

# **Antibodies to Ribosomal P Proteins in Lupus Nephritis**

Thesis

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By

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## **Abstract:**

**Objective:** Lupus nephritis is an immune complex glomerulonephritis that develops as a frequent complication of SLE and is the major predictor of poor prognosis. Since anti-ds DNA is not a universal finding in patients with lupus nephritis, increasing attention has been paid to the relationship of autoantibodies to anti-Rib P with lupus nephritis. The aim of this study is to determine the association of anti-Rib P antibodies and lupus nephritis.

**Patients and Methods:** sixty three female SLE patients with nephritis served as case and twenty five female SLE patients without nephritis served as control were selected from the in-patient section of Rheumatology and Rehabilitation department, Faculty of Medicine, Cairo University hospitals. All patients and controls fulfilled the ACR revised criteria for classification of SLE. A complete routine laboratory evaluation and clinical examination were performed in each patient and control. Anti-Rib P antibodies were detected by ELISA using ORG 517 Anti-Rib-P kit. ANA and anti-dsDNA were done. Disease activity was assessed using SLEDAI.

**Results:** anti- Rib P antibodies in SLE were found to be associated with higher disease activity ( $P=0.02$ ), fever ( $P<0.001$ ), and alopecia ( $P=0.017$ ) but were not associated with lupus nephritis ( $P=0.4$ ).

**Conclusion:** there was no association between anti-Rib P antibodies and lupus nephritis. However, there was association between anti-Rib P antibodies and higher disease activity, fever, and alopecia.

**Keywords:** Systemic lupus erythematosus- lupus nephritis- anti-ribosomal P antibodies - disease activity.

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## List of abbreviations

<b>ACE-I</b>	Angiotensin converting enzyme inhibitors
<b>ACR</b>	American Colleague of Rheumatology
<b>AIH</b>	Autoimmune hepatitis
<b>ALMS</b>	Aspreva Lupus Management Study
<b>ALT</b>	Alanine transaminases
<b>ANA</b>	Antinuclear antibody
<b>AVN</b>	Avascular necrosis
<b>anti-RNP</b>	Anti-ribonucleoprotein
<b>anti-Sm</b>	Anti-smith
<b>APCs</b>	Antigen presenting cells
<b>ARBs</b>	Angiotensin receptor blockers
<b>ARBs</b>	Angiotensin receptor blockers
<b>AST</b>	Aspartate aminotransferase
<b>AZA</b>	Azathioprine
<b>BAFF</b>	B-cell activating factor
<b>BlyS</b>	B lymphocyte stimulator
<b>BP</b>	Blood Pressure
<b>BSA</b>	Body surface area
<b>C</b>	Complement
<b>CAMs</b>	Cell adhesion molecules
<b>CBC</b>	Complete blood picture
<b>CCR5</b>	C-C chemokine receptor type 5
<b>CD</b>	Cluster of differentiation
<b>CKD</b>	Chronic kidney disease
<b>CRP</b>	C-reactive protein
<b>CSF</b>	Cerebrospinal fluid
<b>CYC</b>	Cyclophosphamide
<b>DCs</b>	Dendritic cells
<b>DN</b>	Double negative
<b>DNA</b>	Deoxyriboneuclic acid
<b>ds-DNA</b>	Double stranded deoxyriboneuclic acid
<b>DVT</b>	Deep venous thrombosis
<b>ENAS</b>	Extractable nuclear antigen antibodies
<b>ESR</b>	Erythrocyte sedimentation rate
<b>ESRD</b>	End-stage renal disease
<b>Fcγ</b>	The constant fragment of antibody gamma
<b>fhT</b>	Follicular helper T cells
<b>GBM</b>	Glomerular base membrane
<b>GDCN</b>	Glomerular Disease Collaborative Network
<b>GM-CSF</b>	Granulocyte macrophage colony stimulating factor
<b>GN</b>	Glomerulonephritis

