ROLE OF URINARY MONOCYTE CHEMOATTRACTANT PROTEIN-1 AS A BIOMARKER FOR LUPUS NEPHRITIS ACTIVITY

Thesis

Submitted for Fulfillment of the Master Degree In Internal Medicine

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Dedication

I Dedicate This Work To

The soul of my beloved father

My mother for her endless love, care & support

through all my life stages

My sisters for their help & love.

My family

My supervisors

And to my lovely friends

Abstract

Objective: Renal biopsy is the “gold standard” to determine renal activity in systemic lupus erythematosus (SLE), but it is expensive, invasive, and carries risk. Monocyte chemoattractant protein-1 (MCP-1), a chemotactic cytokine involved in the progression of glomerular and tubulointerstitial injury. We investigated urinary MCP-1(u MCP-1) as potential biomarker for lupus nephritis.

Patients and methods: In 73 SLE patients, and in 23 healthy volunteers, urinary levels of MCP-1 were measured. Disease activity was assessed by total SLE disease activity index (tSLEDAI), and renal activity by renal SLE disease activity index (rSLEDAI), and both were correlated with uMCP-1. Sensitivity, specificity, and predictive values of MCP-1 to predict lupus nephritis (LN) were also calculated.

Results: Significantly higher levels of uMCP-1 were observed in SLE patients with LN compared with those without LN, (MCP-1 $P<0.001$). Other significantly higher levels were observed in SLE patients with LN compared to control subjects (MCP-1, $P<0.001$). Positive correlations were observed between rSLEDAI and MCP-1 ($r=0.635$, $p<0.001$).

Conclusion: The lack of availability of urine biomarkers has impeded development of new therapies for LN. Urinary levels of MCP-1 positively correlate with renal involvement as assessed by rSLEDAI with reasonable sensitivity, specificity and predictive values to detect LN.

Keywords:

(Biomarkers - Lupus Nephritis - Monocyte Chemoattractant Protein-1)

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List of Abbreviations

AGP	**1-acid-glycoprotein**
AKI	**Acute kidney injury**
AH	**Alveolar hemorrhage**
ACR	**American College of Rheumatology**
ARA	**American Rheumatism Association**
ACE	**Angiotensin converting enzyme**
ANA	**Anti neuclear antibody**
Anti dsDNA	**Antibody to double-stranded DNA**
Anti Sm	**Antibody to Sm nuclear antigen**
ARBs	**Angiotensin receptor blockers**
anti-C1q	**Antibodies to complement component C1q**
CNS	**Central nervous system**
CSF	**Cerebrospinal fluid**
CP	**Ceruloplasmin**
CCR	**Chemokine receptor**
CRP	**C reactive protein**
CYC	**Cyclophosphamide**
DC	**Denderitic cells**
DN	**Diabetic nephropathy**
ds	**Double stranded**
ESRD	**End stage renal disease**
ESR	**Erythrocyte sedimentation rate**
FSGS	**Focal segmental glomerulosclerosis**
GWAS	**Genome-wide association study**
GCs	**Glucocorticoids**
GBM	**Glomerular basement membrane**
GM-CSF	**Granulocyte macrophage colony stimulating factor**
HPF	**High-power field**
HLA	**Human leukocyte antigens**
HCQ	**Hydroxychloroquine**
IC	**Immune complex**
(ISN/RPS)	**International Society of Nephrology and the Renal Pathology Society**
ICAM	**Intercellular adhesion molecules**
IL	**Interleukin**
IV	**Intravenous**
KCS	**Keratoconjunctivitis sicca**
KT	**Kidney transplant**
L-FABP	**Liver-type fatty acid binding protein**

L-PGDS	**Lipocalin-type prostaglandin D-synthetase**
LN	**Lupus nephritis**
MHC	**Major histocompatibility**
mEPCR	**Membrane endothelial protein C receptor**
MCP-1	**Monocyte chemoattaractant protein**
MMF	**Mycophenolate mofetil**
NK	**Natural killer**
NGAL	**Neutrophil gelatinase-associated lipocalin**
NO	**Nitric oxide**
OPG	**Osteoprotegerin**
ROC	**Receiver operator characteristic**
RBC	**Red blood cells**
RANTES	**Regulated upon Activation Normal T-cell Expressed & Secreted cytokines**
rSLEDAI	**Renal systemic lupus disease activity index**
RRT	**Renal replacement therapy**
RAAS	**Renin-angiotensin-aldosterone system**
SS	**Single stranded**
SD	**Standard deviation**
SLE	**Systemic lupus erythrmatosus**
TLRs	**Toll-like receptors**
tSLEDAI	**Total systemic lupus disease activity index**
TF	**Transferrin**
TNF	**Tumor necrosis factor**
TAM	**Tumor-associated-macrophage**
UV	**Ultraviolet**
U MCP-1	**Urinary monocyte chemoattaractant protein-1**
WBCs	**White blood cells**
WHO	**World Health Organization**
VEGF	**Vascular endothelial growth factor**

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SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder affecting almost all organs and tissues, including the skin, joints, kidneys, lungs, nervous system and serous membrane ***(Alunno et al., 2012)***.

