

Study of Some Lymphocyte Subsets in Children with Newly Diagnosed Primary Immune Thrombocytopenia

Thesis

*Submitted for Partial Fulfillment of Master Degree
in Pediatrics*

Presented by

Amna Helmy Awad

M.B.B.Ch, Ain Shams University 2008

Under Supervision of

Prof. Wafaa Ezzat Ibrahim

Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Marwa Ahmed Shams

Lecturer of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Shaimaa Abdelmalik Pessar

Lecturer of Clinical and Chemical Pathology

Faculty of Medicine – Ain Shams University

Faculty of Medicine

Ain Shams University

2017



سورة البقرة الآية: ٣٢



First thanks to **ALLAH** to whom I relate any success in achieving any work in my life.

I wish to express my deepest thanks, gratitude and appreciation to Prof. Wafaa Ezzat Ibrahim, Professor of Pediatrics Faculty of Medicine – Ain Shams University for her meticulous supervision, kind guidance, valuable instructions and generous help.

Special thanks are due to Dr. Marwa Ahmed Shams, Lecturer of Pediatrics Faculty of Medicine – Ain Shams University for her sincere efforts, fruitful encouragement.

I am deeply thankful to Dr. Shaimaa Abdelmalik Pessar, Lecturer of Clinical and Chemical Pathology Faculty of Medicine – Ain Shams University for her great help, outstanding support, active participation and guidance.

Last but not least my sincere thanks and appreciation to all patients participated in this study from Hematology Clinic, Children Hospital, Ain shams University.

Amna Helmy Awad



Dedication

*To the memory of my father Helmy
Awad, you left fingerprints in my heart,
you'll always be with me.*

List of Contents

Title	Page No.
List of Tables	6
List of Figures.....	8
List of Abbreviations	10
Introduction	1
Aim of the Work	15
Review of Literature	
▪ Immune Thrombocytopenia	16
▪ Pathogenesis.....	28
▪ Management of childhood ITP	40
Subjects and Methods.....	57
Results	65
Summary	93
Conclusion	95
Recommendation.....	96
References	97
Arabic summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Bleeding score of bleeding symptoms at presentation and at each subsequent evaluation:	21
Table (2):	Frequent Examples of Differential Diagnosis of ITP and Potential Alternative Causes of Thrombocytopenia Identified By Patient History.	26
Table (3):	Grade of severity and management of patients with ITP.	44
Table (4):	First - line /initial treatment in children with ITP.	46
Table (5):	Treatment options in children with persistent or chronic ITP.....	47
Table (6):	Comparison of acute immune thrombocytopenic purpura (ITP) treatment regimen in children.	48
Table (7):	Patients characteristics, History, Examination and bleeding score results of studied group.....	65
Table (8):	Basic laboratory findings of studied group.....	67
Table (9):	Follow up results of studied group	68
Table (10):	Response results of studied group	69
Table (11):	Comparison of CD markers and age results of studied patients as regards their response using One way ANOVA Test.....	70
Table (12):	Comparison of follow up results of studied patients as regards their response using Oneway ANOVA Test	72
Table (13):	Comparison of history and examination findings of studied patients as regards their response using Chisquare Test.....	76
Table (14):	Comparison of follow up results of studied patients using Paired t Test	77

List of Tables cont...

Table No.	Title	Page No.
Table (15):	CD markers value of studied group.....	79
Table (16):	Relation between CD markers of studied group and their response to therapy using One way ANOVA Test	81
Table (17):	Correlation of follow up results of studied patients as regards CD markers using one way ANOVA Test	82

