Assessment of CMV Infection Positivity on the Prognosis of Idiopathic Thrombocytopenic Pupura

Thesis
Submitted for Partial fulfillment of Master Degree
in pediatrics

BY
Ahmed Mohamed Mohamed Afifi
MB.BCh., Dec (, , ,)

Supervised by **Prof. Galila Mohamed Mokhtar**

Professor of Pediatrics, Faculty of Medicine Ain Shams university.

Dr. Iman Ahmed Ragab

Lecturer of Pediatrics Faculty of Medicine Ain Shams University

Faculty of Medicine Ain Shams University



First of all, great thanks to God who enabled us to complete this work, hoping to provide a useful guide to the scope of cytomegalovirus associated ITP.

No words can express my deepest appreciation and profound respect to **Prof. Dr. Galila Mohamed Mokhtar,** Professor of Pediatrics, Ain Shams University, for her continuous guidance, support and constructive criticism through the work. She has generously devoted much of her time and her effort for planning and supervision of this study.

Also, my profound gratitude to **Dr. Iman Ahmed Ragab**, Lecturer of Pediatrics, Ain Shams University, for her kind supervision and support. It was great honor to work under her supervision.

Also, my profound gratitude to Dr. Salwa Bakr Ragab, Lecturer of Clinical Pathology, El Fayoum University for her great effort in the laboratory part of the study it was great honor to work with her.

I'd like to thank the patients and parents who participate in this study, without whom any medical progress would never be completed.

Lastly, I don't forget my family, the best helper for me, their full support, prayers and wishes were a great motive to accomplish this work. My deepest gratitude to them and thanks will never appreciate what I owe them.



Afifi

Contents

Title	Page	
List of Abbreviation		I
List of Figures		IV
List of Tables		VI
Abstract		X
Introduction		
Aim of the Work		
Review of Literature:		
Chapter (): Immune Thrombocytopenia		
Chapter (): Cytomegalovirus		
Chapter (): CMV Associated ITP		
Methodology	• • • • • • • • • • • • • • • • • • • •	
Results		
Discussion		
Summary		
Conclusion.		
Recommendations	• • • • • • • • • • • • • • • • • • • •	
References		
Arabic Summary		

List of Abbreviations

Abb.	Meaning
ACA	Anticardiolipin antibody
ADHD	Attention deficit-hyperactivity disorder
AHA	Auto immune hemolytic anemia
AIDS	Acquired immunodeficiency SYNDROM
ALPAs	Antiphospholipid antibodies
ALPS	Autoimmune lymphoproliferative
	syndrome
ALT	Alanine aminotransferase
ANA	Antinuclear antibodies
ANCA	Anti-neutrophil cytoplasmic antibodie
APS	Antiphospholipid syndrome
BAFF	Tumor necrosis factor ligand
BMT	Bone marrow transplantation
BSER	Brain stem-evoked response
CMV	Cytomegalovirus
CMVR	Cytomegalovirus retinitis
$\mathbf{C}\mathbf{R}$	Complete response
CVID	Common variable immune deficiency
DNA	Deoxyribonucleic acid
DPT	Diphtheria, Pertussis (whooping cough)
10	and Tetanus
E	Early
EBV	Epstein-Barr virus
ES	Evans syndrome
ESR	Erythrocyte sedimentation rate
G PD	Glucosephosphate dehydrogenase
	deficiency
gB	Glycoprotein B
GIT	Gastrointestinal tract

Abb.	Meaning
GVHD	Graft-versus-host disease
HAART	Highly active antiretroviral therapy
Hb	Hemoglobin
HBV	Hepatitis B virus
HCMV	Human cytomegalovirus
HCV	Hepatitis C virus
HDMP	High dose methyl prednisolone
HIV	Human immunodeficiency virus
HL	Hodgkin LYMPHOMA
HP	Helicobacter pylori
HTN	Hypertension
IE	Immediate early
IFN-g	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
\mathbf{IL}	Interleukin
ITP	Immune thrombocytopenic purpura
IVIG	Intravenous immunoglobulin
IWG	International Working Group
\mathbf{L}	Late
LAC	Lupus anticoagulant
LDH	Lactate dehydrogenase
M/E ratio	Myeloid to erythroid ratio
MCV	Mean corpuscular volume
MHC	Major histocompatibility complex
MMR	Measles-mumps-rubella
MPV	Mean platelet volume
mRNA	Messenger Ribonucleic acids
N	Number
NR	No response
OPV	Oral polio vaccine

