Significance of Antichromatin Antibodies in Egyptian Patients with Systemic Lupus Erythematosus

Thesis

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List of abbreviations

ACR..... American college of rheumatology
ALT ..... Alanine transminase
ANA ..... Antinuclear antibody
APCs ..... Antigen presenting cells
AST ..... Aspartate transaminase
BILAG..... British isles assessment group
BLyS ..... B-lymphocyte stimulator
BUN ..... Blood urea nitrogen
CD ..... Cluster of differentiation
CIC ..... Circulating immune complexes
CNS ..... Central nervous system
COX-2 ..... Cyclo-oxygenase 2
CPK ..... Creatine phosphokinase
Cr. Clearance... Creatinine clearance
CTLA ..... Cytotoxic T-lymphocyte-associated protein
DHEA..... Dehydroepiandrosterone
DLE ..... Discoid lupus erythematosus
DNA ..... Deoxyribonucleic acid
DNP..... Deoxyribonucleic protein
ds-DNA ..... Double stranded DNA
ECLAM..... European Consensus Lupus Activity Measurement
ELISA ..... Enzyme linked immunosorbent assay
EM..... Electron microscopy
ESR ..... Erythrocyte sedimentation rate

ESRD..... End stage renal disease
GBM..... Glomerular basement membrane
GFR..... Glomerular filtration rate
GI..... Gastro-intestinal
Hb..... Hemoglobin
HLA..... Human leucocytic antigen
IF ..... Immunofluoresence
IL ..... Interleukin
IMT ..... Intima-media thickness
INR..... International normalized ratio
LE..... Lupus erythematosus
LM..... Light microscopy
LN ..... Lupus nephritis
MCTD ..... Mixed connective tissue disease
MMF ..... Mycophenolate mofetil
MR..... Magnetic resonance
NIH..... National institute of health
NSAIDs..... Non-steriodal anti-inflammatory drugs
PBC ..... Primary biliary cirrhosis
PLT..... Platelets
RNA ..... Ribonucleic acid
SAD..... Systemic autoimmune diseases
SCLE..... Subacute cutaneous lupus erythematosus
S. creat..... Serum creatinine
SLAM..... Systemic lupus activity measures
SLE..... Systemic lupus erythematosus

SLEDAI ..... Systemic lupus erythematosus disease activity index.

Sm ..... Smith

SnRNP..... Small nuclear ribonucleoprotein

ssDNA..... Single stranded DNA

UV ..... Ultra violet

WBC..... White blood cells

WHO ..... World Health Organization

Introduction

SLE is a non-organ specific autoimmune disease characterized by widespread inflammation, affecting virtually every organ and/or system in the body, and by the production of various autoantibodies, in particular antinuclear autoantibodies ***(Saisoong et al., 2006)***.

Lupus nephritis refers to a spectrum of glomerulopathies that range from minor focal scarring to diffuse proliferative destruction of glomeruli with active inflammation and immune complex deposition ***(Peter and Merkel, 2001)***.

It is one of the most serious manifestations of SLE and is associated with considerable morbidity and even mortality. Treatment remains problematic, particularly in terms of controlling the underlying disease process while at the same time preventing unacceptable side effects of therapy ***(Agrawal et al., 2006)***.

ANAs are autoantibodies directed against chromatin and its individual components including double-stranded DNA (ds-DNA) and histones, and some ribonucleoproteins ***(Saisoong et al., 2006)***.

In the last few years, several reports have shown that chromatin represents the main autoantigen-immunogen in SLE and that specific antibodies are an important marker of the disease ***(Villalta et al., 2005)***.

Antinucleosome antibody could be an early and sensitive tool in the diagnosis and assessment of disease activity in SLE patients who are negative for anti-dsDNA antibodies ***(Saisoong et al., 2006)***.

The measurement of antichromatin antibodies appears to be a useful addition to the lab tests that can help in the diagnosis and treatment of SLE. These antibodies are both sensitive and specific for SLE, and are a useful marker for an increased risk of lupus nephritis ***(Cervera et al., 2003)***.

Aim of the work:

So the aim of this study was to assess the value of antichromatin antibodies in diagnosing SLE, specially anti-ds-DNA negative patients, assessing disease activity and to determine the association of these antibodies with lupus nephritis, in Egyptian SLE patients.

Systemic Lupus Erythematosus

Introduction:

SLE lacks a single, unifying pathognomonic marker It is a disease with a complex set of immunologic abnormalities that appear to involve multiple mechanisms of dysregulation and that may be linked to more than twenty different genetic determinants. Early in the disease course, the signs may be subtle or may be suggestive of other entities, such as rheumatoid arthritis, palindromic rheumatism, antiphospholipid syndrome, Still's disease, rheumatic fever, sarcoidosis, Lyme disease, multiple sclerosis, thrombotic thrombocytopenic purpura, cryoglobulinemia, viral illnesses, vasculitis and even fibromyalgia ***(Edworthy, 2005).***

Epidemiology: SLE varies among age, race and sex. Lupus affects both men and women; however, women aged 15-45 years are affected more frequently than men ***(Lash and Lusk, 2004)***.

The female: male ratio is 9: 1 (or greater) ***(Petri, 2006)***.

Age at onset can range from infancy to advanced age; in both pediatric and older-onset patients, the female:male ratio is approximately 2:1. ***(Ward et al., 1995).***

Because of empiric research and better diagnosing, the cases of SLE are increasing. In one study the occurrence of SLE was found to be 7.3 per 100,000 cases ***(Ruiz-Irastorza et al., 2001)***.

Race-based statistics show a higher incidence in African American women than in white women ***(Walsh et al., 1995)***.

Being of black ethnicity also increases the severity of SLE, leading to greater morbidity and mortality ***(Bongu et al., 2002)***.

Although women are affected more often than men, men who have SLE develop more severe pathophysiology outcomes related to hematologic, neurologic, renal and vascular disease ***(Petri, 2005)***.

SLE is predominantly a postpubertal female disease. In women with SLE, systemic DHEA supplementation allowed for greater prednisone reduction and fewer flares. In men with SLE, androgens worsen disease ***(Petri, 2006)***.

Although the cause of SLE remains unknown, certain genetic, hormonal, and environmental factors clearly influence the course and severity of disease expression. A genetic predisposition for the development of SLE is supported by the following:

- A high concordance rate among monozygotic twins.
- The significant percentage of relatives of patients with SLE who develop the disease (5% to 12%).
- The high frequency of certain HLA genotypes (e.g., HLA-B8, DR3, and DQW1) ***(Schur, 1995).***

Environmental factors other than estrogens also may affect the occurrence and expression of SLE. The classic environmental precipitant of SLE is UV light. UV-B can be blocked by sun blocks and by glass and UV-A from computer and television screens by plastic. Smoking is a risk factor for lupus ***(Costenbader et al., 2004)***.

In patients already diagnosed with lupus, smoking increases the risk of discoid lupus and reduces the efficacy of antimalarial drugs. Infections have long been suspected to play a role in SLE: pet dogs of patients with lupus are more likely to have lupus ***(Chiou et al., 2004)***.

Laboratory personnel preparing lupus sera are more likely to develop lupus auto antibodies. The strongest case

for an infectious precipitant is the high association of Epstein-Barr viral infection with SLE in both children and adults in multi case SLE families ***(James et al., 2001)***.

Antibodies of Epstein-Barr occur before the onset of lupus-specific autoantibodies. Toxic exposures including silica and mercury, have also been associated with SLE ***(Cooper et al., 2004)***.

Pathogenesis:

Although numerous immunologic abnormalities have been noted in patients with SLE, it is unclear which factors are directly related to the pathogenesis of the disease itself and which factors are epiphenomena. SLE is a disease in which abnormalities of immune regulation lead to a loss of self-tolerance and subsequent autoimmune responses ***(Kotzin, 1996).***

The main immunologic dysfunction of SLE is the deposition of immune complex in various cells that precipitates inflammation with eventual organ pathology. Vasculopathy and vasculitis are related to the sequelae of endothelial cell damage. The similarities in pathologic responses seen in most organ system dysregulation are abnormality in cellular basement membranes, cellular proliferation, inflammation, and the deposition of IgM, IgG,

IgA into cells and tissues. Complement components are also activated. Activated complement consists of membrane and plasma proteins that modify cell membranes and promote inflammation ***(Atkinson and Liszewski, 2001)***.

Inflammation of endothelial cells and deposits of immune complex stimulate inflammation in blood vessels, leading to venous and arterial thrombosis. Auto antibodies common in SLE may stimulate coagulopathic responses. Specific autoantibodies known to trigger clot formation are lupus anticoagulants, anticardiolipin antibody and antiphospholipid antibody ***(Pisetsky, 2001)***.

SLE has been associated with a decreased number of cytotoxic and suppressor T cells, increased helper (CD4+) T cells, polyclonal activation of B cells, defective B cell tolerance and dysfunctional T cell signaling. Some of these abnormalities contribute to the activation and clonal expansion of CD4+ T cells, which, by means of cytokine release, cause activation of autoreactive B cells, leading to their proliferation and differentiation into cells that produce excess antibodies against nuclear antigens ***(Elkon, 1995).***

Ultimately, unique idiotypic autoantibodies are produced by clones of B cells, leading to high levels of

antibodies directed against nuclear antigens such as ANA, DNA, Sm, RNA, Ro and La ***(Chabre et al., 1995).***

The formation of circulating immune complexes and their deposition with complement activation are important in certain patterns of glomerular damage. Immune complexes are detectable in the skin at the dermal-epidermal junction and in the choroid plexus, pericardium and pleural spaces. The propensity of these immune complexes to cause disease depends not only on size and charge but also on the rate of clearance by Fc receptors in the liver and spleen ***(Appel et al., 2000).***

Diagnosis: In 1971, criteria were developed to deal with a growing concern that epidemiologic studies and clinical trials involving lupus patients, would be difficult to compare with one another if there was no documented consensus on what constituted the findings of systemic lupus. The revised American College of Rheumatology classification criteria were published eleven years later, before the demonstration of the antiphospholipid syndrome and also before the plethora of studies on new autoantigens was reported. One of the driving forces of the revised criteria, was to incorporate the antinuclear antibody tests, that had become widely available and that had essentially replaced the LE cell preparation for detecting the

characteristic laboratory finding of lupus. Another factor was to rely less heavily on invasive diagnostic procedures, such as the kidney biopsy. A third consideration was to remove elements such as Raynaud's phenomenon and alopecia because the low specificity of these criteria combined with the low prevalence of SLE in the population will cause a high false-positive rate of classification ***(Hochberg, 1997).***

Changes have been recommended to two of the criteria. Dr. Marc Hochberg called for the deletion of the LE cell preparation criteria and established the accepted methods for identifying antiphospholipid antibodies based on anticardiolipin antibodies, lupus anticoagulant testing or a false positive serologic test for syphilis ***(Edworthy, 2005)***.

The classification criteria, however, are heavily weighted toward cutaneous manifestations, and are limited to psychosis and seizures in the neurologic criterion. The ACR has established definitions of neuropsychiatric manifestations of SLE. In terms of laboratory tests, the classification criteria omit hypocomplementemia, one of the most useful laboratory manifestations. An effort is now under way to revise and update the ACR classification criteria ***(Petri, 2006)***.

Table(II-1**) :** Revised criteria of the ACR for the classification of SLE.

1-Malar rash	Fixed erythema flat or raised over the malar eminences tending to spare the nasolabial folds.
2- Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging. Atrophic scarring may occur in older lesions.
3- Photosensivity	Skin rash as a result of unusual reaction to the sunlight by patient history or physician observation.
4- Oral ulcers	Oral or nasopharyngeal ulceration usually painless observed by physician.
5- Arthritis	Nonerosive arthritis involving two or more peripheral joints characterized by tenderness and swelling or effusion.
6- Serositis	Pleuritis - Convincing history of pleuritic pain or rub heard by physician or evidence of pleural effusion. Pericarditis - Documented by ECG or rub or evidence of pericardial effusion.
7- Renal disorders	a) Persistent proteinuria greater than 0.5 gm/ day or greater than 3+ b) Cellular casts may be red cell, hemoglobin, granular, tubular or mixed.
8- Neurological disorders	a) Seizure in the absence of drugs or known derangement e.g. uremia, or electrolyte imbalance. b) Psychosis in the absence of drugs or known derangement e.g uremia, or electrolyte imbalance.
9- Hematologic disorders	**a)** Hemolytic anemia with reticulocytosis, or **b)** Leukopenia less than 4,000/cubic mm total on two or more occasions or lymphopenia less than 1,500/cubic mm on two or more occasions. Or **c)** Thrombocytopenia less than 100,000/cubic mm in the absence of offending drugs.
10- Immunologic disorders	**a)** Anti DNA: antibody to native DNA in abnormal titers or **b)** Anti Sm: presence of antibody to Sm nuclear antigen or **c)** Positive antiphospholipid antibodies.
11- Antinuclear antibody	An abnormal titer of antibody by immuno-fluorescence or an equivalent assay at any point of time and in the absence of drugs known to be associated with "drug induced lupus syndrome".

Clinical Findings:

Commonly Involved Organ Systems:

Mucocutaneous:

The cutaneous system is one of the most commonly affected, approaching 80%-90% ***(Tsakonas et al., 1998)***.

Examination of the skin should include inspection for malar rash, discoid rash, Raynaud's phenomenon, acral cyanosis, periungual erythema, livedo reticularis and maculo-papular rashes of the trunk or extremity. Of these, the most strongly associated with SLE is malar rash ***(Werth et al., 1997).***

Notably, four of the 11 criteria can be fulfilled in this system alone. SLE-specific skin lesions are classified into three types-chronic, subacute, and acute-based strictly on clinical appearance and duration, without considering the extracutaneous manifestations or laboratory features of the overall disease ***(Buyon, 2001).***

The most common form of chronic disease is discoid lupus (DLE), which occurs in 15%-30% of people with SLE. It can occur as part of the systemic disease or it can exist in isolation in the absence of any autoantibodies (2%-10% will develop SLE). DLE lesions are discrete plaques, often erythematous, covered by scales that extend into dilated

hair follicles. These lesions most typically occur on the face, scalp, in the pinnae, behind the ears, and on the neck. They can be seen in areas not exposed to the sun. The lesions can progress, with active indurated erythema at the periphery. Central atrophic scarring is characteristic. Irreversible alopecia can result from follicular destruction ***(Hahn, 2001)***.

Alopecia, particularly when it is associated with inflammatory changes in the scalp, is a sign of lupus. Hair thinning often occurs when patients have suffered a flare of lupus and may also be associated with immunosuppressant drugs. Hairs at the anterior margin of the scalp may have a stiff, spike appearance (lupus hairs) and allow one to evaluate the patient's claims of hair problems ***(Sontheimer, 1996).***

Lupus panniculitis-lupus profundus is a less-common form of chronic disease. These lesions spare the epidermis and represent involvement of the deep dermis and subcutaneous fat. The lesions of lupus panniculitis are firm nodules generally without surface changes. In time, the overlying skin becomes attached to the subcutaneous nodular lesions and is drawn inward, resulting in deep depressions ***(Hahn, 2001)***.

Subcute cutaneous lupus erythematosus (SCLE) lesions are seen in 7%-27% of patients. SCLE primarily affects caucasian women. The lesions are typically symmetric, widespread, superficial, and nonscarring, and are most often present in sun-exposed areas. The lesions begin as small, erythematous, scaly papules or plaques that can evolve into papulosquamous (psoriasiform) or annular polycyclic forms. The latter often coalesce to produce large confluent areas with central hypopigmentation. Generally, both forms are nonscarring. Antibodies to SSA/Ro ribonucleoproteins are commonly found in people with SCLE ***(Buyon, 2001)***.

Perhaps the most classic of all the rashes in SLE is the malar, or butterfly, rash, which is categorized among the acute rashes. It occurs in 30%-60% of all patients. This erythematous and edematous eruption simulates the shape of a butterfly with its body bridging over the base of the nose and wings spreading out over the malar eminences. At times the same-rash can be seen on the forehead and chin, but it classically spares the nasolabial folds. The absence of discrete papules and pustules distinguishes it from acne rosacea. The rash is abrupt in onset and can last for days. Postinflammatory changes are common, particularly in patients with pigmented skin. The butterfly rash is often initiated and/or exacerbated by exposure to sunlight.

However, patients can have a photosensitive erythematous rash. The criteria for photosensitivity and butterfly rash are thus independent of each other ***(Hahn, 2001)***.

Mucosal lesions are also part of the clinical spectrum of SLE and can affect the mouth (most commonly), nose, and anogenital area. Although oral lesions can be seen on the buccal mucosa and tongue, sores on the upper palate are particularly characteristic. They typically are described as painless, but need not be ***(Buyon, 2001)***.

Vasculitis is another component of skin disease in SLE. It may be manifest as urticaria, palpable purpura, nail-fold or digital ulcerations, erythematous papules of the pulps of the fingers and palms, or splinter hemorrhages ***(Buyon, 2001)***.

Musculoskeletal Involvement:

Arthritis is the most common sign that patients exhibit over time, although there may be an age-dependent distribution of the symptom that affects the prevalence of the finding at disease onset as well as over the course of the disease. Arthritis that progresses to erosions and deformity is rare. The non-erosive deforming arthropathy found in SLE, which carries the eponym Jaccoud's arthropathy, involves the same distribution as rheumatoid arthritis, including the metacarpophalangeal joints, wrists and meta-

tarsophalangeal joints. More common is the finding of arthralgia in these joints that migrates during a 24-to 48 hour period across several joint regions, including both the large and small joints of the appendicular skeleton. On occasion, one can feel warmth in these joints, but typically there is little evidence of inflammation aside from some mild soft tissue swelling around the joints. This degree of inflammation is typically not debilitating. In fact, it is usually the associated fatigue that patients complain is the most debilitating symptoms ***(Richter Cohen et al., 1998).***

Rheumatic complaints localized to the hips should raise serious consideration of osteonecrosis, the frequency of which has been reported at 5%-10%. Although the femoral head is the most common site of involvement, other sites include the femoral condyles, talus, humeral head, and occasionally the metatarsal heads, radial head, carpal bones, and metacarpal bones. Bilaterality is frequent but not necessarily simultaneous. Most cases are associated with the use of corticosteroids, but causality has also been attributed to Raynaud's phenomenon, small vessel vasculitis, fat emboli, or the presence of anti-phospholipid antibodies. Typically, people with osteonecrosis complain of persistent painful motion localized to a single joint, often relieved by rest ***(Buyon, 2001)***.

Respiratory System:

The lung and contiguous structures involved in normal respiration are commonly affected by SLE. More than 30% of patients have some form of pleural disease in their lifetime, either as pleuritis with chest pain or frank effusion. Pleurisy is a more common feature of serositis than pericarditis. The pain of pleuritis can be quite severe and must be distinguished from pulmonary embolus or infection. Pleural rubs are less common than either clinical pleurisy or radiographic abnormalities. Pleural effusions are most often small and bilateral. The fluid is usually clear and exudative, has increased protein, and normal glucose. The WBC count is elevated but <10,000 with a predominance of neutrophils or lymphocytes, and decreased levels of complement ***(Buyon, 2001).***

A clue to pulmonary involvement may be cough or chest pain on breathing; however, neither has a high positive predictive value. Chest pain occurs in approximately 50% of patients, but this can often be demonstrated to be chest wall pain involving musculo-skeletal elements ***(Edworthy, 2005).***

Cough is most often associated with upper respiratory tract infection of a viral etiology. Nevertheless,

pulmonary involvement in lupus must be carefully assessed to ensure that acute lupus pneumonitis, characterized by pleurisy, dyspnea, cough, and fever with radiologic findings of pulmonary infiltrates, is not present. Once infection has been ruled out, it is imperative that immunosuppressant therapy and supportive respiratory care be given. Prognosis is poor (50 percent mortality) and a sequela for survivors is severe restrictive lung disease ***(Edworthy, 2005).***

Interstitial pneumonitis leading to fibrosis occurs occasionally; the inflammatory phase may respond to treatment; the fibrosis does not ***(Hahn, 2001).***

The lung also exhibits an unusual "shrinking" phenomenon in some patients, which could be due to either a weakened or fibrotic diaphragmatic muscle or involvement of the phrenic nerve ***(Rubin and Urowitz, 1983).***

Hematologic Involvement:

Rarely is anemia, leukopenia or thrombocytopenia the presenting feature of SLE without concomitant problems of the skin, joints, CNS or cardiopulmonary systems. Once SLE has been identified and routine monitoring of patients is undertaken, it is common to find anemia, often with low mean corpuscular volume, of chronic

inflammatory processes that inhibit iron transfer. Coomb's-positive anemia, indicating an antibody-mediated process, is relatively uncommon when patients are observed longitudinally.It is usually responsive to high-dose glucocorticoids; resistant cases may respond to splenectomy ***(Keeling and Isenberg, 1993)***.

