Clinical Utility of CD154 and Monocyte Chemoattractant Protein-1 in Patients with Acute Ischemic Stroke

Thesis

Submitted for the partial fulfillment of
M.D. in Clinical and Chemical Pathology

By

Heba M. Adel Tawfik Abou Zaghla
M.B.B.Ch & M.Sc. Ain Shams University

Supervised by

Professor/ Sawsan Said Hafez
Professor of Clinical and Chemical Pathology
Faculty of Medicine-Ain Shams University

Professor/ Mona Mohamed Zaki
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Doctor/ Abeer I. Abd El-Mageed
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Doctor/ Eman Saleh El-Hadidi
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Professor/ Hany Mohamed Amin Aref
Professor of Neurology
Faculty of Medicine-Ain Shams University

Faculty of Medicine
Ain Shams University
2012

الأهمية الإكلينيكية لقياس CD154 و MCP-1 في مرضى السكتة الدماغية

رسالة

توطئة للحصول علي درجة الدكتوراه

في الباثولوچيا الإكلينيكية والكيميائية

مقدمة من

الطبيبة / هبه محمد عادل توفيق أبو زغلة

بكالوريوس الطب والجراحة

وماجستير الباثولوجيا الإكلينيكية والكيميائية جامعة عين شمس

تحت إشراف

الأستاذ الدكتور/ سوسن سعيد حافظ

أستاذ الباثولوچيا الإكلينيكية والكيميائية

كلية الطب – جامعة عين شمس

الأستاذ الدكتور/ منى محمد زكى

أستاذ الباثولوچيا الإكلينيكية والكيميائية

كلية الطب – جامعة عين شمس

الدكتور/ عبير إبراهيم عبد المجيد

أستاذ مساعد الباثولوچيا الإكلينيكية والكيميائية

كلية الطب ـ جامعة عين شمس

الدكتور/ إيمان صالح الحديدي

أستاذ مساعد الباثولوجيا الإكلينيكية والكيميائية

كلية الطب ـ جامعة عين شمس

الأستاذ الدكتور/ هانى محمد أمين عارف

أستاذ الأمراض العصبية

كلية الطب – جامعة عين شمس

كلية الطب

جامعة عين شمس

2012

Acknowledgement

Before all, thanks to ALLAH *who granted me the power to accomplish this work.*

My deep appreciation and sincere gratitude goes to ***Professor/ Sawsan Said Hafez****, Professor of Clinical and Chemical Pathology, Ain Shams University, who honored me by her supervision of this work and who unselfishly, spent much of her valuable time and effort trying to get the best out of it. I owe a lot to her meticulous supervision and expert touches.*

I am also indebted to ***Professor/ Mona Mohamed Zaki****, Professor of Clinical and Chemical Pathology, Ain Shams University, for her indispensable guidance and her accurate instructions; her keen advice made a great difference in this work.*

My particular and deepest thanks are expressed to ***Doctor/ Abeer I. Abd El-Mageed****, Assistant Professor of Clinical and Chemical Pathology, Ain Shams University, who provided me a great of her time, continuous advice, directions and effort to fulfill this work. I would like to take this opportunity to thank her for her continuous encouragement and support throughout the whole work.*

My sincere gratitude and thanks to ***Doctor/ Eman Saleh El-Hadidi,*** *Assistant Professor of Clinical and Chemical Pathology, Ain Shams University, for giving me the privilege of working under her supervision and for her constant help and encouragement.*

I am also greatful to ***Professor/ Hany Mohamed Amin Aref****, Professor of Neurology, Ain Shams University, for his valuable help, faithful supervision and great cooperation.*

My deepest appreciation and grateful thanks are to ***Professor/ Aza Abd El-Naser Abd El-aziz,*** *Professor of Neurology, Ain Shams University, for her kind advices and her great efforts throughout this work.*

I am truly grateful to ***Professor/ Manal A. Shams Eldin Eltelbany,*** *Professor of Clinical Pathology, Ain Shams University, for her close supervision, sincere help, valuable suggestions and continuous encouragement throughout the whole work.*

Lastly, I would be remiss if I failed to acknowledge ***my mother and the soul of my father****, the dearest persons in my life, many thanks.*

