Introduction

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease in which diverse immunological events can lead to a similar clinical picture, characterized by a wide range of clinical manifestations and many target organinvolvement with unpredictable flares and remissions that eventually lead to permanent injury ***(Salgado and Herrera-Diaz,2012).***

Lupus nephritis (LN) occurs in up to 50% of SLE patients. It is one of the most serious SLE complications associated with significant morbidity and mortality and it is the major predictor of poor prognosis. Severe LN has been reported to result in end-stage kidney disease at a rate of 10-26%, which may be a result of the difficulty in recognizing a flare early enough to affect the course of the disease since prompt diagnosis and early treatment lead to better outcomes ***(Rubinstein et al., 2008).***

Current laboratory markers for LN such as proteinuria, urine protein-to-creatinine ratio, creatinine clearance, anti-dsDNA antibodies, and complement levels are unsatisfactory. They lack sensitivity and specificity for differentiating renal activity and damage in LN. Significant kidney damage can occur before renal function is impaired and before the first detection by laboratory parameters ***(Mok, 2010).***

Although the activity scores, **[**e.g., Systemic Lupus Erythematosus Disease Activity Index **(SLEDAI)** and Systemic Lupus Activity Index **(SLAM)]** have diagnostic utility for disease activity, they do not accurately assess either severity or changes in nephritic activity, and serologic evaluations alone are insufficient. Ideally, biomarkers should indicate the severity of nephritis and

guide therapy at various stages of disease ***(Manoharan and Michael, 2010).***

Thus, novel biomarkers that are able to discriminate lupus renal activity and its severity, predict renal flares, and monitor treatment response and disease progress are clearly necessary. Urinary biomarkers are easily obtained and probably are the best at reflecting the current renal status, as they specifically represent local inflammatory activity ***(Mok, 2010).***

The TNF superfamily cytokine "Tumor necrosis factor–like weak inducer of apoptosis" (TWEAK) induces mesangial cells, podocytes, and endothelial cells to secrete pro-inflammatory chemokines including Monocyte Chemoattractant Protein-1**(MCP-1)**, Interferon inducible Protein-10 **(IP-10)** and Regulated on Activation, Normal T Cell Expressed and Secreted **(RANTES)**, which are crucial in the pathogenesis of LN ***(Noa et al., 2006).***

The major role of TWEAK in LN is centered on its pro-inflammatory and chemokine inducing effects ***(Campbell et al., 2006).***

Schwartz and his colleagues (2009) supported a role for TWEAK in the pathogenesis of LN, and provided strong evidence for urinary TWEAK (uTWEAK) as a candidate clinical biomarker for LN.

Blocking TWEAK/Fn14 interactions may be a promising therapeutic target in immune-mediated renal diseases ***(Gao et al., 2009).***

Aim of the Work

To clarify the role of uTWEAK in diagnosis of lupus nephritis and its correlation -if any- to the disease activity.

Systemic lupus erythematosus and Lupus nephritis

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease in which diverse immunological events can lead to a similar clinical picture, characterized by a wide range of clinical manifestations and target organs affection (phenotypes) with unpredictable flares and remissions that eventually lead to permanent injury.Lupus nephritis (LN) is one of the most serious SLE complications since it is the major predictor of poor prognosis ***(Wang et al., 2008).***

About 50-80% of patients with lupus suffer from LN & 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 ***(Werth et al., 1997)***.

Predisposing Factors of SLE:

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

1- Environmental triggers:

These factors may not only exacerbate existing lupus conditions but also trigger the initial onset. They include certain medications (such as some antidepressants and antibiotics), extreme stress, and exposure to sunlight, hormones, and infections. These stimuli cause the destruction of cells and expose

their DNA, histones, and other proteins, particularly parts of the cell nucleus ***(Wang et al., 2008).***

2- Genetics of SLE:

Research indicates that SLE may have a genetic link. Lupus does run in families, but no single "lupus gene" has yet been identified. Instead, multiple genes appear to influence a person's chance of developing lupus when triggered by environmental factors. The most important genes are located in the human leucocyte antigen (HLA) region; especially Class II (DR, DQ, DP) and class III (C2, C4,) on chromosome 6, where mutations may occur randomly (*de novo*) or may be inherited. Other genes which contain risk variants for SLE are shown in **Table (1)** ***(Hahn et al., 2008).***