Leukopenia is common but is rarely associated with recurrent infections and does not require treatment ***(Hahn, 2001).***

Thrombocytopenia is often documented, and although it is of some concern with respect to bleeding propensity if levels drop below 30,000, the association with antiphospho-lipid syndrome and the sequelae of thromboembolic disease is of greatest concern ***(Jorfen et al., 1998).***

Severe thrombocytopenia should be treated with high-dose glucocorticoids. Short term improvement can be achieved by administration of intravenous gamma globulin. If the platelet count fails to reach acceptable levels in 2 weeks, addition of cytotoxic drugs, cyclosporine, danazole, and/or splenectomy should be considered ***(Hahn, 2001).***

Ocular Involvement:

With regard to the eye itself, cotton-wool spots in the retina are generally cited as being the most common lesion,

followed in frequency by corneal and conjunctival involvement, with patients only rarely exhibiting uveitis or scleritis. Although also quite uncommon, retinal damage from antimalarials used in treating SLE is probably a greater cause of vision loss than is retinal involvement occurring in the natural course of the disease. Cotton-wool spots (an ophthalmologic term) result from focal ischemia and are not pathognomonic for lupus. They occur preferentially in the posterior part of the retina and often involve the optic nerve head. Cytoid bodies refer to the histologic features of the cotton-wool spot ***(Buyon, 2001).***

Knowledge about the mechanism of ocular involvement and pathogenesis in SLE is limited. This is an important issue, because the ocular symptoms in this disease could be potentially sight threatening in acute cases. Reports indicated an involvement of anti-phospholipid antibodies in the retinal and choroidal vasculopathy in SLE, although their precise role in this process is uncertain ***(Nag and Wadhwa, 2006)***.

Nervous System involvement:

The pathogenesis of neuropsychiatric systemic lupus erythematosus has been attributed to autoantibody-

mediated neural dysfunction, vasculopathy, and coagulaopthy ***(Valesini et al., 2006)***.

Approximately two-third of people with SLE have neuropsychiatric manifestations***(Boumpas et al., 1995).***

Neuropsychiatric systemic lupus includes neurologic syndromes of the central, peripheral, and autonomic nervous systems, and psychiatric disorders in which other causes have been excluded. These manifestations may occur as single or multiple events in the same person. Symptoms can be present concomitantly with activity in other systems, or exist in isolation ***(Hahn, 2001).***

A variety of psychiatric disorders are reported and include mood disorders, anxiety, and psychosis. Unequivocal attribution to lupus is difficult because such disorders may be related to the stress of having a major chronic illness, or may be due to drugs, infections, or metabolic disorders. Patients can demonstrate significant cognitive defects such as attention deficit, poor concentration, impaired memory, and difficulty in word finding. These abnormalities are best documented by neuropsychological testing and a decline from a higher former level of functioning. Another syndrome of diffuse neurologic dysfunction is acute confusional state, defined as disturbance of consciousness or level of arousal with

reduced ability to focus, maintain, or shift attention, accompanied by cognitive disturbance and/or change in mood, behavior, or affect. The syndrome often develops over a brief time-frame, fluctuates over the day, and covers a wide spectrum ranging from mild alterations of consciousness to coma ***(Buyon, 2001)***.

Inclusive in the neurologic manifestations of the central nervous system are seizures, which may be focal or generalized. Headache is a common complaint in patients, but there is still debate as to whether this is a feature attributable to SLE. The "lupus headache" has been defined as severe, disabling, persistent, and not responsive to narcotic analgesics ***(Hahn, 2001).***

Chorea is the most common movement disorder observed in SLE. This and cerebrovascular accidents have been related to the presence of antiphospholipid antibodies. Disturbances of the cranial nerves can result in visual defects, blindness, papilledema, nystagmus or ptosis, tinnitus and vertigo, and facial palsy. Peripheral neuropathy may be motor, sensory, mixed motor-sensory, or mononeuritis multiplex. Transverse myelitis presenting with lower extremity paralysis, sensory deficits, and loss of sphincter control has been observed in a limited number of people with SLE. An acute inflammatory demyelinating

polyradiculoneuropathy (Guillain-Barre syndrome) has been described ***(Buyon, 2001)***.

Examination of the cerebrospinal fluid is useful for ruling out infection. However, with regard to neuropsychiatric lupus, the findings often are nonspecific. Elevated cell counts, protein levels, or both, are found in only about one-third of patients. The fluid may be completely normal in the face of acute disease. Computerized tomography is sufficient for the initial diagnosis of most mass lesions and intracranial hemorrhages. The findings of magnetic resonance imaging (MR) reflect the histopathologic findings of vascular injury and may involve the white or gray matter ***(West et al., 1995).***

Cardiovascular involvement:

A variety of cardiac complications are seen in SLE, but the most common is pericarditis. Symptoms may either be mild or severe. A pericardial rub may or may not be present and can be heard in an asymptomatic patient. Although the electrocardiogram may show the typical T-wave abnormalities, echocardiography is the best diagnostic test. Most effusions are small to moderate. The pericardial fluid is straw-colored to serosanguinous, exudative, and can have a high WBC count (up to 30,000

cells/mm^3) with a predominance of neutrophils. LE cells can be seen in the centrifuged cell sediment. Cardiac tamponade is rare, as is constrictive pericarditis ***(Buyon, 2001)***.

The patient may have fever, dyspnea, palpitations, heart murmurs, sinus tachycardia, ventricular arrhythmias, conduction abnormalities, or congestive heart failure. It is now well recognized that hemodynamically and clinically significant valvular disease occurs and may require prosthetic valve replacement. Aortic insufficiency represents the most commonly reported lesion ***(Roldan et al., 1996).***

Libman-Sacks "atypical verrucous endocarditis", the classic cardiac lesion of SLE, is comprised of verrucous vegetations ranging from 1 mm to 4 mm in diameter, initially reported to be present on the tricuspid and mitral valves. Interestingly, it has been noted that neither the usual clinical and immunologic markers of lupus activity, nor its treatment, are temporally related to the presence of or changes in valvular disease. Prophylactic antibiotics for surgical and dental procedures are recommended for all people with SLE ***(Roldan et al., 1996).***

Accelerated atherosclerosis has received considerable attention. It is an important cause of morbidity and mortality from myocardial infarction and is approximately 10 times greater in people with SLE than in the general age- and sex- matched population ***(Urowitz and Gladmann, 2000).***

Studies have identified hypercholesterolemia, hypertension, and lupus itself as risk factors in these patients ***(Petri, 2000).***

Corticosteroid therapy contributes to the elevation of plasma lipids, while antimalarials may result in a reduction of plasma cholesterol low-density lipoprotein, and very low-density lipoprotein. Coronary arteritis is rare and may coexist with atherosclerotic disease. Studies of clinical outcomes for atherosclerotic disease, including angina and myocardial infarction, have shown a prevalence of 6%-12% in a number of SLE cohort ***(Urowitz and Gladmann, 2000).***

More sensitive investigations, including carotid plaque and intima-medial wall thickness (IMT) measured by B-mode ultrasound, revealed that 40% of 175 women with SLE had focal plaque ***(Manzi et al., 1999).***

Gastrointestinal involvement:

Gastrointestinal symptoms are often difficult to interpret in patients with systemic lupus erythematosus. Symptoms can develop either from symptomatic auto-immune tissue injury, complications of lupus-related organ dysfunction, infections, thromboembolic manifestations of anti-phospholipid antibody syndrome, medication or unrelated disorders ***(Witt et al., 2006)***.

Common GI symptoms include nausea vomiting, rebound tenderness, fever, diarrhea and vague discomfort. Symptoms may result from lupus peritonitis and may herald a flare of SLE. Vasculitis of the intestine is the most dangerous manifestation, presenting with acute crampy abdominal pain, vomiting and diarrhea. Intestinal perforation can occur and usually requires immediate surgery. Patients with pseudoobstruction have abdominal pain, x-rays show dilated loops of small bowel which may be edematous; surgery should be avoided unless frank obstruction is present. Glucocorticoid therapy is useful for all these GI syndromes ***(Hahn, 2001).***

Abdominal pain in SLE can also be caused by pancreatitis. A number of lupus patients develop episodes of acute "idiopathic" pancreatitis, unrelated to the known causes of mechanical obstruction of the pancreatic duct or

toxic-metabolic etiologies. This lupus-associated pancreatitis is rare ***(Breuer et al., 2006)***.

Rectal bleeding can be present in mesenteric vasculitis. Protein-losing enteropathy is quite uncommon, but should be considered in the face of low serum albumin, pedal edema and the absence of proteinuria . The peritoneum is the least likely of the serosal linings to be affected in SLE ***(Buyon, 2001).***

Abnormalities of liver function are not included in the diagnostic criteria of SLE, and the liver is generally not a major target organ for damage in patients with SLE. Nonetheless, patients with SLE have a 25% to 50% chance of developing abnormal results on liver tests at some point.The percentage doesn't include unconjugated hyperbilirubinemia due to hemolysis associated with SLE, or elevated AST caused by SLE-associated myositis ***(Van Hoek, 1996).***

Disease Activity:

Remission of all lupus activity is unusual. Control of activity with medications such as corticosteroids, immunosuppressants, and antimalarials can be achieved with some risk of iatrogenesis. Decisions regarding therapy are made while taking into consideration the degree of

damage that may result from untreated disease activity. Therefore, determining activity in a systematic fashion is a prerequisite to optimal clinical care ***(Edworthy, 2005).***

Laboratory Markers of Disease Activity:

Of the laboratory markers of disease activity of SLE, no single test has been proved sufficiently reliable, sensitive and specific to be used in isolation ***(Isenberg et al., 1984).***

The erythrocyte sedimentation rate (ESR) is probably the most useful non specific laboratory marker for distinguishing between active and inactive disease, but may be normal even in the presence of major organ involvement and, conversely, may remain raised in remission, being maintained by changes in immunoglobulin and lipids ***(Morrow et al., 1982).***

ESR is not useful in differentiating SLE activity from an intercurrent infection ***(Hughes and Khamashta, 1994).***

C-reactive protein, the classical acute phase protein, is seldom raised in acute disease unless the exacerbation is accompanied by serositis or infection ***(Borg et al., 1990).***

Of the more specific tests for SLE, raised titers of antibodies to DNA and low complement usually indicate active disease. In particular if significant changes in these parameters are seen, they often predict the development of renal disease ***(Borg et al., 1990).***

Assessment of Disease Activity:

Clinical activity scales:

The course of SLE is characterized by exacerbations (flare) and remissions, usually in response to treatment. A clinical activity index would enable standardization of the definition of flare and measurement of the reduction in disease activity in response to treatment. Such scales could then be used to indicate the need for, or response to, treatments in clinical trials, or to monitor the course of the disease in longitudinal studies of outcome ***(Hay et al., 1993). Liang et al., (1989)*** reported more than 60 scales for assessment of SLE activity.

Three of the currently available activity scales have been shown to be reliable (BILAG, SLAM, SLEDAI) ***(Hay et al., 1993).***

1. The British isles assessment group (BILAG) index:

In 1984, a group of British rheumatologists convened with the aim of developing and testing a disease activity

index which would score disease activity in different organ systems separately, and which would by suitable for use in studies designed to follow up patients longitudinally. It incorporates treatment decisions into the assessment ***(Symmons et al., 1988).***

2. Systemic lupus activity measures (SLAM):

Liang et al. (1989) in Boston developed the systemic lupus activity measure (SLAM), which contains disease manifestations originally derived from a review of publications and refined by clinical judgment using a consensus technique. It covers symptoms that occurred over the previous month and includes 24 clinical manifestations and 8 laboratory parameters to evaluate organ systems that could not otherwise be assessed, parameters of immune function are not included.

3. Systemic lupus disease activity index (SLEDAI):

The SLE disease activity index (SLEDAI) was developed in 1985 at the conference in prognostic studies in lupus convened in Toronto ***(Bombardier et al., 1992).***

SLEDAI consists of 24 variables covering nine organ systems (including some immunological tests) scored according to weights derived using multiple regression techniques. A final weighted total SLEDAI score is then calculated. This generated a weighted index of 9 organ

systems for disease activity in SLE, the SLEDAI, as follows:

8 for central nervous system and vascular,

4 for renal and musculoskeletal,

2 for dermal, serosal, and immunologic,

1 for constitutional and haematologic.

The maximum theoretical score is 105.

It was demonstrated that this index performs satisfactorily when compared with expert's global ratings of disease activity ***(Bombardier et al., 1992).***

Table (II-2): SLEDAI scoring system.

Weight	SLEDAI score	Descriptor	Definition
8	-----	Seizure	Recent onset. Exclude metabolic infectious or drug causes
8	-----	Psychosis	Altered ability to function in normal activity due to sever disturbance in the perception or reality. Include hallucination incoherence, marked loose association, marked illogical thinking, bizarre, disorganized or catatonic behavior. Exclude uremia and drug causes.
8	-----	Organic brain syndrome	Altered mental function with impaired orientation, memory or other intellectual function, with rapid onset and fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain alteration in the environment, plus at least two of the following perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.
8	-----	Visual disturbance	Retinal changes of SLE. Include cited bodies, retinal hemorrhages, serious exude or hemorrhages in the chord, or optic neuritis. Exclude hypertension, infection or drug causes.
8	-----	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	-----	Lupus headache	Severe persistent headache, may be migranous, but must be non responsive to narcotic analgesia
8	-----	CVA	New onset of cerebrovascular accidents. Exclude atherosclerosis.
8	-----	Vasculitis	Ulceration gangerene, tender finger nodules, periungual infarction, splinter hemorrhages or biopsy or angiogram proof of vasculitis
4	-----	Arthritis	More than two points with pain and signs of inflammation (i.e. tenderness swelling or effusion)
4	-----	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase aldolase or electromyogram changes or a biopsy showing myositis
4	-----	Urinary casts	Heme, granular or red blood cell casts
4	-----	Hematuria	>5 red blood cell/high power field. Exclude stone, infection, or other cause

4	-----	Proteinuria	>0.5 gm 24 hours. New onset or recent increase of more than 0.5gm /24 hours
4	-----	Pyuria	>5 white blood cells/ high power field. Exclude infection
2	-----	New rash	New onset or recurrence of inflammatory type rash
2	-----	Alopecia	New onset or recurrence of abnormal patchy or diffuse loss of hair
2	-----	Mucosal ulcers	New onset or recurrence of oral or nasal ulceration
2	-----	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening
2	-----	Pericarditis	Pericardial pain with at least one of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2	-----	Low complement	Decrease in CH50, C3, or C4 below the lower normal of testing laboratory
2	-----	Increased DNA binding	> than 25% binding by farr assay or above normal range for testing laboratory
1	-----	Fever	>38C. Exclude infectious cause
1	-----	Thrombocytopenia	<100,000 platelets/cubic mm.
1	-----	Leucopenia	<3,000 white blood cell/cubic mm. exclude drug cause.
Total score			

Management of SLE

Because most therapeutic interventions in patients with SLE are associated with significant undesirable side effects, the physician must first decide whether a patient needs treatment and if so, whether conservative management is sufficient or aggressive immunosuppression is necessary. In general, patients with manifestations of SLE that are not life-threatening and are unlikely to be associated with organ damage should be treated conservatively. On the other hand, if the disease is life threatening or if major organ systems are at high risk for irreversible damage aggressive intervention is mandatory ***(Hahn, 2005).***

Arthritis Arthralgia and Myalgia:

Arthritis, arthralgia and myalgia are the most common manifestations of SLE. For patients with mild symptoms, administration of analgesics, NSAIDS or salicylates may provide adequate relief although non of these is as effective as glucocorticoids ***(Wallace, 2002).***

The effectiveness of NSAIDs varies for individual patients, and the choice of agent is dictated by cost, availability, tolerance and effectiveness. Adverse effects of NSAIDs on the kidney, liver and CNS may be confused

with worsening lupus activity ***(Ostensen and Villiger, 2000).***

Gastrointestinal toxicities are the most common side effects of the nonselective cyclooxygenase (COX) inhibitors at doses that effectively control inflammation. Although there are no published studies specifically examining the safety and efficacy of the selective COX-2 inhibitors in SLE, these agents are expected to reduce gastrointestinal side effects, as they have in other populations ***(Manzi, 2001).***

In many SLE patients, musculoskeletal symptoms are not well controlled by NSAIDs alone. A trial of antimalarial drugs may be useful in such individuals ***(Wallace, 2002).***

Combinations of antimalarials are thought to be synergistic and are commonly used when one agent alone is not effective. Particular benefit, including steroid-sparing effect, has been reported with hydroxychloroquine (200-400 mg/d). It is estimated that only 10% of patients have difficulties tolerating hydroxychloroquine. Mild side effects include gastric upset, headache, myalgia, or rash. Much of the concern over use of antimalarial agents centers around the risk of ophthalmologic toxicity which is relatively rare

at the recommended low doses used to treat SLE ***(Manzi, 2001).***

We should have baseline retinal examination before starting this therapy. If hydroxychloroquine is used for more than 6 months, regular examination for retinal damage is mandatory ***(Hahn, 2005).***

Evaluation should include visual acuity, slit-lamp, fundoscopic, and visual-field testing ***(Manzi, 2001).***

Cutaneous Lupus:

Photosensitive patients should minimize their exposure to UV light by wearing protective clothing, using tinted glass in car windows, avoiding direct exposure and applying sunscreens ***(Ting and Sontheimer, 2001)***.

