

Urinary Biomarkers in lupus Nephritis

Thesis
Submitted for Partial Fulfillment of the M.D. in Internal Medicine
By

AlKhatib Kadry Moustafa AlKemary

Assistant lecturer of Internal Medicine

Cairo University

Supervised by

Dr. Dawlat M. Abdel Hamid Belal

Professor of Internal Medicine & Nephrology

Cairo University

Dr. Mohammed Hamdi El-Akkad

Professor of Internal Medicine
Cairo University

Dr. Mohamed El-Khatib

Assist. Professor of Internal Medicine&Nephrology

Cairo University

Dr. Amal Rashad El- Shahabe

Assist. Professor of Biochemistry

Cairo University

Faculty of Medicine Cairo University 2011

ABSTRACT

This work aimed to study whether urinary tumor necrosis factor (TNF)-like weak inducer of apoptosis (uTWEAK) level has a predictive and/or diagnostic value in lupus nephritis (LN).

73 patients with systemic lupus erythematosus (SLE) fulfilling the 1997 revised criteria for the classification of SLE and 23 age/ sexmatched healthy control included in this study were subjected to full history taking, clinical examination, routine laboratory investigations as well as measurement of uTWEAK level.

uTWEAK levels were significantly higher in SLE patients with active LN compared to those without or with inactive renal disease and normal healthy subjects

Keywords: - Systemic lupus erythematosus - Lupus nephritis

- Urinary TNF-like weak inducer of apoptosis (uTWEAK)

<u>ACKNOWLEDGEMENTS</u>

I would like to express my deepest gratitude and thankfulness; first to Allah for giving me the will and strength to fulfill this work then to my mother, father and my wife for their continuous support, endless help and encouragement.

I would like to express my deepest appreciation to **Dr. Dawlat M.**Abdel Hamid Belal, Professor of Internal Medicine & Nephrology,

Cairo University for her keen interest in the progress of this work.

Special thanks to **Dr. Mohammed Hamdi El-Akkad,** Professor of Internal Medicine, Cairo University, for his great assistance and precious suggestions.

Sincere thanks to **Dr. Mohamed El-Khatib**, assist. Professor of Internal Medicine & Nephrology, Cairo University, for his great patience in reading and revising the manuscripts, and his great help and valuable ideas throughout the work.

No words can express my gratitude to **Dr. Amal Rashad El-Shahabe**, Assist. Professor of Biochemistry, Cairo University for her great assistance. She was very generous in providing me with her knowledge and scientific materials.

Last but not least, it gives me a great pleasure to thank all my family members and my patients for their assistance and faithful encouragement.

CONTENTS

	Page
LIST OF ABBREVIATIONS	
LIST OF FIGURES	
LIST OF TABLES	
■ INTRODUCTION AND AIM OF THE WORK	
■ REVIEW OF LITERATURE	1
Chapter 1: Pathogenesis Of Lupus Nephritis	1
- Immune complex formation	3
- Anti-C1q and anti-α-actinin antibodies: relevance in lupus nephritis	5
- Cytokines and their roles in the pathogenesis of lupus nephritis	9
Chapter 2: Pathology Of Lupus Nephritis	23
- Renal pathologic changes	23
- ISN/RPS classification	28
- Histopathological classifications and prognostic value	34
- Other types of renal diseases	35
- Antiphospholipid syndrome nephropathy	39
- Silent Lupus Nephritis	40
Chapter 3: Diagnosis Of Lupus Nephritis	41
-Criteria for diagnosis of lupus nephritis	42
-Clinical Presentation of Nephritis	42
-Urinary investigations	45
-Serological investigations	48
-Tests for monitoring patients with lupus nephritis	49
-Renal biopsy	51
-Lupus disease activity and damage instruments	61
Chapter 4: Biomarkers In Lupus Nephritis	63
-Introduction	63
-Serum biomarkers in lupus nephritis	66
-Urinary biomarkers in lupus nephritis	68
Chapter 5: Tumor necrosis factor-like weak inducer of apoptosis (TWEAK)	79
-Introduction	79
- Structure	79
-Function	84
-TWEAK is a multifunctional cytokine	85