List of Abbreviations

4-AAP 4-Aminoantipyrine
AcPAO ..... Acetylpolyamine oxidase
ADC ..... Analog to digital converter
ADP..... Adenosine diphosphate
AIS ..... Acute ischemic stroke
AP..... Alkaline phosphatase
APC..... Antigen presenting cells
ASA..... Acetylsalicylic acid
ATP ..... Adenosine triphosphate
BBB..... Blood–brain barrier
B-FABP..... Brain-fatty acid binding protein
cAMP ..... Cyclic adenosine monophosphate
CAT..... Computed axial tomography
CCchemokine..... Cysteine-cysteine chemokine
CCL2..... Cysteine-cysteine ligand 2
CCR2..... Cysteine-cysteine receptor 2
CCR4..... Cysteine-cysteine receptor 4
CD40L..... Clusters of differentiation 40 ligand
CE..... Cholesterol esterase
CEA..... Carcinoembryonic antigen
CEP ..... Cytokine expression profiling
CHD risk %..... Coronary heart disease risk percentage
CK-MB..... Creatine Kinase MB fraction
CNS..... Central nervous system
CSF..... Cerebrospinal fluid
CT..... Computerized tomography
CTA..... Computed tomographic angiography
DAG..... Diacylglycerol
dC..... Delta change
DNA..... Deoxyribonucleic acid
DSA..... Digital subtraction angiography
ECG..... Electrocardiogram
EDTA ..... Ethylene diamine tetraacetic acid
ELISA ..... Enzyme linked immunosorbent assay

List of Abbreviations (Cont.)

ELISpot ..... Enzyme-linked immunospot
ESPS-2 ..... European stroke prevention study 2
ETS..... Environmental tobacco smoke
FABP..... Fatty acid binding protein
FCM ..... Flow cytometry
FITC ..... Fluorescein isothiocyanate
FL ..... Fluorescence
FS ..... Forward Scatter
GFAP ..... Glial fibrillary acidic protein
GP..... Glycoprotein
GP IIb/IIIa ..... Glycoprotein IIb/IIIa complex
GPIb–IX–V ..... Glycoprotein Ib–IX–V complex
GPO..... Glycerophosphate oxidase
HDL-C ..... High density lipoprotein cholesterol
HEPES ..... 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
H-FABP ..... Heart-fatty acid binding protein
HMG-CoA 3-Hydroxy-3-methylglutaryl-CoA
HPLC ..... High performance liquid chromatography
HRP..... Horseradish peroxidase
HRT..... Hormonal replacement therapy
hsCRP..... High-sensitivity C-reactive protein
HT ..... hemorrhagic transformation
ICAM-1..... Intercellular adhesion molecule-1
ICH..... Intracerebral hemorrhage
IgM..... Immunoglobulin M
IL-1ß..... Interleukin-1ß
IL2..... Interleukin-2
IP3 ..... Inositol triphosphate
IQR..... Interquartile range
IS ..... Ischemic stroke
KDa ..... Kilodalton
LACI ..... Lacunar infarct
LAPSS..... Los Angeles prehospital stroke screen

List of Abbreviations (Cont.)

LDL-C ..... Low density lipoprotein cholesterol
LPS ..... Lipopolysaccaride
LVH ..... Left ventricular hypertrophy
MAB ..... Monoclonal antibody
MAP ..... Mitogen-activated protein
MBP ..... Myelin basic protein
MCA ..... Middle cerebral artery
MCAF ..... Monocyte chemotactic and activating factor
MCP-1 ..... Monocyte chemotactic protein-1
MESF ..... Molecules of equivalent soluble fluorochrome
MFI ..... Mean fluorescence intensity
MHC class II ..... Major histocompatibility complex
MI ..... Myocardial infarction
MIP-1β ..... Macrophage inflammatory protein-1beta
MMP-2 ..... Matrix metalloproteinases-2
MMPs ..... Matrix metalloproteinases
MRA ..... Magnetic resonance angiography
MRI ..... Magnetic resonance imaging
mRNA ..... Messenger ribonucleic acid
NDKA ..... Nucleotide diphosphate kinase A
NDP ..... Nucleoside diphosphate
NFκB ..... Nuclear factor kappa B
NIHSS ..... National institute of health stroke scale
NK ..... Natural killer
NMDA ..... N-methyl-D-aspartic acid
NMR ..... Nuclear magnetic resonance
NO ..... Nitric oxide
NSE ..... Neuron specific enolase
OCSP ..... Oxford community stroke project
PAB ..... Polyclonal antibody
PACI ..... Partial anterior circulation infarct
PDGF ..... Platelet-derived growth factor
PE ..... Phycoerythrin

List of Abbreviations (Cont.)