Costicosteroids are effective in the management of many different manifestations of SLE. Topical or intralesional preparations often are used for cutaneous lesions ***(Manzi, 2001).***

Antimalarial agents are useful in many patients with lupus dermatitis, whether the lesions are those of SLE, subacute cutaneous lupus or discoid lupus. Antimalarials

have multiple sunblocking, anti-inflammatory and immunosuppressive effects ***(Wallace, 2002).***

Thalidomide can be effective for the treatment of discoid lupus. Maintenance doses of 25-50 mg/d resulted in partial remission in 30% and complete remission in 60% of 30 lupus patients with discoid lesions refractory to corticosteroid and antimalarial therapy ***(Godfrey et al., 1998).***

The well-recognized risk of teratogenicity prevents the use of this agent in women planning pregnancy ***(Manzi, 2001).***

Serositis:

Episodes of chest and abdominal pain may be secondary to lupus serositis. In some patients, complaints respond to salicylates, NSAIDs (indomethacin may be best), or antimalarial therapies, or to low doses of systemic glucocorticoids. In others, systemic gluco-corticoids must be given in high doses to achieve disease control ***(Hahn,2005).***

Aggressive Therapy:

Institution of aggressive therapy, beginning with high dose glucocorticoids, should be used whenever a

patient has life-threatening SLE that is likely to respond to steroids, such as: Vasculitis, polyserositis, Myocarditis, hemolytic anemia, thrombocytopenia and glomerulo-nephritis. Infectious causes of the manifestations interpreted as lupus must be carefully excluded before instituting or increasing glucocorticoid therapy. The physician must also consider the presence of comorbid conditions that increase the risk of glucocorticoid therapy, such as infection, hypertension, diabetes mellitus, obesity, osteoporosis, and psychiatric disorders **.** Several problems arise during treatment with glucocorticoids such as: the toxic side effects, failure of some patients to respond and relapse of some patients when the dose is tapered. The criteria for response should include both clinical and laboratory parameters. If the desired effect is not obtained within the appropriate time frame, the next decision is whether to change the glucocorticoid dose, to introduce additional therapy, or to stop immunosuppression ***(Hahn, 2005).***

Several experts have studied the efficacy of administering methylprednisolone (10 to 30 mg/kg, 500-1000 mg/dose), in single high intravenous doses for three to six doses, then maintaining responses with high doses of

daily oral prednisone (40-60 mg) which are rapidly tapered ***(Wallace, 2002).***

Combination therapy with glucocorticods and cytotoxic drugs is probably superior to glucocorticoids alone in controlling acute severe SLE, reducing irreversible tissue damage, minimizing maintenance glucocorticoid requirements and prolonging survival ***(Bansal and Beto, 1997).***

The two drugs most frequently used are azathioprine and cyclophosphamide; of the two cyclophosphamide is more effective especially in lupus nephritis, however it is more toxic ***(Fox and McCune, 1994).***

Azathioprine is a purine analogue that inhibits nucleic-acid synthesis and affects both cellular and humoral immune function. The most common side effects are bone marrow and gastrointestinal toxicity. Regular monitoring of complete blood counts during therapy is recommended. Because of hepatic metabolism and urinary excretion, renal and liver function should be checked periodically. Dosage adjustment may be necessary in patients with renal or hepatic dysfunction. An increased risk of malignancy, such as non-Hodgkin's lymphoma has been reported following treatment with azathioprine ***(Manzi, 2001).***

Cyclophosphamide has been one of the most extensively studied immunosuppressive agents for the treatment of SLE in the past several decades ***(Ortmann and Kippel, 2000).***

Nausea and vomiting are common side effects of cyclophosphamide treatment, but effective antiemetic regimens minimize the gastrointestinal toxicity. Hair loss, which occasionally can be significant, typically reverses with cessation of therapy. Patients treated with cyclophosphamide are at increased risk of bacterial, fungal, and viral infections, with particular susceptibility to herpes zoster ***(Manzi, 2001).***

Gonadal toxicity from cyclophosphamide can result in gonadal failure or azoospermia ***(Slater et al., 1999).***

Acrolein, a drug metabolite of cyclophosphamide, is a bladder irritant and has been incriminated in the hemorrhagic cystitis, fibrosis, and transitional squamous cell carcinoma seen in people on long-term cyclophosphamide therapy. Daily oral cyclophosphamide poses the highest risk and should be accompanied by generous daily fluid intake. In patients treated with intravenous bolus cyclophosphamide, adequate hydration and administration of mesna, an acrolein binder, are common practices, particularly in patients with a history of

cystitis. Patients treated with cyclophosphamide should be screened routinely for bladder carcinoma, which can develop years after discontinuation of therapy***(Manzi, 2001).***

We are about to enter a new era in the treatment of patients with SLE. For the past 40 years hydroxychloroquine sulfate and corticosteroids, together with varying combinations of immunosuppressive drugs, have been the main treatments for SLE. Although effective for many patients, some patients fail to respond to these drugs and even more suffer from major side effects due to the generalized nature of the immunosuppression. There has been a remarkable confluence of new therapies ranging from newer immunosuppressive drugs with fewer side effects, such as mycophenolate mofetil, to the more targeted approaches offered by biological agents. These agents have been designed to block molecules such as CD20, CD22 and interleukin-10 that are thought to have an integral part in the development of SLE ***(Isenberg and Rahman, 2006)***.

The presence of auto-antibodies produced by B lymphocytes is a pathological hallmark of SLE. Development of high-affinity antibodies depends on the interaction of B cells with CD4 T-helper cells. Auto-antigen-specific T cells are found in patients with SLE and can interact with B cells to stimulate auto-antibody release.

The T cells themselves are stimulated in response to antigen on the surface of specialized antigen-presenting cells (APCs), which also provide costimulatory interactions to promote T-cell activation. The recognition of the central roles played by both B-cells and T cells in SLE lead to the idea that treatment strategies could target either of these cell types or the interaction between them. Thus, one might seek to deplete all B lymphocytes, using anti-CD20 or anti-CD22 monoclonal antibodies, or to specifically target B cells secreting anti-DNA antibodies using abetimus sodium. Alternatively, it is possible to interfere with B-cell activation and survival by using treatments that target the powerful B-lymphocyte stimulator (BLyS; also known as tumor necrosis factor ligand superfamily, member 13b) protein system. One could target T-helper cells specifically, using MMF, or could modulate the activation of T cells stimulated by APCs using cytotoxic T-lymphocyte associated protein 4 (CTLA4)-immunoglobulin or anti-CD40 ligand (CD40L) antibody ***(Isenberg and Rahman, 2006)***.

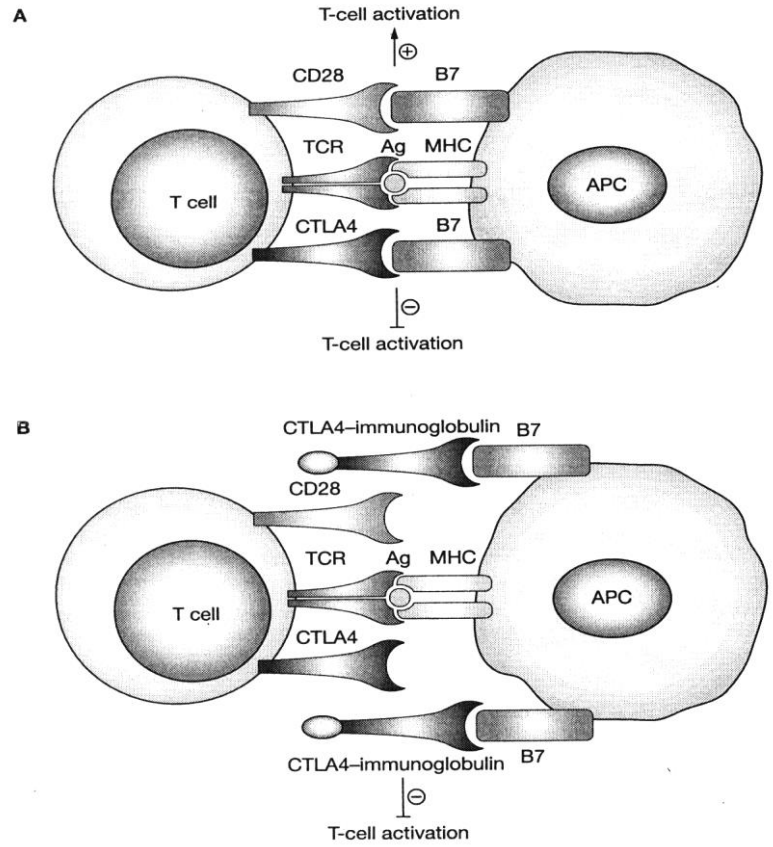

Fig.(II -1): Interactions between T cells and antigen-presenting cells. (A) The T-cell receptor recognizes the antigen-major histocompatibility complex on the surface of antigen-presenting cells. Interaction between CD28 on the T cell and B7 on the antigen-presenting cell provides a positive costimulatory signal, whereas interaction between CTLA4 and B7 is inhibitory to T-cell activation. If the positive signal through CD28-B7 interaction dominates, the T cell is activated resulting in cytokine production, provision of B-cell help and inflammation. If the CTLA4-B7 interaction dominates, T-cell activation is suppressed. (B) CTLA4-immunoglobulin is a fusion protein comprised of the extracellular domain of CTLA4 and the immunoglobulin constant region. CTLA4-immunoglobulin binds to B7 with a higher affinity than CD28 and thereby inhibits the CD28-B7 interaction, preventing T-cell activation. Ag, antigen; APC, antigen-presenting cell; CTLA4; cytotoxic T-lymphocyte-associated protein 4; MHC, major histocompatibility complex; TCR, T-cell receptor**.**

Cytokines are molecules secreted by cells that regulate immune responses, and act on target tissues and cells of the immune system. Designing treatments that interfere with cytokine function could therefore be effective in modulating immunity and controlling tissue inflammation at specific sites ***(Isenberg and Rahman, 2006)***.

B cells have a major role in the etiopathogenesis of SLE, principally by virtue of the auto-antibodies they produce, and possible through their function as APCs. B cells are characterized by specific markers, including CD-20 and CD-22. CD20 is present on all B-cell subsets except pre-B cells and plasma cells. The possibility of treating SLE using the monoclonal antibody rituximab, which binds specifically to CD20, in combination with other more established drugs such as corticosteroids and cyclophosphamide, is an attractive proposition ***(Looney et al., 2006)***.

Monoclonal antibodies to CD40L have been used to treat patients with SLE, but enthusiasm for their use has been tempered by the tendency for patients treated with this antibody to experience thromboembolic complications ***(Wallace, 2002)***.

By contrast, use of the fusion protein CTLA4-immunoglobulin, which blocks the binding of the

costimulatory B7 molecules expressed on APCs with CD28 on activated T cells, seems more promising and less problematic ***(Davidson et al., 2005)***.

Blocking the final common pathway C5-C9 of both the classical complement pathway and the alternative complement pathway could be an attractive approach for treating patients with SLE because both these pathways are activated in this disease ***(Schur and Klickstein, 2002)***.

Immune ablation followed by autologous stem cell transplantation or allogeneic stem cell transplantation has been used for many years to treat a variety of hematologic malignancies. This rather aggressive approach has also been used in the US and Europe for patients with severe SLE ***(Jayne et al., 2004)***.

Lupus Nephritis

Lupus nephritis (LN) is both a frequent and potentially serious complication of systemic lupus erythematosus (SLE). Serious kidney disease may influence morbidity and mortality both directly and indirectly through complications of therapy ***(Lenz and Contreras, 2004)***.

Epidemiology of Lupus Nephritis:

SLE is more likely to be associated with severe nephritis in children, and less likely to be associated with it in the elderly ***(McKundy et al., 1992).***

SLE appears to be both more common and have more severe renal involvement in the African-American population, although the precise roles of biologic-genetic factors versus socioeconomic factors have not been clearly defined ***(Barr et al., 2003).***

Lupus nephritis is present in approximately 25-50% of patients at the time of diagnosis and eventually develops in up to 60% of adults and 80% of children ***(Cameron, 1999)***.

The incidence of renal involvement is variable depending not only on the populations studied and the diagnostic criteria but also on whether involvement is defined by renal biopsy or clinical features, how vigorously clinical findings (such as urinary sediment changes) are sought for, and whether small amounts of immune deposits in the kidney indicate nephritis or merely the presence of systemic disease ***(Appel et al., 2000).***

Pathogenesis of Lupus Nephritis:

Lupus nephritis is an autoimmune disease. Its immunopathogenesis is characterized by the loss of self-tolerance. There is ample evidence to suggest a pathogenic role of nephritogenic autoantibodies. These antibodies cross react with nucleosomal epitopes ***(Tang et al., 2005)***.

Histones bind to the glomerular basement membrane (GBM) as well and may facilitate antinuclear antibody localization. Once deposited, the complement cascade is activated, leading to complement-mediated damage, activation of procoagulant factors, leukocyte infiltration, release of proteolytic enzymes, and various cytokines regulating glomerular cellular proliferation and matrix synthesis ***(Appel et al., 2000).***

Depression of classic complement pathway components and high titers of anti-DNA, anti-nucleosome, or anti-C1q antibodies identify patients at-increased risk of renal involvement or flares of nephritis ***(Balow, 2005)***.

Glomerular and vascular damage may be potentiated by hypertension and coagulation abnormalities. Glomerular and vascular lesions can be potentiated by the presence of antiphospholipid antibodies, directed against a phospholipid B_2-glycoprotein complex, and by their attendant alterations in endothelial and platelet function. These alterations include reduced production of prostacyclin and other endothelial anticoagulant factors, plasminogen activation, protein C or S inhibition, and enhanced platelet aggregation ***(Appel et al., 2000).***

Pathology of Lupus Nephritis:

The glomerular pathology of lupus nephritis is the result of diverse immune insults ***(Lewis and Schwartz, 2005)***.

This diversity of disease expression is evident when comparing adjacent glomeruli in a single biopsy or the biopsy findings of different patients. Moreover, the histo-pathologic features of LN have the capacity to transform or evolve from one pattern to another either spontaneously or

as a result of therapy. Initial attempts to classify biopsies of patients with SLE were hindered by the variable expression of the disease, its ability to transform, and the lack of a well-defined clinical pathologic correlation that reflected prognosis ***(Appel et al., 2000).***

Originally developed in the mid-1970s, the World Health Organization (WHO) Classification of Lupus Nephritis is now widely accepted by pathologists, nephrologists, and rheumatologists (Table II-**3**). It combines light microscopic (LM) findings with the immuno-fluorescence (IF) and electron microscopic (EM) findings to present a uniform classification system that is both accurate and precise ***(Churg and Sobin, 1982).***

Despite its widespread use and advantages over older classifications, the WHO system still has limitations. It is based on an evaluation of the glomerular lesions and does not integrate changes in extraglomerular structures such as the tubulo-interstitium and vasculature, which can influence both prognosis and therapy ***(Schwartz and Lewis, 2002)***.

Nevertheless, the system has proven effective in prognostication and has served as a useful guide for therapy. In the WHO system, biopsies are classified into

one of six categories according to glomerular changes by LM, IF and EM ***(Appel et al., 2000).***

This classification system has recently been revised by the international society of nephropathology and the renal pathology society ***(Weening et al., 2004)***.

Table (II-**3):** Modified WHO Classification of Lupus Nephritis.

Categories	Description
I	Normal glomeruli (by LM, IF, EM)
II	Mesangial glomerulonephritis
a.	Normal by LM, mesangial deposits by IF and/or EM
b.	Mesangial hypercellularity and deposits by IF and/or EM
III	Focal segmental glomerulonephritis
a.	Active necrotizing lesions
b.	Active sclerosing lesions
c.	Sclerosing lesions
IV	Diffuse glomerulonephritis (severe mesangial, endocapillary, or mesangiocapillary proliferation and/or extensive subendothelial deposits)
a.	Without segmental lesions
b.	With active necrotizing lesions
c.	With active and sclerosing lesions
d.	With sclerosing lesions
V	Diffuse membranous glomerulonephritis
a.	Pure membranous glomerulonephritis
b.	Associated with lesions of category II (a or b)
c.	Associated with lesions of category III (a-c)
d.	Associated with lesions of category IV (a-d)
VI	Advanced sclerosing glomerulonephritis

LM = light microscopy, IF = immunofluorescence, EM = electron microscopy. ***(Churg and Sobin, 1982)***

WHO class I biopsies have normal glomeruli by LM, IF and EM. Class I is rarely reported in series of biopsied lupus patient ***(Chabre et al., 1995).***

WHO class II biopsies show lesions confined to the mesangium. In WHO class IIa mesangial immune deposits are only detectable by IF or EM, and glomeruli are normal by LM. WHO class IIb biopsies have LM findings of mesangial hypercellularity in association with immune deposits in the mesangium. Mesangial proliferation and immune deposits are commonly found in all of the more severe patterns of LN. Mesangial proliferation, even if severe and diffuse, must be differentiated from true diffuse proliferative LN (WHO class IV) in which there are diffuse subendothelial immune deposits along the peripheral capillary walls ***(Appel et al., 2000).***

In WHO class III LN there is focal and segmental endocapillary proliferation ***(D'Agati, 1998).***

Some definitions of class III require that less than 50% of glomeruli be affected by segmental endocapillary proliferation. Others define class III as involvement of less than 50% of the total glomerular surface area by endocapillary proliferation lesions. By this definition,

endocapillary proliferation may be focal and global, or diffuse and segmental. Some patients with severe class III lesions clearly have a clinical picture and course akin to those with class IV lesions. This is especially true for biopsies showing a relatively high percentage of affected glomeruli and abundant necrotizing lesions and crescents. However, other patients with a small percentage of affected glomeruli and milder lesions (e.g., without necrotizing features or crescent formation) may have a better prognosis ***(Appel et al., 2000).***

Patients with WHO class IV diffuse proliferative LN have glomeruli with endocapillary glomerular proliferation qualitatively similar to those with class III, but the proliferation involves more than 50% of the glomerular capillary surface area ***(Cameron, 1997).***

In class IV, glomerular endocapillary proliferative lesions are usually diffuse and global. Biopsies reveal more extensive peripheral capillary wall subendothelial immune deposition, and extracapillary proliferation in the form of crescents is not uncommon in severe cases ***(Appel et al., 2000).***

Lesions of WHO class V, membranous glomerulo-nephritis, are similar in many ways to idiopathic

membranous nephropathy, with subepithelial glomerular immune deposits (class Va) ***(Pasquali et al., 1993).***

However, the coexistence of mesangial immune deposits and mesangial hypercellularity is encountered in most examples of membranous LN (Class Vb). As in idiopathic membranous glomerulopathy evaluated by LM, early class V biopsies may have no identifiable abnormalities. In well-developed membranous glomerulo-nephritis, there is typically a thickening of the glomerular capillary walls and "spike" formation. The presence of significant proliferative lesions beyond mesangial hyper-cellularity or more than sparse subendothelial immune deposits warrants a designation of combined class V and III or V and IV LN (also called class Vc or Vd) ***(Appel et al., 2000).***

In the revised WHO classification, there is a sixth class of LN (WHO class VI) reserved for those biopsy specimens with advanced sclerosing features. It may be difficult in such biopsies to even establish the diagnosis of LN without the identification of residual glomerular and extraglomerular immune deposits by IF and EM ***(Appel et al., 2000).***

The activity and chronicity indices:

The Bethesda group has proposed the so-called chronicity and activity indices, obtained by summing the scores attributed to certain histological features.Patients with a high chronicity index have much poorer preservation of renal function at 5 years than those with a low index ***(Esdaile et al., 1989)***.

The activity index is important for assessing the presence of lesions that can potentially progress to irreversible renal damage. However these lesions can be reversed by the prompt appropriate treatment. Thus the activity index does not have an absolute prognostic significance; rather it is particularly useful in deciding whether a treatment should be aggressive or not.

Table (II-**4):** Renal pathology scoring system in lupus nephritis*.

Activity index	Chronicity index
Glomerular abnormalities	1. Glomerular sclerosis.
1. Cellular proliferation.	2. Fibrous crescents
2. Fibrinoid necrosis, karyorrhexis	
3. Cellular crescents	
4. Hyaline thrombi, wire loops	1. Interstitial fibrosis
5. Leucocyte infiltration	2. Tubular atrophy
Tubulointerstitial abnormalities	
1. Mononuclear-cell infiltration	

Each factor is scored from 0 to 3.*Fibrinoid necrosis and cellular crescents are weighted by a factor of 2. Maximum score of the activity index is 24 , and of the chronicity index is 12.

Clinical Manifestations:

The diversity of clinical presentations of lupus nephritis parallel the deiversity of pathologic lesions seen in the kidneys of patients with SLE. Renal manifestations range from asymptomatic hematuria or proteinuria to overt nephritic and nephrotic syndromes, rapidly progressive glomerulonephritis, and chronic renal failure. Subclinical nephropathy both during presentation and during monitoring of disease activity is frequently missed because of the notorious unreliability of routine screening urinalyses performed in clinical pathology laboratories ***(Balow, 2005)***.

