# LEVELS OF ANGIOPOIETIN-2 IN PLASMA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND ITS CORRELATION WITH VARIOUS DISEASE PARAMETERS

**Thesis**

***Submitted for the Complete Fulfillment of the Master Degree (M.Sc) in Medical Biochemistry***

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**2010**

# *Acknowledgment*

*First and foremost, thanks to **ALLAH**, who is most beneficial and most merciful.*

*I had the honor to accomplish my work under the supervision of Professor Doctor **Maysa Kamal Salama** Professor of Medical Biochemistry, Faculty of Medicine, Cairo University. I am really indebted to her for her unlimited support and kind encouragement during this work.*

*I wish to express my sincere thanks and gratitude to Doctor **Fatma Mohamed Taha**, Lecturer of Medical Biochemistry Faculty of Medicine, Cairo University for her valuable help and expert advice throughout this study.*

*I also wish to express my deep thanks to Doctor **Hanan El-Sayad Ali Darwish**, Lecturer of Rheumatology, Faculty of Medicine, Cairo University for her help throughout this study.*

*I would like to express my deep thanks to all staff members and colleagues in the Medical Biochemistry Department, Cairo University for their help and support.*

*Finally, no words can express my deep thanks and appreciation to my **family** for their continuous support and outstanding encouragement during this work.*

# ABSTRACT

Systemic lupus erythematosus is one of autoimmune diseases characterized by multisystem involvement associated with autoantibody and immune complex vasculitis with endothelial cell damage.

The aim of the present work was to study the possible role of Angiopoietin - 2 as a recent inflammatory and angiogenic mediator in the pathogenesis of SLE and its correlation with the state of another inflammatory marker P-Selectin as well as various markers of the disease activity .

The present study included 3 main groups: active SLE patients(group I), inactive SLE patients( group II) and healthy normal control subjects ( group III). All groups were subjected to measurement of plasma Angiopoietin-2 and P-Selectin by ELISA in addition to various laboratory investigations diagnosing SLE and all parameters of disease activity as: Hb, WBC´s, Platelets, ESR, serum creatinine ,C3 ,C4 and 24 hour urine protein .

The mean level of Plasma Ang-2 showed a high significant increase in active and inactive SLE patients than control subjects ( $p< 0.001$) and in active than inactive SLE patients( $p< 0.001$) . Mean level of Plasma P-Selectin showed a high significant increase in active SLE patients than inactive SLE patients and control subjects( $p< 0.001$) . There was a significant positive correlation between Ang-2 ,P-Selectin, and each of SLEDAI ,24 h urine protein , ESR in all SLE patients as well as in active group .A significant negative correlation was found between Ang-2, P-Selectin and each of C3 ,C4 in all SLE patients as well as in active group.

**In conclusion ,** plasma Ang-2 could be considered as a strong indicator of disease activity .Ang-2 does not primarily affect endothelial cell inflammation by itself but facilitates endothelial activation and subsequent leucocyte transmigration in the presence of various inflammatory stimuli as P-Selectin. There is possible involvement of Ang-2 in the pathogenesis of renal lupus.

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# List of abbreviations

| | |
|---|---|
| **aa** | aminoacid |
| **ABIN- 2** | A20-binding inhibitor of nuclear factor (NF)-kB |
| **ACR** | American College of Rheumatology |
| **ALT** | Alanine transaminase |
| **ANA** | Antinuclear antibodies |
| **Anti-Ro/SSA** | Anti-Ro Sjogren's syndrome antibodies |
| **Anti-Sm** | Anti-Smith |
| **APL** | Anti-phospholipid |
| **AST** | Aspartate transaminase |
| **BUN** | Blood urea nitrogen |
| **C3** | Complement 3 |
| **C4** | Complement 4 |
| **Clq** | Complement subcomponent |
| **CPK** | Creatine phosphokinase |
| **CRP** | C-Reactive Protein |
| **Da** | Dalton |
| **ds-DNA** | Double-stranded DNA |
| **EC** | Endothelial cell |
| **EDTA** | Ethylene diamine tetraacetic acid |
| **ELSA** | Enzyme Linked Immuno Sorbent Assay |
| **ESR** | Erythrocyte sedimentation rate |
| **FANA** | Fluorescent antinuclear antibody |
| **FGF** | Fibroblast growth factor |
| **FKHR-1** | Forkhead transcription factor |
| **FOXO1** | Forkhead Box subfamily O |

| | |
|---|---|
| **GMP-140** | Granule Membrane Protein 140 |
| **GPIbα** | Glycoprotein Ibα |
| **Hb** | Hemoglobin |
| **HLA** | Human leucocyte antigen |
| **HSP** | Heat-shock proteins |
| **ICAM-1** | Intercellular adhesion molecule |
| **IgG** | Immunoglobulin G |
| **IL-8** | Interleukin 8 |
| **KDa** | Kilodalton |
| **LPS** | lipopolysaccharide |
| **MAdCAM-1** | Mucosal addressin cellular adhesion molecule-1 |
| **MCP** | Metacarpo-pharyngeal |
| **MHC** | Major histocomptability complex |
| **MMP-2** | Matrix metalloprotease |
| **MPs** | Microparticles |
| **MTP** | Metatarsopharyngeal |
| **NSAIDs** | Nonsteroidal anti-inflammatory drugs |
| **NSCLC** | Non small cell lung cancer |
| **PADGEM** | Platelet Activation-Dependent Granule to External Membrane Protein |
| **PBMCs** | Peripheral blood mononuclear cells |
| **PKB** | Protein kinase B |
| **PLA2** | Phospholipase A2 |
| **PMA** | Phorbol12-myristate-13-acetate |
| **PSGL-1** | P-Selectin glycoprotein ligand-1 |
| **RNP** | Ribonucleoprotein |
| **SCLE** | Subacute cutaneous lupus erythematosus |
| **SLE** | Systemic lupus erythematosus |

| | |
|---|---|
| **SLEDAI** | SLE disease activity index |
| **SMC** | Smooth muscle cell |
| **TEMs** | Tumor endothelium markers |
| **TNF-α** | Tumor necrosis factor |
| **UV** | Ultraviolet |
| **VCAM-1** | Vascular cell adhesion molecule-1 |
| **VEGF** | Vascular endothelial growth factor |
| **WBC´s** | White blood cells, |
| **WPBs** | Weibel–Palade bodies |

# INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex disease with variable presentations, course and prognosis **(*Bertsias et al., 2008*)**. It is a chronic multifactorial inflammatory disorder caused by changes in the immune system with genetic, environmental and hormonal interactions. Its diagnosis is made by means of clinical history, physical examination and complemented by tests, such as routine laboratory tests (complete blood count, erythrocyte sedimentation rate, and urine analysis),and histopathology **(*Murphy, 2004)*.**

SLE is a progressive autoimmune disease with a wide range of clinical and immunological abnormalities. The clinical expression of SLE is the consequence of its complex immunopathology, involving the production of autoantibodies and immune complex vasculitis with endothelial cell damage **(*Belmont et al., 1996*).**

***Riccieri and his colleagues (2005)*** reported that microvascular involvement is an important feature in SLE.A pathologic hallmark of SLE is the appearance of diverse vascular lesions **(*Font et al., 2001*).**

Angiopoietins are a novel class of angiogenic growth factors that act selectively on endothelial cells (ECs) (***Orfanos et al., 2007)***. The Angiopoietin family includes four ligands (Ang-1,Ang-2 ,Ang- 3 and 4), three of which are expressed in humans, namely Angiopoietin (Ang)-1, 2,and 4.All of which act on two corresponding tyrosine kinase receptors (Tie 1 and Tie 2) ***(Jones et al .,2001a).*** Ang1 and Ang2 are antagonistic ligands which bind with

similar affinity to the extracellular domain of the tyrosine kinase with Ig-like and epidermal growth factor-like domains 2 (Tie2) receptor, which is almost exclusively expressed by endothelial cells ***(Wakui et al., 2006).***

Non redundant constitutively operational Ang1/Tie2 signalling maintains vessel integrity, inhibits vascular leakage, suppresses inflammatory gene expression and prevents recruitment and transmigration of leukocytes ***(Thurston et al., 2000).*** In contrast, binding of Ang-2 disrupts protective Ang1/Tie2 signaling and facilitates endothelial inflammation ***(Parikh et al., 2006)***. Ang-2 has been regarded as the dynamic regulator within the Ang/Tie system, since it constitutes a Weibel–Palade body-stored molecule (WPB), which is rapidly released and induced upon endothelial stimulation.

Recent investigations provided evidence that inflammation exists in a mutually dependent association with angiogenesis ***(Dvorak et al., 1995*****) .**During inflammatory processes, newly formed vessels supply the inflamed tissues with nutrients and oxygen allowing the transport of inflammatory cells. Among these, neutrophils are the first cells recruited in the angiogenic bed and provide cytokines, growth factors, and proteolytic enzymes, which together contribute to regulate angiogenesis ***(Shaw et al., 2003)***. Neutrophils tethering, rolling, and firm adhesion to endothelial cells (ECs) require the interaction of different adhesion molecules between ECs and neutrophils. Stimulation of ECs with inflammatory mediators including thrombin, histamine, and VEGF can promote a rapid and transient P-selectin translocation at their surface ***(Sako et***

***al.,1993*)** .P -selectin is then able to interact with its high-affinity counterreceptor, P-selectin glycoprotein ligand-1 (PSGL-1), expressed on neutrophils and promote their rolling and transient adhesion ***(Rollin et al., 2004*** **).**

***Lemieux et al., (2005)*** have proved that Ang -1 and 2 could induce P-Selectin translocation and neutrophil adhesion on to endothelial cells in an in vitro study.

# AIM OF THE PRESENT WORK

The aim of this work is to study the possible role of Angiopoietin - 2 as a recent inflammatory and angiogenic mediator in the pathogenesis of SLE and its correlation with the state of another inflammatory marker P-Selectin as well as various markers of the disease activity.

# SYSTEMIC LUPUS ERYTHEMATOSUS

## Definition and history:

**Systemic lupus erythematosus SLE or lupus**, is a chronic inflammatory autoimmune disease of unknown etiology, with variable manifestations, course and prognosis ***(Gladman et al., 1999***). As occurs in other autoimmune diseases, the immune system attacks the body's cells and tissue, resulting in inflammation and tissue damage. SLE most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. The course of the disease is unpredictable, with periods of illness (called *flares*) alternating with remissions ***(Schur, 2000).***

The term lupus was used in the 18$^{th}$ century to describe a variety of skin conditions. However, the first historical account of was *by* Biett, in 1833 **(*Smith and Cyr, 1988).*** For a number of decades the disease was considered a chronic dermatologic disorder, but in 1872 Kaposi described the systemic nature of lupus erythematosus. The recognition of the antinuclear factor provided some insight into pathogenesis of the disease. Whereas recognition of the inflammatory and immunologic features of the disease provided insight into therapeutic modalities. Subsequently, late causes of mortality and morbidiy not immediately related to immunologic abnormalities of SLE has been described, such as premature atherosclerosis, neurocognitive dysfunction and avascular necrosis **(*Gladman and Urowitz, 1987)***.