<b>HB</b>	Hemoglobin
<b>HCQ</b>	Hydroxychloroquine
<b>HLA</b>	Human leukocyte antigen
<b>HRP</b>	Horseradish peroxidase
<b>ICAM-1</b>	Intracellular adhesion molecule-1
<b>ICs</b>	Immune complexes
<b>IFN-<math>\alpha</math></b>	Interferon alpha
<b>IPF</b>	interstitial pulmonary fibrosis
<b>Ig</b>	Immunoglobulin
<b>IL</b>	Interleukin
<b>i-NOS</b>	Inducible nitric oxide synthase
<b>IPF</b>	Interstitial pulmonary fibrosis
<b>ISN/ RPS</b>	International Society of Nephrology/ Renal Pathology Society
<b>IV</b>	Intravenous
<b>IVIG</b>	Intravenous immunoglobulin
<b>L Th17</b>	Th17 Lymphocytes
<b>LN</b>	Lupus nephritis
<b>LPL</b>	lipoprotein lipase
<b>LUNAR</b>	The Lupus Nephritis Assessment with Rituximab
<b>mAb</b>	Monoclonal antibodies
<b>MAC</b>	Complement membrane attack complex
<b>MAPK</b>	Mitogen activated protein kinase
<b>MCP-1</b>	Monocyte chemotactic protein-1
<b>MCPs</b>	Metacarpophalangeals
<b>MCV</b>	mean corpuscular volume
<b>mDCs</b>	Myeloid DCs
<b>MMF</b>	Mycophenolate mofetil
<b>MP</b>	Methyl prednisolone
<b>MPA</b>	Mycophenolic acid
<b>MTPs</b>	Metatarsophalangeals
<b>NFAT</b>	Nuclear factor of activated T cells
<b>NF<math>\kappa\beta</math></b>	Nuclear factor kappa
<b>NIH</b>	National Institutes of Health
<b>NKs</b>	Natural killer T cells
<b>NO</b>	Nitric oxide
<b>NPSLE</b>	Neuropsychiatric systemic lupus erythematosus
<b>NZB</b>	New Zealand Black
<b>PDGF</b>	Platelet-derived growth factor
<b>PIPs</b>	Proximal interphalangeals
<b>PLT</b>	Platelet
<b>PMNs</b>	Polymorph nuclear leucocytes
<b>PTEC</b>	Proximal tubular epithelial cells

<b>RAAS</b>	Renin angiotensin aldosterone system
<b>Rag 1</b>	Recombination activating gene 1
<b>RANTES</b>	Regulated on activation, normal T cell expressed and secreted
<b>RCTs</b>	Randomized controlled trials
<b>ROR</b>	Retinoid-related orphan receptors
<b>ROS</b>	Reactive oxygen species
<b>SLE</b>	Systemic lupus erythematosus
<b>SLEDAI</b>	Systemic Lupus Erythematosus Disease Activity Index
<b>ss-DNA</b>	Single stranded deoxyriboneuclic acid
<b>TCR</b>	T cell receptor
<b>TGF</b>	Transforming growth factor
<b>TGF<math>\beta</math></b>	Transforming growth factor beta
<b>Th1</b>	T helper 1
<b>Th17</b>	T helper 17
<b>Th2</b>	T helper 2
<b>TIAS</b>	Transient ischemic attacks
<b>TLC</b>	Total leucocyte count
<b>TLRs</b>	Toll-like receptors
<b>TNF</b>	Tumor necrosis factor
<b>TTP</b>	Thrombotic thrombocytopenic purpura
<b>TWEAK</b>	TNF-related weak inducer of apoptosis
<b>VCAM-1</b>	Vascular cell adhesion molecule-1
<b>WHO</b>	World Health Organization

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## **Introduction**

Lupus nephritis is an immune complex glomerulonephritis that develops as a frequent complication of SLE (**Lech and Anders, 2013**).

It is one of the most serious SLE complications since it is the major predictor of poor prognosis (**Salgado and Herrera-Diaz, 2012**).

Currently, anti-ds DNA is the best available biomarker for lupus nephritis since it correlates well with renal activity, worse prognosis and histology severity (**Dooley, 2007**). But as it is not a universal finding in patients with lupus nephritis, increasing attention has been paid to the relationship of autoantibodies to anti-Rib P with lupus nephritis (**Toubi and Shoenfeld, 2007**) (**Kiss and Shoenfeld, 2007**).