Renal involvement often develops concurrently or shortly following the onset of SLE and may follow a protracted course with periods of remissions and exacerbations. Clinical renal involvement usually correlates well with the degree of glomerular involvement in SLE. However, some patients may have severe vascular or tubulointerstitial disease leading to major clinical manifestations, despite a benign pattern of glomerular involvement ***(Appel et al., 2000).***

Those rare patients who have a totally normal renal biopsy by LM, IF and EM, according to WHO class I criteria, will have no evidence of clinical renal disease. Likewise, most patients with disease confined to the mesangial regions of the glomeruli (WHO class II) have mild or minimal clinical renal findings ***(Berden, 1997).***

They may have active-lupus serologic tests (e.g., a high anti-DNA antibody titer and low serum complement), but urinary sediment is inactive, hypertension is infrequent and easily controlled when present, proteinuria is usually less than 1 g daily, and the serum creatinine and glomerular filtration rate (GFR) are usually normal ***(Appel et al., 2000).***

Focal segmental proliferation LN (WHO class III) is often associated with active lupus serology, although the degree of serologic activity does not correlate with the severity or extent of the histologic damage ***(Grishman and Churg, 1982).***

Hypertension and active urinary sediment are commonly present in focal segmental proliferative LN. Proteinuria is usually present, often more than 1 g daily, and as many as one fourth to one third of patients with

focal LN will have the nephritic syndrome at presentation. As many as one quarter of these patients will have an elevated serum creatinine at presentation. Patients with less extensive involvement of the glomeruli by the focal proliferative process and with fewer necrotizing features and without crescents are more likely to have less hypertension and preserved renal function. Most treatment trials deal largely with patients with diffuse proliferative disease that presents with the most active and severe clinical features. Patients with this biopsy pattern of involvement typically have high anti-DNA antibody titers; low serum complement levels; and very active urinary sediment, with erythrocytes and red cell and other casts present on urinalysis.Virtually all patients have proteinuria, and in most series as many as half of the patients will have nephritic syndrome. Hypertension also is common. Renal dysfunction is typical, and even when the serum creatinine appears normal, the GFR is usually depressed ***(Appel et al., 2000).***

In most series, patients with lupus membranous nephropathy present with proteinuria, edema, and other manifestations of nephrotic syndrome ***(Pasquali et al., 1993).***

Although serologic activity is often mild, when present, up to 60% of patients will have a low serum complement and an elevated anti-DNA antibody titer. Likewise, active urine sediment, hypertension, and renal dysfunction may all occur in patients with lupus membranous nephropathy, without superimposed proliferative lesions. Patients with lupus membranous nephropathy may present with heavy proteinuria and what appears to be idiopathic nephrotic syndrome before developing other clinical and laboratory manifestations of SLE ***(Appel et al., 1976).***

As with idiopathic membranous nephropathy, patients with lupus membranous nephropathy are predisposed to developing thombotic complications such as renal vein thrombosis and pulmonary emboli ***(Houssiau, 2004).***

Advanced sclerosing glomerulonephritis (WHO class VI) is usually the result of "burnt out" LN of long duration. It is often the end result of years of lupus flare-ups, alternating with periods of inactivity. Much of the renal histologic damage may represent nonimmunologic progression of disease in remaining glomeruli as a result of reduced numbers of functioning nephrons. Although the lesions are sclerosing and fibrotic without activity on biopsy results, these patients still may have microhematuria and

chronically low levels of proteinuria. Virtually all such patients have both hypertension and a decreased GFR. Levels of anti-DNA antibodies and serum complement levels have usually normalized by the time these patients develop class VI lesions ***(Cameron, 1997).***

Course and Prognosis of Lupus Nephritis:

Lupus nephritis still has a negative impact on lupus patients' survival as indicated by the long-term data collected between 1990 and 2000 by the investigators of the European Working Party on Systemic lupus erythematosus in a prospective series of 1000 European patients, whose overall survival rate at 10 years was 88 and 94% for patients with and without renal involvement, respectively ***(Cervera et al., 2003)***.

Although this course is partly defined by the initial pattern and severity of renal involvement, it is modified by therapy, exacerbations of the disease, and complications of treatment. Moreover, the prognosis, even for patients with severe renal disease, has improved in recent decades. This relates to recognition of patients with milder renal involvement as well as the wider and more judicious use of immunosuppressive agents other than corticosteroids in those patients with severe proliferative disease ***(Appel et al., 2000).***

Patients with lesions limited to the renal mesangium generally have an excellent course and prognosis. Those who do not transform into other patterns are unlikely to develop progressive renal failure, and mortality is generally the result of extrarenal manifestations of SLE and complications of therapy. It is not known why some patients will progress to more serious renal disease, whereas others remain with a benign mesangial pattern over a lifetime. Focal proliferative disease has an extremely varied course. Patients with mild proliferative disease in only some glomeruli appear similar to those with mesangial lesions. They respond well to therapy, and fewer than 5% progress to renal failure over 5 year of follow-up. Patients with greater involvement of glomeruli with proliferation, those with only focal involvement but with areas of necrosis and karyorrhexis, and those with crescent formation have a prognosis more akin to patients with WHO class IV diffuse proliferative disease. Some class III patients will transform or evolve to WHO class IV over time ***(Berden, 1997).***

Patients with diffuse proliferative disease have had the least favorable prognosis in virtually every series. Nevertheless, the prognosis for this group has markedly improved in recent years ***(Appel and D'Agati, 1995).***

In some studies, serologic abnormalities, such as elevated anti-DNA antibody titers and low serum

complement levels, or high levels of CIC, also have defined a population more likely to have progressive renal disease. However, serologic abnormalities may revert with therapy, and in many prognostic studies they have not correlated with long-term prognosis ***(Dooley et al., 1997).***

In several studies, anemia has been a poor prognostic finding, regardless of the underlying cause ***(Austin et al., 1994).***

Renal dysfunction, as noted by an elevated serum creatinine or decreased GFR or by heavy proteinuria and nephritic syndrome, is also indicative of a poor renal prognosis in many series ***(Esdaile et al., 1991).***

Finally, histologic features such as WHO class, the degree of active proliferative features, or features of chronic irreversible renal damage on biopsy have been associated with prognostic value ***(Mok, 2005).***

Therapeutic goals in lupus nephritis:

Optimal management of LN remains a challenge because of the heterogeneity of the disease at presentation and its unpredictable course ***(Houssiau, 2004)***.

Most clinicians will agree on the following therapeutic goals for a patient with newly diagnosed lupus nephritis:

1. To achieve prompt renal remission.
2. To avoid renal flares.
3. To avoid chronic renal impairment.
4. To fulfill these objectives with minimal toxicity.

Although patient and renal survival rates have improved over the past decade, it should be stressed that current immunosuppressive regimens still achieve suboptimal results. First, the rate of renal remission after a first-line therapy is at best 81% in recent prospective studies ***(Contreras et al., 2004)***.

Second, renal relapses occur in one third of lupus nephritis patients, mostly when patients are still immunosuppressed ***(El-Hachmi et al., 2003)***.

Third, between 10 and 20% of lupus nephritis patients experience end-stage renal disease 5 to 10 years after disease onset, although these figures are lower in recent studies ***(Houssiau et al., 2002)***.

Treatment of Lupus Nephritis:

Patients with WHO class I biopsies and those with only mesangial lesions have an excellent renal prognosis. They need no therapy directed at the kidney and should be treated only for extrarenal manifestations of SLE. There is no general consensus on the treatment of patients with

focal proliferative LN, WHO Class III lesions. Patients with only moderate proliferative lesions involving only a few glomeruli, with no necrotizing features and no crescent formation, have a good prognosis and often will respond to a short course of high-dose corticosteroid therapy. Patients with larger amounts of the glomerular surface area involved, with necrotizing features and crescent formation, require more vigorous therapy, similar to that of patients with diffuse proliferative LN ***(Appel et al., 2000).***

Patients with diffuse proliferative disease (WHO class IV lesions) require aggressive treatment to avoid irreversible renal damage and the ultimate outcome of ESRD. The precise form of aggressive treatment is still debated, but a variety of immunosuppressive regimens already are in use ***(Cameron, 1997).***

Currently used agents in treating severe lupus nephritis include the following:

Corticosteroids:

A number of uncontrolled clinical trials performed between 1976 and 1982 suggested improvement of renal function with steroids alone ***(Ponticelli et al., 1982)***.

However, subsequent randomized clinical trials performed at the National Institute of Health (NIH) clearly demonstrated that corticosteroids alone were inferior to cyclophosphamide in the treatment of proliferative lupus nephritis ***(Steinberg and Steinberg, 1991).***

Follow-up studies demonstrated that the combination of pulse methylprednisolone and cyclophosphamide conveyed an additional benefit, and this regimen has become the standard of care in many centres ***(Illei et al., 2001).***

Cyclophosphamide:

Cyclophosphamide has been used as a therapy for lupus nephritis since the 1960s, and a retrospective analysis published in 1984 suggested a benefit in regards to progression towards end-stage renal disease and the incidence of death ***(Felson and Anderson, 1984).***

Based on currently published clinical trials induction therapy with cyclophosphamide combined with pulse steroids is the best treatment option to achieve remission. Currently there is not enough data to suggest that either oral or intravenous cyclophosphamide induction is more efficacious ***(Lenz and Contreras, 2004).***

The smallest effective dose and the shortest duration of Cyclophosphamide treatment should be chosen to avoid

adverse events, in particular gonadal failure and infections, which can be achieved by using sequential therapy with either azathioprine or mycophenolate mofetil in the maintenance phase ***(Lenz and Contreras, 2004).***

Azathioprine:

Azathioprine inhibits purine synthesis in all replicating cells, which leads to a decreased number of circulating B and T-lymphocytes as well as decreased antibody production and interleukin (IL-2) expression. Currently, azathioprine is most commonly used in sequential regimens and in combination with corticosteroids to treat severe lupus nephritis during pregnancy ***(Lenz and Contreras, 2004).***

Azathioprine, appears to have a cyclophosphamide sparing effect, resulting in fewer adverse events ***(Bansal and Beto, 1997).***

Mycophenolate mofetil (MMF):

MMF, like azathioprine, inhibits purine synthesis, however it's effect is selective for lymphocytes. There is accumulating evidence that the combined use of mycophenolate mofetil and corticosteroid presents an effective treatment for severe proliferative lupus nephritis, and is associated with much fewer adverse effects compared with cyclophosphamide-based regimens ***(Chan, 2005).***

Sequential therapies including Cyclophosphamide, Azathioprine and MMF:

In the last decade, sequential immunosuppressive regimens for the treatment of proliferative lupus nephritis have been used in uncontrolled and controlled clinical trials ***(Contreras et al., 2004; Houssiau et al., 2002).***

The goals of these studies were to achieve remission with a limited exposure to cyclophosphamide during the induction phase and to suppress renal flares during the maintenance phase. Both are important, since failure to achieve remission and frequent nephritic relapses have been associated with poor outcome and progression of renal disease ***(Moroni et al., 1996).***

The sequential regimen most widely used is cyclophosphamide induction followed by azathioprine maintenance, or more recently MMF maintenance ***(Lenz and Contreras, 2004).***

For relapsing patients, a new induction course with IV cyclophosphamide is probably justified, followed by maintenance therapy with another immunosuppressant than the one on which the patient failed ***(Houssiau, 2004)***.

Non-specific therapies:

The goal of non-specific therapies is to slow the progression of renal disease and avert co-morbidities such as cardiovascular disease, thromboembolic complications, and bone loss. All patients require tight blood pressure control ***(Bakris et al., 2000)*** the use of angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers, correction of dyslipidemia, smoking cessation, and anticoagulation to an INR of 3-4 if symptomatic antiphospholipid syndrome is present ***(Clark and Moist, 1998).***

Patients requiring prolonged corticosteroid therapy have a high risk to develop osteopenia and osteoporosis and should receive preventive treatment for bone loss, including calcium, vitamin D, and antiresorptive agents ***(De Dieus et al., 2003).***

Autoantibodies in SLE

Currently, the sine qua non of established lupus is the antinuclear antibody test; however, the results of current laboratory testing for antinuclear antibodies may vary considerably ***(Tan et al., 1997).***

As a group, the molecules targeted by (ANA) anti-nuclear antibody are highly conserved among species, serve important cellular functions, and exist inside cells as part of large complexes comprised of various protein and nucleic-acid components (e.g. nucleosomes). Antibodies to certain nuclear antigens (e.g. DNA and histones) frequently occur together, a phenomenon known as linkage. Linkage suggests that the complex, rather than the individual components, serves as the target of autoreactivity, as well as its driving antigen ***(Buyon, 2001)***.

With the widespread availability of antinuclear antibody tests and the general knowledge that nonspecific symptoms may be associated with connective tissue diseases, it has become common practice for general practitioners to order antinuclear antibody tests in a broad range of patients. This is ordered to rule out the diagnosis when the results are negative rather than as a confirmatory test. A positive antinuclear antibody response points toward a connective tissue disease, among which

SLE is the most common, but it may also represent a nonspecific finding ***(Suarez - Almazor et al., 1998).***

Among ANA specificities in SLE, two appear unique to this disease. Antibodies to double-stranded(ds) DNA and a nuclear antigen called Sm essentially are found only in people with SLE, and are included as serologic criteria in the classification of SLE. Although both **anti-DNA** and **anti-Sm** are serologic markers, they differ in their pattern of expression and clinical associations. These antibodies are produced independently by patients, and although anti-DNA levels frequently fluctuate over time and may disappear with disease quiescence, anti-Sm levels remain more constant. The anti-Sm and anti-DNA responses also differ in the nature of their target antigens ***(Buyon, 2001)***.

Perhaps the most remarkable feature of the anti-DNA response is its association with immunopathologic events in SLE, especially glomerulonephritis. This role has been established by correlating anti-DNA serum levels with periods of disease activity, isolating anti-DNA in enriched from glomerular eluates of patients with active nephritis, and inducing nephritis by administering anti-DNA antibodies to normal animals. The relationship between levels of anti-DNA and active renal disease is not invariable, some patients with active nephritis may lack serum anti-DNA and others with high levels of anti-DNA

are clinically discordant and escape nephritis ***(Pisetsky, 1998)***.

Autoantibodies that bind double stranded DNA are present in approximately 70% of patients with SLE and are found in the kidneys of patients with lupus nephritis, but not with other types of nephritis ***(Mason et al., 2005).***

The occurrence of nephritis without anti-DNA may be explained by the pathogenicity of other autoantibody specificities (e.g. **anti-Ro** or anti-Sm). The converse situation of clinical quiescence despite serologic activity suggests that only some anti-DNA provoke glomerulonephritis. Antibodies with this property are denoted as pathogenic or nephritogenic. Studies to delineate the basis of renal pathogenicity initially focused on the role of antibodies to single-stranded DNA and dsDNA. Although anti-dsDNA antibodies essentially are exclusive to SLE, **anti-ssDNA** antibodies have wider expression among inflammatory and infectious diseases. Both specificities frequently coexist in SLE, however, because many anti-DNA antibodies bind a common antigenic determinant present on both ss-DNA and dsDNA. Since renal eluates show antibody activity to both DNA forms, it appears likely that both anti-dsDNA and antibodies to ss-DNA have similar pathogenic roles ***(Buyon, 2001)***.

Studies correlating the properties of anti-DNA antibodies with nephritis suggest that several features promote pathogenicity. These features include isotype, charge, ability to fix complement, and capacity to bind glomerular preparations ***(Lefkowith and Gilkeson, 1996)***.

In this regard, anti-DNA antibodies appear to be a subset of pathogenic antibodies that bind to nucleosomes, the likely form of DNA in the circulation as well as in immune deposits. These antibodies can bind to such components as DNA or histones, or to higher-order structures requiring an intact nucleosome or various components together ***(Amoura et al., 1999)***.

In contrast to their role in nephritis, anti-DNA antibodies have not been clearly associated with other clinical events. Although many ANAs never have been adequately evaluated for pathogenicity, there is evidence that certain auto antibodies other than anti-DNA have a clinical impact ***(Buyon, 2001)***.

The contribution of ANAs to clinical events in SLE has been difficult to understand because the intracellular location of the target antigens should protect them from antibody interactions. The location of these antigens may not be fixed, however, and some antigens may translocate

to the membrane and become accessible to antibody attack. Damage to cells by ultraviolet radiation, for example, may lead to such movement. There also is evidence to suggest that ANAs may enter cells, bind nuclear antigens, and disturb cell function ***(Reichlin, 1995)***.

In addition to ds-DNA, a number of specific epitopes within the nucleus have been shown to give rise to positive test results in lupus patients. These include, SS-A/Ro, SS-B/La, the nuclear matrix, the nucleolar organizing region, the chromosomal coat protein and histones ***(Cabral and Alarcon-Segovia, 1997).***

Other epitopes that are targeted by antibodies in the sera of SLE patients are the phospholipid moieties on platelets and other tissues, lymphocyte markers and red blood cell antigens. Although thought to be more specific for scleroderma, both the centromere and topoisomerase antibodies can be found in lupus patients without concurrent scleroderma.SLE Patients with anti-centromere antibody do not represent a different clinical subgroup ***(Respaldiza et al., 2006)***.

Pathogenic autoantibodies:

The pathogenesis of SLE is complex. Target tissue damage is caused primarily by pathogenic autoantibodies

and immune complexes. The abnormal immune response that permits persistence of pathogenic B and T cells has multiple components that include processing of increased quantities of self antigens by antigen-presenting cells, hyperactivation of T and B cells, and failure of multiple regulatory networks to interrupt this process ***(Tsao, 2002)***.

Pathogenic Ig molecules are often highly mutated, particularly in the hypervariable regions of their heavy and light chains ***(Peeva et al., 2002)***.

Several features of autoantibodies influence their pathogenic potential, including what antigens they bind, avidity for those antigens, the net charge of the Ig molecule and the immune complex it forms with antigen, the presence in the Ig molecule of charged amino acids that interact with opposite charges on cell membranes of DNA, presence in the Ig molecules of sequences recognized by helper T cells, and ability to fix and activate complement ***(Hahn, 1998)***.

Even though we understand many of these principles, it is difficult to predict that a given monoclonal anti-self antibody will be a pathogen. For example, among monoclonal human antibodies to DNA, some caused proteinuria upon transfer to severe combined

immunodeficiency mice, whereas others did not ***(Ehrenstein et al., 1995)***.

In terms of antigens bound, it is convenient to think of the autoantibodies of SLE as belonging to one of several groups directed against DNA/protein complex, RNA/protein complex, 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 (ds)-DNA ***(Hahn et al., 2005)***.

All individuals produce numerous antibodies that react with self molecules. The characteristics of the back-ground anti-self normal repertoire are that most of the antibodies are immunoglobulin IgM, they have weak avidity for self antigens and they tend to be widely cross-reactive with multiple antigens. Pathogenic antibodies are different. They tend to be IgG, have high avidity for self antigens and have restricted specificity ***(Peeva et al., 2002).***

It is likely that at least some pathogenic anti-dsDNA antibodies bind directly or in complexes to renal structures including heparan suflate, histone, laminin alpha-actinin

and collagen in glomerular basement membranes ***(Chan et al.,2002).***

Sera from patients with lupus nephritis contain antibodies that bind to glomeruli; some bind DNA and others don't. Non-DNA binding autoantibodies that probably can cause nephritis include antibodies to Clq, nucleosomes and Ro/SS-A. Other autoantibodies that directly cause disease include those that coat platelets or erythrocytes. Interestingly, antibodies against platelets in lupus patients with thrombocytopenia recognize a wider variety of antigens than do antibodies in patients with idiopathic thrombocytopenic purpura. In both diseases, antibodies are directed against surface glycoprotein II and III antigens on intact platelets; but in SLE there are additional antibodies against cytoplasmic antigens that come to the surface of activated platelets and against phospholipids, suggesting that activated, damaged cells and cells undergoing apoptosis (in which antigenic portions of phospholipids in cell membranes are directed outward rather than inward) are important in stimulating pathogenic responses in SLE ***(Rioux et al., 1995).***

Antibodies against proteins associated with the Ro particle are probably also direct pathogens particularly in congenital heart block ***(Tran et al., 2002)***. IgG anti-Ro

crosses the placenta, can bind to certain areas of fetal heart conduction system tissue, alter myosin-actin function and probably cause heart block. Furthermore, once a woman has produced a fetus with congenital heart block, the chances of subsequent fetuses having the same problem are greatly increased. Most babies with congenital heart block have mothers with anti-Ro (or rarely anti-ribonucleoprotein (RNP) or anti-La); many of those mothers are healthy at the time of this occurrence, but some develop SLE or lupus-like syndromes later ***(Buyon, 1999).***

Antibodies against phospholipids (either anti-cardiolipin or the lupus anticoagulant) increase the risk for venous or arterial thrombosis, for fetal loss and for thrombocytopenia ***(McCarty, 2002).***

Autoantibodies to ribosomal P proteins in patients with SLE have been recognized for more than a decade. They 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 kd, 19 kd and 17kd respectively ***(Yalaoui et al., 2002).***

These antibodies occur in approximately 10% of randomly selected SLE patients but in upto 40% of patients

with active disease. They are higher in Asian patients and have a relatively lower prevalence in black and Caucasian patients. They appear to be a highly specific diagnostic marker for SLE because they are rarely detected in other multisystem autoimmune disorders ***(Gerli and Caponi., 2005).***

Antibodies to ribosomal P protein are more prevalent in juvenile-onset SLE than in adult-onset SLE. Levels of antibodies to ribosomal P protein vary with the clinical disease activity as measured by the SLEDAI, often in concordance with the levels of anti-ds-DNA ***(Reichlin et al., 1999).***

They identify a subset of SLE patients at higher risk for liver and kidney involvement, in addition to the risk for neuropsychiatric disease ***(Mahler et al., 2006).***

The mechanism by which they cause disease is unclear but may be related to the fact that they, like some antibodies to DNA and RNP, can penetrate membranes and alter cell function ***(Reichlin, 1999)***.

