Acute Hemolytic Anemia as an Initial Presentation of Wilson's Disease

Thesis
Submitted for Fulfillment
of Master Degree in Pediatrics

Presented by
Amerah Mohamed Ahmed ElShahawy
M.B., B.Ch.

Under Supervision of

Prof. Dr. Mona El-Said El-Raziky
Professor of Pediatrics
Faculty of Medicine-Cairo University

Prof. Dr. Mona Mohamed Hamdy
Ass. Professor of Pediatrics
Faculty of Medicine-Cairo University

Prof. Dr. Amal Abdel Hamid Ali
Ass. Consultant of Pediatrics
National Hepatology and Tropical Medicine Institute

Faculty of Medicine
Cairo University
2012

Acknowledgement

“FIRST OF ALL WE THANK ALLAH”

I thank Allah for all his blessings and givings, particularly the blessing of being surrounded by loving and supportive professors, family and friends.

I would like to express my deepest gratitude and admiration to **Prof. Dr. Mona El-Said El-Raziky**, Professor of Pediatrics, Cairo University, for her continuous support, encouragement and kindness, her help can never be forgotten and working under her supervision had been a great honor and indeed a great privilege.

I would like to express my sincere thanks to **Prof. Dr. Mona Mohamed Hamdy**, Assistant Professor of Pediatrics, Cairo University, for her guidance, encouragement and patience, her sincere attitude and gentleness has been inspiring to me.

I'm deeply indebted to **Dr. Amal Abdelhamid Ali**, Assistant consultant of Pediatrics, National Hepatology and Tropical Medicine Institute, for her continuous support, cooperation and precise supervision.

Many thanks to **Dr. Hanan Mina** Fellow of Pediatrics, National Hepatology and Tropical Medicine Institute, for her sincere and kind help.

Also, I would like to express my sincere gratitude to my husband and family, for their kind help, support to me during working in this thesis.

Finally, I would like dedicate this work to my late father, Allah bless his soul.

Abstract

Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism resulting in pathological accumulation of copper in many organs and tissues. Hemolytic anemia (HA) in WD occurs in up to 17% of patients at some point in time during the natural course of illness.

The aim of current study was to screen for WD among children presenting HA. The study was carried out on cases with combs negative HA attending the pediatric hematology clinic of Cairo University. The twenty studied children were screened for WD by serum ceruloplasmin level, 24-hr urinary copper before and after D-penicillamine challenge test and slit lamp examination for detecting Kayser-Fleisher ring. Our results showed that according to the scoring system used, we found one case has definite WD and 7 cases that were likely to have WD. These 8 cases were referred to as +ve cases. Their mean age was 9.9 $\pm$ 3.1 years. Comparison between the +ve and -ve cases for WD revealed that +ve cases have; a significantly lower weight percentile (p= 0.003), a significantly lower hemoglobin, MCV and MCH (p= 0.04, 0.001 and 0.04) respectively and a significantly higher urinary copper after penicillamine (p= 0.000) and finally a significantly lower retics (p= 0.04) than negative cases. Our results showed that 40% of cases presented by combs' negative HA were likely to have WD and that they all had elevated levels of urinary copper after penicillamine.

We concluded that WD in not uncommon in children with hemolytic anemia after exclusion of common causes.

Keywords: Wilson's disease – hemolytic anemia – initial presentation - Coombs'' negative

Table of Contents

List of Tables

List of Figures

List of Abbreviations

^{51}Cr	Chromium-51
AGLT	Acidified glycerol lysis test
AIHA	Autoimmune hemolytic anemia
ALT	Alanine transaminase
AST	Aspartate aminotransferase
CD20	Cluster of Differentiation 20
CLL	Chronic lymphocytic leukemia
CNS	Central nervous system
CPM-like	Central pontine myelinolysis-like in MRI
CSF	Cerebro-spinal fluid
Ctr1	Copper transporter 1
DAT	Direct antiglobulin test
DIC	Disseminated intravascular coagulation
EPO	Erythropoietin
G6PD	Glucose-6-phosphate dehydrogenase
GIT	Gastro –intestinal tract
HA	Hemolytic anemia
HbA2	Hemoglobin A2
HbF	Fetal hemoglobin
HS	Hereditary Spherocytosis
HUS	Hemolytic uremic syndrome
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	International normalized ratio
IRMA	Immunoradiometric assay
IVIG	Intravenous immunoglobulin
LDH	Lactic acid dehydrogenase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

MRI	Magnetic resonance imaging
MT	Metallothionein
OF	Osmotic fragility
PCT	Penicillamine challenge test
PET	Positron emission tomography
PNH	Paroxysmal nocturnal hemoglobinuria
PT	Prothrombin Time
RBC	Red blood cells
RDW	Red blood cell distribution width
REM	Rapid eye movement
SD	standard deviation
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SLE	Systemic lupus erythematosus
SPSS	Statistical Package for Social Science
TTP	Thrombotic thrombocytopenic purpura
WD	Wilson disease
XIAP	X-linked inhibitor of apoptosis

