Occult Hemorrhage in Egyptian Children with Immune Thrombocytopenia

Thesis

Submitted for Partial Fulfilment of Master Degree in Pediatrics

By

Ahmed El Tayeb Hassan

M.B., B.ch.

Under Supervision of

Prof. Azza Abd El Gawad Tantawy

Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Nayera Hazaa El Sherif

Assistant Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Mahmoud Adel Kenny

Lecturer of Pediatrics

Faculty of Medicine – Ain Shams University

Faculty of Medicine - Ain Shams University

2019

Acknowledgment

First and foremost, I feel always indebted to **ALLAH**, *the Most Kind and Most Merciful.*

I'd like to express my respectful thanks and profound gratitude to **Prof. Azza Abd El Gawad Tantawy,** *Professor of Pediatrics - Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to **Dr. Nayera Hazaa El Sherif,** *Assistant Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

I am deeply thankful to **Dr. Mahmoud Adel Kenny,** *Lecturer of Pediatrics, Faculty of Medicine, Ain Shams University, for his great help, active participation and guidance.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Ahmed El Tayeb Hassan

Dedication

This work is dedicated to . . .

My beloved father, to whom I owe everything I ever did in my life and will achieve and making me the man, I am now.

My mother for always being there for me.

My brothers and my sisters for their support and encouragement.

Finally **my wife** for her support and continuous encouragement from step to other higher step.

List of Contents

Title	Page No.
List of Tables	i
List of Figures	iii
List of Abbreviations	iv
Abstract	V
Introduction	1
Aim of the Work	8
Review of Literature	9
Subjects and Methods	31
Results	35
Discussion	49
Summary	57
Conclusion	61
Recommendations	62
References	63
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Enumeration of bleeding sites in ITP patients	15
Table (2):	Test to evaluate occult hemorrhage in ITP	18
Table (3):	Grades based on worst incident episodes since last visit (SMOG)	20
Table (4):	Demographic and clinical data of the studied ITP patients	35
Table (5):	Laboratory and radiological data of the studied ITP patients.	36
Table (6):	Quality of life of both child and parents among the studied ITP patients and the effect 0f the disease in the parents life:	37
Table (7):	Comparison between types of disease (acute, persistent and chronic) ITP as regards the demographic data, clinical data, bleeding score, treatment and response to treatment	38
Table (8):	Comparison between types of the disease (acute, persistent and chronic) as regards laboratory and radiological data.	39
Table (9):	Comparison between types of the disease (acute, persistent and chronic) ITP as regards child and parental quality of life.	40
Table (10):	Comparison between Patients with Occult Bleeding and those without as regards demographic data, clinical data, bleeding score, treatment and response to treatment	41
Table (11):	Comparison between patients with occult bleeding and those without as regards Laboratory data	42

List of Tables (Cont...)

Table No.	Title	Page No.
Table (12):	Comparison between patients with occult bleeding and those without as regards both child and parental quality of life and effect of the disease on the parents' life	43
Table (13):	Correlation between the child quality of life and the disease duration, bleeding score, initial platelet count as well as the mean platelet count over the disease duration.	44
Table (14):	Correlation between the parent's quality of life and the disease duration, bleeding score, initial platelet count as well as the mean platelet count over the disease duration in years	45
Table (15):	Correlation between initial platelet count and the mean platelet count over the disease duration and the bleeding score and the duration of the disease	47
Table (16):	Demographic, clinical, laboratory, radiological and quality of life data of patients with occult bleeding	48

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Summary of the multiple defects associated with ITP pathogenesis including B- and plasma cell-defects as well as T cell-impairment.	12
Figure (2):	Correlation between the child quality of life and the bleeding score.	44
Figure (3):	There was a significantly negative correlation between bleeding score and the parental quality of life.	46
Figure (4):	There was a significantly positive correlation between mean platelet count over the disease duration and the parentalquality of life	46

List of Abbreviations

Abb.	Full term

APC .....*Antigen presenting cells*

CD.....*Cluster differentiation*

Fe .....*Iron*

H2O2 .....*Hydrogen peroxide*

IBLS .....*ITP Bleeding Scale*

ICH .....*Intracranial hemorrhage*

ITP .....*Immune thrombocytopenia*

IVIg .....*Intravenous immunoglobulin*

MCD .....*Minute cerebral capillary bleeding*

MKs .....*Megakaryocytes*

MR .....*Magnetic resonance*

MRI.....*Magnetic resonance imaging*

PLT .....*Platelet*

RES .....*Reticuloendothelial system*

Tc .....*T cells*

TPO .....*Thrombopoietin*

ABSTRACT

Background: Occult hemorrhage can occur in any internal organ in ITP patients. Four sites of occult hemorrhage deserve special attention including microscopic hematuria, fecal occult blood, retinal hemorrhage, and silent intracranial hemorrhage.