The alpha-actinin-binding antibodies are significantly associated with glomerulonephritis in SLE. Whether such autoantibodies may anticipate the development of this

complication of SLE remains to be verified ***(Renaudineau et al., 2006)***.

Several autoantibodies specificities have been reported in serum and cerebrospinal fluid of neuropsychiatric systemic lupus erythematosus patients (i.e., antineuronal, antiribosomal P/proteins, antiglial fibrillary acidic proteins, anti-phospholipid and anti-endothelial antibodies). Recently, ***Valesini et al., 2006*** demonstrated an association between serum anti-endothelial antibodies and psychosis or depression in patients with SLE.

Guanosine is the most immunologically active nucleoside of DNA. Circulating levels of antiguanosine antibodies in patients with SLE correlate closely with disease activity and may be pathogenically important in SLE by interfering with signal transduction, inactivating mitochondrial and cell function ***(Colburn and Green, 2006)***.

Pathogenic immune complexes:

Like autoreactive antibodies, some immune complexes are pathogenic and others are not. Size of the immune complex is important; large immune complexes are cleared by the mononuclear phagocytic cell system on their first "pass" through the circulation; small immune complexes are more likely to deposit in tissue. Excessive quantities of immune complexes overwhelm the mechanisms used to clear them. Finally, some immune complexes are "tissue tropic" and prone to bind to tissues because they have a net cationic charge or because the antibodies they contain are directed against tissue components. It is well accepted that immune complexes that fix complement are responsible for much of the tissue damage that characterizes SLE ***(Carroll, 2000).***

Antichromatin Antibodies

Systemic lupus erythematosus is characterized by the presence of antinuclear autoantibodies. Native DNA, histone proteins and small nuclear ribonulceoproteins (snRNP) are the main targets of these ANA-s, but some of them may derive against the entire chromatin, which is composed of the above listed elements, and also against its

fundamental unit, that is nucleosomes ***(Koutozov et al., 2004).***

Definition: The nucleosome is a large protein-nucleic acid complex involved in DNA packing and in controlling genetic information ***(Decker, 2006)***.

Nucleosomes are the repeating unit of histone and DNA that make up chromatin. Thus polynucleosomes and chromatin are identical. Chromatin, the native complex of histones and DNA found in the cell nucleus of eukaryotes, is comprised of approximately 40% DNA, 40% histones and 20% non-histone proteins, RNA and other macromolecules. The periodic arrangement of histones along the DNA gives chromatin a 'beads-on-a string' appearance in electron micrographs. The 'beads' can be isolated by digesting the linker DNA between them with micrococcal nuclease, yielding nucleosomes ***(Burlingame and Cervera, 2002).***

Nucleosome is composed of 180 basepairs (bp) DNA and histones (two copies of the core histones H2A, H2B, H3 and H4 and one copy of the linker histone H1). The core histones are organized as a histone octamer (containing two H2A-H2B dimers and one H3-H4 tetramer) around which 146bp of DNA are wrapped, forming the core particle. This structure is stabilized by histone H1 which is outside the nucleosome. Nucleosomes are separated by linker DNA.

The crystal structure of the nucleosome core particle has been resolved at high resolution. This subnucleosomal structure contains only 146 DNA and no histone H1. In this particle, the 146-bp DNA are wrapped around the histone octamer in 1.65 turns of a left-handed superhelix. N-terminal histone tails cross the DNA superhelix and are exposed at the surface of the core particle. Interestingly, B and T-helper (th) autoepitopes are often localized in histone tails or histone regions exposed at the surface of the nucleosome in solution ***(Decker, 2006)***.

Nucleosomes are generated during cell apoptosis by cleavage of the chromatin by endonucleases. In physiologic conditions, these apoptotic cells are recognized and engulfed by phacocytes to prevent any release of cell constituents in the extracellular milieu ***(Dieker et al., 2002).***

Under certain circumstances, nucleosomes are released into the extracellular milieu and are then early accessible to cells of the immune system ***(Decker, 2006)***.

Anti-chromatin antibodies are defined as antibodies that react with the portion of histone exposed in chromatin, the structure of DNA found in chromatin, or an epitope comprised of the native histone-DNA complex. Specifically excluded are antibodies reacting with non-histone proteins, with epitopes on histones that are buried in chromatin, and with structures of DNA such as A,C & Z forms, that are not

present in chromatin. Thus, some, but not all, histone and DNA reactive antibodies have anti-chromatin reactivity ***(Burlingame and Cervera, 2002).***

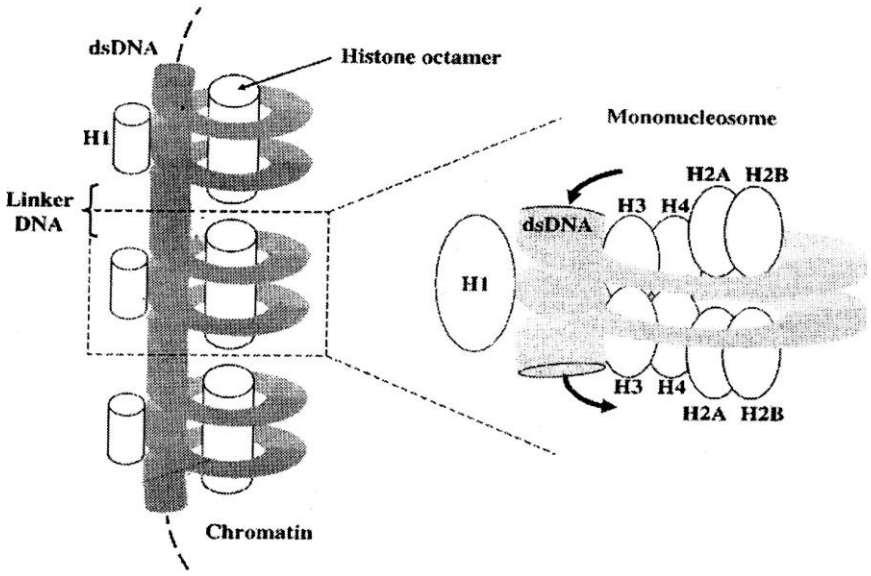

Fig. (II-**2):** The nucleosome is the fundamental repeating unit of chromatin. The nucleosome core particle is composed of a central core tetramer of two molecules each of histone H3 and H4, flanked by two dimers of histones H2A and H2B, surrounded by two superhelical turns of 146 basepairs of histone-free DNA. Histone H1 is located at the point where DNA enters and exits the nucleosome ***(Amoura et al., 1997).***

Nucleosomes and Autoantibody Production in SLE:

Systemic lupus erythematosus is characterized by the production of both antigen-driven antibodies, like anti-double-standard DNA (dsDNA) and antihistone antibodies, and polyclonal unspecific autoantibodies ***(Amoura et al., 2000).***

Evidence is to date accumulating to suggest that the nucleosome, the fundamental unit of chromatin and ubiquitous product of cell apoptosis, plays a key role in the pathogenesis of SLE ***(Amoura and Piette, 2003)***.

In the last few years, several reports have shown that chromatin represents the main autoantigen-immunogen in SLE and that specific antibodies are an important marker of the disease ***(Villalta et al., 2005)***.

Polynucleosomes bind to activated monocytes and could be presented by antigen-presenting cells such as monocytes and this presentation could induce an antigen-driven response. Kinetic analysis of autoantibody production in several lupus-prone mouse strains revealed the presence of antibodies directed to the native nucleosome

particle but not to its individual components, DNA and histones ***(Amoura et al., 1994).***

These nucleosome-restricted or specific antibodies emerge before the occurrence of anti-dsDNA and antihistone antibodies. Both the kinetics of autoantibody emergence and the immunoglobulin (Ig) subclass distribution are similar to those of antibodies mediated by an exogenous T-dependent antigen and suggest that the production of nucleosome-specific, anti-dsDNA and antihistone antibodies involves an immunization by the nucleosome. Nucleosome-specific CD4+ T cells are detected long before lupus mice produce pathogenic autoantibodies, suggesting that these cells play a role in triggering the disease ***(Cervera et al., 2003).***

On the other hand, studies showed that nucleosomes may elicit production of interleukin-6 and stimulation of lympho-proliferation and IgG synthesis by splenic B cells that could result in a polyclonal activation ***(Amoura et al., 1997).***

Increased amounts of oligonucleosomes have been found in the circulation of SLE patients raising the possibility that circulating nucleosomes could trigger both a

specific (nucleosome-driven) and an unspecific antibody production ***(Amoura et al., 1997).***

Because apoptosis is the source of nucleosome production it has been suggested that programmed cell death could be increased in SLE, leading to an increased nucleosome release. Indeed lymphocytes of both SLE patients and lupus mice have been shown to undergo increased rate of apoptosis ***(Emlen et al., 1994).***

Apoptotic defects and impaired removal of apoptotic cells contribute to an overload of autoantigens that become available to initiate an auto-immune response ***(Ballestar et al., 2006)***.

But in vitro apoptosis of peripheral blood mononuclear cells has also been observed in other chronic inflammatory diseases and thus was not specific to lupus patients ***(Lorenz et al., 1997).***

Defective phagocytosis of apoptotic cells seems to be another explanation for the presence of increased circulating nucleosome levels in SLE ***(Hermann et al., 1998).***

Interestingly, nucleosome per se has been found to inhibit removal of apoptotic cells by murine lupus macrophages. Because during apoptosis nucleosomes co-

cluster in apoptotic bodies with other lupus auto-antigens (ribonucleoprotein) it is tempting to speculate that in SLE it is the persistence of circulating apoptotic cells that triggers autoantibody production ***(Amoura et al., 2000).***

Antichromatin chromatin immune complexes can bind to the glomerular basement membrane in vivo. Antichromatin antibodies are a ubiquitous feature of murine lupus, and are necessary but not sufficient for the development of glomerulonephritis in one strain of mouse ***(Mason et al., 2005)***.

The nucleosome as the in vivo target of lupus anti-dsDNA and antihistone antibodies:

Specific studies of lupus polyclonal sera or monoclonal antibodies by enzyme-linked immunosorbent assay using nucleosomes as substrates has strengthened the notion that nucleosomes may be in vivo targets of anti-dsDNA lupus antibodies ***(Amoura et al., 2000).***

Analysis of serum antibody activity against nucleosomes and dsDNA showed both in human and murine lupus that a serum anti-dsDNA reactivity is almost always associated with an antinucleosome reactivity ***(Chabre et al., 1995).***

An early study indicated that in SLE antihistone antibodies were found concomitantly with anti-dsDNA antibodies in the same individual, suggesting that these two autoantibody populations had the same target, composed of dsDNAS and histone, i.e., the nucleosome.

Studies of lupus antihistone antibodies showed that they were predominantly directed against H1 and H2B, the most accessible histones in the nucleosome particle, and that they recognized restricted histone epitopes located in amino and carboxyl-termini portions exposed outside the nucleosome ***(Amoura et al., 2000).***

The broad antinucleosome antibody family:

There is now strong evidence that the nucleosome is both the driving immunogen and the in vivo target of lupus anti-dsDNA antibodies and antihistone antibodies specially in drug-induced lupus ***(Decker, 2006)***.

In light of the overall findings a new concept has emerged in lupus leading to the definition of the broad antinucleosome antibody family that includes the nucleosome-specific antibodies (antinucleosome antibodies without anti-dsDNA and antihistone reactivities), the antinucleosome antibodies with anti-dsDNA reactivity, and the antinucleosome antibodies with antihistone reactivity ***(Amoura et al., 2000).***

Relationship to LE cell factor:

In 1948, Hargraves published his seminal paper describing the LE cell ***(Hargraves et al., 1948)***. Because immunofluorescent and enzymatic based tests to detect antibodies were not available at this time, the cell based LE cell test was the best test available. Eventually the LE cell test became a standard procedure that was performed in labs throughout the world to help diagnose SLE. Over the next 2 decades a large amount of research was performed to discover the specificity of the antibodies that caused the LE cell phenomenon. At that time the most well-accepted technique to identify the antigenic-specificity of an antibody was immuno-adsorption, since western blot and ELISA techniques were not available. Also, the biochemical properties of chromatin were not well understood then. A crude preparation called deoxyribonucleoprotein (DNP) was the only soluble form of chromatin known. Nonetheless, a number of groups demonstrated that (DNP), but not its individual components i.e. DNA or histone, could remove from serum the antibody that caused LE cell formation ***(Lachman, 1961).***

A relatively more recent paper used a well-defined form of chromatin called nucleosomes to remove LE cell reactivity from serum ***(Rekvig and Hannestad, 1981).***

So it was generally accepted that anti-chromatin antibodies caused the LE cell phenomenon. Performing the LE cell test is very time consuming, requires live cells and shows large lab-to-lab variation. Thus, as techniques for measuring autoantibodies improved, labs began to move away from performing the LE cell test ***(Burlingame and Cervera, 2002).***

Even though other techniques to measure anti-chromatin antibodies were developed, such as agglutination of chromatin-coated latex beads and immunoprecipitation, these tests never became common in clinical laboratories. One reason was that both DNA itself and anti-DNA antibodies were found in the sera of patients with SLE, and anti-DNA was found to be a clinically important marker of disease ***(Tan et al., 1966).***

At the same time a number of methods to detect anti-DNA were developed. The two most common methods were immunoprecipitation of radiolabeled DNA, commonly called the Farr assay ***(Word et al., 1968)*** and an immuno-fluorescent technique using crithidia luciliae as substrate ***(Aarden et al., 1975).***

Because whole chromatin is insoluble in physiological salt solutions but pure DNA is soluble, the physical and biochemical properties of DNA make it much more

conducive to immunological tests than chromatin. Additionally, the DNA based tests were more reliable and reproducible than the early Chromatin based tests. Eventually an anti-DNA ELISA became available ***(Karsh et al., 1982).***

The switch from performing the LE cell prep that measured anti-chromatin antibodies to the Farr, Crithidia Luciliae and ELISA assays that measured anti-DNA antibodies was gradual ***(Burlingame and Cervera, 2002).***

Both the preliminary criteria ***(Cohen et al., 1971)*** and the 1982 revised criteria for the classification of SLE ***(Tan et al., 1983)*** showed that a positive LE cell test was highly helpful in diagnosing SLE, and was more sensitive than anti-DNA in SLE patients. Nonetheless, the latest recommendations from the American College of Rheumatology (ACR) on diagnosing SLE removed a positive LE cell test as a criterion because it is not routinely performed anymore ***(Hochberg, 1997).***

In the new criteria a positive LE cell test was replaced by a positive anticardiolipin test. Over the years anti-DNA antibodies have replaced a positive LE cell prep as the hallmark of patients with SLE ***(Burlingame and Cervera, 2002).***

Different names used for the same antibody-specificity:

One factor that has led to confusion concerning antichromatin antibodies is that many different names have been used for these antibodies. Old publications called them LE cell factors ***(Holman and Deicher, 1959)*** and anti-DNP ***(Karsh et al., 1982)*** or anti-SNP ***(Robitaille and Tan, 1973).***

Modern publications have called them anti-chromatin ***(Burlingame et al., 1994)***, anti-nucleosome ***(Schlumberger et al., 2002)*** and anti- (H2A-H2B)-DNA ***(Wallace et al., 1994).***

Clinical sensitivity and specificity:

The 2 most important recurring observations concerning antichromatin antibodies are that this antibody is sensitive and specific for SLE, and the presence of anti-chromatin is often correlated with glomerulonephritis in patients with SLE ***(Cervera et al, 2003).***

Evidence has accumulated in recent years that anti-chromatin autoantibodies are correlated even better with lupus nephritis than anti-dsDNA ***(Koutouzov et al., 2004)***.

Anti-nucleosome antibodies are a useful tool for the diagnosis of SLE, especially at early stages of the disease, and a useful marker of diseases activity, particularly in patients who are negative for anti-dsDNA antibodies ***(Saisoong et al., 2006)***.

Clinical relevance of antinucleosome antibodies:

There are now indications that the broad anti-nucleosome antibody population could be of clinical relevance. Study of the prevalence of antinucleosome IgG in 4 connective tissue diseases showed that their presence was limited to SLE, scleroderma and mixed connective tissue disease ***(Servais et al., 2001).***

Antinucleosome antibodies are present in active but also in inactive SLE patients (most of whom are anti-dsDNA negative). Thus, antinucleosome antibodies are a sensitive marker of anti-DNA negative SLE patients, additionally several studies have shown that antinucleosome antibody titers are correlated more closely or in a similar extent with the systemic lupus Erythematosus Disease Activity Index (SLEDAI), a validated index of SLE activity ***(Simon et al., 2004).***

High titers of anti-nucleosome antibodies identify patients who are at increased risk of renal involvement or flares of nephritis ***(Balow, 2005)***.

Recent findings have indicated that assessment of antinucleo-some antibody isotype are of interest and that antinucleosome of the IgG3 isotype likely plays a major pathogenetic role in SLE. First, comparison of the antinucleosome IgG subclass distribution between SLE, mixed connective tissue disease, and scleroderma showed a selective and significant increase of antinucleosome IgG3 in active SLE. Second, sequential determinations of antinucleosome isotypes within the same SLE individuals at different states of disease showed that antinucleosome IgG3 levels were significantly augmented during flares. Third, antinucleosome antibodies of the IgG3 isotype were the only ones to positively correlate with the SLEDAI. Fourth, SLE nephritis was marked by a selective and significant augmentation of antinucleosome antibodies of the IgG3 subclass, a feature that was seen only during active nephritis. Interestingly, when examining the bone fide anti-dsDNA antibody population, we found an absence of correlation between IgG3 anti-dsDNA and SLE disease activity, and no difference in the percentage of IgG3 anti-dsDNA between patients with active disease who did and those who did not have nephritis. Therefore, determination

of antinucleosome IgG3 could be of clinical relevance in the differential diagnosis of connective tissue diseases. Antinucleosome antibodies of the IgG3 isotype constitute a selective biologic marker of active SLE in particular of lupus nephritis ***(Amoura et al., 2000).***

There has been a renewed interest in anti-chromatin antibodies in the last few years. A number of research papers have demonstrated the clinical utility of this antibody to help diagnose systemic lupus erythematosus and drug induced lupus. It may seem that antichromatin antibodies have only recently been discovered, but they were actually one of the first autoantibodies discovered, and at one point the measurement of antichromatin was one of the most common immunological tests performed in clinical laboratories ***(Burlingame and Cervera, 2002).***

The development of an immunotherapy based on nucleosome-derived synthetic peptides has been suggested in order to modulate the reactivity of nucleosome-specific Th lymphocytes and thus modify the cytokine secretion pattern and/or Ab production by B lymphocytes. Recently, such a therapy has been tried in lupus mice and resulted in a decrease in autoantibody levels, prolonged life span by delaying nephritis and the development of regulatory T-lymphocytes ***(Kang et al., 2005)***.

