gene even within the non-coding introns or within dinucleotides at intron-exon junction (Fig. 3) ***(Sadiq et al., 2001)***.

Mutations may affect every step in the pathway of globin gene expression, transcription, RNA processing, translation and posttranslational integrity of the β-polypeptide chain. These result in either absence of β-globin chains (β^0-thalassemia) or reduction of synthesis (β^+-thalassemia) (table 1) ***(Weatherall and Clegg, 1999).***

Both splicing of mRNA precursor and ineffective cleavage of mRNA transcript result in β-thalassemia. Mutations that interfere with translation involve the initiation, elongation or termination of globin-chain production and result in β-thalassemia. Approximately half of all mutations interfere with translation. These include frame-shift and nonsense mutatins which introduce premature termination codons ***(Olivieri, 1999)***.

Mutations involving exon 3, result in the production of unstable globin chains of varying lengths that together with excess of α-globin chains, precipitate in red cell precursors leading to in effective erythroposiesis even in the heterozygous state. This is the molecular basis for dominantly inherited β-thalassemia ***(Olivieri, 1999)***.

β-thalassemia mutations differ greatly in their phenotypic effects from hereditary persistence of fetal hemoglobin, and the silent carrier to the various clinical syndromes. Thalassemia major is a homozygous form of β-thalassemia it is a clinically severe disorder due to the presence of two identical or dissimilar mutations one on each chromosome 11. As a sequence of reduced Hb A synthesis, the circulating RBCs are thin, small, distorted and contain markedly reduced amounts of hemoglobin. The hypoochromic anaemi of thalassemia major is so severe that dependency on blood transfusions is usually established at an early age (***Clarke and Higgins, 2000).***

Thalassemia intermedia is a moderate clinical disorder not requiring chronic transfusion therapy. It is usually due to two- β-thalassemia mutations or occasionally to other genetic combinations, such as the combination of single β-thalassemia defect and an excess of normal α-globin genes ***(Group, 1997).***