Abb.	Meaning
PCR	Polymerase chain reaction
PTDM	Post-transplant diabetes mellitus
\mathbf{R}	Response
RBCs	Red blood cells
SCID	Severe combined immunodeficiency
	syndrome
SCT	Stem cell transplantation
SLE	Systemic lupus erythematosus
TAR	Thrombocytopenia-absent radius
TORCH	toxoplasmosis, rubella, cytomegalovirus,
	herpes simplex, and HIV
TPO	Thrombopoietin
VZV	Varicella zoster virus
WBC	White blood cell

List of Figures

Figure	Title	Page
Fig. (1)	Estimated fraction of the various forms	
	of secondary ITP based on clinical	
	experience of the authors	
Fig. (۲)	Structure of human cytomegalovirus	
	(HCMV, human herpesvirus)	
Fig. (*)	CMV-induced immunopathology in	
	various groups of patients-previously healthy subjects, immunodepressed	
	transplant recipients, and patients with	
	autoimmune disorders	
Fig. (1)	B-cell tolerance checkpoints and loss of	
	self-tolerance in different forms of	
	secondary ITP	
Fig. (*)	Ethidium bromide-stained agarose gel	
	electrophoresis: identification of CMV	
	DNA with p and p primers. Lane	
	M. bp DNA marker (Sinagen, Iran)	
	lane positive control lane positive	
	patient samples lane and negative patient samples lane negative	
	control. The size of gB product is	
	Bp.	
Fig. (٦)	Ethidium bromide-stained agarose gel	
	electrophoresis: identification of CMV	
	DNA with p and p primers. Lane	
	M. bp DNA marker (Sinagen, Iran)	
	lane positive control lane positive patient samples lane and negative	
	patient samples lane negative patient samples lane negative	

Figure	Title	Page
	control. The size of gB product is Bp.	
Fig. (Y)	Ethidium bromide-stained agarose gel	
	electrophoresis: identification of CMV	
	DNA with p and p primers. Lane	
	M. bp DNA marker (Sinagen, Iran)	
	lane positive control lane positive	
	patient samples lane and negative	
	patient samples lane negative	
	control. The size of gB product is	
	Bp.	
Fig. (^)	Agreement between serology and PCR	
	testing for diagnosis of CMV infection	
Fig. (٩)	Age at diagnosis by type of ITP	
Fig. $(\cdot \cdot)$	Gender distribution by type of ITP.	
Fig. (۱۱)	Last vaccination received by type of ITP.	
Fig. (۱۲)	Neutrophil count at sampling by type of	
	ITP.	
Fig. (۱۳)	Platelet count at sampling by type of	
	ITP.	
Fig. (\ \ \)	Prevalence of positive serology testing	
(·)	for CMV by type of ITP.	
Fig. (10)	Prevalence of positive PCR testing for CMV by type of ITP.	
Fig. (۱٦)	Prevalence of positive serology testing	
	for EBV by type of ITP.	
Fig. (۱۷)	First-line treatment by type of ITP.	

Figure	Title	Page
Fig. (۱۸)	Response to first-line treatment by type	
	of ITP.	
Fig. (19)	Response of treatment by type of ITP.	

List of Tables

Table	Title	Page
Tab. (١)	Proposed definitions of disease	
Tab. (۲)	Frequent examples of differential	
	diagnosis of ITP and potential	
	alternative causes of	
	thrombocytopenia identified by	
	patient history	
Tab. (٣)	Recommendations for the diagnosis of	
	ITP in children and adults	
Tab. (٤)	First-line/initial treatment in children	
	with ITP	
Tab. (•)	Treatment options in children with	
	persistent or chronic ITP	
Tab. (٦)	Definitions of response to treatment	
	by ITP	
Tab. (٧)	Definitions of time to and duration of	
	response, and the time to initial and	
	peak response for different ITP	
m 1 (1)	treatments	
Tab. (^)	Signs and symptoms that are possibly suggestive of the diagnosis of CMV-	
	related ITP	
Tab. (٩)	Possible mechanisms of how	
	viruses/bacteria cause ITP	
Tab.	Comparison of demographic	
(\·-A)	characteristics and history by CMV	
	PCR positivity: numerical data	