Patients and Methods

The present study was conducted on 80 persons who were divided into three groups:

I. Group I: which included 40 SLE patients all fulfilling at least 4 of the criteria of the American college of rheumatology for SLE diagnosis ***(Tan et al., 1982)***.

They were further subdivided according to whether or not they had nephritis into two subgroups:

I a: this included 24 patients with lupus nephritis, which was diagnosed by proteinuria exceeding 500mg/d and/or presence of cellular casts (erythrocyte, granular, tubular or mixed) and was confirmed by renal biopsy ***(Cervera et al.,2003)***.

I b: this included 16 patients without lupus nephritis.

The SLE disease activity was assessed using SLEDAI score and accordingly SLE patients were sub-classified into two groups.

All SLE patients once diagnosed started therapy with steroids ± other immunosuppressive drugs.

II. Group II (disease control group): this included 20 patients, with matched age and sex, having other systemic autoimmune diseases.

Which included 5 patients with rheumatoid arthritis, 5 with systemic sclerosis, 4 with MCTD, 2 with juvenile rheumatoid arthritis, 2 with Behcet's disease and 2 with adult onset still's disease.

All patients were chosen from the Ain Shams University Hospital, internal medicine department and rheumatology outpatient clinic during the period from 11/2005 up to 9/2006.

Exclusion criteria:

Patients with evidence of:

- Pre-existing renal disease.
- Malignancy.
- Concurrent infection.
- Drug history of nephrotoxic drug use were excluded from this study.

III. Group III (healthy control group): This included 20 healthy volunteers with matched age and sex.

A verbal consent was obtained from all subjects participating in this study, after explaining its nature.

Methods:

Patients of group I and II were subjected to the following:

1. Complete history taking and thorough clinical examination.
2. Complete blood picture was done by coulter ***(Britten et al., 1969)***.
3. ESR was measured by the **Westergen (1921)** method.
4. Serum anti-DNA antibody titre was detected by ELISA supplied by Calbiotech, Inc (CA).
5. Serum antichromatin antibody titers were measured by quantitative determination using ELISA. Kits provided by Quanta Lite, USA.
6. Pelvi-abdominal ultrasound.

Patients of group I only were subjected to the following:

7. Serum C3 and C4: measured using radial immundiffusion for SLE patients only to assess disease activity.
8. Antinuclear antibodies: assayed by indirect immunofluorescent technique by a kit obtained from inova diagnostics, USA ***(Aarden et al., 1975)***.

9. Creatinine clearance : assayed according to **Wootton (1974).** 24-hrs urinary protein was measured by the method of ***(Henery et al., 1956)*** and serum creatinine was determined by ASTRA.
10. Renal biopsy was done for SLE patients with evidence of nephritis. Specimens were examined by light microscopy, immunofluorescent microscopy if the latter was normal.

The healthy control group underwent the following investigations: CBC, ESR, liver and kidney function tests, complete urine analysis, chest x-ray, ECG, anti-DNA and antichromatin antibody titre.

Principles of the test:

It is an enzyme linked immunosorbent assay (ELISA).

Highly purified calf thymus chromatin consisting of DNA wrapped around the core histone $(H2A\text{-}H2B\text{-}H3\text{-}H4)_2$ octamer is bound to the wells of a micro well plate. Histone H1 and non-histone proteins have been removed from the chromatin during the purification process. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any chromatin antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added

to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

Reagents:

1. Polystyrene microwell ELISA plate coated with a purified chromatin antigen (12-1x8 wells), with holder in foil package containing dessicants.
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to chromatin, prediluted 1.2ml.
3. Chromatin ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to chromatin, prediluted, 1.2ml.
4. Chromatin ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to chromatin, prediluted, 1.2 ml.

5. HRP Sample Diluent, 1 vial-colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50ml.
6. HRP Wash Concentrate, 1 vial of 40x concentrate-colored red containing Tris-buffered saline and Tween 20, 25ml.
7. HRP IgG Conjugate, (goat), anti-human IgG, 1 vial-colored blue containing buffer, protein stabilizers and preservative, 10ml.
8. TMB Chromogen, 1 vial containing stabilizers, 10ml.
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial-colorless, 10 ml.

Preparation of reagent:

The HRP Wash Concentrate was diluted 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975ml of distilled or deionized water.

The patient sample was diluted by adding 5µl of the sample to 500µl of HRP Sample Diluent. The diluted samples were used within 8hours of preparation.

Assay procedure:

1. 100µl of the prediluted Chromatin ELISA Low Positive. High Positive, Negative Control and the

diluted patient samples were added to the wells. The wells were covered and incubated for 30min. at room temperature.

2. Wash step: 200-300µl of the diluted HRP Wash buffer were added to all wells them aspirated. This was repeated 3 times.
3. 100µl of the HRP IgG Conjugate was added to each well and were incubated for 30 min at room temperature.
4. The Wash step was repeated.
5. 100µl of TMB Chromogen was added to each well and incubated in the dark for 30min. at room temperature.
6. 100µl of HRP Stop Solution was added to each well.
7. The absorbance (OD) of each well was read at 450nm within one hour of stopping the reaction.

Calculation of results:

The reactivity for each sample was calculated by dividing the average OD of the sample by the average OD of the Chromatin ELISA Low Positive. The result was multiplied by the number of units assigned to the Chromatin ELISA Low Positive found on the label.

$$\text{Sample value (units)} = \frac{\text{Sample OD}}{\text{Chromatin ELISA Low Positive OD}} \times \text{chromatin ELISA low positive (units)}$$

Interpretation of results:

The sample was considered negative if <20 units

Moderately, positive 20-60 units.

Strongly positive > 60 units

Statistical Methodology:

Data was analyzed on an IBM personal computer, using Statistical Package for Special Science(SPSS) software computer version 13. Data were described using mean, ± standard deviation (SD) and frequencies according if they are quantitative or qualitative respectively.Chi-square test was used for comparison of qualitative variables.Student t test of two independent samples was used for comparison of quantitative variables.One-way ANOVA test was used to compare more than two groups as regard quantitative data. Correlation between continuous variables was performed using Pearson correlation coefficient.

Sensitivity= True positive/True positive+False negative. =ability of the test to detect positive cases.

Specificity=True negative/True negative+False positive. = ability of the test to exclude negative cases.

Positive Predictive Value(PPV): % of true positive cases to all positive cases.

= True positive/True positive+False positive.

Negative Predictive Value(NPV): % of true negative cases to all negative cases.

= True negative/True negative+False negative.

Significance level (P) value:

P > 0.05 is insignificant (NS).

P <0.05 is significant (S).

P <0.01 is highly significant (HS).

P <0.001 is very highly significant (VHS).

Results

Our study was conducted on 80 subjects, who were divided into the following three groups:

Group I: 40 patients with SLE, each of them fulfilling at least 4 criteria of the American college of rheumatology revised criteria for classification of SLE ***(Tan et al., 1982)***.

They were 36 (90%) females and 4 (10%) males with a ratio of 9: 1. Their ages ranged from 15-59 years with a mean of 27.75 ± 10.75, 15 of them had a disease duration of a year or less.The mean disease duration was 3.4(± 3.5)

Group II: 20 patients with other systemic autoimmune diseases , 5 had rheumatoid arthritis, 5 with systemic sclerosis, 4 with MCTD, 2 with Behcet's, 2 with adult still's disease and 2 with juvenile rheumatoid arthritis.

They were 15 (75%) females and 5 (25%) males. Their ages ranged from 14-50 years with a mean of 28.6 ± 9.06.

Group III: 20 healthy controls.

They were 14 (70%) females and 6 (30%) males. Their ages ranged from 15-42 years, with a mean of 28.75 ± 8.55.

SLE patients of Group I were further subdivided according to presence of renal disease into:

Group I a: 24 (60%) patients with lupus nephritis. They were 2 males and 22 females. Their ages ranged from 15-48 years.

Group I b: 16 (40%) patients without lupus nephritis. They were 2 males and 14 females. Their ages ranged from 18-59 years.

Table (IV-1): Comparison between group I, II and III as regards age.

		GroupI 40	**GroupII 20**	**GroupIII 20**	**P-value**	**Sig.**
Age	**No**					
	Mean (S.D.)	27.8 (10.8)	28.6 (9.1)	28.8 (8.6)	>0.05	NS
	Range	13-59	14-50	15-42		

There was no statistically significant difference between groups I, II and III as regards age ($P > 0.05$).

Table (IV-2): Comparison between group I, II and III as regards sex.

Sex	Group I		GroupII		GroupIII		P-value	Sig.
	No	%	No	%	No	%		
M	4	10	5	25	6	30	>0.05	NS
F	36	90	15	75	14	70		

There was no statistically significant difference between group I, II and III as regards sex distribution ($P > 0.05$).

Table (IV-3): Clinical presentation of the 40 SLE patients (group I) arranged in order of frequency.

Parameter	No	%
Nephritis	24	60
Rash	21	52.5
Alopecia	19	47.5
Arthritis	18	45
Oral ulcers	14	35
Fever	13	32.5
Vasculitis	7	17.5
Myositis	3	7.5
Photosensitivity	1	2.5
Seizures	1	2.5
Organic brain syndrome	1	2.5

Table (IV-4): Descriptive laboratory data of SLE patients(40).

Parameter	Minimum	Maximum	Mean	Std. Deviation
Age(years)	13	59	28	10.6
Duration (years)	0.05	17	3.4	3.5
ESR (1st hr.)	8	140	64.4	37.8
$C_{3(mg/dl)}$	5.5	180	75	36.7
$C_{4(mg/dl)}$	2.4	42	21	10.6
SLEDAI score	2	42	14.4	10.1
S.creat(mg/dl)	0.4	6.9	1.0	0.8
BUN(mg/dl)	6	120	23.8	23.1
Cr.Clearance(ml/min)	2	124	88.5	30.8
Prot./ 24hrs(gm)	0.1	12	2.2	2.7
Hematuria	1	55	4.8	10.9
WBC($10^3/mm^3$)	1.4	14.3	6.6	2.9
Hb(g/dl)	5.4	13.1	9.9	2.1
Plt($10^3/mm^3$)	44	438	227.5	95.1
Antichromatin(units/ml)	6.2	200	113.5	68.4
Anti-DNA (Iu/ml)	9	120	41.4	29.1

Table (IV-5): Descriptive data of patients in group II (SAD).

	Minimum	Maximum	Mean	Std. deviation
ESR(1st hr.)	19	130	74.5	32.5
WBC(10^3/mm^3)	5.1	13.6	9.4	2.1
Hb(g/dl)	8.0	12.5	10.4	1.4
Plt(10^3/mm^3)	113	459	241.9	85.3
S. creat(mg/dl)	0.5	1.9	1.1	0.4
AST (Iu/ml)	17	60	32.3	9.6
ALT (Iu/ml)	17	49	30.5	8.7
Antichromatin(units/ml)	4.9	18	10.3	4.4
Anti-dsDNA(Iu/ml)	3.0	80	20.9	22.1

Table (IV-6): Data of healthy controls (group III).

	Minimum	Maximum	Mean	Std. deviation
ESR(1st hr)	5.0	20	10.9	3.8
WBC(10^3/mm^3)	3.6	10	7.2	1.8
Hb(g/dl)	10.3	15	12.6	1.1
Plt(10^3/mm^3)	155	414	271	77
S. creat(mg/dl)	0.5	1.3	0.9	0.2
AST (Iu/ml)	16	40	26.8	7.4
ALT (Iu/ml)	13	40	29	7.8
Antichromatin(units/ml)	4.2	12	7.2	2.3
Anti-dsDNA(Iu/ml)	2.0	34	16.6	9.3

Table (IV-7): Descriptive data of (24) patients with nephritis (group Ia).

Parameter	Minimum	Maximum	Mean	Std. deviation
Age (years)	13	48	25.6	10.2
Duration (years)	0.02	10	2.6	2.7
ESR(1st hr)	17	140	78.3	35.1
$C_{3(mg/dl)}$	19	121	67.5	26.9
$C_{4(mg/dl)}$	2.4	40	19.9	10.6
SLEDAI score	2.0	42.0	17.9	10.7
S. creat (mg/dl)	0.4	6.9	1.3	1.4
BUN(mg/dl)	10	120	27.7	27.7
Cr.Clearance(ml/min)	2	123	82.0	37
Prot/24hrs(gm)	0.3	12	3.5	2.8
Hematuria	1.0	60	5.7	12.9
Activity Ind.	2.0	16	8.6	3.7
Chronicity Ind.	2.0	6	3.0	1.2
WBC($10^3/mm^3$)	1.4	12.7	6.3	2.8
Hb(g/dl)	6.2	13.1	9.2	1.9
Plt($10^3/mm^3$)	80	438	228.5	92.9
Antichromatin(units/ml)	12	200	130.1	63.5
Anti- DNA (Iu/ml)	9	120	48.3	34.7

Table (IV-8): Descriptive laboratory data of 16 SLE patients without nephritis (group Ib).

Parameter	Minimum	Maximum	Mean	Std. deviation
Age (years)	18	59	30.9	11.1
Duration (years)	0.17	17	4.6	4.3
ESR (1st hr)	8	115	43.5	32.4
C_3 (mg/dl)	5.5	180	86.2	46.6
C_4 (mg/dl)	8.0	42	22.7	10.8
SLEDAI score	2.0	25	9.2	6.5
S. creat (mg/dl)	0.5	1.0	0.8	0.2
BUN (mg/dl)	6	60	18	12.1
Cr. Clear (ml/min)	83	124	98.3	14.1
WBC $10^3/mm^3$	3.3	14.3	7.1	2.9
Hb g/dl	5.4	13.0	11.1	1.9
Plt $10^3/mm^3$	44	346	225.9	101.3
Antichromatin (units/ml)	6.2	190	88.6	69.8
Anti- DNA (Iu/ml)	18	61	31.2	12.8

Table (IV-9): Comparison between group I, II and III as regards antichromatin antibodies.

Antichromatin	Group I	Group II	Group III	P-value	Sig.
Mean (S.D.)	113.49 (68.40)	10.34 (4.37)	7.2 (2.32)	< 0.001	VHS
+ve	34 (85%)	0 (0%)	0 (0%)		
-ve	6 (15%)	20 (100%)	20 (100%)		
Total	40 (100%)	20 (100%)	20 (100%)		

The prevalence of antichromatin antibodies in SLE patients was 85% (34/40) versus 0% (0/40) in group II and III (P < 0.001).

There was a statistically very highly significant difference between group I, II and III as regards antichromatin antibody titer, being highest in group I (P<0.001)

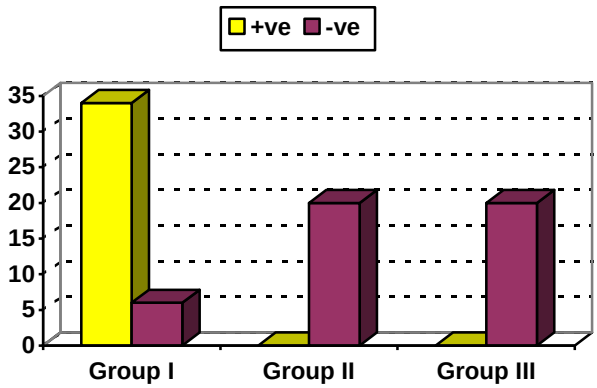

Fig.(IV-1): Prevalence of antichromatin antibodies in group I, II and III.

Table (IV-10): Comparison between group I, II and III as regards anti-DNA antibodies.

Anti-DNA	Group I	Group II	Group III	P-value	Sig.
Mean (S.D.)	41.4 (29.1)	20.9 (22.1)	16.6 (9.3)	< 0.001	VHS
+ve	34 (85%)	4 (20%)	0 (0%)		
-ve	6 (15%)	16 (80%)	20 (100%)		
Total	40 (100%)	20 (100%)	20 (100%)		

The prevalence of anti-DNA antibodies in SLE patients was 85% (34/40), at the time of disease diagnosis,versus 20% (4/20) in group II and 0% (0/20) in group III.

There was a statistically very highly significant difference between group I, II and III as regards anti-DNA titer, being highest in group I (P < 0.001).

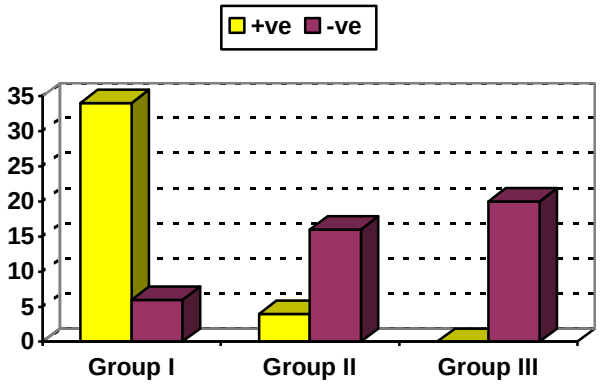

Fig. (IV-2): Prevalence of anti-DNA antibodies in groups I, II and III.

Table (IV-11): Prevalence of antichromatin antibodies in anti-DNA negative SLE patients.

Antrichromatin	No	%
+ve	6	100
-ve	0	0
Total	6	100

Table (IV-12): Value of Antichromatin versus anti-DNA in diagnosing SLE.

	Antrichromatin	Anti-DNA
Sensitivity	85%	85%
Specificity	100%	80%
PPV	100%	89.4%
NPV	76.9%	72.7%

Antichromatin and anti_dsDNA both showed equal sensitivity (85%) for SLE. However antichromatin had a higher specificity and PPV.

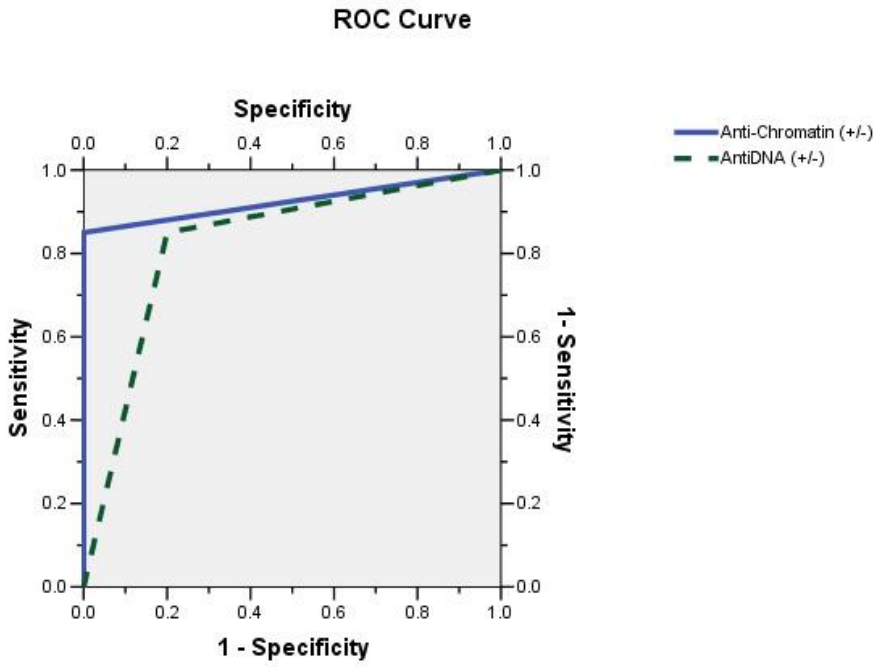

Fig. (IV-3): Receiver Operator Characteristic curve (ROC) for the overall sensitivity and specificity of antichromatin antibodies for SLE at a cutoff value of 20U/ml.

Table (IV-13): Comparison between antichromatin positive and negative patients as regards clinical presentation.

