

# **Urinary Neutrophil Gelatinase Associated Lipocalin as a Novel Biomarker of Disease Activity in Lupus Nephritis**

*Thesis*

Submitted for Partial Fulfillment of Master Degree in Internal Medicine

*BY*

**Michael Atef Napoleon**

M.B.B.Ch.

Faculty of Medicine - Tanta University.

*under the Supervision of*

**Dr. Dalia Fayez Mohamed**

Professor of Internal Medicine and Rheumatology

Faculty of Medicine - Ain Shams University

**Dr. Howaida El Sayed Mansour**

Professor of Internal Medicine and Rheumatology

Faculty of Medicine - Ain Shams University

**Dr. Sherin Mohamed Hosny Hamza**

Assistant Professor of Internal Medicine and Rheumatology

Faculty of Medicine - Ain Shams University

**Cairo**

**2012**

---

# Acknowledgement

Thanks to **ALLAH** for the strength and insistence that I was given during the achievement of this work.

I find no words by which I express my thankfulness, gratitude and appreciation to ***Prof. Dr. Dalia Fayez Mohamed*** Professor of Internal Medicine and Rheumatology, Faculty of Medicine Ain Shams University, for her support, guidance, advice, generous help and encouragement during supervising this study.

I would like to express my extreme appreciation and thanks to ***Prof. Dr. Howaida Elsayed Mansour*** Professor of Internal Medicine and Rheumatology, Faculty of Medicine Ain Shams University, for her great support and valuable unlimited help and supervision throughout the study.

Also I want to thank ***Dr. Sherin Mohamed Hosny Hamza***, Assistant Professor of Internal Medicine and Rheumatology for her guidance, precious advice and continuous support, in every step in this study.

---

# Contents

Title	Page
• List of Tables .....	III
• List of Figures .....	V
• List of Abbreviations .....	VII
• Introduction and Aim of the Study .....	1
• Review of literature	
○ Systemic Lupus Erythematosus .....	4
○ Lupus nephritis .....	66
○ Neutrophil Gelatinase associated Lipocalin (uNGAL).....	106
• Subjects and Methods .....	132
• Results .....	140
• Discussion .....	158
• Summary .....	167
• Conclusion & Recommendations.....	170
• References .....	171
• Arabic Summary	

---

# List of Tables

## Tables of Review

<b>Table No.</b>	<b>Subject</b>	<b>Page</b>
<b>Table (1):</b>	Frequency of various manifestations of SLE at disease onset and during the disease course.....	11
<b>Table (2):</b>	Neuropsychiatric Syndromes of SLE.....	25
<b>Table (3):</b>	Systemic lupus erythematosus hepatitis versus autoimmune hepatitis.....	30
<b>Table (4):</b>	American College of Rheumatology Criteria for the Classification of SLE.....	35
<b>Table (5):</b>	Systemic Lupus Activity Measure (SLAM).....	37
<b>Table (6):</b>	Autoantibodies and Clinical Feature.....	44
<b>Table (7):</b>	Imaging Findings in SLE.....	49
<b>Table (8):</b>	Recommended monitoring of cytotoxic drug therapy in systemic lupus erythematosus.....	65
<b>Table (9):</b>	WHO Classification of lupus nephritis.....	73
<b>Table (10):</b>	International Society of Nephrology/Renal Pathology Society 2003 classification of LN.....	73
<b>Table (11):</b>	Activity and chronicity indices in lupus nephritis (National Institutes of Health “NIH”).....	90

---

## Tables of Results

<b>Table No.</b>	<b>Subject</b>	<b>Page</b>
<b>Table (A):</b>	The distribution of demographic data among SLE patients.....	142
<b>Table (B):</b>	Descriptive analysis of the commonest clinical findings among the SLE patients.....	142
<b>Table (C):</b>	Disease activity as measured by SLAM score among the studied SLE patients.....	143
<b>Table (D):</b>	Distribution of LN cases as regard disease activity with SLAM score and their percentage of SLE cases with the mean uNGAL values of each category.....	144
<b>Table (E):</b>	Descriptive analysis of some laboratory data among the studied SLE patients.....	145
<b>Table (F):</b>	Descriptive analysis of renal functions among the studied SLE group.....	146
<b>Table (G):</b>	Comparison between patients and controls as regard some demographic data.....	147
<b>Table (H):</b>	Comparison between patients and controls as regard renal functions.....	147
<b>Table (I):</b>	Comparison between all the SLE patients and controls as regards the level of uNGAL.....	148
<b>Table (J):</b>	Comparison between the SLE patients without lupus nephritis and controls as regards the level of uNGAL.....	148
<b>Table (K):</b>	Comparison between patients with lupus nephritis and controls as regards the level of uNGAL.....	149
<b>Table (L):</b>	Comparison between SLE patients with and without lupus nephritis as regards the level of uNGAL.....	149