The concordance rate for lupus is 25% among monozygotic twins and approximately 2% among dizygotic twins, these rates indicate that a genetic contribution is important, but it is not sufficient to cause the disease. Many genes that probably contribute to lupus have been identified by means of whole-genome scans from families in which multiple members have lupus ***(Namjou et al., 2007).***

Table (1): The currently known genes and gene regions associated with SLE ***(Hahn et al., 2008)***.

Genes involved in human lupus
1.HLA genes
Extended haplotypes predispose to lupus -HLA-B8/DRB1*0301/DQB1*0201/C4AQO(anti Ro) -HLA-DRB1*1501/DQB1*0602(nephritis, low levels of tumor necrosis factor-α) -HLA-DRB1*0101/DQB1*0402 -HLA-A10/B18/C4A4/C4B2/BFS(associated with C2 deficiency) -DR2 -DR3 -DR2/DQw1(anti Ro) -DR3/DQw2(anti Ro plus anti La) -DR2 with DQw6 or DQw7 and others (anti-Sm) -DR4,DR7 with DQw7,DQw8,DQw6, and others(lupus anti coagulant) -Homozygous deficiency of early complement components(C2,C4)
2.Non HLA genes
-Protein tyrosine phosphatase 22(PTPN22) polymorphism C chromosome1. -C1q (chromosome 1). -FcγRIIA receptor allele (chromosome 1). -Promotor polymorphisms of interleukin-10(chromosome1).

Further studies have shown that the genetic basis of LN predisposition exists in two aspects. On one hand, some susceptibility alleles of candidate genes are associated with LN disease severity. On the other hand, there exists a set of kidney-specific genes that are likely to amplify or sensitize patients to autoimmune pathology of LN. Association studies evaluate candidate genes based on their function in the immune system or their aberrant expression in lupus patients. Some important candidate genes associated with LN are summarized in **Table (2)** ***(Morel, 2007)*****.**

Table (2): Candidate genes associated with LN ***(Morel, 2007)***

Gene	Full name	Variation
Kidney-specific targeting		
FCGR3A	Fcγreceptor III-A	V/F158
FCGR3B	Fc γ receptor III-B	Copy number variation (CNV)
ACE	Angiotensin converting enzyme	Alu I/D
MCP-1	Monocyte chemo attractant protein-1	A–2518G
AGT	Angiotensinogen	M235T
IL-8	Interleukin-8	T–845C
PAI-1	Plasminogen activator inhibitor-1	675 4G4G indel
eNOS	Endothelial nitric oxide synthase	Intron 4 repeat
EPCR	Endothelial protein C receptor	A6936G
Amplification of the autoimmune pathology		
CCR5	C-C chemokine receptor 5	D32
SPP1	Osteopontin	C707T
HLA-DQA	DQ alpha	DQA\0101
HLA-DQB	DQ beta	DQB\0201
PDCD1	Programmed cell death 1	PD1.3G/A
ER	Estrogen receptor	PpXx
MBL2	Mannose binding lectin 2	Gly54Asp
UG	Uteroglobin	A38G
IFNG	IFNγ	Allele 114

3- Hormonal Factors in SLE:

The strongest risk factor for development of SLE appears, to be female sex. The female to male sex ratio of 9:1 in SLE is observed during the peak reproductive years, with a gradual decline in the ratio after menopause ***(Petri, 2002)***.

Lupus flares are caused by use of oral contraceptives, administration of estrogen, and ovulation induction regimens suggesting that sex hormones modulate the incidence or severity of disease in patients with SLE. Conversely, ovarian failure (and presumably, reduced estrogen concentrations) has been associated with reduced rate of lupus flares ***(Mok et al., 1999).***

On the other hand, androgens tend to be immunosuppressive. Serum levels of didehydroepiandrosterone (DHEA), an intermediate compound in testosterone synthesis, are found to be low nearly in all patients with SLE, this might be mediated by impaired interleukin-2 (IL-2) production in SLE patients ***(Suzuki et al., 1995).*** Experimental studies demonstrated that lupus can be ameliorated by oophorectomy or treatment with male hormones (***Schur, 2002***).