Introduction

Wilson's disease (WD) is a rare autosomal recessive disorder with incidence of 1 in 40,000. It occurs due to deficiency in ATP7b protein leading to impairment of biliary copper excretion, hepatic copper accumulation and copper toxicity from oxidant damage (***Ferenci, 2005; Czionkowska et al., 2010).***

Over 80% of patients present first disease symptoms within the first 3 decades of life and about 40–70% of overall initial WD manifestations involve the liver. The hepatic manifestations of WD are diverse and may include asymptomatic elevation of aminotransferase, chronic hepatitis, cirrhosis or acute/fulminant hepatic failure. The diagnosis of Wilson's disease is often delayed as the major difficulty in establishing the diagnosis lies in the fact that this disorder is very uncommon (***Kong et al., 1996; Ala et al., 2007).***

Hemolytic anemia in Wilson's disease occurs in up 17% of patients at some point in time during the natural course of illness, it is uncommon as the initial presentation. It occurs as a result of oxidative injury, altered erythrocyte metabolism and severely compromised antioxidant status caused by toxic effects of copper that is released from necrotic hepatocytes (***Attri et al., 2006).***

Wilson's disease is quite uncommon and it is also a lethal condition in the absence of adequate treatment. Thus, identifying WD at a pre-symptomatic or early clinical stage has enormous implications for the outcome of patients with this condition; hence, WD should certainly be ruled

out in any young patient presenting with findings suggestive of hemolytic anemia of uncertain etiology, as the disease can be successfully treated in the early stages (***Singh et al., 2009).***

Aim of work

This thesis aims at screening for the cases of Wilson's disease among those presenting with hemolytic anemia.

Chapter I: Wilson Disease

Wilson disease is an autosomal recessive inherited disorder of copper metabolism resulting in pathological accumulation of copper in many organs and tissues. The incidence of Wilson disease was estimated to be at least 1:30,000–50,000. ATP7B is the gene product of the Wilson disease gene located on chromosome 13 and resides in hepatocytes in the trans-Golgi network (***Bull et al., 1993).***Other genes may be involved in the pathogenesis of Wilson disease ***(De Bie et al., 2007).***

Pathogenesis

Mutations in ATP7B cause impaired biliary copper excretion that leads to progressive accumulation of copper in the liver followed by subsequent deposition in other organs and defective hepatocyte incorporation of copper into ceruloplasmin. By the end of the first or into the second decade of life, the hepatic burden of copper is exceeded; causing release of free copper into the circulation that penetrates other tissues. During this time, hepatic copper may actually decrease in concentration, whereas brain, kidney and ocular copper increase ***(Scheinberg & Sternlieb, 1984.)***

Serum ceruloplasmin levels are low in Wilson's disease because of decreased synthesis of holoceruloplasmin and rapid clearance of apoceruloplasmin ***(Scheinberg & Sternlieb, 1984).***

Ceruloplasmin may also be abnormally low in other conditions (Menkes' disease, aceruloplasminemia, sprue, nephritic syndrome, protein-losing enteropathy) and in chronic liver disease of any cause ***(Pfeiffer, 2004)***. In contrast, as an acute phase reactant, ceruloplasmin may become transiently

elevated into the normal range in Wilson's disease patients by infection or inflammation or by oral contraceptive pills or steroid ingestion (***Sternlieb & Scheinberg, 1972).***

Hepatic Copper Metabolism and the Role of ATP7B

Copper is an essential nutrient needed for such diverse processes as mitochondrial respiration (cytochrome C), melanin biosynthesis (tyrosinase), dopamine metabolism (DOPA-β-monooxygenase), iron homeostasis (ceruloplasmin), antioxidant defense (superoxide dismutase), connective tissue formation (lysyl oxidase) and peptide amidation (***Ferenci,2005).***

Approximately 10% of dietary copper (about 2mg/day) is absorbed in the upper intestine, transported in the blood loosely bound to albumin, certain amino acids and peptides. Finally, most of the ingested copper is taken up by the liver. Copper homeostasis is critically dependent on the liver, within the hepatic parenchyma, the uptake and storage of copper occurs in hepatocytes which regulate the excretion of this metal into the bile as an unabsorbable complex, so there is no enterohepatic circulation of this metal. Biliary excretion is the only mechanism for copper elimination and the amount of copper excreted in the bile is directly proportional to the size of the hepatic copper pool. The hepatic uptake of diet-derived copper occurs via the copper transporter 1 (Ctr1) which transports copper with high affinity in a metal-specific, saturable fashion at the hepatocyte plasma membrane (***Klomp et al., 2002; Lee et al., 2001).***

After uptake copper is bound to metallothionein (MT), a cytosolic, low molecular weight, cystein-rich, metal binding protein. MT I and MT II are totally expressed in all cell types including hepatocytes and have a critical