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Thrombocytopenia. Reduced levels of thrombocytes in the blood.....	17
Figure (2):	Role of T –Lymphocyte in ITP.....	37
Figure (3):	Mechanism of action of therapies for Immune Thrombocytopenic Purpura.....	45
Figure (4):	Sex distribution among studied patients with newly diagnosed ITP (33% FEMALES, 67% MALES).....	66
Figure (5):	The characteristic of patients according to history and examination.....	66
Figure (6):	Response of patients to steroid therapy after follow up for 4 months.....	69
Figure (7):	Comparison of age of studied group as regard their response	71
Figure (8):	Comparison of bleeding score of studied group after follow up and their response to therapy.....	74
Figure (9):	Comparison of platelets of studied group after follow up as regards their response to therapy	74
Figure (10):	Comparison of total leucocytic count of studied group at diagnosis as regards their response to therapy	75
Figure (11):	Comparison of neutrophil after follow up of studied group at diagnosis as regards their response to therapy.	75
Figure (12):	Comparison of follow up of studied group.	78
Figure (13):	CD value of studied group.....	80
Figure (14):	Relation between CD markers and response to therapy among studied group.	81
Figure (15):	Correlation between platelet after follow up and CD4 markers	84
Figure (16):	Correlation between total leucocytic count at start and CD3 markers	84

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (17):	Correlation between lymphocytes at diagnosis and CD3 markers.	85
Figure (18):	Correlation between lymphocyte at diagnosis and CD4	85
Figure (19):	Correlation between lymphocytes at diagnosis and CD8 86	86
Figure (20):	Correlation between lymphocytes after follow up and CD4/CD8 ratio.	86

List of Abbreviations

Abb.	Full term
<i>APCS</i>	<i>Antigen presenting cells</i>
<i>CBC</i>	<i>Complete blood count</i>
<i>CD</i>	<i>Cluster of differentiation</i>
<i>CPGs</i>	<i>Clinical practice guidelines</i>
<i>CR</i>	<i>Complete response</i>
<i>CTL</i>	<i>Cytotoxic T lymphocytes</i>
<i>CTLA</i>	<i>Cytotoxic T cell-associated antigen</i>
<i>DC</i>	<i>Dendritic cells</i>
<i>EAE</i>	<i>Experimental autoimmune encephalomyelitis</i>
<i>EDTA</i>	<i>Ethylene diamine tetra-acetic acid</i>
<i>EGF</i>	<i>Epidermal growth factor</i>
<i>FOXp3</i>	<i>Forkhead-box p3</i>
<i>FSC</i>	<i>Forward scatter</i>
<i>GI bleeding</i>	<i>Gastrointestinal bleeding</i>
<i>GITR</i>	<i>Glucocorticoid-induced tumour necrosis factor receptor</i>
<i>GP</i>	<i>Glycoprotein</i>
<i>HCV</i>	<i>HEPATITIS c virus</i>
<i>HIV</i>	<i>Human immunodeficiency virus</i>
<i>I Tregs</i>	<i>Induced Regulatory T cells</i>
<i>ICH</i>	<i>Intracranial hemorrhage</i>
<i>ICIS</i>	<i>International cooperative ITP study group</i>
<i>ITP</i>	<i>Immune thrombocytopenic purpura</i>
<i>IgA</i>	<i>Immunoglobulin A</i>
<i>IgG</i>	<i>Immunoglobulin G</i>
<i>IgM</i>	<i>Immunoglobulin M</i>
<i>IL</i>	<i>Interleukin</i>
<i>INF</i>	<i>Interferon</i>

List of Abbreviations Cont...

Abb.	Full term
ISTH BAT.....	<i>International society of thrombosis / scientific and standardization committee bleeding assessment tool</i>
IVIG.....	<i>Intra venous immunoglobulin</i>
mAbs	<i>Monoclonal antibodies</i>
M-CSf.....	<i>Macrophage colony stimulating factor</i>
MHC	<i>Major histocompatibility complex</i>
MMR.....	<i>Measles –mumps-rubella</i>
MYH9.....	<i>Myosin heavy chain 9</i>
<i>n</i> Tregs.....	<i>Naturally occurring Regulatory T cells</i>
NAIT.....	<i>Neonatal alloimmune thrombocytopenia</i>
NR.....	<i>No response</i>
PC	<i>Portable computer</i>
PLN.....	<i>Proliferative lymphoid nodules</i>
R.....	<i>Response</i>
RH	<i>Rhesus factor</i>
SLE.....	<i>Systemic lupus erythematosus</i>
SPSS.....	<i>Statistical package for Social Science</i>
T regs	<i>Regulatory T cells</i>
TAR.....	<i>Thrombocytopenia absent radius</i>
TCR.....	<i>T cell receptor</i>
Th.....	<i>T helper</i>
TNF.....	<i>Tumor necrosis factor</i>
TPO.....	<i>Thrombopoietin receptor</i>