A better understanding of hormonal relationships in SLE could lead to novel and improved application of hormonal immunotherapy ***(McMurray et al., 2003).***

4- Abnormalities in Immune tolerance:

Highly autoreactive B lymphocytes and T lymphocytes are deleted, inactivated, or suppressed in healthy individuals by immune tolerance. Mechanisms of tolerance include deletion (B cells and T cells), anergy (B cells and T cells), B-cell receptor (BCR) editing (achange in the light chain of the antibody expressed by an autoreactive B lymphocyte), cytokine shifts (Th1

to Th2 cytokine shifts during T cell development), and induction of regulatory cells (suppressing B cells and T cells). Tolerance steps occur at several points along cell development, beginning with immature or naive cells in the thymus (T cells) or bone marrow (B cells) and extending to peripheral lymphoid organs (B cells and T) ***(Jacobi et al., 2005).***

There are several points in B cell development during which deletion of autoreactive cells can occur. It was found that 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 ***(Dorner et al., 2001).***

The intrinsic check points in B cell development are influenced by sex hormones; second signals (especially CD40/CD40L, both of which are overexpressed in SLE B cells and T cells); BAFF (B-cell activating factor), which is elevated in some SLE patients; and cytokines that are B cell growth factors, such as IL-6 and IL-10 (both are increased in some SLE patients). Ability of this intrinsic B cell tolerance to proceed in an orderly fashion is altered by external factors, and by genetic background and the tolerogenic or activated states of antigen presenting cells (APC_s), including dendritic cells (DCs) ***(Jacobi et al., 2005).***

Pathogenesis of SLE:

The pathogenesis of SLE is complex. It depends on the co-ordinated interactions bridging innate and adaptive effector arms of the immune system **(Fig. 1 & 2*) (Hicks and Bullard, 2006 & Walter et al., 2012).***

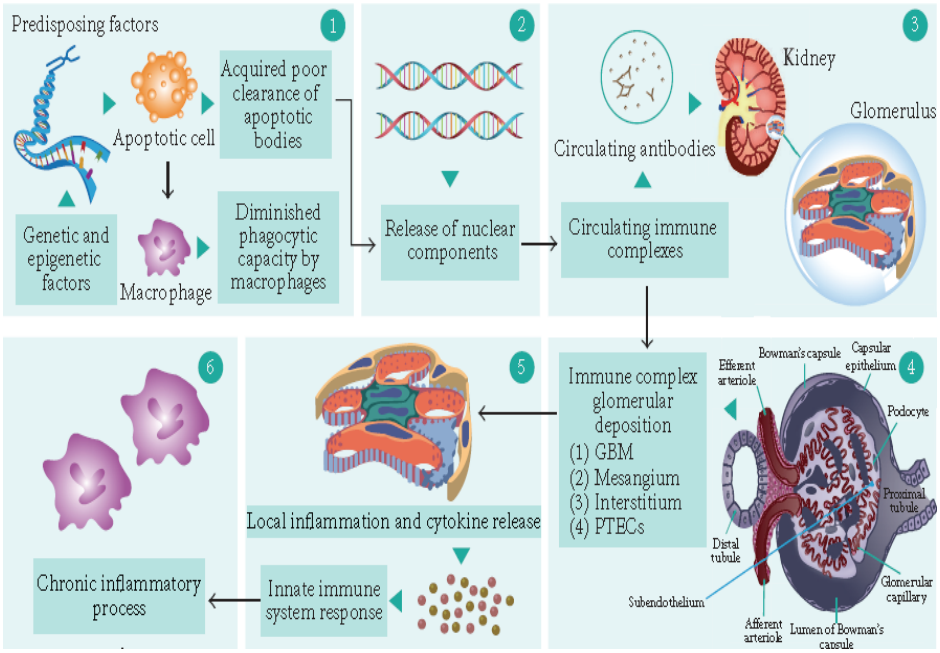

Fig. (1): Lupus nephritis: an imbalance between cytokine homeostasis and IC deposition ***(Hicks and Bullard, 2006).***