Anti-Rib P are directed to 3 phosphoproteins (P0, P1, and P2), which are located on the larger 60S subunit of eukaryotic ribosomes, and have molecular weights of 38, 19, and 17 kDa, respectively. They have been shown to react a common linear determinant that is present in the carboxyl-terminal 22-amino-acid sequence (**Elkon et al, 1986**).

## **Aim of Work**

The aim of this study is to determine the association of anti-Rib P antibodies and lupus nephritis.

## **Pathogenesis of Lupus Nephritis**

Lupus nephritis (LN) is a potentially devastating complication of systemic lupus erythematosus (SLE) (**Schwartz et al, 2014**).

Although all renal compartments including glomerular, tubulointerstitial, and vascular components may be injured by the disease; however, the term “lupus nephritis” is mainly used to define the immune complex-mediated glomerulonephritis (GN) (**Kiremitci and Ensari, 2014**).

It is a serious potential feature of SLE. Though SLE typically cycles through periods of flares and remission, patients often eventually succumb to end-stage kidney or cardiovascular damage (**Sterner et al, 2014**).

The incidence and prevalence of LN varies depending on the studied population. The LN incidence is higher in people of Asian (55%), African (51%), and Hispanic (43%) ancestry compared with Caucasians (14%) (**Ortega et al, 2010**).

Up to 25% of these patients still develop end-stage renal disease (ESRD) 10 years after onset of renal compromise. In terms of outcome, the 5- and 10-year renal survival rates of LN in the 1990s ranged between 83–93% and 74–84%, respectively (**Mok, 2010**).

In addition, LN develops early in the course of SLE thus becoming a major predictor of poor prognosis (**Anaya et al, 2011**). However, in about 5% of the cases, LN may appear several years after the onset of SLE (i.e., delayed LN) (**Varela et al, 2008**).

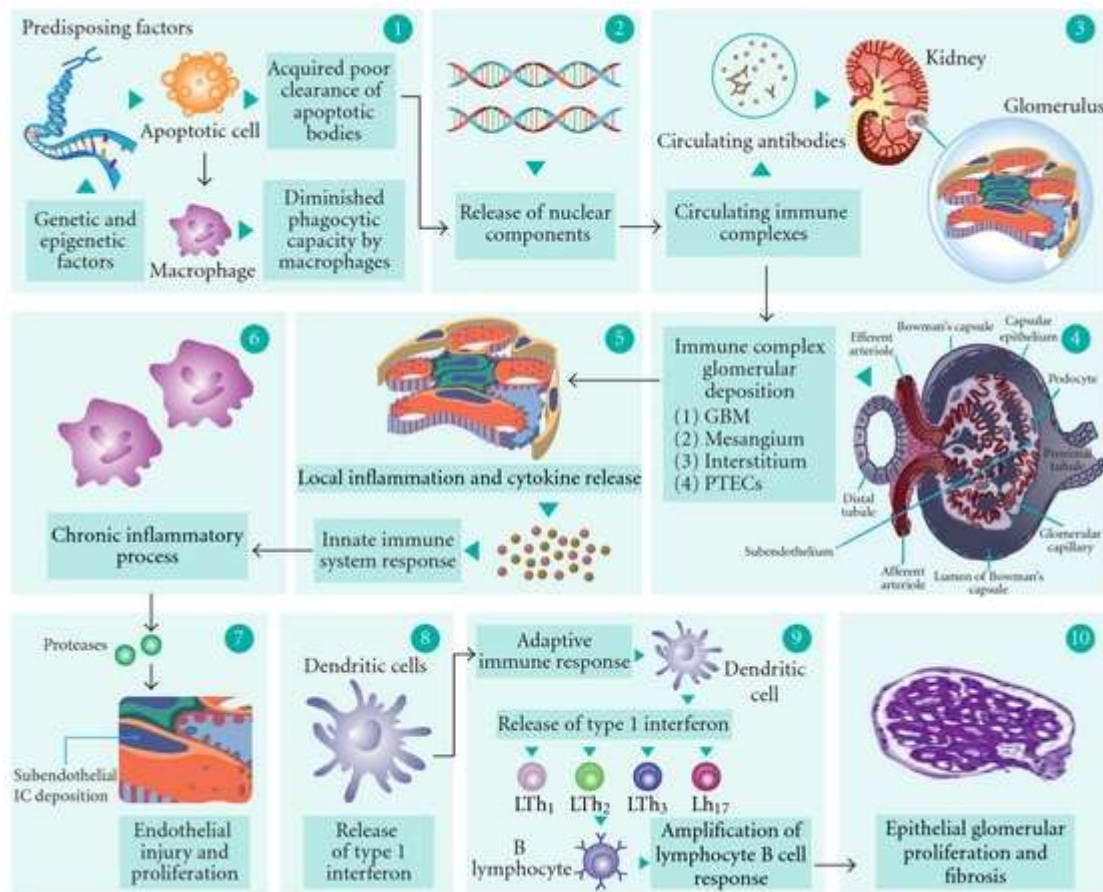
In susceptible individuals suffering of SLE, in situ formation and deposit of immune complexes (ICs) from apoptotic bodies occur in the kidneys as a result of an amplified epitope immunological response (**Salgado and Herrera-Diaz, 2012**).

