

Evaluation of the role of ADAMTS13 activity and Anti-ADAMTS13 autoantibodies in the pathogenesis and prognosis of thrombotic microangiopathy patients on plasma exchange therapy.

**Thesis submitted for partial fulfillment of (M.D)
degree in Clinical and Chemical Pathology.**

By

Ahmed Mohamed Ali Abdel Hafez
(M.B., B.CH., M.Sc.)

Supervised by

Prof. Dr. Azza Ahmed Aboul-Enein

Professor of Clinical & Chemical Pathology
Faculty of Medicine
Cairo University

Prof. Dr. Osama Ahmed Khalafallah

Professor of Clinical & Chemical Pathology
Faculty of Medicine
Cairo University

Prof. Dr. Nermeen Ahmed El-Desouki

Professor of Clinical & Chemical Pathology
Faculty of Medicine
Cairo University

Dr. Hala Mahmoud Abdelhamid

Assistant Professor of Internal medicine
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University

2012

ACKNOWLEDGEMENT

First and foremost thanks to ALLAH the most kind and merciful.

I was absolutely lucky to be supervised by this group of our professors who taught me, not only the basis of research, but also many of good morals. I would like to express my endless appreciation to **Dr. Azza Ahmed Abo Elenin, Dr. Osama Ahmed Khalaf Allah, Dr. Nermeen Ahmed ElDousoky and Dr. Hala Mahmoud AbdelHamid** for their continuous instructions, valuable criticism and sincere advice.

I would like to express my appreciation to **Dr. Iman Abdel Wahab** and all the staff members and employee of Plasma exchange unit in the central blood bank of Al kasr Al Aini hospitals.

I wish to express my deep feelings and profound gratitude to my dear **wife, my kids and my parents** who without their support and love I would not be able to continue this work.

List of Content

<i>Introduction.....</i>	<i>1</i>
<i>Aim of the work.....</i>	<i>4</i>
<i>Chapter I: <u>ADAMTS family</u>.....</i>	<i>5</i>
Nomenclature.....	6
The domain structure of the ADAMTS proteins.....	7
Regulation and expression of the ADAMTSs.....	14
Regulation of ADAMTS gene expression.....	16
ADAMTS functions.....	19
ADAMTSL molecules.....	29
<i>Chapter II: <u>Thrombotic Microangiopathies</u>.....</i>	<i>30</i>
Introduction.....	30
Types of microangiopathic hemolytic anemias.....	31
I-Thrombotic thrombocytopenic purpura.....	32
☒ Pathophysiology.....	32
☒ Types of Thrombotic Thrombocytopenic Purpura.....	35
A-Hereditary thrombocytopenic purpura.....	35
B-Acquired TTP.....	37
☒ Clinical Features.....	38
☒ Laboratory Findings.....	40
☒ ADAMTS13, Von Willebrand factor multimers and antigen levels.....	42

☒ Differential Diagnosis.....	43
☒ Management and Prognosis.....	46
II-Shiga toxin-associated haemolytic uremic syndrome.....	50
☒ Etiology.....	50
☒ Pathophysiology.....	51
☒ Clinical Manifestations.....	52
☒ Laboratory Findings.....	53
☒ Management and Prognosis.....	55
Atypical hemolytic uremic syndrome.....	56
Platelet damage by abnormal vascular surface.....	61
Miscellaneous forms of non-immunologic platelet destruction.....	63
<i>ChapterIII: <u>Plasmapheresis</u></i>	65
Different Techniques of plasmapheresis Therapy.....	66
Component Collection.....	69
Platelets pheresis.....	70
- Donor selection and monitoring.....	71
- Laboratory testing.....	73
Plasma.....	74
Red cells.....	76
Granulocytes.....	76
- Storage and Infusion.....	78
Hematopoietic Progenitor Cells.....	79
Therapeutic apheresis.....	79
Vascular Access.....	80

Removal of Pathologic Substances.....	81
Removal of Normal Plasma Constituents.....	82
Replacement Fluids.....	84
Complications.....	85
Indications for therapeutic apheresis.....	90
<i>Subjects and Methods</i>	99
<i>Results</i>	113
<i>Discussion</i>	149
<i>Summary and recommendation</i>	161
<i>References</i>	163
الملخص العربي.....	1