Table	Title	Page
Tab.	Comparison of demographic	
(1 · -B)	characteristics by CMV PCR	
	positivity: categorical data	
Tab.	Comparison of different presentations	
(1 · -C)	according to CMV-PCR positivity:	
	categorical data	
Tab.	Comparison of important points in	
(1 · -D)	history according to CMV-PCR	
	results: categorical data	
Tab. (۱۱)	Comparison of results of clinical	
	examination by CMV PCR positivity:	
	categorical data	
Tab. (۱۲)	Comparison of initial hematologic	
	indices, and biochemical results by	
	CMV PCR positivity	
Tab. (۱۳)	Comparison of bleeding score by CMV	
	PCR positivity	
Tab. (14)	Comparison of hematologic indices at	
	time of sampling and bone marrow	
	examination, by CMV PCR positivity	
Tab. (۱٥)	Comparison of atypical lymphocytes	
	in CBC and prevalence of EBV	
	infection by CMV PCR positivity	
Tab. (۱٦)	Comparison of lines of treatment,	
, ,	response, and refractoriness to	
	treatment by CMV PCR positivity	

Table	Title	Page
Tab. (۱۷)	Diagnostic value of serology and its	
	agreement with PCR for diagnosis of	
	CMV infection	
Tab.	Comparison of demographic	
(\ \ - A)	characteristics and history by type of	
	ITP: skewed numerical data	
Tab.	Comparison of different presentations	
(, y-B)	according to acute and chronic ITP	
	results: categorical data	
Tab.	Comparison of important points in	
(1 V-C)	history according to acute and chronic	
	ITP results: categorical data	
Tab. (۱۹)	Comparison of findings on clinical	
	examination between acute and	
m 1 (4)	chronic ITP patients	
Tab. (۲۰)	Comparison of hematologic indices,	
	and biochemical results by type of ITP: skewed numerical data	
Tab.	Comparison of bleeding score and	
(Y \ -A)	hematologic indices at time of	
(11)	sampling and result of bone marrow	
	examination: normally distributed	
	numerical data	
Tab.	Comparison of hematologic indices at	
(1.B)	time of sampling, result of bone	
	marrow examination, and bleeding	
	score after first-line treatment:	
	skewed numerical data	

Table	Title	Page
Tab.	Comparison of results of CBC in acute	
(1.C)	and chronic ITP patients: categorical	
	data	
Tab. (۲۲)	Comparison of virological studies in	
	acute and chronic ITP patients	
Tab. (۲۳)	Lines of treatment, response, and	
	refractoriness to treatment	

Abstract

Human cytomegalovirus (CMV) modulates host immunity, and CMV-infected patients often develop signs of immune dysfunction; Several reports have implicated cytomegalovirus (CMV) in the pathogenesis of ITP. Our Study aimed to evaluate CMV positivity prevalence among pediatric patients with acute and chronic ITP.

And it's impact on severity of bleeding, response to treatment and development of chronic disease.

Methods: A cross-sectional study was conducted including Patients with ITP diagnosed and/or following at Ain-Shams university pediatrics hospital hematology unit in the period between August and April

. All patients were subjected to history taking emphasizing on age, sex, clinical presentation suggestive of viral infection e.g.pneumonitis, hepatitis, petechial rashes, mononucleosis-like syndrome (characterized by fatigue, malaise, myalgia, headache, fever, hepatosplenomegaly, elevated liver enzymes, and atypical lymphocytosis), any persistent fever, morbilliform rash. Patients were then subjected to physical examination for bleeding signs and type of bleeding (including retinal hemorrhages), Severity of bleeding was calculated using bleeding score (**Edslev et al.** Y··V), any organomegaly and presence of dysmorphic features. Data about treatment received and outcome including lines of treatment, initial response (after st line or more than one line needed) were included in the study. CMV-PCR was done for all patients.

Results: Our study results showed that CMV prevalence in patients with patient acute ITP and patient chronic ITP). (/), (Patients with CMV-PCR positive had a median age of diagnosis months compared to months in CMV- PCR negative patients with no significant difference (p> of patients with CMV- PCR). negative had a chronic ITP compared to in CMV -PCR positive), and no gender difference. No difference in the initial patients (p= clinical presentation or bleeding scores according to CMV-PCR status. Two patients with CMV-PCR positive had an intracranial bleeding however, no significant difference were encountered in other bleeding