INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmunity affecting many systems. Both antibodies and autoreactive T cells play significant roles in its pathogenesis. Experimental data and clinical observations indicate that autoimmunity and end organ damage are under separate genetic controls and that there are significant interactions between these two pathways. Experimental evidence has been obtained to support the hypothesis that autoantibodies and autoreactive T effector cells may be initiated by environmental factors through molecular mimicry and the inherent polyreactive nature of antigen receptors. A unified hypothesis has been postulated for the pathogenesis of SLE that has practical implications ***(Lewis et al., 2013).***

Renal damage is common in patients with SLE. Up to 60% of adults and 80% of children with lupus develop overt renal abnormalities over the entire course of the disease. Importantly, the renal domain of the Systemic Lupus International Collaborative Clinics (SLICC) damage index is known to be independently associated with a shorter time to death, suggesting that preventing renal damage in lupus patients has long-term prognostic implications ***(Romero-Diaz, 2011).***

Tubulointerstitial injury is characterized by tubular atrophy and interstitial damage is better correlated with impaired renal function than the degree of glomerular injury in

patients with chronic kidney disease (CKD) ***(Robriguez-Iturbe et al., 2005).***

The transforming growth factor B (TGF-B) is a family of cytokines that includes three closely related members: TGF-B1, TGF-B2 and TGF-B3. TGF-B1 is the most abundant form in lymphoid organs.A defective TGF-B1 signalling pathway leads to the activation of a self-targeted immune response ***(Yong et al., 2013).***

It is known that the production of TGF-BI by lymphocytes is reduced in patients with SLE. It was suggested that lower serum levels of TGF-B1 might be the most consistent cytokine abnormality in SLE. They found lower levels of TGF-B1 to correlate with disease activity and severe organ damage ***(Becker-Merock et al., 2010).***

In this study, we investigated serum TGF-B1 levels in patients with lupus nephritis and analyze the association between serum TGF-B1 and clinical features in LN patients.

AIM OF THE WORK

To assess the serum levels of TGF-B1 in lupus nephritis patients and its relation to disease activity and renal damage.

Chapter One

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmunity affecting many systems. Both antibodies and autoreactive T cells play significant roles in its pathogenesis. Experimental data and clinical observations indicate that autoimmunity and end organ damage are under separate genetic controls and that there are significant interactions between these two pathways .SLE is characterized by unpredictable flares of disease activity and irreversible damage ***(Lewis et al., 2014).***

SLE is characterized by a variety of clinical and laboratory abnormalities, including rash, arthritis, leucopenia, thrombocytopenia, alopecia, fever, nephritis, and neurologic disease. Most or all of the symptoms of acute lupus are attributable to immunologic attack on the affected organs. Many complications of long-term disease are attributable to both the disease and to its treatment ***(Zonana-Nacach et al., 2011)***.

Epidemiology:

Sex:

SLE is a disease of women, particularly during their reproductive period. Female to male ratio 12:1**,** between menarche and menopause is 3:1 in younger and older

(Schwartzman-Morris & Putterman, 2012). This ratio support the hypothesis that hormonal factors may be involved in the pathogenesis of the disease, these were supported by increase risk of development of SLE in men with Klinefelter Syndrome, menopausal women treated with hormonal replacement therapy and women exposed to estrogen containing oral contraceptives ***(Costenbader, 2007).***

The annual SLE prevalence in United States is 67.90-89.35 cases per 100,000 general populations ***(Furst et al., 2011).*** In women, prevalence rates vary from 164 (white) to 406 (African American) per 100,000 ***(Pons-Estel et al., 2010).***

Age:

There is a relationship between age and disease frequency observed in women with SLE, which was correlated with levels of female sex hormones which suggests being a key role in SLE etiology ***(Danchenko et al., 2006).***

Disease incidence is higher among women between 14-55 years. Female children and postmenopausal women at equal rates to developed SLE. Males, in contrast to females, do not have an age-related peak incidence ***(Amador-Patarroyo et al., 2012).***

Pregnancy:

There is increased in SLE disease activity during pregnancy which increases the risk of spontaneous abortion, intrauterine fetal death, preeclampsia, intrauterine growth retardation and preterm birth because of increased levels of estrogen, prolactin, and T–helper cell 2 cytokines ***(Chen et al., 2004).*** SLE Prognosis for both mother and child are best when SLE is quiescent for at least 6 months before the pregnancy and when the mother's underlying renal function is stable and normal or near normal. Lupus nephritis can get worse during pregnancy ***(Gladman et al., 2010).***