List of abbreviation

<i>AABB:</i>	American association of blood banking.
<i>ACE:</i>	Angiotensin converting enzyme.
<i>ADAM:</i>	a disintegrin and metalloproteinase like.
<i>ADAMTS13:</i>	A disintegrin and metalloprotease thrombospondin type13
<i>ADAMTSL:</i>	A disintegrin and metalloprotease thrombospondin like.
<i>ANCA:</i>	Anti neutrophil cytoplasmic antibody.
<i>ASFA:</i>	American society for apheresis.
<i>AT:</i>	Apheresis technician.
<i>BF:</i>	Complement factor B.
<i>BM:</i>	Bone marrow.
<i>C3:</i>	Complement 3.
<i>C. elegans:</i>	Caenorhabditis elegans.
<i>CD:</i>	Cluster of differentiation.
<i>CFH:</i>	Complement factor H.
<i>CIPD:</i>	Chronic inflammatory demyelinating polyneuropathy.
<i>CT:</i>	Computed tomography.
<i>CUB:</i>	complement subcomponent C1r/C1s/embryonic sea urchin protein.
<i>DNA:</i>	Deoxyribose nucleic acid.
<i>ECG:</i>	Electrocardiogram.
<i>ECM:</i>	Extra cellular matrix.
<i>EDTA:</i>	Ethylene diamine tetra acetic acid.

<i>EDS:</i>	Ehler-Danlos syndrome.
<i>ELISA:</i>	Enzyme linked immunosorbant assay.
<i>ERK:</i>	extracellularsignal- regulated kinase.
<i>ESR:</i>	Erythrocyte sedimentation rate.
<i>FDA:</i>	Food and drug administration.
<i>FFP:</i>	Fresh frozen plasma.
<i>GAGs:</i>	Glycated aminoglycans.
<i>Gb3:</i>	globotriosyl ceramide.
<i>G-CSF:</i>	Granulocyte-colony stimulating factor.
<i>Hb:</i>	Haemoglobin.
<i>HBsAg:</i>	Hepatitis B surface antigen.
<i>HCV-Abs:</i>	Hepatitis C virus antibodies.
<i>HELLP:</i>	Hemolysis, elevated liver enzyme, low platelet count.
<i>HES:</i>	Hydroxyethyl starch.
<i>HIV:</i>	Human immunodeficiency virus.
<i>HLA:</i>	Human leucocyte antigen.
<i>HP:</i>	Haemapheresis practitioner.
<i>HUS:</i>	Haemolytic uraemic syndrome.
<i>IF:</i>	Complement factor I.
<i>IGD:</i>	Intraglobular domain.
<i>IgG:</i>	Immunoglobulin G.
<i>IL:</i>	Interleukin.
<i>INH:</i>	Inhibitor.
<i>ITP:</i>	Idiopathic thrombocytopenic purpura.

<i>JNK:</i>	c-Jun N-terminal kinase.
<i>KDa:</i>	Kilo Dalton.
<i>LDH:</i>	Lactate dehydrogenase.
<i>MCP:</i>	Membrane cofactor protein.
<i>MGUS:</i>	Monoclonal gammopathy of unknown significance.
<i>MMPs:</i>	Matrix metalloproteinase.
<i>MRI:</i>	Magnetic resonance imaging.
<i>m-RNA:</i>	Messenger- ribose nucleic acid.
<i>PACE:</i>	paired basic amino acid converting enzyme.
<i>PBS:</i>	Phosphate buffer saline.
<i>PCINP:</i>	pro-collagen I N-proteinase.
<i>PCR:</i>	Polymerase chain reaction.
<i>PE:</i>	Plasma exchange.
<i>PLAC:</i>	Protease and lacunin domaine.
<i>PLT:</i>	Platelets.
<i>PNH:</i>	Paroxysmal nocturnal haemoglobinuria.
<i>POEMS:</i>	Polyneuropathy, organomegaly, endocrinopathy, MGUS and skin change.
<i>RBCs:</i>	Red blood cells.
<i>RPGN:</i>	Rapidly progressive glomerulonephritis.
<i>Rtx:</i>	Reticulocytic count.
<i>SCR-7:</i>	Short consensus repeat-7.
<i>SD:</i>	Standard deviation.
<i>SPC:</i>	subtilisin-like proprotein convertase.

<i>T3:</i>	Tri-iodothyronine.
<i>TIMP:</i>	Tissue inhibitor of metalloproteinase.
<i>TMA:</i>	Thrombotic microangiopathies.
<i>TMB:</i>	Tetramethyl benzidine.
<i>TPE:</i>	Therapeutic plasma exchange.
<i>TSP1:</i>	thrombospondin type I-like).
<i>Uegf:</i>	Urchin epidermal growth factor.
<i>ULVWF:</i>	Ultralarge von- willebrand facto.r
<i>VEGF:</i>	Vascular endothelial growth factor.
<i>vWF:</i>	von Willebrand factor.
<i>vWFCP:</i>	von Willebrand factor carrier protein.
<i>WMS:</i>	Weill-Marchesani syndrome.