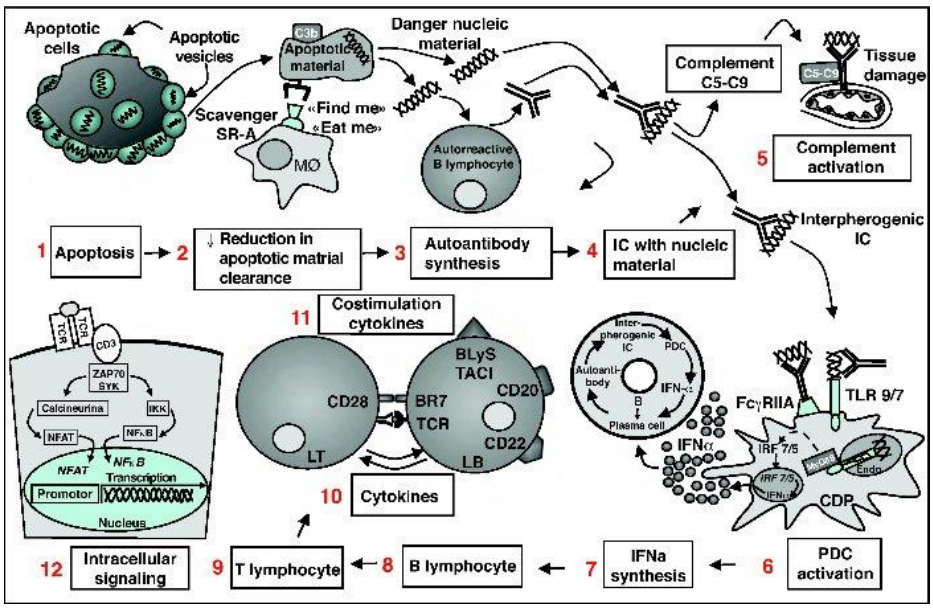

Fig. (2): Panoramic vision of pathogenesis of SLE ***(Walter et al., 2012).***

1- Apoptosis as a source of Autoantigens in SLE:

One manifestation of lupus is abnormalities in apoptosis, (a type of programmed cell death in which aging or damaged cells are neatly disposed of as a part of normal growth or functioning).The obvious source of nucleosomes is the cellular debris released as a result of apoptosis. During apoptosis, blebs of cellular material form on the surface of the dying cell. Antigens thatare normally buried within the cells are exposed on the surface of these blebs; and they may trigger an immune response. These exposed antigens include nucleosomes, Ro 62, Ro 50, La, and anionic phospholipids **(Fig. 3)** ***(Rahman et al., 2008)***.

The clearance of early apoptotic cells is an important function in multicellular organisms. If this ability is disturbed, the apoptosis process progress and finally secondary necrosis of the cells occurs. Necrotic cells release nuclear fragments as potential autoantigens as well as internal danger signals, inducing maturation of DCs, since they have lost their membranes integrity. Increased apoptosis also leads to inefficient clearance that leads to maturation of DCs and also to the presentation of intracellular antigens of late apoptotic or secondary necrotic cells, via MHC molecules. Autoimmunity possibly results by the extended exposure to these nuclear and intracellular autoantigens. In this case B and T cell tolerance for apoptotic cells is abrogated, and the lymphocytes get activated by these autoantigens; inflammation and the production of autoantibodies by plasma cells is then initiated ***(Gaipl et al., 2007).***

Impaired clearance of dying cells is a potential pathway for the development of this systemic autoimmune disease. A clearance deficiency in the skin for apoptotic cells has also been observed in patients with cutaneous lupus erythematosus (CLE).