Aim: The aim of this study was to investigate for the frequency of subclinical bleeding in Egyptian children with ITP and its relation to different clinical and laboratory parameters of the disease including bleeding score and health quality of life.

Methods: This cross sectional study included 40 ITP patients recruited from the Pediatric Hematology & Oncology unit, Children Hospital, Ain Shams University. Occult blood in stools and urine analysis, fundus examination, and non-contrast brain MRI, for brain microbleed, were done.

Results: The total number of patients with occult bleeds was eleven. Two patients had occult blood in stool, five had microscopic hematuria, one had retinal bleeds and three patients had brain microbleeds. Their mean age was 10.23 ± 4.18 and their mean initial bleeding score was 2.55 ± 0.82. Nine patients with occult bleeding were chronic, one persistent and one acute ITP patients. There was no significant differences between patients with occult bleeding and those without as regards the initial bleeding score, the platelet counts & hemoglobin level, as well as the mean platelet counts & mean hemoglobin level over the disease duration. Although the scoring of the effect on the parent's life, Child and parents quality of life was low in 3 out of 11 patients with occult bleeding, we did not find a significant difference between patients with occult bleeding and those without as regards the ITP health quality of life items.

Conclusion: Our results suggest that subclinical bleeding is a potential risk in children with ITP, more commonly chronic ITP patients. We could not demonstrate a significant relation of occult bleeding to the laboratory findings, bleeding score, and the ITP health quality of life; nevertheless, the significance of the routine assessment of occult bleeding in ITP and the identification of high-risk patients require additional studies.

Keywords: Occult Hemorrhage - Immune Thrombocytopenia

INTRODUCTION

Immune thrombocytopenia (ITP) is a hematological disorder characterized by a reduced number of circulating platelets and an increased risk of bleeding. The platelet count is most often used to assess disease status and response to therapy; however, bleeding is the most clinically important outcome because it has a direct impact on morbidity, mortality, quality of life and treatment decisions ***(Neunert et al., 2015).***

In children, ITP typically presents in otherwise healthy patients, often resolving spontaneously or following therapy within 6–12 months of diagnosis. However, approximately 20%–25% of children with newly diagnosed ITP ultimately develop chronic disease ***(Jung et al., 2016).***

Bleeding manifestations in patients with ITP range from mild skin bruises to life-threatening intracranial hemorrhage (ICH). Severe bleeding is distinctly uncommon when the platelet count is $>30 \times 10^9$/L and usually only occurs when the platelet count falls $<20 \times 10^9$/L. Based on estimates from clinical studies, ITP registries and administrative, the frequency of ICH in patients with ITP is ~0.5% in children and 1.5% in adults ***(Arnold, 2015).***

Four sites of occult hemorrhage deserve special attention, ie, microscopic hematuria which can be detected by urine analysis, fecal occult blood can be detected by stool analysis, retinal hemorrhage detected by fundus examination, and silent (subclinical) ICH can be detected by MRI ***(Flores and Buchanan, 2013).***

ICH is the most devastating complication of ITP in children, and prevention of ICH is the primary goal of ITP treatment. However, the great majority of patients with ITP, even those with very low platelet counts, do not experience severe bleedingand ICH occurs in less than 1 in 100 children with ITP ***(Psailaet al., 2009).***

After ICH, hemosiderin-containing deposits stored in macrophages and glial cells cause focal changes in the magnetic resonance (MR) signal due to their magnetic properties. Such "brain microbleeds" appear dark on T2-weighted spin-echo sequences, appearing enhanced when relying on gradient-echo sequence. Studies in adults with unruptured cerebral aneurysms or hypertension have shown that brain microbleeds can be detected as early as 3 weeks and for as long as 18 months after the onset of localized hemosiderin deposition ***(Flores and Buchanan, 2016).***

AIM OF THE WORK

The aim of this study wasto investigate for the frequency of occult (subclinical) bleeding in Egyptian children with ITP and relation to different clinico-epidemological aspects of the disease including bleeding score and health quality of life.