PerCP ..... Peridinin chlorophyll protein
PGI_2 ..... Prostaglandin I_2
PIP2 ..... Phosphatidylinositol (4,5)-bisphosphate
PKC ..... Protein kinase C
PLC ..... Phospholipase C
PMT ..... Photomultiplier tube
RNA ..... Ribonucleic acid
sCD40L ..... Soluble CD40 ligand
SCYA2 ..... Small inducible cytokine A2
SD ..... Standard deviation
SIG ..... Small inducible gene
SMC ..... .Smooth muscle cells
SMO ..... Spermine oxidase
SPSS ..... Statistical package for the social sciences
SS ..... Side Scatter
SST ..... Serum separator tube
TACI ..... Total anterior circulation infarct
TC ..... Total cholesterol
TCD ..... Transcranial doppler
TF ..... Tissue factor
TG ..... Triglycerides
TGF ..... Transforming growth factor
TH1 ..... T helper1
TIA ..... Transient ischemic attack
TNF ..... Tumor necrosis factor
TNFSF ..... Tumor necrosis factor superfamily
TOAST ..... Trial of organization 10172 in acute stroke treatment
Tpa ..... Tissue plasminogen activator
TRAP ..... Thrombin receptor activating peptide
TxA_2 ..... Thromboxane A_2
VCAM-1 ..... Vascular cell adhesion molecule-1
VLDL-C ..... Very low density lipoprotein cholesterol
VWF ..... Von Willebrand factor

List of Tables

Table No.	Table Title	Page No.
1	Descriptive statistics of the different studied parameters in all studied patients and patients ‘ subgroups.	108
2	Descriptive and comparative statistics using Wilcoxon signed rank test for comparison between CD40L and MCP1 before and after 1 week of treatment and comparison between MRS after 1 week and after 3 months of treatment in all patients.	109
3	Descriptive and comparative statistics of different studied parameters between small vessel and large vessel ischemic stroke groups using Student t test for parametric data and Wilcoxon Rank Sum test for non-parametric data.	110
4	Descriptive and comparative statistics of different studied parameters between diabetic and non-diabetic groups using Student t test for parametric data and Wilcoxon Rank Sum test for non-parametric data.	111
5	Descriptive and comparative statistics of different studied parameters between hypertensive and non-hypertensive groups using Student t test for parametric data and Wilcoxon Rank Sum test for non-parametric data.	112

Table No.	Table Title	Page No.
6	Descriptive and comparative statistics of different studied parameters between patients′ groups with and without coronary heart disease risk (male <35% and female <40%) using Student t test for parametric data and Wilcoxon Rank Sum test for non-parametric data.	113
7	Descriptive and comparative statistics of different studied parameters between carotid stenosis (≥70%stenosis) and non-carotid stenosis (<70%stenosis) groups using Student t test for parametric data and Wilcoxon Rank Sum test for non-parametric data.	114
8	Descriptive and comparative statistics of different studied parameters between non-recurrent and death groups using Student t test for parametric data and Wilcoxon Rank Sum test for non-parametric data.	115
9	Correlation study using Spearman rank correlation test in all patients.	116

List of Figures

Figure No.	Figure title	Page No.
1	Endothelial injury by risk factors results in lipid and inflammatory cell deposition	9
2	Mechanism of thrombus initiation and thrombus propagation	10
3	CT scan of a patient who has had left middle cerebral artery stroke	24
4	MRI of a patient who has had stroke of the left hemisphere of the brain	25
5	An angiogram showing one of the major blood vessels of the brain that is blocked causing a stroke	26
6	Gelatinase expression after hemorrhagic transformation	32
7	Role of CD40L in the biochemical mechanisms linking cerebrovascular risk factors, inflammation, lipid peroxidation and platelet activation	40
8	Mechanical thrombectomy	44
9	Location of CD40L gene on the long (q) arm of the X chromosome at position 26	54

List of Figures (Cont.)