The impaired clearance may be attributed to deficient phagocytic activity and scant serum components (e.g., complement factors, C-reactive protein (CRP)) in addition to increased apoptosis ***(http://wapedia.mobi/en/ Systemic_ lupus_erythematosus.2009).***

In vitro studies proved that the removal of apoptotic debris is abnormal in patients with lupus. Phagocytes from patients with lupus were shown to engulf far less apoptotic material than phagocytes from healthy people during a 7-day culture period ***(Herrmann et al., 1998).***

C1q plays a role in phagocytosis by binding to cell debris, which can then be engulfed by macrophages that have surface C1q receptors. Thus, a deficiency of complement may be an important reason for the poor waste disposal seen in lupus. Homozygous deficiencies of C1q, C2, and C4 are rare disorders, but the presence of any of these genetic conditions was proved to be a strong predisposing factor for lupus ***(Walport, 2002).***

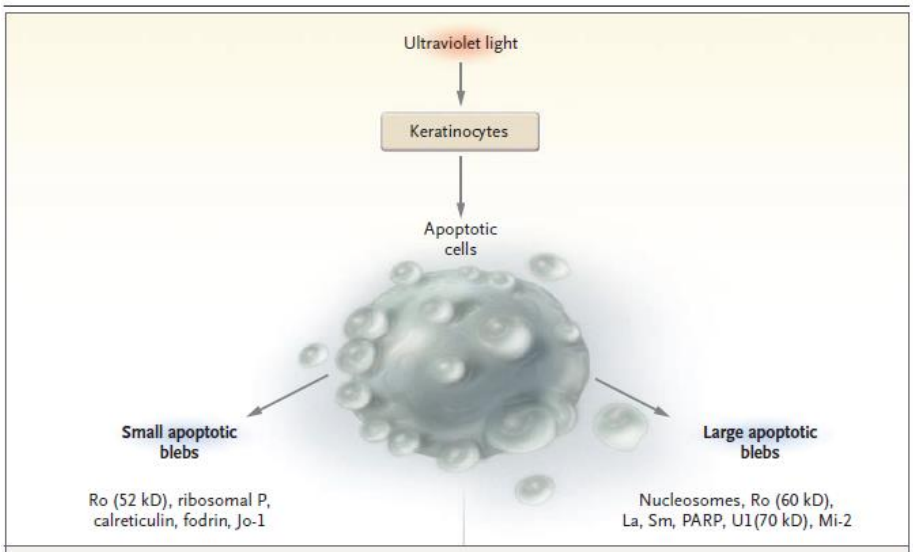

Fig. (3): Induction of Surface Blebs during Apoptosis (PARP denotes poly–ADP–ribose polymerase) ***(Rahman et al., 2008)***.

In SLE, monocytes isolated from whole blood of patients show reduced expression of CD44 surface molecules involved in the uptake of apoptotic cells. Most of the monocytes and tangible body macrophages (TBM), which are found in the germinal centres (GC) of lymph nodes and responsible for removal of apoptotic bodies, even show a definitely different morphology in patients with SLE; they are smaller or scarce and die earlier. Serum components like complement factors, CRP -which are decisively important for an efficiently operating phagocytosis- are often missing, diminished, or inefficient **(Fig. 4)** ***(http://en.wikipedia.org /wiki/ Systemic_lupus_ erythematosus# cite_note18. 2009)***.

