THE VALUE OF LIPOCALIN-2 IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Thesis

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List of Abbreviations

Abb.	Full Term
ACR	American College of Rheumatology
ANA	Anti nuclear antibody
Anti-Sm	Anti Smith antibody
APCs	Antigen presenting cells
AUC	Area Under the Curve
BUN	Blood urea nitrogen
C	Complement
CBC	Complete blood picture
CD	Cluster of differentiation
ds-DNA	Double stranded deoxyribonucleic acid
EBV	Ebstein Barr virus
ELISA	Enzyme linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
ESRD	End stage renal disease
Fc	Fraction of crystallizable
GFR	Glomerular filtration rate
Hb	Heamoglobin
HLA	Human leucocytic antigen
IFN	Interferone
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LN	Lupus nephritis
MMF	Mycofenolate mofitel

Abb.	*Full Term*
MMP	Matrix metallopeptidase
NGAL	Neutrophil gelatinase associated lipocalin-2
NK	Natural killer
NO	Nitric axide
p value	Probability value
R	Pearson correlation coefficient
RBC	Red blood cell
RNA	Ribonucleic acind
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
SD	Standard deviation
SLEDAI	Systemic lupus erythematosus disease activity index
SLICC/ACR-DI	Systemic lupus International Collaborating clinics/ American College of Rheumatology damage index.
TLR	Toll like receptor
TNF	Tumor necrosis factor
Upro/crea	Urinary protein/ creatinine ratio
UV	Ultra violet
WBCs	White blood cells
WHO	World Health Organization

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ABSTRACT

Objective: the aim of this study is to determine the role of urinary and serum lipocalin-2 as a biomarker in the systemic lupus erythematosus (SLE).

Methods: thirty patients with SLE were divided and selected into fifteen patients with lupus nephritis (LN), and fifiteen SLE patients without LN.All patients were subjected to full history taking, full examination, complete urine analysis and detection of all the laboratory and radiological examination needed for SLE diagnosis. SLE Disease Activity Index (SLEDAI) score were evaluated to all SLE patients.Results were matched with fifiteen control participents with normal urine analysis, all were age and sex matched.

Results: Urinary lipocalin-2 showed highly significant statisical difference in comparing all SLE patients and the controls (*p<0.001).*The LN group showed higher mean levels of urinary lipocalin-2 than the compared groups (*p<0.001)*. Urinary lipocalin-2 correlated significantly with blood urea nitrogen, serun creatinine, 24/hr urin protien , total SLEDAI and the renal SLEDAI. No correlation was found between urinary lipocalin-2 and the extra-renal SLEDAI, disease duration, anti-dsDNA, C3 and C4. The levels of serum lipocalin-2 did not show significant statistical difference between all SLE patients and the control, and also no significant statistical difference between (LN) group, and the non-LN group. Also no correlation was found as regards the urinary and the serum lipocalin-2.

Conclusion: Urinary lipocalin-2 could be used as a biomarker of SLE disease activity. Serum lipocalin-2 did not prove its usage in the assessement of renal involvement in SLE.

Key Words: Systemic Lupus Erythematosus (SLE), Lupus nephritis (LN), Urinary and serum lipocalin-2.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune multisystem disease predominantly affecting women in the childbearing period **(Shankar and Behera 2014)**. The majority of the pathology in SLE is related to deposits of immune complexes in various organs, which triggers complement and other mediators of inflammation **(Cojocaru et al., 2011).**

SLE is characterized by a very large spectrum of clinical manifestations accompanied by prototypic abnormalities of the immune system. While recent advances in therapeutic approaches have taken place, SLE still has a profound impact on the quality of life and life expectancy of affected persons **(Chizzolini et al., 2009).**

Renal involvement occurs in 40–70% of all SLE patients and is a major cause of morbidity and hospital admissions **(Bertsias et al., 2012).** Its clinical presentations are highly variable, ranging from mild asymptomatic proteinuria and/or hematuria to rapidly progressive uremia. Early diagnosis and prompt treatment may dramatically modify the course of renal disease and improve the long term survival **(Molino et al., 2009).**Approximately 10 to 30 % of patients with lupus nephritis progress to end-stage renal disease (ESRD) **(Bomback and Gerald, 2013).**

The accepted routine measures of assessing patients with SLE includes acute phase markers, erythrocyte sedimentation rate (ESR), C-reactive protein, plasma/serum complement component 3 (C3) and component 4 (C4) and presence of antibodies to double-stranded DNA (anti-dsDNA) **(Elwy et al., 2010)**.Such markers help in a variety of ways, including early detection of flare, distinction between flare and chronic damage and monitoring response to therapy. However, improved new markers are required to assist clinicians in diagnosis of lupus patients. Among these new markers, lipocalin-2 is a promising one **(Adhya et al., 2011).**

The lipocalin protein family is a large group of mostly secreted soluble proteins that carrying small molecules to specific cells. Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin (NGAL) is expressed in neutrophils and in low levels in the kidneys **(Sharifipour et al., 2013)**.