SLE is protean in its manifestations and follows a relapsing and remitting course **(*Bartels et al., 2006)***. While the etiology of SLE is thought to be multifactorial, the disease is characterized by the production of autoantibodies which leads to immune complex(IC) deposition, inflammation and eventually, permanent organ damage ***(Lam and Petri, 2005)***.

In genetically predisposed subjects, environmental factors, such as viral infections and smoking, induce the breakdown of self-tolerance eventually triggering autoimmune response ***(Alunno et al., 2012)***.

SLE manifestations are caused by autoantibodies and ICs that activate the complement system in various tissues. This results in acute and chronic inflammation and tissue damage ***(Munoz et al., 2010)***.

It is characterized by a loss of tolerance to nuclear antigens and various immunological abnormalities, including deregulated activation of both T and B lymphocytes and subsequent polyclonal activation of circulating B lymphocytes which produces a large quantity of autoreactive antibodies and the formation of ICs causing tissue and organ damage ***(Shui-Lian et al., 2012)***.

The clinical course of SLE is variable and may be characterized by periods of remissions and chronic or acute relapses. Women, especially in their 20s and 30s, are affected more frequently than men ***(Cervera et al., 2003)***.

In genetically predisposed subjects, environmental factors, such as viral infections and smoking, induce the breakdown of self-tolerance eventually triggering autoimmune response ***(Alunno et al., 2012)***.

EPIDEMIOLOGY

The reported prevalence of SLE in the population is 20 to 150 cases per 100,000 ***(Lawrence et al., 1998)***. In women, prevalence rates vary from 164 (white) to 406 (African American) per 100,000. Due to improved detection of mild disease, the incidence nearly tripled in the last 40 years of the 20th century ***(Chakravarty et al., 2007)***.

Geographic and racial distribution

- The disease appears to be more common in urban than rural areas ***(Pons-Estel et al., 2010)***.
- The prevalence of SLE is higher among Asians, Afro-Americans, Afro-Caribbeans, and Hispanic Americans compared with Americans of European decent in the United States, and among Asian Indians compared with Caucasians in Great Britain. In comparison, SLE occurs infrequently in Blacks in Africa ***(Rus et al., 2007)***.

Gender

SLE principally affects women during childbearing years. The female-to-male ratio is around 9:1. Although virtually all patients have skin and joint disease, between 30 and 50% will also develop renal, lung, heart and central nervous system (CNS) involvement ***(Li & Isenberg et al., 2005)***.

The increased frequency of SLE among women has been attributed in part to an estrogen hormonal effect ***(Chung et al., 2009)***.

- In children, in whom sex hormonal effects are presumably minimal, the female-to-male ratio is 3:1 ***(Costenbader et al., 2007)***.
- In adults, the ratio ranges from 7:1 to 15:1 ***(Chakravarty et al., 2007)***.
- In support of the potential role of estrogens in predisposing to SLE, the Nurse's Health study showed that women with early menarche, or treated with estrogen-containing regimens, such as oral contraceptives or postmenopausal hormone replacement therapies, have a significantly increased risk for SLE ***(Cooper et al., 2008)***.

Factors related to the X chromosome may also be important in predisposing women to SLE. At least three predisposing genes are located on X chromosomes ***(Lahita, 2009)***. There is also evidence for a gene dose effect, since the prevalence of XXY (Klinefelter's syndrome) is increased 14-fold in men with SLE when compared with the general population of men, whereas XO (Turner's syndrome) is underrepresented in women ***(Buyon et al., 2005)***.

Age of onset:

Sixty-five percent of patients with SLE have disease onset between the ages of 16 and 55 ***(Ballestar et al., 2006)***. Of the remaining cases, 20 percent present before age 16, and 15 percent after age 55***(Lockshin, 2006)***.

Etiopathogenesis

The etiology of SLE remains unknown and is clearly multifactorial. Many observations suggest a role for genetic, hormonal, immunologic, and environmental factors (Fig. 1) . Recent advances that could improve the treatment of SLE include the identification of genetic variations ***(Gualtierotti et al., 2010)***.