CONTENTS

-Physiological role of TWEAK	88
- Pathogenic role of TWEAK	90
-The role of tweak in the pathogenesis of lupus nephritis	97
- TWEAK/Fn14 interactions and cancer	101
Chapter 6: Treatment Of Lupus Nephritis	105
-General approach	105
-Medications	105
-Therapies for specific types of LN based on renal pathology	106
-Experimental drugs	107
-End-stage renal disease	112
-Prognosis	112
■ SUBJECTS AND METHODS	114
■ RESULTS	126
■ DISCUSSION	140
■ SUMMARY AND CONCLUSIONS	146
■ RECOMMENDATIONS	149
■ REFERENCES	150
■ ARABIC SUMMARY	

LIST OF ABBREVIATIONS

aa	Amino acid
ACR	American College of Rheumatology
Ag-Ab	Antigen-antibody
AGP	Alpha-1-acid-glycoprotein
AKI	Acute kidney injury
ALT	Alanine transaminases
ANA	Antinuclear antibody
ANCA	Antineutrophil cytoplasmic autoantibodies
APCs	antigen-presenting cells
aPL	Antiphospholipid antibodies
APRIL	A proliferation-inducing ligand
APS	antiphospholipid syndrome
AST	Aspartate transaminases
BAFF-R	B-cell activating factor receptor
ВСМА	B-cell maturation protein
BILAG	The British Isles Lupus Assessment Group
BLys	B lymphocyte stimulator
BUN	Blood urea nitrogen
С	Complement
СВС	Complete blood count
CD	Cluster of differentiation
cGVH	Chronic graft-versus- host
CKD	Chronic kidney disease

CNS	Central nervous system
СР	Ceruloplasmin
СрG	Cytosine-phosphateguanine
CTLA-4	cytotoxic T-lymphocyte antigen 4
CVA	Cerebrovascular accident
CXCL	C-X-C chemokine ligand
DCs	dendritic cells
DD	Death domain
ds DNA	Double stranded deoxyriboneuclic acid
DTPA	diethylene triamine pentaacetic acid
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immunsorbant Assay
EM	Electron microscopy
ESR	Erythrocyte sedimentation rate
ESRD	End-stage renal disease
FasL	Fas ligand
Fc	The constant fragment of antibody
FeET-1	fractional excretion of ET-1
Fn14	Fibroblast growth factor-inducible 14
FOXP3	Forkhead family transcription factor 3
g	gram
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GN	Glomerulonephritis

gp	Glycoproteins
НВ	Hemoglobin
HCQ	Hydroxychloroquine
Hp-Hb	Haptoglobin-haemoglobin
HUS	Hemolytic Uremic Syndrome
IC	Immune complex
ICAM 1	Intercellular adhesion molecule 1
IF	Immunofluorescence
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
IP-10	Interferon-γ-inducible protein
ISN	International Society of Nephrology
ΙκΒs	Inhibitory kappa B subunits
JAK1	Janus kinase-1
kD	KiloDalton
LM	Light microscopy
LN	Lupus nephritis
LPGDS	Lipocalin-type prostaglandin D-synthetase
m TWEAK	membrane-bound TWEAK
МАРК	Mitogen-activated protein kinase
MCSF	Macrophage colony stimulating factor
MCSF	Macrophage colony stimulating factor
mg	Milligram

MIF	macrophage migration inhibitor factor
mm	Millimeter
MMPs	matrix metalloproteinases
MPO	myeloper-oxidase
mRNA	Messenger ribonucleic acid protein
MS	multiple sclerosis
NFĸB	nuclear factor kappa light chain enhancer of activated B cells
NGAL	Neutrophil gelatinase-associated lipocalin
NK	natural killer
NSAIDs	Non-steroidal anti-inflammatory drugs
OPG	Osteoprotegerin
PAH	Para-aminohippurate
PAMPs	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PDC	Plasmacytoid dendritic cells
PDGF	Platelet derived growth factor
PR3	Proteinase 3
R	Receptor
RA	Rheumatoid arthritis
RANTES	Regulated upon Activation, Normal T cell Expressed, and Secreted
RBCs	Red blood cells
RIG-I	retinoic acid inducible gene I
ROC	Receiver Operating Characteristic