List of Tables

<u>Table</u>		<u>Page</u>
Table 1	Differential diagnosis of TTP	46
Table 2	Colloid replacement fluids for therapeutic plasma exchange..	84
Table 3	Sex distribution among cases and controls.....	115
Table 4	Pathogenesis of TTP among studied cases.....	116
Table 5	Outcome of studied cases.....	117
Table 6	Number of plasmapheresis sessions among studied cases.....	118
Table 7	Comparison between number of sessions and pathogenesis of TTP among cases.....	118
Table 8	Fragmented RBCs before and after plasmapheresis Sessions among studied cases.....	118
Table 9	Lab results before and after plasmapheresis sessions among studied cases.....	119
Table 10	Activity and inhibitor before and after plasmapheresis in Cases and Controls.....	122

Table 11	Activity and inhibitor before and after plasmapheresis in Cases.....	124
Table 12	Percent change in laboratory results after plasmapheresis sessions in Cases.....	125
Table 13	Relation between sex and outcome of the disease.....	126
Table 14	Relation between age and outcome of the disease.....	126
Table 15	Relation between age and outcome of the disease.....	127
Table 16	Relation between the platelet count and the outcome of the disease.....	128
Table 17	Platelets count among primary versus secondary cases within group I.....	129
Table 18	Platelets count among primary versus secondary cases within group II.....	130
Table 19	Relation between LDH level and outcome of the disease the studied cases.....	131
Table 20	LDH among primary versus secondary cases within group I.....	132
Table 21	LDH among primary versus secondary cases within group II.....	133

Table 22	Relation between ADAMTS13 Activity (%) and outcome of the disease in the studied cases.....	134
Table 23	ADAMTS13 % Activity among primary versus secondary cases within group I.....	135
Table 24	ADAMTS13 % Activity among primary versus secondary cases within group II.....	136
Table 25	Relation between inhibitor (IU/ml) and the outcome of the disease in the studied cases.....	137
Table 26	ADAMTS13 inhibitor among primary versus secondary cases within group I.....	138
Table 27	ADAMTS13 inhibitor among primary versus secondary cases within group II.....	139
Table 28	Relation between Number of sessions and the outcome of the disease.....	140
Table 29	Comparison between gender and pathogenesis of TTP among cases.....	141
Table 30	Comparison between different variables and pathogenesis of TTP among our cases.....	142
Table 31	Frequency of drug IV addicts among cases.....	145
Table 32	Statistical analysis of addict/non addict cases within the secondary group.....	146

<i>Table 33</i>	Effect of plasmapheresis on ADAMTS13 within the addict /non addict cases in the secondary group.....	146
<i>Table 34</i>	Correlation between different variables and Sessions number	147
<i>Table 35</i>	Comparison between Sex and different variables.....	148
<i>Table 36</i>	Modified Rose severity scoring system of patients with TTP.	150
<i>Table 37</i>	Results of various studies.....	156

List of Figures

Figure		Page
Figure 1	Domain structure of the ADAMTS proteins.....	10
Figure 2	The phylogeny tree and physiological role of ADAMTS members.....	28
Figure 3	Pathogenesis of TTP.....	33
Figure 4	Scheme of management of TTP	49
Figure 5	Centrifugal separation.....	67
Figure 6	Centrifugal separator versus and membrane filtration system	69
Figure 7	Central vascular access	80
Figure 8	Simplified graphical presentation of plasmapheresis /haemodialysis.....	98
Figure 9	ADAMTS13 Inhibitor.....	102
Figure 10	Standard curve of ADAMTS13 INH ELISA.....	104
Figure 11	ADAMTS13 activity calibration curve.....	110
Figure 12	ADAMTS13 Activity.....	110
Figure 13	Sex distribution among cases and controls.....	115
Figure 14	Pathogenesis of TTP among studied cases.....	116
Figure 15	Outcome of studied cases.....	117
Figure 16-a	Platelets “x10 ⁹ /l” before and after plasmapheresis sessions among studied cases.....	120

Figure 16-b	Hemoglobin (gm %) before and after plasmapheresis sessions among studied cases.....	120
Figure 16-c	Reticulocytic count before and after plasmapheresis sessions among studied cases.....	121
Figure 16-d	LDH before and after plasmapheresis sessions among studied cases.....	121
Figure 17	Activity (%) before plasmapheresis sessions among cases versus.....	123
Figure 18	Activity (%) after plasmapheresis sessions among cases versus controls.....	123
Figure 19	Inhibitor before plasmapheresis sessions among cases versus controls.....	123
Figure 20	Inhibitor after plasmapheresis sessions among cases versus controls.....	123
Figure 21-a	Activity before versus after plasmapheresis sessions.....	124
Figure 21-b	Inhibitor before versus after plasmapheresis sessions among cases.....	124
Figure 22	Relation between platelet count and outcome of the disease.	128
Figure 23	Platelets count among primary versus secondary	129
Figure 24	Platelets count among primary versus secondary cases within favorable cases.....	130
Figure 25	Relation between LDH level and outcome of the disease.....	131
Figure 26	LDH among primary versus secondary cases within group I...	132
Figure 27	LDH among primary versus secondary cases within group II..	133