Figure No.	Figure title	Page No.
10	Secondary structure of CD40L	55
11	Scheme showing trimeric stucture of CD40 ligand	56
12	Role of CD40/CD40L interaction in T cell and macrophage activation	60
13	Role of CD40L in the complex interplay between inflammation, endothelial activation/dysfunction and platelet/coagulative activation	64
14	The building blocks of a flow cytometer	67
15	Flow cytometers use the principle of hydrodynamic focusing for presenting cells to laser beam	68
16	One-parameter histogram	70
17	Two parameter histogram	70
18	MCP-1 monomer and MCP-1 dimer	80
19	Three dimensional structure of chemokines	82
20	Shows flow cytometry analysis and gating of platelets	97

List of Figures (Cont.)

Figure No.	Figure title	Page No.
21	Shows flow cytometry analysis and quantification of gated platelets using phycoerythrin-conjugated anti-CD42a	98
22	Shows flow cytometry analysis and quantification of non-specific binding of isotypic control on platelets	99
23	Shows flow cytometry analysis and quantification of CD40L on platelets.	99

Contents

Page

Acknowledgement I
List of Abbreviations III
List of Tables VII
List of Figures IX

- Introduction and Aim of the Work.....1
- Review of Literature
  - ***Chapter 1:*** **ACUTE ISCHEMIC STROKE**. 4

A. Definition 4
B. Epidemiolog.....5
C. Causes and Classifications of Acute Ischemic Stroke 5
D. Pathophysiological Mechanism of Acute Ischemic stroke.....7
   1. Inflammatory Profile of Atherosclerosis 7
   2. Mechanism of Intraluminal Thrombosis 9
   3. Pathophysiological Role of Platelets in AIS.....11
   4. Effects of Acute Ischemic stroke 12
E. Risk Factors of AIS 15
   1. Non-Modifiable Risk Factors 15
      a) Age.....15
      b) Gender 16
      c) Ethnicity and heredity.....16
      d) Prior personal history of stroke or TIA 17
   2. Major Risk Factors which are Amenable to Modification 17
      a) Hypertension.....17
      b) Diabetes mellitus 17
      c) Blood lipids.....18
      d) Smoking 18
   3. Other Likely Risk Factors which are Amenable to Modification 19
      a) Diet and nutrition.....19
      b) Physical activity 19
      c) Obesity.....20

Contents (Cont.)

Page

F. Diagnosis of Acute Ischemic Stroke....20
1. History....20
2. Clinical Presentation....21
3. Physical and Neurological Examination....23
4. Investigations....23
a) Imaging....23
i) Computed axial tomography....24
ii) Magnetic resonance imaging....25
b) Tests that view the blood vessels supplying the brain....25
i) Carotid ultrasound....25
ii) Cerebral angiography....25
iii) Computed tomographic angiography....26
iv) Magnetic resonance angiography....26
v) Transcranial Doppler....26
c) Test for the underlying etiology....27
d) Diagnostic laboratory markers of stroke....27
i) C-Reactive protein....28
ii) Fibrinogen....29
iii) Cell adhesion molecules....30
iv) Matrix metalloproteinase-9....30
v) Oxygen free radicals....32
vi) Fatty acid binding protein in Stroke....33
vii) Polyamine oxidase and acrolein....34
viii) PARK7 and nucleoside diphosphate kinase A....34
ix) Circulating plasma nucleic acid....35
x) Myelin basic protein....36
xi) Protein S100-B subunit....37
xii) Glial fibrillary acidic protein....38
xiii) Soluble CD40 ligand....39
xiv) CD40 ligand....40
xv) Monocyte chemoattractant protein-1....40
G. Prognosis of Acute Ischemic Stroke....41
H. Treatment of Acute Ischemic Stroke....42
1. Thrombolysis....43
2. Mechanical Thrombectomy....44
3. Conventional Medications with Antiinflammatory Effects....45
a) Statins....45