RPGN	rapidly progressive glomerulonephritis
RPS	the Renal Pathology Society
rSLEDAI	renal Systemic Lupus Erythematosus Disease Activity Index
S.D.	Standard deviation
SELDI-TOF MS	surface-enhanced laser desorption/ionization time-of- flight mass spectrometry
SLAM	the Systemic Lupus Activity Measure
SLE	Systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index,
SLICC/ACR	The Systemic Lupus International Collaborating Clinics/ American College of Rheumatology
sTWEAK	soluble TWEAK
TACI	transmembrane activator and calcium modulating cyclophilin ligand interactor
TCR	T cell receptor
TF	transferrin
TGF-β	Transforming growth factor-beta
Th	T-helper
TLC	Total leucocytic count
TLRs	Toll-like receptors
TMA	Thrombotic microangiopathy
TNFRSF	Tumor necrosis factor receptor superfamily
TNF-α	Tumor necrosis factor-α
TRAF	TNFR-associated factor
TRAILR	TNF-related apoptosis-inducing ligand receptor

Tregs	regulatory T cells
tSLEDAI	total Systemic Lupus Erythematosus Disease Activity Index
TTP	Thrombotic Thrombocytopenic Purpura
TWEAK	Tumor necrosis factor-like weak inducer of apoptosis
u-MCP-1	urinary monocyte chemoattractant protein-1
VCAM- 1	Vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
WBC	White blood cells
WHO	World health organization

LIST OF FIGURES

		Page
Fig.(I)	A simplified scheme of binding of anti-C1q antibodies to molecules of C1q	6
Fig.(2)	Schematic diagram to show the complex interaction between various immune cells and cytokines which lead to the pathogenesis of SLE	18
Fig.(3)	Schematic representation of the location of ICs in glomeruli	26
Fig.(4)	Mesangial GN	31
Fig.(5)	Diffuse proliferative GN	31
Fig.(6)	Membranous GN.	31
Fig.(7)	The incidence of the different forms of LN	34
Fig.(8)	Structure of TWEAK and Fn14	80
Fig.(9)	TWEAK actions regulation	82
Fig.(10)	Tweak receptors	83
Fig.(11)	TWEAK is a multifunctional cytokine	85
Fig.(12)	Model for the role of TWEAK/Fn14 pathway in health and disease	86
Fig.(13)	Model of cellular and molecular mechanisms underlying the physiological and pathological roles of TWEAK	89
Fig.(14)	TWEAK the facilitator	92
Fig.(15)	Inflammation and parenchymal cell death are key features of renal injury	93
Fig.(16)	Therapeutic approaches to manipulating the TWEAK/Fn14 pathway to treat cancer	102
Fig.(17)	Functions of TWEAK and TNF	104
Fig.(18)	Biological agents	109
Fig.(19)	Therapeutic targets in SLE	110

Fig.(20)	Comparison between tSLEDAI and rSLEDAI in group I and group II	129
Fig.(21)	Comparison between urine proteins, and serum creatinine in group I and group II	130
Fig.(22)	Comparison between C3 and C4 in group I and group II	130
Fig.(23)	Comparison between TWEAK of all SLE patients included in the study	131
Fig.(24)	22a, showing the positive correlations between urinary TWEAK and renal disease activity index	134
	22b showing the negative correlation between urinary TWEAK levels in SLE patients and renal biopsy grading	
	22c, 22d showing the negative correlation between TWEAK levels vs. C3 and C4	
Fig.(25)	Roc curve of TWEAK of SLE patients with nephritis (1) versus no nephritis (0)	135

LIST OF TABLES

		Page
Table (1)	Important cytokines in SLE	19
Table (2)	The 1995 WHO classification for LN	24
Table (3)	Integration of LM, IF, and EM Findings by WHO Class	26
Table (4)	ISN/RPS 2003 classification of LN	29
Table (5)	The definition for diagnostic terms	30
Table (6)	Renal biopsy activity and chronicity indexes	33
Table (7)	Proposed features of APS nephropathy	39
Table (8)	Clinical characteristics of various forms of lupus nephritis	44
Table (9)	Suggested indications for performance of a kidney biopsy in lupus nephritis	54
Table (10)	Considerations regarding renal biopsy in lupus nephritis	59
Table (11)	SLE Disease Activity Index domains	123
Table (12)	Demographic characteristics of studied SLE patients	126
Table (13)	Clinical variables of group I	127
Table (14)	Laboratory variables of group I	128
Table (15)	Clinical variables of group II	129
Table (16)	Laboratory variables of group II	131
Table (17)	Comparison between clinical variables in group I and group II	132
Table (18)	Comparison between laboratory variables in group I and Group II	133
Table (19)	Comparison between mean uTWEAK levels in group I, group II and group III	135
Table (20)	Correlation between uTWEAK and (demographic, clinical, laboratory and treatment) data of SLE patients with nephritis	136- 137