Abstract

There was significant positive correlation between total leucocytic count at diagnosis and CD3, And significant positive correlation between platelets after follow up and CD4 And high significant positive correlation between lymphocyte at diagnosis and CD3, CD4, CD8. And high negative significant correlation between lymphocytes after follow up and CD4/CD8 ratio.

There was no significant relation between CD3⁺, CD8⁺, CD4⁺ T lymphocytes and response to therapy, however it was observed that there is increase in expression of CD4⁺ T lymphocytes among the complete response compared with the response group but this was not significant, which may need further evaluation.

Understanding the role of T cell subsets will permit a better control of autoimmunity.

Keywords: *Regulatory T cells- T cell receptor- T helper - Tumor necrosis factor- Measles –mumps-rubella*

Introduction

Childhood immune thrombocytopenic purpura (ITP) is a common acquired bleeding disorder that is characterized by isolated thrombocytopenia (peripheral blood platelet count $<100 \times 10^3/\mu\text{L}$) in the absence of other causes of thrombocytopenia. It is an autoimmune-mediated condition that results from antibody-mediated destruction of platelets and impaired megakaryocyte platelet production (*Neunert , 2013*). Newly diagnosed ITP may follow within a few weeks after an antigenic challenge such as infection or vaccination (*Stasi and Newland, 2011*).

The trigger also may be a loss of tolerance due to molecular mimicry with cross-reaction of antibodies arising from infectious agents or drugs, genetic factors, and/or platelet Toll receptors. This loss of tolerance activates autoreactive effector B and T lymphocytes, which in turn initiates platelet destruction, mediated by cytotoxic T lymphocytes and the release of pro-inflammatory cytokines (IL-2/IL-17) by T helper (Th) cells (Th1/Th17). Th2 (anti-inflammatory) and regulatory B (Breg) and Treg cells are also inhibited (with decrease in IL-10/TGF- β), which leads to the disease becoming chronic. Some isotypes of autoantibodies may increase the bleeding risk (*Perera et al., 2016*).

T cells have an important role in modulating the immune system's direction towards disease. Dysregulation of T cell

activity and cytokine abnormalities have been found to be a contributing factor to many autoimmune diseases, including ITP (*Hu et al., 2012*).

The Th1/Th2 cytokine axis is critical for autoimmune reactivity. For example, the Th1/Th2 imbalance leading to autoreactive B cell differentiation seen in ITP was shown to be restored to a protective Th2 population after treatment with glucocorticoid (*Guo et al., 2012*).

Management of newly diagnosed ITP consists of careful observation, regardless of platelet count (*Schultz et al., 2014*), Severe bleeding, which occurs in only 3%–5% of children, requires treatment with corticosteroids, intravenous immunoglobulin (IVIG), or anti-Rhesus-D immunoglobulin (*Neunert et al., 2011*).

Most children with ITP have an acute presentation of purpura and bruising, and 80%–90% of the cases recover spontaneously or with therapy. However, in 10–20% of newly diagnosed children, ITP has a chronic course that persists beyond 12 months (*Schultz et al., 2014*). Because of the high impact of ITP on a child's everyday life and activities, identification of prognostic factors would be beneficial for reducing stress and improving quality of life for both these children and their parents (*Heitink et al., 2014*).

AIM OF THE WORK

To evaluate levels of CD3+ (T lymphocytes), CD4+ helper T lymphocytes and CD8+ cytotoxic T lymphocytes in patients with newly diagnosed immune thrombocytopenia and its impact on disease outcome.