Parameter	Antichromatin +ve(34)		Antichromatin -ve(6)		X^2	P	Sig.
	No	%	No	%			
Photo-sensitivity	0	0	1	16.7	5.8	<0.05	S
Rash	17	50	4	66.7	0.57	>0.05	NS
Lupus headache	1	2.9	0	0	0.18	>0.05	NS
Alopecia	17	50	2	33.3	0.57	>0.05	NS
Arthritis	16	47.1	2	33.3	0.39	>0.05	NS
Fever	13	38.2	0	0	3.40	>0.05	NS
Oral ulcers	12	35.3	2	33.3	0.01	>0.05	NS
Vasculitis	7	20.6	0	0	1.50	>0.05	NS
Myositis	3	8.8	0	0	0.57	>0.05	NS
Seizures	1	2.9	0	0	0.18	>0.05	NS
Organic brain syndrome	1	2.9	0	0	0.18	>0.05	NS
Nephritis	22	64.7	2	33	2.10	>0.05	NS

There was a statistically significant difference ($P< 0.05$) between antichromatin positive and negative patients as regards photosensitivity.

There was no statistically significant difference ($P < 0.05$) between them however as regards other clinical manifestations including nephritis.

To study the significance of antichromatin antibodies in nephritis, we divided our patients into two groups according to high or low antichromatin titers which was defined as values above and below 70U/ml. 25/40(62.5%) of patients had a high titer, 15/40 (37.5%) had a low titer.

Table (IV-14): Comparison between patients with high antichromatin titer and low titer as regards prevalence of nephritis.

	Antichromatin		**X^2**	**p-value**	**Sig.**
	High titer(25)	**Low titer(15)**			
Nephritis	18	6	4.0	<0.05	S
Non nephritis	7	9			

There was a statistically significant difference between patients with high and low titer as regards prevalence of nephritis, being more prevalent in the former ($P<0.05$).

There was a statistically significant difference between antichromatin positive and negative patients as regards C_3, Hb and anti-DNA titer ($P < 0.05$).

There was a statistically highly significant difference between the two groups as regards ESR being higher in antichromatin positive patients($P < 0.01$).

There was a statistically very highly significant difference between the two groups as regards SLEDAI and poteinuria, both being higher in antichromatin positive patients ($P < 0.01$).

There was no statistically significant difference as regards other parameters ($P > 0.05$).

Table (IV-15): Comparison between patients with high antichromatin titer and low antichromatin titer as regards lab parameters.

Parameter	High titer(25)		Low titer(15)		t	P-value	Sig.
	Mean	S.D.	Mean	S.D.			
ESR(1st hr)	82.7	33.6	33.9	21.2	-5.0	<0.001	VHS
C3(mg/dl)	62.1	30.9	96.4	36.4	3.2	<0.01	HS
C4(mg/dl)	17	9.6	9.0	9.0	3.5	<0.001	VHS
SLEDAI score	19.3	9.2	5.5	5.5	-4.9	<0.001	VHS
S.creat(mg/dl)	1.3	1.4	0.7	0.2	-2.0	>0.05	NS
BUN(mg/dl)	26.1	27.6	20.1	12.4	-0.8	>0.05	NS
Cr.Clearance(ml/min)	82.4	32.7	98.6	25.1	1.6	>0.05	NS
Prot/24hrs(gm)	3.1	3.0	0.8	1.2	-3.4	<0.01	HS
WBC($10^3/mm^3$)	5.8	2.6	7.8	3.0	2.2	<0.05	S
Hb(g/dl)	9.3	2.2	11.0	1.3	3.0	<0.01	HS
Plt ($10^3/mm^3$)	215.8	104.7	247	75.8	1.0	>0.05	NS
Activity index	9.7	3.3	5.3	2.9	-2.9	<0.01	HS
Chronicity index	3.1	1.3	2.7	1.0	-0.8	>0.05	NS

Results

There was a statistically significant difference between the two groups as regards WBC ($P<0.05$).

There was a statistically highly significant difference between the two groups as regards C3, proteinuria, Hb and activity index ($P<0.01$).

There was a statistically very highly significant difference between the two groups as regards ESR ,C4 and SLEDAI ($P<0.001$).

Out of the 40 SLE patients, 28(70%) tested positive for both antichromatin and anti-DNA antibodies, 6(15%) tested positive for antichromatin only and 6(15%) tested positive for anti-DNA only.

Of the 34 patients who had positive anti-DNA, 28 were antichromatin positive(82.4%).

Table (IV-16): Comparison between patients with antichromatin +ve only, anti-DNA +ve only and patients having both antibodies as regards clinical data.

Parameter	Antichromatin only(6)		Anti-DNA only(6)		Both (28)		X^2	P-value	Sig.
	No	%	No	%	No	%			
Photosensitivity	0	0	1	16.7	0	0	5.81	>0.05	NS
Rash	5	83.3	4	66.7	12	42.9	3.81	>0.05	NS
Lupus headache	0	0	0	0	1	3.6	0.44	>0.05	NS
Alopecia	3	50	2	33.3	14	50	0.57	>0.05	NS
Arthritis	2	33.3	2	33.3	14	50	0.94	>0.05	NS
Fever	1	16.7	0	0	12	42.9	4.94	>0.05	NS
Oral ulcers	4	66.7	2	33.3	8	28.6	3.16	>0.05	NS
Vasculitis	1	16.7	0	0	6	21.4	1.58	>0.05	NS
Myositis	1	16.7	0	0	2	7.1	1.22	>0.05	NS
Seizures	0	0	0	0	1	3.6	0.44	>0.05	NS
Organic brain syndrome	0	0	0	0	1	3.6	0.44	>0.05	NS
Nephritis	4	66.7	2	33.3	18	64.3	2.10	>0.05	NS

There was no statistically significant difference between the three groups as regards clinical symptoms (P> 0.05).

Despite showing a statistically insignificant difference between the three groups, the prevalence of nephritis was higher in patients with reactivity to antichromatin alone(66.7%) than in patients with

reactivity to both antibodies (64.3%). Patients with only anti-dsDNA reactivity had the least prevalence (33.3%).

Table (IV-17): Comparison between patients with antichromatin +ve only, anti-DNA+ve only and patients having both antibodies as regards laboratory data.

Parameter	Antichromatin only(6)		Anti-DNA only(6)		Both (28)		F	p-value	Sig.
	Mean	S.D.	Mean	S.D.	Mean	S.D.			
Age(years)	29.7	12.3	29	10.9	27.4	10.5	0.14	>0.05	NS
Duration (years)	3.0	2.5	5.9	5.7	2.9	3.0	1.8	>0.05	NS
ESR(1st hr)	66.3	49.2	28.2	24.5	71.8	34.0	3.8	<0.05	S
C3(mg/dl)	70.7	31.6	102.2	30.5	70.1	37.3	2.0	>0.05	NS
C4(mg/dl)	19.4	11.3	28.1	11.7	19.9	10.1	1.6	>0.05	NS
SLEDAI score	13.2	9.5	4.7	3.9	16.8	10.1	4.2	<0.05	S
S.creat(mg/dl)	0.9	0.3	0.7	0.1	1.2	1.3	0.8	>0.05	NS
BUN(mg/dl)	15.8	9.0	15.8	4.0	27.25	26.6	0.9	>0.05	NS
Cr.Clearance(ml/min)	83.3	28.1	96.8	20.5	87.8	33.5	1.2	>0.05	NS
Prot/24hrs(gm)	2.8	2.5	0.5	0.6	2.5	2.9	0.3	>0.05	NS
WBC(10^3/mm^3)	6.3	1.7	7.5	3.3	6.5	3.0	0.35	>0.05	NS
Hb(g/dl)	9.4	2.5	10.9	0.9	9.9	2.2	0.89	>0.05	NS
Plt (10^3/mm^3)	168.5	109	238.7	79.1	237.7	93.8	1.4	>0.05	NS

There was a statistically significant difference between the three groups as regards SLEDAI and ESR, being highest in patients having both antibodies ($P < 0.05$).

There was no statistically significant difference between the three groups as regards other laboratory parameters (P> 0.05).

Table (IV-18): Comparison between group Ia (Nephritis) and Ib (non-nephritis) as regards laboratory parameters.

Parameter	Group Ia(24)		Group Ib(16)		t	P	Sig.
	Mean	S.D.	Mean	S.D.			
Duration (years)	2.6	2.7	4.6	4.3	1.9	>0.05	NS
ESR(1st hr)	78.3	35.1	43.5	32.4	-3.17	<0.01	HS
C3(mg/dl)	67.5	26.9	86.2	46.6	1.60	>0.05	NS
C4(mg/dl)	19.9	10.6	22.7	10.8	0.81	>0.05	NS
SLEDAI score	17.9	10.7	9.2	6.5	-2.91	<0.01	HS
S. creat(mg/dl)	1.3	1.4	0.8	0.2	-1.9	>0.05	NS
BUN(mg/dl)	27.7	27.7	18	12.1	-1.5	>0.05	NS
Cr.Clearance(ml/min)	66.8	49.4	94.7	27.6	2.8	<0.05	S
WBC(10^3/mm^3)	6.3	2.8	7.1	3.0	0.83	>0.05	NS
HB(g/dl)	9.2	1.9	11.1	1.9	3.07	<0.01	HS
Plt (10^3/mm^3)	228.5	92.9	225.9	101.3	-0.08	>0.05	NS
Antichromatin(units/ml)	130.1	63.5	88.6	69.8	-1.95	<0.05	S
Anti- DNA(Iu/ml)	48.3	34.7	31.2	12.8	-2.1	<0.05	S

There was a statistically highly significant difference between the two groups as regards ESR, SLEDAI and Hb (P < 0.01).

There was a statistically significant difference between the two groups as regards antichromatin, anti-DNA and creatinine clearance ($P < 0.05$).

There was no statistically significant difference between the two groups as regards other lab parameters ($P > 0.05$).

Table (IV-19): Prevalence of antichromatin and anti-DNA antibody in lupus nephritis patients.

	Antichromatin		**Anti-DNA**	
	No	**%**	**No**	**%**
Lupus nephritis	22	91.7	20	83.3

Prevalence of antrichromatin was 91.7% (22/24), while that of anti-DNA was 83.3% (20/24**).**

Table (IV-20): Value of antichromatin and anti-DNA antibodies reactivity in diagnosis of lupus nephritis.

	Antichromatin	**Anti-DNA**
Sensitivity	91%	83.3%
Specificity	12.5%	12.5%

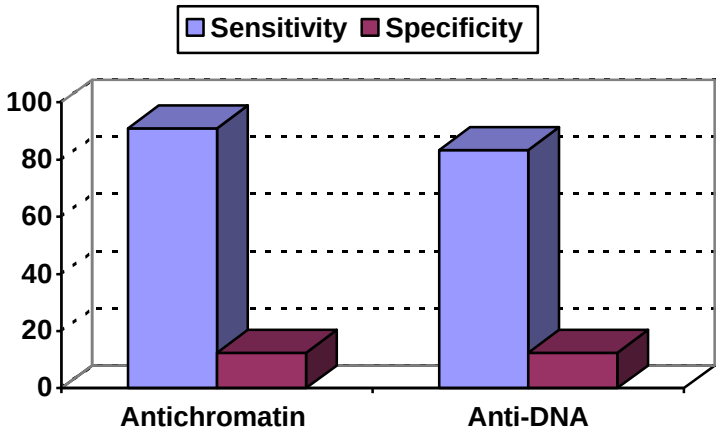

Fig. (IV-4): Value of antichromatin and anti-DNA antibodies in diagnosis of lupus nephritis.

Table (IV-21): Value of high antichromatin titer in diagnosis of lupus nephritis.

	Antichromatin
Sensitivity	75%
Specificity	56%
PPV	72%
NPV	60%

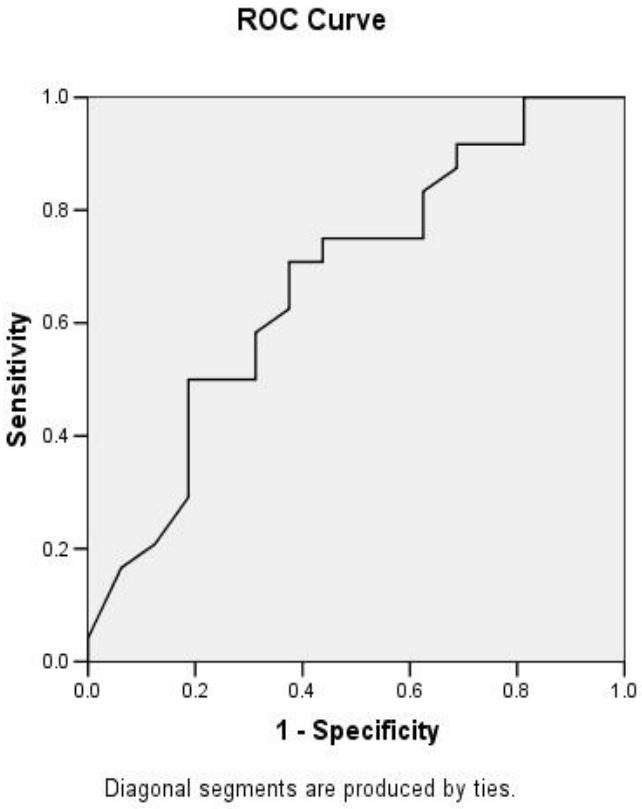

Fig. (IV-5): Receiver Operator Characteristic curve(ROC) for the overall sensitivity and specificity of antichromatin antibodies for SLE at a cutoff value of 70U/ml

The most prevalent class of nephritis in our study group was class IV, followed by class II, III and V with a prevalence of 50%, 20.8%, 16.7% and 12.5% respectively. None of the patients had class I or VI lupus nephritis.

Table (IV-22): Comparison between different classes of lupus nephritis as regards anti chromatin titer.

	Antichromatin		**P-value**	**Sig.**
Class	**Mean**	**S.D.**		
II	118.8	77.9		
III	131	57.5	> 0.05	NS
IV	129.1	70.9		
V	152	24.3		

There was no statistically significant difference between the different classes of nephritis and antichromatin titer (P> 0.05).

Table (IV-23): Comparison between different classes of lupus nephritis as regards anti-DNA titer.

	AntiDNA		**P-value**	**Sig.**
Class	**Mean**	**S.D.**		
II	44.4	43.2		
III	44.8	32.1	> 0.05	NS
IV	44.9	34.9		
V	72.7	29.4		

There was no statistically significant difference between the different classes of nephritis and anti-DNA titer (P > 0.05).

SLE patients were further subdivided into active and inactive according to SLEDAI score. They were 8/40 (20%) with inactive disease and 32/40 (80%) with active disease.

The prevalence of antichromatin antibody in active SLE patients was 93.7%(30/32) versus 50% (4/8) in inactive disease.However the prevalence of anti-dsDNA in active SLE was34.3% (11/32) vs.0%(0/8) in inactive SLE.

Table (IV-24): Comparison between active and inactive patients as regards anti-DNA and antichromatin titer.

	Antichromatin		**P**	**Sig.**	**Anti-DNA titer**		**P**	**Sig.**
	Mean	**S.D.**			**Mean**	**S.D.**		
In active	35.8	30.1	<0.001	VHS	22.1	10.1	<0.01	HS
Active	132.9	61.1			46.3	30.3		

There was a statistically very highly significant difference between active and inactive patients as regards antichromatin titer, being higher in active patients (p < 0.001).

There was a statistically highly significant difference between active and inactive patients as regards anti-DNA titer, being higher in active patients ($p < 0.01$).

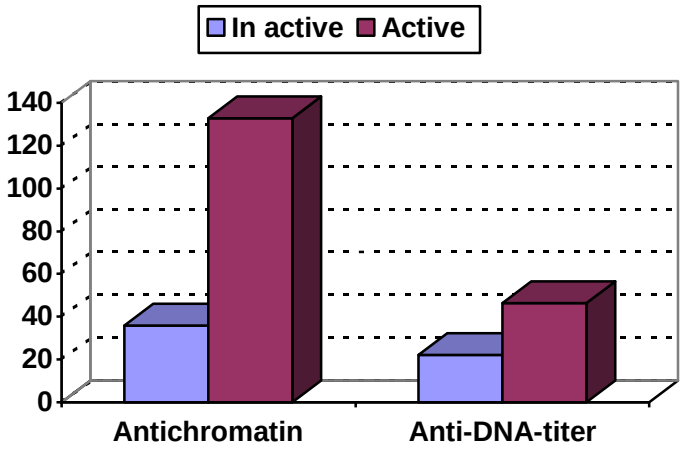

Fig. (IV-6): Antichromatin and anti-dsDNA titer in active and inactive SLE.

Table (IV-25): Value of antichromatin and anti-DNA antibodies in disease activity.

	Antichromatin	**Anti-DNA**
Sensitivity	93.7%	78%
Specificity	50%	12.5%

Table (IV-26): Correlation between antichromatin antibody titer and lab parameters of renal disease.

Parameter	r	P-value	Sig.
S. creat	0.29	>0.05	NS
BUN	0.18	>0.05	NS
Cr. Clearance	-0.26	>0.05	NS
Proteinuria	0.53	<0.001	VHS
Activity index	0.60	<0.01	HS
Chronicity index	0.12	>0.05	NS

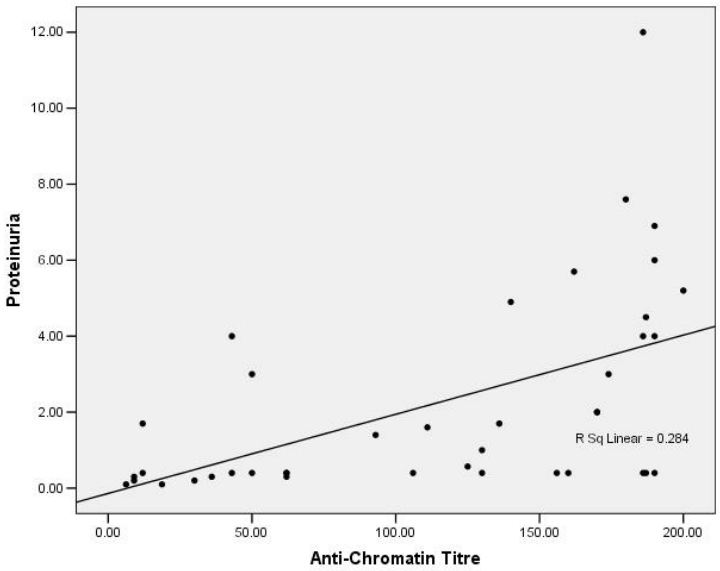

Fig (IV-7): Correlation between antichromatin titer and proteinuria.

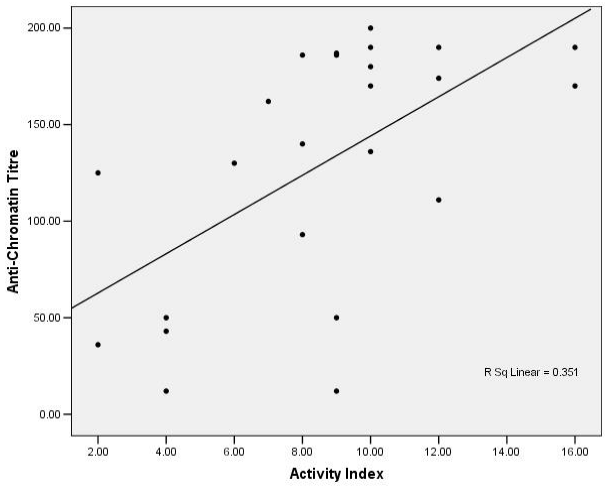

Fig (IV-8): Correlation between antichromatin titer and activity index.

Table (IV-27): Correlation between antichromatin antibody titer and parameters of disease activity.

Parameter	r	P-value	Sig.
SLEDAI	0.66	<0.01	HS
C3	-0.46	<0.01	HS
C4	-0.50	<0.01	HS
ESR	0.61	<0.01	HS
Anti-DNA	0.44	<0.01	HS

There was a statistically highly significant negative correlation between antichromatin antibody titer C3 and C4 titers ($p < 0.01$).

There was a statistically highly significant positive correlation between antichromatin antibody titer and SLEDAI, ESR and anti-DNA titer ($p < 0.01$).