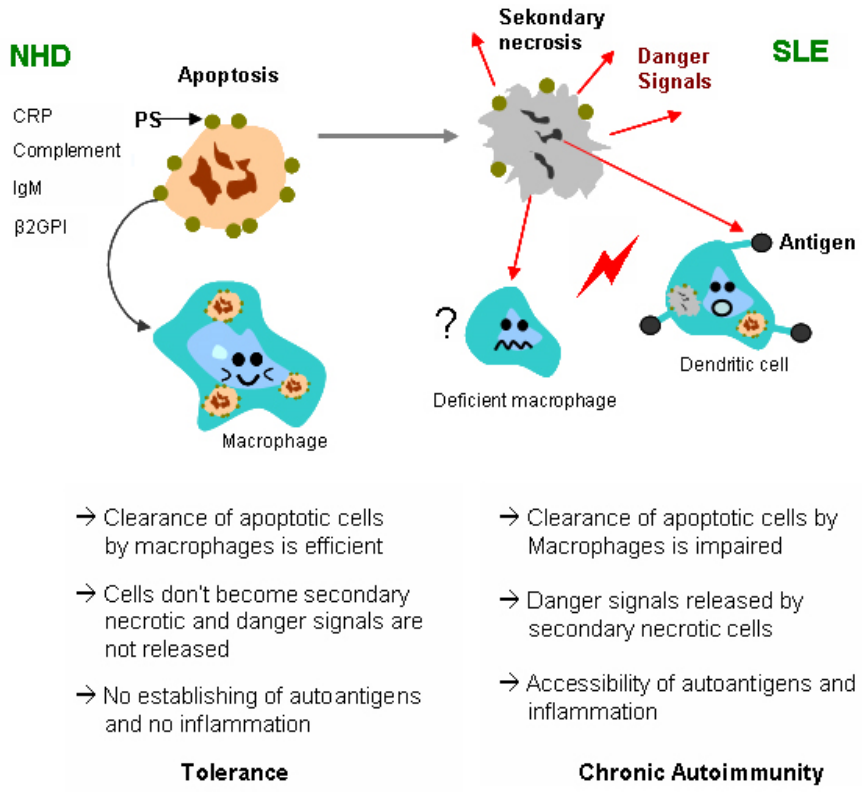

Fig. (4): Defects in the clearance of apoptotic cells ***(http://en.wikipedia.org/wiki/Systemic_lupus_erythematosus#cite-note-_18.2009)***.

TBMs are large phagocytic cells in the germinal centers of secondary lymph nodes; they express CD68 protein (is a glycoprotein which binds to low density lipoprotein. It is expressed on monocytes/macrophages and is used to identify macrophages and giant cells).These cells normally engulf B cells that have undergone apoptosis after somatic hypermutation. In some patients with SLE, significantly fewer TBMs were found, where they rarely contain material from apoptotic B cells. Also, uningested apoptotic nuclei were found outside of TBMs. This material may present a threat to the tolerization of B cells and T cells. Dendritic cells in the GC may endocytose such antigenic material and present it to T cells, activating them. Also, apoptotic chromatin and nuclei may attach to the surfaces of follicular dendritic cells (FDCs) and make this material available for activating other B cells that may have randomly acquired self-specificity through somatic hypermutation. These autoreactive B cells migrate into the GC light zone ***(Gaipl et al., 2006).***

Autoreactive B cells, maturated coincidentally, normally do not receive survival signals by antigen planted on FDCs, and perish by apoptosis. In the case of clearance deficiency, apoptotic nuclear debris accumulates in the light zone of GC and gets attached to FDC. This serves as a germinal centre survival signal for autoreactive B-cells. After migration into the mantle zone, autoreactive B cells require further survival signals from autoreactive helper T cells, which promote the maturation of autoantibody-producing plasma cells and B memory cells. In the presence of autoreactive T cells, a chronic autoimmune disease may then be the consequence ***(http://en.wikipedia.org /wiki/Systemic_ lupus_ erythematosus#cite_note-18. 2009).***

Moreover, ***Gaipl et al. (2006)*** showed that apoptosis is increased in monocytes and keratinocytes in lupus patients. In addition, they showed that the expression of Fas by B cells and T cells is increased and there were correlations between the apoptotic rates of lymphocytes and disease activity.

2- Autoantibodies in SLE (Table 3):

All individuals produce numerous antibodies that react with self-molecules. Characteristics of the normal background antiself repertoire include the following: most of the antibodies are IgM, they have weak avidity for self-antigens, and they are widely cross-reactive with multiple antigens. Pathogenic autoantibodies are different. They are usually IgG, have high avidity for self-antigens, and have restricted specificity. Pathogenic immunoglobulin molecules are often highly mutated, particularly in the hyper variable (complementarity determining) regions of their heavy and light chains. The autoantibodies of SLE are directed against DNA/protein complexes, RNA/protein complexes, cell membrane structures, and intracellular molecules that reach cell surfaces during cell activation. The antibodies considered to be the hallmark of SLE are IgG antibodies to double-stranded DNA (ds-DNA) ***(Hahn et al., 2008).***