The incidence of exacerbations during pregnancy and the postpartum period, especially in women in remission at the beginning of pregnancy, has been progressively diminishing in the last 30 years. Possible causes for flare-ups during the postpartum period include decreased levels of anti-inflammatory steroid, elevated levels of prolactin (which is a proinflammatory hormone) and changes in the neuroendocrine axis ***(Stojan & Baer, 2012).***

Mortality \ Morbidity:

The unpredictable nature of the disease and wide spread potential harm lead to variation in the clinical course of the disease ranging from relatively benign to rapidly progressive or even fatal disease. Organ damage in SLE begins with the

formation of scar tissue in response to inflammation and ischemia and progress over time ***(Edworthy, 2005).***

A Cohort study done by ***Cervera et al. in (2003)*** found that within 10 years of diagnosis, 48.1% patients presented 1 or more episodes of arthritis, 31.1% patients had malar rash, 27.9% active nephropathy, 19.4% neurologic involvement, 16.6% fever, 16.3% Raynaud phenomenon, 16.0% serositis, 13.4% thrombocytopenia, and 9.2% thrombosis.

Remission of SLE is not uncommon but it is often punctuated by flares**.** Since the 1950 s, the 5 year survival rates of patients with SLE had increased from 50% to a range of 91 to 97% ***(Gill et al., 2003)***, this increase in survival rate is due to improved management, advancement in general medical care, versus diagnosis of earlier and milder disease ***(Kasitanon et al., 2006).***

A retrospective cohort study used data from the National Health Insurance Research Database of Taiwan on female patients newly diagnosed with SLE from 2001 to 2004 had found that female patients with late-onset (>50years) SLE carried a higher risk of mortality than those with adult-onset disease (18-50 years) in the presence of co-morbidities. Juvenile-onset SLE patients (<18 years) were at greatest risk of mortality, which is probably due to disease severity ***(Chen et al., 2013).***

Infections and disease of the cardiovascular, renal, pulmonary, and central nervous systems are the most frequent causes of death in patients with SLE ***(Heller et al., 2007).*** The highest mortality rate was estimated in a patient group characterized by female sex, younger age, SLE duration <1 year or black/African American race ***(Bernatsky et al., 2006).***

Premature coronary artery disease, related to accelerated atherosclerotic disease, represent a significant source of morbidity and mortality in lupus patients because of number of factors, including endothelial cell injury during active SLE, vasospasm, Raynaud's phenomenon, vasculitis, corticosteroid-induced proatherogenesis, dyslipidemia related to renal disease, hypertension, obesity and sedentary lifestyle ***(Telles et al., 2010).*** Women with SLE aged 35-44 years were found to have a rate of myocardial infarction 50 times that of women of the same age without SLE ***(Gladman & Urowitz, 2007).***

Despite the improvement in the overall survival rate, patients with SLE still have a death rate 3-5 times higher than that of general population ***(Brent, 2010).*** The LUMINA study in the United States on SLE patients reported that both disease activity and poverty predicted higher mortality ***(Alarcón et al., 2001).***

Pathophysiology and Pathogenesis:

SLE is an autoimmune, complex, rheumatic, heterogeneous disease, the pathogenesis of which remains

something of a mystery. In recent years understanding the pathogenesis of the disease had been advanced due to the development of novel genetic and immunological techniques ***(Pathak & Mohan, 2011).***

1. Immune dysfunction in SLE:

B cells and T cells dysfunction:

Central to the immune dysfunction seen in SLE is the existence of overactive B cells, which produce autoantibodies, associated with inappropriate T cell suppression (high ratio of $CD4^+$ to $CD8^+$ T cells), defects in immune cell tolerance, and dysfunctional signaling by immune cells ***(Nagy et al., 2005).*** B cell activators, such as protein B-lymphocyte stimulator (BLyS), appear to be unregulated in lupus, further encouraging B-cell survival ***(Gerl et al., 2009).***

Immune cell signaling is abnormal in SLE. Stimulation of lupus B and T cells results in abnormally high free intracellular calcium concentrations with increased production of tyrosine phosphorylated proteins. This inappropriate response may account in part for the 'overzealous' behavior of these cells ***(Anolik, 2007).***

Disturbance of the immune response:

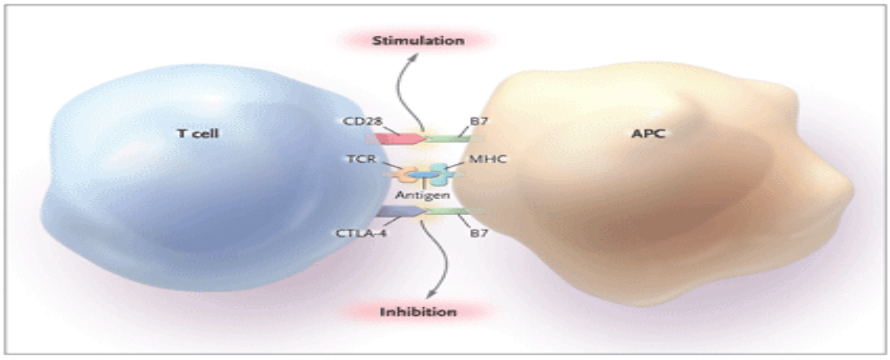

Figure (1): Interaction between a T cell and an Antigen-Presenting Cell (APC) ***(Rahman & Isenberg, 2008):***

The antigen-presenting cell binds antigen in a complex with a molecule from the major histocompatibility complex (MHC) on its surface.This complex interacts with the T-cell receptor (TCR). The effect on the T cell depends on the interaction between other molecules on the surfaces of the two cells ***(Rahman & Isenberg, 2008).***

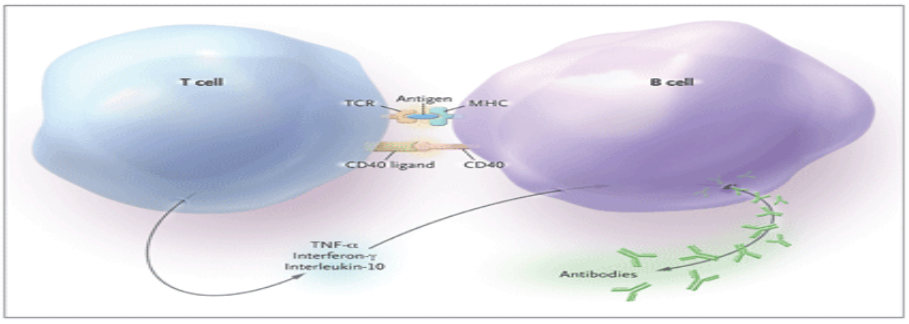

Figure (2): T cell -B cell Interaction ***(Rahman & Isenberg, 2008).***

B cell and a T cell interacting and stimulating each other. T-cell cytokines affect B cells by stimulating cell division,

switching antibody production from IgM to IgG, and promoting a change in the molecular sequence of the secreted antibody so that it binds more strongly to the driving antigen. Thus, T-cell help makes possible the production of high-affinity IgG autoantibodies. These kinds of antibodies are closely linked to tissue damage in lupus ***(Rahman, 2004).***

The autoantigen-specific B cells and T cells that interact to produce injurious autoantibodies are absent in healthy people. Several mechanisms could account for the absence of such cells. These mechanisms include removal (deletion) of the autoreactive B cells, inactivation of the cells so that they remain in the body but are anergic, or a change in the light chain of the antibody expressed by an autoreactive B lymphocyte (so-called receptor editing) such that the antibody loses the ability to bind autoantigen. The use of certain light-chain genes by populations of B cells from patients with lupus indeed differs from the light-chain repertoire in healthy people; this difference could be due to aberrant receptor editing ***(Panigrahi et al., 2008).***

B cell activation is abnormal in patients with SLE. The number of B cells at all stages of activation is increased in the peripheral blood of patients with active SLE**.** These B cell abnormalities can precede the development of SLE ***(Nagy et al., 2005).***

Autoantibodies:

B lymphocytes of SLE patients display lack of self-tolerance, and inappropriate overproduction of antibodies which penetrate membranes of living cells, bind to cytoplasmic or nuclear structures, and alter cell function ***(Graham et al., 2004).*** The presence of antinuclear antibodies (ANA) is the immunological hallmark of SLE, in clinical practice; ANA testing is often used as a part of an initial investigative screen. A positive ANA is a sensitive test, found in 98% SLE patients ***(Lyons et al., 2005),*** but the presence of anti-DNA antibodies is a much more specific finding as it is present in about 60% of SLE patients ***(Hirabayashi, 2006).*** Serial serum concentrations of these antibodies reflect disease activity in many patients, but not all. It is now clear that anti-DNA antibodies are, in some way, directly pathogenic by direct binding or in complexes to DNA deposited in glomerular basement membranes and other renal structures ***(Pavlovic et al., 2010).***