The following steps have been suggested:

1. Genetic predisposition.
2. Gender as an additional predisposing factor.
3. Environmental stimuli which start immune responses.
4. Appearance of autoantibodies.
5. Regulation of the autoantibodies, T and B cell fails with the development of the clinical disease.
6. Chronic inflammation and oxidative damage as causes of tissue damage influencing morbidity ***(Gualtierotti et al., 2010)***.

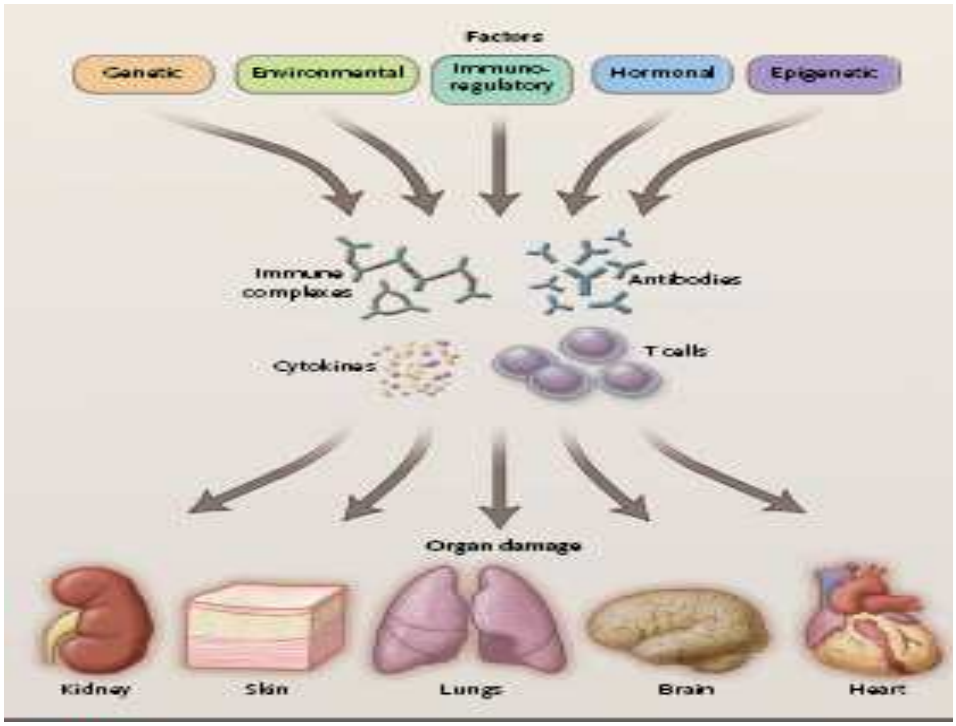

Figure (1): Over view of the pathogenesis of SLE **(Gualtierotti et al., 2010).**

1) Genetic factors

- Studies have demonstrated a greater concordance rate of SLE among monozygotic and dizygotic twins. Concordance of SLE was present in 11 (58%) of 19 monozygotic twins ***(Von Mühlen & Nakamura, 2012)***.

- Five to twelve percent of relatives of patients with SLE have the disease ***(Harley et al., 2006)***.

- The most common genetic predisposition is found at the major histocompatibility locus(MHC). The MHC contains genes for antigen presenting molecules (class I human leukocyte antigens [HLA-A, -B, and -C] and class II HLA molecules [HLA-DR, -DQ, and DP]). The MHC also contains genes for some complement

components, cytokines, and heat shock protein ***(Murashima et al., 2004)***.

Genes on different chromosomes are also associated with clinical subsets such as nephritis (2q34), hemolytic anemia (11q14), discoid lupus and thrombocytopenia (11p13), vitiligo (17p12); to production of certain autoantibodies (e.g., anti double stranded DNA(anti-ds DNA) [19p13.2]); or to increased risk for end stage renal disease ***(Jönsen et al., 2007)***.

2) Hormonal factors

Hormones contribute through unknown mechanisms to the increased prevalence of SLE among women. The X chromosome may contribute independently from hormones because in castrated female and male mice that have been genetically manipulated to express XX, XO (female), XY, or XXY (male) combinations, the presence of two X chromosomes increases the severity of SLE ***(George and Tsokos, 2012)***.

Substantial evidence of the immunoregulatory function of estradiol, testosterone, progesterone and pituitary hormones, including prolactin, has supported the hypothesis that they modulate the incidence and severity of SLE ***(Croker and Kimberly, 2005)***. As examples:

- The use of estrogen-containing contraceptive agents is associated with a 50 percent increase in risk of developing SLE; while either early onset of menarche (age ≤10 years) or administration of estrogen to postmenopausal women doubles their risk ***(Kim-Howard et al., 2010)***.

- SLE has been observed in some males with Klinefelter's syndrome ***(Li et al., 2005)***.