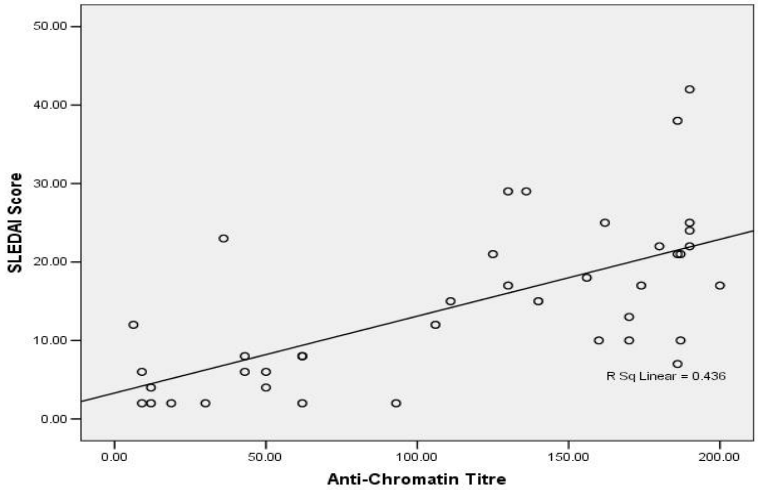

Fig.(IV-9): Correlation between antichromatin titer and SLEDAI score.

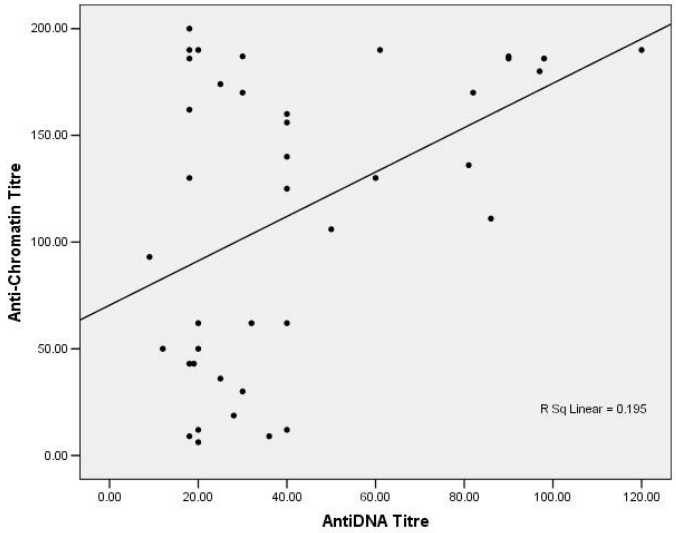

Fig. (IV-10): Correlation between antichromatin titer and anti-dsDNA.

Discussion

Systemic lupus erythematosus is an autoimmune disease that causes inflammation in the tissues of the brain, endothelial cells, gastrointestinal/genitourinary, joints, kidneys, muscles and skin. Lupus comprises a range of multisystem disorders involving the deposition of aberrant immune complexes into tissues. Inflammation occurs as a result of autoantibodies attacking organ systems ***(Childs, 2006).***

Lupus nephritis presents insidiously, and if it is not detected early, the risk of progression to renal impairment is high ***(D'Cruz, 2006).***

Autoantibodies may be present for many years before the clinical onset of the disease, and there may be increasing numbers of antibodies just before symptoms develop ***(Arbuckle et al., 2003).***

For a long time, ds-DNA has been believed to be the major autoantigen in SLE. As a consequence, most of the studies on sensitivity and specificity have focused on anti-dsDNA antibodies ***(Saisoong et al., 2006).***

Discussion

Antichromatin antibodies are actually detected very early during the disease, before anti-dsDNA ***(Amoura et al., 1994).***

Importantly, about 30% of SLE patients have no anti-dsDNA antibodies. It has been suggested that antichromatin antibodies could be a better disease marker than anti-dsDNA, as they are detected in both active and inactive SLE in contrast to anti-dsDNA antibodies which were nearly exclusively detected in active patients ***(Decker, 2006).***

The two most important recurring observations concerning antichromatin antibodies are that this antibody is sensitive and specific for SLE, and the presence of antichromatin is often correlated with glomerulonephritis in patients with SLE ***(Burlingame and Cervera, 2002).***

Our study was therefore performed to assess the value of antichromatin antibodies in diagnosing SLE, specially in anti-dsDNA negative patients , assessing disease activity, and to determine the association of these antibodies with lupus nephritis, in Egyptian SLE patients.

Discussion

It included 40 SLE patients who fulfilled at least four of the ACR 1982 revised criteria, 20 patients with other SAD as a disease control group, in addition to 20 healthy controls with matched age and sex, as the healthy control group.

In our study antichromatin antibodies were detected in 34/40(85%) of SLE patients and in non of the other SAD patients or healthy controls, making it 85% sensitive and 100% specific for SLE.

The mean titer showed a statistically very highly significant difference between the three groups ($P<0.001$), being higher in SLE patients as compared to the disease and healthy control groups.

Anti-DNA had the same prevalence and sensitivity for SLE 34/40(85%) but a lower specificity 80%, as it was present in 20% of the disease control group, but in non of the healthy controls.

There was a statistically very highly significant difference between the three groups as regards anti-dsDNA titer ($P<0.001$), being higher in SLE patients as compared to the two other groups. The levels however, were lower

than those of antichromatin antibodies, which supports the hypothesis that the nucleosome and not free DNA is the autoantigen which drives the autoimmune response.

Cervera et al., 2003, performed a study on 100 SLE patients, 140 SAD and 100 healthy controls, to assess the prevalence and clinical associations of antichromatin antibodies in SLE. He reported a prevalence of 69% in SLE patients, 8% in other SAD and 0% in healthy controls.

Our results were in agreement with ***Simon et al., 2004.*** This study aimed at comparing the utility of different antichromatin antibodies in diagnosing SLE and as markers of disease activity. It was performed on 73 SLE patients of recent onset, 261 patients with SAD and 130 healthy controls. He reported that the prevalence of antinucleosome antibodies was 100% in SLE patients and only 3% in healthy controls, with a sensitivity and specificity of 100% and 97% respectively. In comparison, Anti-DNA was prevalent in only 63% of SLE patients and 5% of healthy controls.

The study by ***Bruns et al., 2000,*** was the first to study the prevalence of antinucleosome antibodies in other

SAD, it included 136 SLE patients and 309 with other SAD. This study reported a prevalence of 56 % in SLE patients and 3% in other SAD.

Our results were closely similar to those of ***Amoura et al., 2000,*** who studied the frequency and disease specificity of antinucleosome antibody reactivity in diverse C.T diseases including SLE. He found that the prevalence of these antibodies was 71.7%.

Min et al., 2002 studied the prevalence and clinical significance of antinucleosome antibodies in 129 SLE patients lacking anti-DNA. He reported a prevalence of 76%.

Finally, ***Haddouk et al., 2005,*** who studied the clinical significance of antinucleosome antibodies in Tunisian SLE patients, stated that the prevalence of antichromatin antibodies was 78.6% .

Other studies reported a much lower prevalence of antinucleosome antibodies in SLE. ***Kiss et al., 2001*** reported that the prevalence of antinucleosome antibodies in SLE patients was 39.2% while that of anti-DNA was 28%. ***Benucci et al., 2003*** found that only 37.5% of SLE

patients showed reactivity to antinucleosome antibodies. Recently, ***Saisoong et al., 2006,*** studied the correlation between antinucleosome, anti-DNA and C3 and C4 in 65 SLE patients. He found that the prevalence of antinucleosome vs. anti-DNA antibodies in SLE patients was 52.3% vs. 36.9%.

Evidence has been accumulating to suggest that nucleosomes are the main autoantigen in lupus and that circulating nucleosomes play a key role in the development of lupus. The high sensitivity and specificity of antichromatin antibodies in our study, which is consistent with most studies, proves this point. The differences observed in some studies as regards the specificity of these antibodies to SLE might result from the difference in race of patients, the study sample size , the treatment used as well as the quality and the characteristics of the nucleosomes used in the assays.

Regarding the significant prevalence of antinucleosome antibody in systemic sclerosis and mixed connective tissue disease found in some studies, as ***Amoura et al., 2000*** and ***Sato et al., 2004,*** discrepancies might be explained by the quality of nucleosome

preparations used. Scl-70 is known to bind to nucleosomes occasionally. Anti-Scl-70 antibodies are detected in most systemic sclerosis patients and in some patients with MCTD. Using Scl-70-free nucleosomes, antinucleosome antibodies were only detected in SLE patients and not in systemic sclerosis patients, suggesting that antinucleosome antibodies are specifically associated with SLE.

15% of our SLE patients were negative for anti-dsDNA antibodies. The prevalence of antichromatin antibodies in these patients was 100%(6/6), again this was in agreement with ***Simon et al., 2004*** who reported the same prevalence. Other studies such as ***Min et al., 2002, Cervera et al., 2003, Haddouk et al., 2005*** and ***Saisoong et al., 2006*** reported a varying prevalence that ranged from 24 -60%. These results support the view that antichromatin antibodies can be a reliable and accurate marker for anti-dsDNA negative SLE patients.

Our study revealed a strong association between antichromatin antibody reactivity and disease activity. We had 34 patients with sera positive for antichromatin and 6 patients with negative sera. Comparison between the two groups revealed the following:

Discussion

There was a statistically significant difference ($P<0.05$) in C3, C4 and hemoglobin levels between antichromatin positive and negative patients, being lower in the former, probably due to the fact they had the higher disease activity.

This was further confirmed by the finding that ESR and SLEDAI score, which are both indicators of disease activity, showed a statistically highly significant difference between the two groups, being higher in antichromatin antibody positive patients as compared to antichromatin negative patients ($P<0.01$).

In agreement with our results ***Benucci et al., 2003*** found that C3, C4 and hemoglobin were statistically significantly lower in antinucleosome positive patients as compared to antinucleosome negative patients.

Out of the 40 SLE patients, 28/40 (70%) showed reactivity to both antichromatin and anti-DNA, 6/40 (15%)were positive for antichromatin only and 6/40 (15%) were positive for anti-DNA only.

Comparison between these groups of patients showed that there was a statistically significant difference between

them as regards ESR and SLEDAI score ($P<0.05$), being higher in patients having both antibodies.

The majority of patients had both antibodies in their sera 28/40 (70%). All patients who were DNA negative were antichromatin positive which proves that the production of anti-dsDNA antibodies is associated with that of antichromatin and that anti-dsDNA antibodies are a subset of antichromatin antibodies. The most likely explanation for the presence of anti-dsDNA antibodies in the absence of antichromatin antibodies, 6/40 (15%), is that some antibodies may react with certain structures of DNA that occur in solution but are absent in the DNA wrapped around the histones in the structure of chromatin used as a substrate in ELISA.

Also, in our study antichromatin antibody was more prevalent in active than in inactive SLE patients, 93.7% (30/32) vs.50%(4/8). It was even more prevalent than anti-DNA antibody, which showed a prevalence of 34.3%(11/32) in active vs. 0%(0/8) in inactive disease.

Antichromatin antibody titers were statistically significantly very high in patients with active disease as

compared to patients in remission (P<0.001). In inactive patients, although the mean titer was relatively low it remained above the cutoff value.

Anti-dsDNA antibody titers were also statistically significantly higher in patients with active disease than those with inactive disease (P<0.01). Titers of anti-dsDNA, however were below the cutoff value in inactive patients. This observation proves that antichromatin antibody remains positive even in remission of the disease unlike anti-dsDNA which can convert to negativity in periods of disease quiescence.

Furthermore, we found in our study that antichromatin antibody titers were correlated to parameters of disease activity, namely ESR, SLEDAI score, C3 and anti-dsDNA titers.

Several other studies studied the correlation between antichromatin antibody titers and parameters of disease activity.

Bruns et al., 2000 found a correlation between disease activity using ECLAM score and antinucleosome antibodies.

Discussion

Saisoong et al., 2006 found that only C3 and not C4 was inversely correlated to antinucleosome while SLEDAI and anti-DNA both correlated with it positively.

Simon et al., 2004, stated in his study that there was a positive correlation between SLEDAI score and antinucleosome antibody titer.

Campos et al., 2006 found that antinucleosome antibodies were associated with a higher SLEDAI score.

Our results suggest that antichromatin antibodies are in fact reliable markers for disease differentiation and activity and that they are related to the pathogenesis of the disease.

Another important finding in our study was the presence of a strong association between antichromatin antibody and lupus nephritis.

Of the 40 SLE patients, 24(60%) had lupus nephritis. The prevalence of antichromatin antibodies in nephritis was 91.7% vs. 83.3% for anti-DNA antibodies.

Discussion

Cervera et al., 2003 found that antichromatin antibodies had a lower prevalence of 58% in patients with nephritis vs. 29% in non-nephritis patients.

Saisoong et al., 2006, reported that the prevalence of antinucleosome antibodies in patients with lupus nephritis was 63.9% vs. 37.9% in non-nephritis patients. There was no significant difference between the two groups as regards anti-DNA.

A higher prevalence (58% vs. 29%) of antichromatin antibodies was found in Spanish SLE patients with nephropathy compared to those without ***(Cervera et al., 2001).***

The mean antichromatin titer in our study was statistically significantly higher in patients with nephritis than non nephritis,130U and 89U respectively ($P<0.05$).

Anti-dsDNA antibody titers were also significantly higher in lupus nephritis as compared to non-nephritis with a mean of 48.3Iu/ml and 31.2Iu/ml respectively. This can be explained by the fact that the patients with nephritis in this study had more active disease than those with no nephritis as proved by the ESR and SLEDAI score which

were both statistically significantly higher in patients with nephritis.

The sensitivity for lupus nephritis in our study was 83.3% for anti-DNA as compared to 91% for antichromatin antibodies. Both antibodies had a specificity of 12.5% for lupus nephritis.

Cervera et al., 2003 stated that the mean titer of antichromatin antibody in nephritis vs. non-nephritis was 68U vs.42U respectively, with a sensitivity and specificity of antichromatin antibodies for lupus nephritis of 81% and 39% respectively as compared to anti-DNA which had a sensitivity of 75% and a specificity of 63%.

In our study, the prevalence of nephritis in antichromatin positive SLE patients was higher than in antichromatin negative SLE patients(64.7% vs.33.3%) which was statistically insignificant.

There was a statistically highly significant difference between the two groups as regard anti-dsDNA ($P<0.01$).

Discussion

There was also a statistically very highly significant difference between the two groups as regards proteinuria ($P<0.001$), being higher in antichromatin positive patients.

There was no statistically significant difference between the two groups as regards other lab parameters.

Considering high and low antichromatin titers using a titer of 70U (strongly positive) as a cutoff, there were 25/40(62.5%) patients with high titer and 15/40 (37.5%) with low titer. Comparison between the two groups revealed that patients with high antichromatin antibody titers had a statistically significantly higher prevalence of nephritis than patients with low titer ($P<0.05$). These patients also had a higher degree of proteinuria and a higher activity index ($P<0.01$).

We also found that high titers of antichromatin antibody have a sensitivity of 75% and specificity of 56% for lupus nephritis.

Simon et al., 2004, agreed with our results when he reported that high titers of antichromatin antibodies were associated with proteinuria and hematuria, both parameters of nephritis.

Cortes-Hernandez et al., 2004, reported that higher levels of anti-DNA and antichromatin antibodies were found in active lupus nephritis than those with active SLE without nephritis.

Renal biopsies performed in patients with lupus nephritis revealed that class IV was the most prevalent class 50%, followed by class II, III and V with a prevalence of 20.8%, 16.7% and 12.5% respectively.

Inspite of the aforementioned findings, there was no statistically significant difference between the different classes as regards reactivity or levels of antichromatin or anti-dsDNA antibodies ($P > 0.05$).

This was in contrast to one study by ***Cortes-Hernandez et al., 2003***, who stated that antinucleosome antibodies were associated more with proliferative nephritis; classes II, III and IV than with membranous nephritis.

Nevertheless, antichromatin antibodies in our study correlated with parameters of renal disease as proteinuria and activity index of renal biopsy. ***Burlingame et al., 1994*** stated that there was a significant correlation

between antichromatin antibodies and proteinuria, but the correlation of anti-DNA and proteinuria did not reach a statistical significance.

While a few studies dismissed the alleged relation of antichromatin to nephritis as ***Campos et al., 2006*** and ***Ghirardello et al., 2004***, most of the other studies, as mentioned above confirmed this association.

Our study also confirmed this association with lupus nephritis which suggests that antichromatin antibodies are nephritogenic antibodies. It seems to depend on a complex interaction between charges associated with the quaternary structure of the nucleosomes and epitope targets in renal tissue. That is, the histones that constitute part of the nucleosomes have a cationic charge, whereas the glomerular basement membrane has an anionic charge, which permits an interaction between them.

We also found that as regards comparison of clinical manifestations between antichromatin positive and negative patients, photosensitivity was statistically significantly lower in patients with antichromatin antibodies as compared to patients lacking these antibodies

(P<0.05). There was no association however between antichromatin and other clinical manifestations.

Unlike our results, ***Simon et al., 2004*** found that these antibodies were associated with other clinical manifestations as malar rash, arthritis and oral ulcers.

Campos et al., 2006 reported that the presence of antinucleosome antibodies were significantly associated with malar erythema and hemolytic anemia.

Ghirardello et al., 2004 stated that there was no relevant relationship between antichromatin antibodies and disease features.

Conclusion & Recommendations

- Several studies, including ours have found that antichromatin antibodies are both sensitive and specific for SLE.
- These antibodies have a higher specificity for SLE than anti-dsDNA.
- Antichromatin antibodies can be a useful tool for diagnosis of SLE and monitoring disease activity in anti-DNA negative patients.
- Anti-DNA antibodies are mostly present in patients with active disease and almost absent in patients with inactive disease.
- Antichromatin antibody titers correlate with disease activity parameters as C3, ESR and SLEDAI score.
- Antichromatin antibody titers are higher in patients with nephritis than non-nephritis. They are correlated with parameters of renal disease as proteinuria and activity index.

Conclusion &Recommendations

In light of the above mentioned findings, we recommend the following:

- Antichromatin antibodies could be used as one of the ACR criteria to diagnose SLE.
- Antichromatin antibodies could be used to differentiate SLE in its early stages from other SAD.
- Antichromatin antibody titers could be used as a marker of disease activity, especially in patients lacking anti-dsDNA antibody.
- Patients with high titers of antichromatin antibodies should have their renal functions closely followed up for fear of development of lupus nephritis.

Summary

Systemic lupus erythematosus is a multisystem disease with diverse clinical manifestations. It is characterized by the presence of antinuclear antibodies.

Lupus nephritis is both a frequent and potentially serious complication of SLE. Serious kidney disease may influence morbidity and mortality both directly and indirectly through complications of therapy.

Chromatin is made up of polynucleosomes. Nucleosomes are the repeating unit of histone and DNA.

Evidence is to date accumulating to suggest that the nucleosome plays a key role in the pathogenesis of SLE.

Several reports have shown that chromatin represents the main autoantigen in SLE and that specific antibodies are an important marker of the disease.

This study was carried out to evaluate the presence of antichromatin antibodies in Egyptian SLE patients, especially in anti-dsDNA negative patients. It assessed the

prevalence of these antibodies in the other SAD and their association with lupus activity and lupus nephritis.

Our study included: 40 patients with SLE, 24 with lupus nephritis and 16 without nephritis, 20 patients with other SAD in addition to 20 healthy controls.Our results showed that antichromatin antibodies are sensitive and even more specific to SLE than anti-dsDNA antibodies. Antichromatin antibodies were present in all patients who were negative for anti-dsDNA.

Titers of antichromatin antibody were statistically significantly higher in patients with nephritis than non-nephritis.

These antibodies positively correlated with ESR, SLEDAI, anti-DNA titer, proteinuria and activity index of renal biopsy. They correlated inversely with C3.

Antichromatin antibodies are a useful tool for the diagnosis of SLE, especially at early stages of the disease, and a useful marker of disease activity, particularly in patients who are negative for anti-ds DNA antibodies.High titers of antichromatin antibodies identify patients who are at increased risk of renal involvement.

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