In addition to anti-DNA antibodies, a variety of other autoantibodies are often detected known as Non-DNA autoantibodies: anti-Sm antibodies ***(Rahman et al., 2008),*** anti-Ro/SS-A associated with an increased risk of the development of a photosensitive rash and also directed against the conducting tissues of the fetal heart from maternal blood ***(Clancy et al., 2004),*** and anti-La/SS-B antibodies, antibodies directed against surface glycoprotein II and III antigens on intact platelets and antibodies against cytoplasmic antigens of

platelets ***(To & Petri, 2005),*** antiphospholipid antibodies (anticardiolipin or lupus anticoagulant)**,** anti-nucleosome antibodies which play a role in cutaneous lupus also have a direct pathogenic effect on renal cell ***(Zheng et al., 2009),*** anti-NMDA *(*N-methyl-D-aspartate) receptor antibodies are present in the brain tissue of patients with cerebral lupus causing cognitive impairment and hippocampal damage ***(Kowal et al., 2006)*** and antibodies against ribosomal P ***(Ersvaer et al., 2004).*** Thus, these resulting in large immune complexes which are cleared by the mononuclear phagocytic cells and small immune complexes which deposit in tissues resulting in widespread tissue damage ***(Carroll, 2000).***

Table (1): Pathogenic Autoantibodies in Systemic Lupus Erythematosus ***(Rahman & Isenberg, 2008):***

Antigen specificity	Prevalence (%)	Main Clinical Effects
Anti-Double stranded DNA	70-80	Kidney & skin disease
Nuleosomes	60-90	Kidney & skin disease
Ro	30-40	Skin & kidney disease, fetal heart problems
La	15-20	Fetal heart problems
Sm	10-30	Kidney disease
NMDA receptor	33-50	Brain disease
Phospholipids	20-30	Thrombosis, pregnancy loss
∝-Actinin	20	Kidney disease
C1q	40-50	Kidney disease

Complement:

Complement is involved in the clearance of immune complexes, and its function is somehow disturbed with the development of lupus. The association between genetic complement deficiencies and the development of lupus triggered early speculation about a possible role for complement in the etiology of SLE**.** It was observed that in patients with SLE, complement consumption, with falling serum concentrations, often mirrors disease activity ***(Karim, 2006).*** With increased interest in apoptosis, defective clearance of apoptotic fragments, aberrant tolerance induction or changes in cytokine regulation may provide the link between complement dysfunction and SLE ***(Gaipl et al., 2006).*** On the other hand, complement also takes part in the inflammatory reaction that gives rise to the tissue and organ damage occurring in SLE ***(Truedsson et al., 2007).***

Cytokines:

Cytokines are low molecular weight structures which act as the chemical modulators of the immune system; it would be a good potential site for dysfunction and convenient therapeutic target ***(Chung et al., 2006).***

* ***Interleukin-6 (IL-6):*** seems to be increased in the cerebrospinal fluid (CSF) of patients with central nervous system (CNS) involvement in SLE but not in patients with SLE who lack neurological symptoms. It is important to

note that cytokine production is changed in patients with SLE with different disease phenotypes ***(Dean et al., 2000).*** An increasing number of studies supporting that raised levels of IL6 in SLE may influence the development of anaemia ***(Ripley et al., 2005).*** Recent study proved that IL-6 is a potential biomarker and therapeutic target in the prevention of joint damage in SLE arthritis ***(Eilertsen et al., 2011).***

* ***Interleukin-10 (IL-10):*** it is secreted by T-helper cells and stimulates B-cell proliferation and antibodies production in SLE ***(Beebe et al., 2002).*** The serum concentration of IL-10 in lupus patients is significantly higher than that seen in normal controls ***(Lacki et al., 1997).*** Studies suggested a genetic basis for elevated levels of IL-10 contributing to the pathogenesis of SLE and correlate with the activity of the disease ***(Gibson et al., 2001).***

* ***Interleukin-12 (IL-12):*** which promotes cell mediated immune responses but exerts some inhibitory activities on humoral responses, is lower in SLE patients than in healthy controls ***(Mok & Lau, 2003).*** IL12 production is also lower in patients with active disease when compared with those with inactive disease ***(Dean et al., 2000).***

* ***Tumour necrosis factor α (TNF α):*** it may be protective factor against lupus ***(Jacob et al., 1990),*** so the development of anti-TNFα drugs has provided a new angel on the hypothesis that blocking TNFα may be involved in the