---

<b>Table (M):</b>	Comparison between various SLE disease activity scores & the level of uNGAL by One-Way ANOVA test.....	150
<b>Table (N):</b>	Comparison between SLE patients with and without major organ affection as regard the level of uNGAL.....	151
<b>Table (O):</b>	Correlation between uNGAL level and different clinical and laboratory parameters among the studied SLE patients.....	152
<b>Table (P):</b>	Relation between lupus nephritis versus different risk factors among SLE patients using likelihood ratio.....	156
<b>Table (Q):</b>	ROC Curve to assess the cutoff value, sensitivity, specificity and predictive values of uNGAL in detection lupus nephritis.....	156

---

# List of Figures

## Figures of Review

Figure No.	Subject	Page
Figure (1):	Localized acute cutaneous lupus erythematosus (malar rash).....	13
Figure (2):	Subacute cutaneous lupus lesions.....	14
Figure (3):	Jaccoud's arthropathy.....	22
Figure (4):	Reflux esophagitis in a patient with SLE.....	48
Figure (5):	Interstitial lupus nephritis.....	71
Figure (6):	Mesangial nephropathy (WHO Class II).....	74
Figure (7):	Membranoproliferative pattern of glomerular injury by LM and IF.....	74
Figure (8):	Membranous pattern with minimal mesangial proliferation (class V pattern).....	75
Figure (9):	Ultrastructural appearance of subepithelial deposits.....	75
Figure (10):	Urine sediment findings in LN.....	80
Figure (11):	Class III/IV induction therapy.....	96
Figure (12):	Treatment of class V LN.....	102
Figure (13):	Structure of the lipocalin fold.....	108
Figure (14):	Schematic model of NGAL-mediated iron traffic.....	113
Figure (15):	The cellular role of neutrophil gelatinase-associated lipocalin.....	115

---

## Figures of Results

<b>Figure No.</b>	<b>Subject</b>	<b>Page</b>
<b>Figure (A):</b>	The percentage of clinical manifestations among the SLE patients.....	143
<b>Figure (B):</b>	SLE disease activity as measured by SLAM score.....	143
<b>Figure (C):</b>	Number of SLE cases in mild to moderate and severe disease activity with SLAM score and number of LN cases in each category.....	144
<b>Figure (D):</b>	Mean uNGAL levels in LN cases with mild to moderate and sever disease activity by SLAM score.....	145
<b>Figure (E):</b>	Comparison between patients and controls as regard renal functions.....	148
<b>Figure (F):</b>	Comparison between controls, all SLE patients, SLE patients without lupus nephritis and lupus nephritis patients as regard the mean level of uNGAL.....	150
<b>Figure (G):</b>	Comparison between SLE patients (mild to moderate and severe disease activity by SLAM score) as regards uNGAL level.....	151
<b>Figure (H):</b>	Comparison between SLE patients with and without nephritis as regards the level of uNGAL.....	152
<b>Figure (I):</b>	Correlation between uNGAL levels and different parameters including BUN, urinary WBCs, protein/creatinine ratio, SLAM score and activity index.....	155
<b>Figure (J):</b>	Receiver Operator Characteristic (ROC) curve for the sensitivity and specificity of uNGAL in detecting lupus nephritis.....	157



---

# List of Abbreviations

° C	Degree Centigrad
° F	Degree Fahrenheit
μ	Micro
APS	Antiphospholipid Antibody Syndrome
AbTPO	Antithyroid Peroxidase Antibody
ACE	Angiotensin-Converting Enzyme
ACR	American College Of Rheumatology
AHA	Autoimmune Hemolytic Anemia
AHF	Acute Heart Failure
AKI	Acute Kidney Injury
AMI	Acute Myocardial Infarction
anti-dsDNA	Anti-Double Stranded Dna Antibody
APC	Antigen Presenting Cell
ARBs	Angiotensin Receptor Blockers
AVN	Avascular Necrosis
AZA	Azathioprine
BAL	Bronchio Alveolar Lavage
BILAG	The British Isles Lupus Assessment Group Index
BLyS	B-Lymphocyte Stimulator
BSA	Body Surface Area
CBC	Complete Blood Count
CHF	Chronic Heart Failure
CLE	Cutaneous Lupus Erythematosus
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
COX	Cyclooxygenase
CPK	Creatine Phosphokinase

---

CQ	Chloroquine Phosphate
CR1, 2	Complement Receptors 1, 2
CR3	Complement Receptor 3
CRP	C Reactive Protein
CS	Corticosteroids
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CVD	Cardio-Vascular Disease
CYC	Cyclophosphamide
DHEA	Dehydroepiandrosterone
DIL	Drug-Induced Lupus
DLE	Discoid Lupus Erythematosus
DNA	Deoxyribonucleic Acid
dsDNA	Double-Stranded DNA
DVT	Deep Venous Thrombosis
E2	Estradiol
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
ECLAM	The European Consensus Lupus Activity Measure
EEG	Electroencephelograms
ELISA	Enzyme-Linked Immunoabsorbent Assay
EM	Electron Microscopy
EMG	Electromyography
ESR	Erythrocyte Sedimentation Rate
ESRD	End-Stage Renal Disease
FA-Kinase	Focal Adhesion Kinase
FcγRIIA	Fcγ Receptor Type IIA
FDA	Food And Drug Administration
G6PD	Glucose-6-Phosphate Dehydrogenase