REVIEW OF LITERATURE

Definition:

Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by platelet destruction by antiplatelet autoantibodies that result in platelet phagocytosis via the reticuloendothelial system (RES). ITP is one of the most common hemorrhagic platelet disorder ***(Tantawy et al., 2010).***

Epidemiology of ITP:

In total, the incidence of ITP is approximately 1.9–6.4 per 100,000 children/year and 3.3–3.9 per 100,000 adults/year, ***(Bennett et al., 2017).***

In Egypt, the estimated incidence of ITP is 100 cases per 1 million persons per year, and about half of these cases occur in children. New cases of chronic refractory ITP comprise approximately 10 cases per 1000,000 per year ***(Mokhtar et al., 2012).***

Pathophysiology of ITP:

Two major mechanisms contribute to the development of ITP: increased platelet destruction and insufficient platelet production. Platelet destruction, the most common mechanism of ITP development, involves loss of self-tolerance of platelet antigens and formation of antibodies that target glycoprotein IIa/IIIa on platelets, causing their destruction by macrophages

or cytotoxic T cells. Impaired function of megakaryocytes and an insufficient level of thrombopoietin (TPO) are two other factors involved in decreased platelet production ***(Khan et al., 2017).***

The initial event(s) leading to anti-platelet autoimmunity remains unclear, but strong evidence exists that autoantibodies and autoreactive $CD8^+$ cytotoxic T cells (Tc) trigger enhanced platelet destruction and impair platelet production by megakaryocytes (MKs) in the bone marrow***(Swinkels et al., 2018).***

Thrombopoietin (TPO) is a growth factor produced primarily by the liver that mediates its effects through the TPO receptor (cMPL) and potent megakaryocyte colony-stimulating factor, along with other cytokines, increase the size and number of marrow megakaryocytes and circulating platelets. In ITP, thrombopoietin levels are normal in 75% of the cases rather than increased. Levels of TPO lower than expected in ITP may be caused by binding to TPO-receptor c-MPL on the increased megakaryocyte mass with subsequent internalization and degradation or secondary to TPO bound to platelets targeted for destruction ***(Mokhtar et al., 2012).***

1-Molecular and cellular mechanisms of the pathogenesis of ITP:

a) B Cells and Autoantibodies

Patients with ITP produce anti-platelet IgG antibodies (and more rarely IgM or IgA antibodies) which bind to platelets

and mark them for phagocytic breakdown in the spleen and liver. These antibodies often bind to very abundant glycoproteins on the platelet surface, particularly GPαIIbβ3 (GPIIbIIIA) and GPIb-IX-V molecules. However, in as many as 30% to 40% of the patients, no detectible antibodies can be found. This could be due to the robustness of the antibody tests used or a purely T cell-mediated mechanism. In those patients positive for anti-platelet antibodies, other antibody specificities beside the classic surface glycoproteins have been found, including cytosolic proteins, which may suggest that platelets undergo protein degradation by antigen presenting cells (APC) followed by antigen presentation to T cells ***(Zufferey et al., 2017).***

b)T-Cell Imbalance in ITP

Abnormal T cells have been described in patients with ITP, including a higher T helper cell reactivity against platelets, a lower frequency of circulating CD4+CD25+FoxP3+ Tregs and CD4+ Th0, and Th1 activation patterns. Cytotoxic CD8+ T cells were found in the circulation of patients. CD8+ T cells are able to directly lyse platelets in vitro and can accumulate in the bone marrow, where they are able to inhibit thrombopoiesis ***(Zhang et al., 2011).***

Furthermore, compared with healthy individuals, CD3+ T cells from patients with ITP have a lower rate of apoptosis and a higher clonal expansion rate, leading to abnormal cytokine secretion, including IL-2, INF-γ, and IL-10, which may be responsible for the lower CD4+CD25+FoxP3+ Treg