NGAL has been evaluated as an early biomarker of acute kidney injury after cardiopulmonary bypass and kidney transplantation **(Abdallah E et al., 2013)**. It is also a candidate biomarker for chronic kidney diseases, such as immunoglobulin a nephropathy, membranous and membranoproliferative glomerulonephritis **(Yang et al., 2012)**.Based on these findings, Lipocalin-2 may be a potential biomarker for renal damage/ inflammation in lupus nephritis.

AIM OF THE WORK

The aim of the present study is to assess serum and urinary lipocalin- 2 level in patients with Systemic lupus erythematosus (SLE) and its correlation to disease activity and lupus nephritis (LN).

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is systemic autoimmune disease characterized by diverse multisystem involvement and the production of an array of autoantibodies. Clinical features in individual patients can be quite variable, ranging from mild joint and skin involvement to severe life-threatening internal organ disease **(Dall'Era and Wofsy, 2013).**

Systemic lupus erythematosus (SLE) preferentially affects the vasculature, joints, skin, hematopoietic system, kidneys, brain and serosal surfaces **(Rahman & Isenberg, 2008).**It predominantly targeting young women in their childbearing years and with the potential to cause significant physical disfigurement, morbidity and occasionally mortality **(Crow, 2013).**

Although the specific cause of SLE is unknown, multiple factors are associated with the development of the disease, including genetic, racial, and environmental factors. Immune complexes form in the microvasculature, leads to complement activation and inflammation. Moreover, antibody-antigen complexes deposit on the basement membranes of skin and kidneys. In active SLE, this process has been confirmed based on the presence of complexes of nuclear antigens such as DNA,

immunoglobulins, and complement proteins at these sites. Serum antinuclear antibodies (ANAs) are found in virtually all individuals with active SLE, and antibodies to native double-stranded DNA (dsDNA) are relatively specific for the diagnosis of SLE **(Rahman and Isenberg, 2008)**.

Sociodemographic factors such as sex and race play an important role in the incidence of the disease, frequency of its manifestations, and therapeutic response **(Salgado and Herrera-Diaz, 2012).**

Pathogenesis of SLE:

The pathogenesis of systemic lupus erythematosus (SLE) is complex.Target tissue damage is caused primarily by pathogenic autoantibodies and immune complexes. The immunologic abnormalities occur in a framework of interactions between multiple susceptibility genes, gender influences; and environmental stimuli, at least one of which ultraviolet [UV] light can induce apoptosis in dermal cells that results in presentation of RNA protein, DNA protein, and phospholipid self-antigens to the immune system **(Hahn and Tsao, 2008), figure (1)**.

Although genetic, environmental, and sex hormonal factors have been implicated in the pathogenesis of SLE, it is known that several cytokines, nitric oxide (NO), free radicals, a

deranged immune system, deficient antioxidant defenses, and toll-like receptors have a significant role both in the initiation and perpetuation of the inflammatory process observed **(Das, 2010).**Once the inflammatory process is triggered, this leads to the production of a variety of proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor-α (TNF-α), interferons (IFNs), macrophage migration inhibitory factor (MIF), HMGB-1 (high-mobility group B1), and possibly a reduction in the elaboration of anti-inflammatory cytokines such as IL-10, IL-4, and transforming growth factor-β (TGF-β) **(Das, 2010).**

This imbalance between the pro- and anti-inflammatory cytokines coupled with increased secretion of free radicals such as superoxide anion (O_2-), hydrogen peroxide ($H2O_2$), inducible nitric oxide (iNO), and other reactive oxygen species (ROS) by activated monocytes, macrophages, polymorphonuclear leukocytes (PMNL), T-cells, Kupffer cells, glial cells in the brain, and other organ-specific reticuloendothelial cells would ultimately cause target tissue/organ damage seen in SLE **(Das, 2010).**