IC glomerular deposits generate release of proinflammatory cytokines and cell adhesion molecules (CAMs) causing inflammation. This leads to monocytes and polymorphonuclear cells chemotaxis. Subsequent release of proteases generates endothelial injury and mesangial proliferation. Presence of ICs promotes adaptive immune response and causes dendritic cells (DCs) to release type I interferon (IFN). This induces maturation and activation of infiltrating T cells, and amplification of T helper 2 (Th2), T helper 1 (Th1) and T helper 17 (Th17) lymphocytes. Each of them, amplify B cells and activates macrophages to release more proinflammatory molecules, generating effector cells that cannot be modulated promoting kidney epithelial proliferation and fibrosis (**figure 1**) (**Salgado and Herrera-Diaz, 2012**).

## **Apoptosis**

Apoptosis is a tightly regulated process of programmed cell death that regulates the late phase of immune responses. Disordered regulation of both apoptosis and the clearance of apoptotic products have been implicated in the pathogenesis of SLE and LN (**Kamradt et al, 2001**).

The idea of impaired apoptosis had gained a crucial role in pathogenesis of SLE. Auto-antigens are found in apoptotic and necrotic material and they are recognized by autoimmune sera from SLE patients (**Muñoz et al, 2005**).



**Figure 1:** Lupus nephritis: an imbalance between cytokine homeostasis and I.C. deposition. In predisposing susceptible individuals who develop SLE, there is an acquired poor clearance of apoptotic bodies and a diminished phagocytic capacity by macrophages (1). Early formation of ICs include antinucleosomes, anti-dsDNA, DNA extractable nuclear antigen antibodies (ENAS), antibodies against C1q complex of the complement system, free DNA, antiribonucleoproteins (anti-RNP), and histones as byproducts of inefficient phagocytosis of apoptotic bodies (2). Circulating ICs are deposited initially at the GBM, mesangium, and interstitial tissue within the PTECs (3) and (4). The deposited ICs initiate the release of proinflammatory cytokines and chemokines such as MCP-1, IL-1, IL-6 and CAMs thus establishing a chronic inflammatory process (5). The resulting overload of the mesangial phagocytic system (innate immune system) leads to deposits of subendothelial ICs becoming an easy target for monocyte migration and infiltration and generating endothelial injury and proliferation (6) and (7). In turn, the adaptive immune system is activated secondary to the presence of ICs and DCs (8), which subsequently trigger release of type 1 IFN and induce maturation and activation of infiltrating T cells. This activation leads to sequential amplification of Th2, Th1, and Th17 (9). Each of these amplifies lymphocyte B cell response and further activates macrophages, generating a second general response, which increases recruitment of effector cells that can no longer be modulated by regulatory T cells and resulting in the end in epithelial glomerular proliferation and fibrosis (10) (Salgado and Herrera-Diaz, 2012).