---

GBM	Glomerular Basement Membranes
GERD	Gastroesophageal Reflux Disease
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
HCQ	Hydroxychloroquine
HIF-1 $\alpha$	Hypoxia-Inducible Factor 1, Alpha Subunit
HLA	Human Leukocyte Antigen
HPA	Hypothalamic-Pituitary-Adrenal
hpf	High Power Field
Hsp90	Heat Shock Protein 90
IF	Immunofluorescence
IFN- $\gamma$	Interferon- $\gamma$
IIM	Idiopathic Inflammatory Myositis
IL	Interlukins
IP-10	Interferon-Producing Protein 10
ISN	International Society Of Nephrology
ITP	Immune Thrombocytopenic Purpura
IVC	Intravenous Cyclophosphamide
IVIG	Intravenous Immunoglobulins
kDa	Kilo Dalton
kg	Kilogram
L	Litre
LAI	The Lupus Activity Index
Lcn-2	lipocalin 2
LKM	Liver Kidney Microsome
LM	Light Microscopy
LN	Lupus Nephritis
LSP	Liver-Specific Lipoprotein
LV	Left Ventricle

---

MAC	Membrane Attack Complex
MBP	Mannose-Binding Protein
MCP	Metacarpophalangeal
MCP-1	Monocyte Chemoattractant Protein -1
MDCK cells	Madin Darby Canine Kidney Cells
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
MMP-9	Matrix Metalloproteinase 9
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NF- $\kappa$ B	Nuclear factor-kappa B
NIH	National Institutes Of Health
NMDA	N-Methyl-D-Aspartic Acid
NP-SLE	Neuropsychiatric Systemic Lupus Erythematosus
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
P/C ratio	Urine Protein to Creatinine Ratio
PBC	Primary Biliary Cirrhosis
PCNA	Proliferating Cell Nuclear Antigen
PET	Positron Emission Tomography
PIP	Proximal Interphalangeal
PLN	Proliferative Lupus Nephritis
PML	Progressive Multifocal Leukoencephalopathy
RBCs	Red Blood Cells
rIFN- $\gamma$	Recombinant Interferon- $\Gamma$
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
ROS	Reactive Oxygen Species
RPM	Round Per Minute
RPS	Renal Pathology Society

---

SCLE	Subacute Cutaneous Lupus Erythematosus
SLAM	Systemic Lupus Activity Measure
SLE	Systemic Lupus Erythematosus
SLEDAI	The Systemic Lupus Erythematosus Disease Activity Index
SMA	Smooth Muscle Antibody
SNPs	Single-Nucleotide Polymorphisms
SPF	Skin Protection Factor
TACI-Ig	Transmembrane Activator and Calcium-Modulator and An Immunoglobulin Chain
TGF- $\beta$	Tumor Growth Factor B
TNF	Tumor Necrosis Factor
TTP	Thrombocytopenic Purpura
uNGAL	Urinary Neutrophil Gelatinase-Associated Lipocalin
UVR	Ultraviolet Radiation
VCAM-1	Vascular Cell Adhesion Protein 1
WBCs	White Blood Cells
WHO	World Health Organization

# **Introduction & Aim of the Study**

Systemic lupus erythematosus (SLE) is a prototype of autoimmune diseases affecting predominantly women. It is characterized by dysregulation of self-reactive B cells leading to autoantibody production against own antigens, immune complex deposition and subsequent complement activation with tissue damage (*Rojas-Villarraga et al., 2010*).

SLE affects multiple organ systems including kidneys, skin, lung, heart, the hematopoietic system and brain. One of the most severe complications of SLE that is associated with significant morbidity and mortality is lupus nephritis, it may lead to persistent proteinuria, chronic renal failure and end stage renal disease (*Bagavant and Fu, 2009*).

Lupus nephritis (LN) occurs in up to 50% of SLE patients. Severe LN has been reported to result in end stage kidney disease at a rate of 10-26%, which may be a result of the difficulty in recognizing a flare early enough to affect the course of the disease

since prompt diagnosis and early treatment lead to better outcomes (*Rubinstein et al., 2010*).

Biomarkers provide the potential to non invasively evaluate and help to manage patients with lupus nephritis. Many candidates have been identified, but they require validation in larger cohorts. It is likely that combinations of biomarker profiles, rather than individual markers, will emerge to help better prediction of the severity of inflammation, the extent of fibrosis, the degree of drug responsiveness, and other variables (*Manoharan and Madaio, 2010*).

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa iron-transporting protein in secondary granules of neutrophils. NGAL is secreted more readily from neutrophils than any other proteins and is considered as a special biomarker of neutrophil degranulation (*Chen et al., 2009*).

NGAL is rapidly accumulating in the kidney tubules and urine after nephrotoxic and ischemic insults so it has been put forward as an early, sensitive, non-invasive biomarker for acute kidney injury (AKI) (*Makris et al., 2009*).