ANTI-NUCLEOSOME ANTIBODIES AS A DISEASE ACTIVITY MARKER IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

by

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بسم الله الرحمن الرحيم

وَقُل رَّبِّ زِدْنِي عِلْمًا {طه,114}

[Taha, 114] O my Lord ! increase me in knowledge.

DEDICATION

I would like to dedicate my thesis to my parents, whose confidence in me has been unwavering, and to my beloved brothers and sisters. Thank you for the moral and financial support that kept me afloat.

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LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ANA	Anti-nuclear antibody
aPL	Anti-phopholipid antibodies
BILGA	British Isles Lupus Assessment Group
C3	Complement-3
C4	Complement-4
CH_{50}	Hemolytic complement-50
CI	Confidence interval
CNS	Central nervous system
CRP	C-reactive protein
CTD	Connective tissue diseases
ds-DNA	Double-standed deoxynucleic acid
ECLAM	European Consensus Lupus Activity Measurement
ELISA	Enzyme linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
FITC	Fluorescein isothiocynate
FTA	Fluorescent treponemal antibody absorption test
GBM	Glomerular basement membrane
HeLa	Henrietta Lacks
HEp	Human epithelial
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
IF	Immunofluorescence
Ig (s)	Immunoglobulin (s)
IL	Interleukin
IQR	Interquartile rang
LE	Lupus erythematosus
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex

n	Number
NC	Negative control
NK	Natutal killer
NPV	Negative predictive value
NSAID	Non steroidal anti-inflammatory
PBS	Phosphate buffer saline
PC	Positive control
PPV	Positive predictive value
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
SLAM	Systemic Lupus Activity Measure
SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
TMP	Tetramethylbenzidine
TPI	Treponema pallidum inhibition
WBC	White blood cell
WHO	World Health Organization

ABSTRACT

The clinical aspect of measuring the level of anti-nucleosome antibodies in patients with systemic lupus erythematosus (SLE) has been well established and it is claimed that their presence is associated with the disease activity. The overall aim of this study was to measure the level of anti-nucleosome antibodies in the SLE patients, to determine the sensitivity as well as the specificity of these antibodies in the diagnosis of the disease. The evaluation of the relationship between the levels of anti-nucleosome antibodies, the disease activity as well as and other markers (anti-dsDNA antibodies, C3, C4 and CRP) were also compared and assessed.

In this cross sectional study, all patients were selected from Hospital Universiti Sains Malaysia (HUSM), who were either attending the medical specialist's clinic or have been admitted to the medical wards. Ninety SLE patients, forty-five other connective tissue diseases (e.g., rheumatoid arthritis, mixed connective tissue disease, scleroderma, Raynaud's syndrome and Sjögren's syndrome) and ninety normal controls were tested for anti-nucleosome antibodies by enzyme linked immunosorbent assay (ELISA), anti-dsDNA and ANA by Immunofluorescence (IF), C3, C4 and CRP by immunoturbidometry techniques. SLE diseases activity was evaluated by using SLE-Disease Activity Index (SLEDAI) score. A patient is defined as having active SLE when SLEDAI score is more than 5.0

Out of 90 SLE patients, anti-nucleosome antibodies were positive in 52% of patients, whereas these antibodies were positive in 7% of patients with other connective tissue diseases. The median concentration of anti-nucleosome antibodies was significantly different between SLE patients and other connective tissue diseases. None of the normal controls was found to be positive for these antibodies. Anti-dsDNA antibodies were positive in 37% SLE patients, whereas these antibodies were positive in 8% of patients with other connective tissue diseases. None of the normal controls was found to be positive for these antibodies. ANA was found to be positive in 81% SLE patients, in 61% of patients with other connective tissue diseases and 7% in normal controls. Anti-nucleosome antibodies had a sensitivity of 52%, whereas anti-dsDNA antibodies had a sensitivity of 37%. The specificity of anti-nucleosome and anti-dsDNA antibodies was 98% and 97% respectively. ANA had sensitivity and specificity of 81% and 75% respectively. Anti-nucleosome antibodies were positive in 98% of patients with active SLE, whereas these antibodies were positive in 14% of patients with inactive SLE. The median concentration of anti-nucleosome antibodies was significantly different between active and inactive SLE patients. Anti-dsDNA antibodies were found to be positive in 61% of patients with active SLE and in 16% of patients with inactive SLE. Anti-nucleosome antibodies had a stronger correlation than anti-dsDNA antibodies with SLEDAI score. The median concentration of C3 and C4 was significantly different between active and inactive SLE patients, and showed an inverse and significant correlation with SLEDAI score, whereas the median concentration of CRP was not significantly different between active and inactive SLE.

In conclusion, anti-nucleosome antibodies test is a recently developed test that may help in diagnosis of SLE patients. Anti-nucleosome antibodies are highly sensitive and specific for the diagnosis of SLE, especially when the anti-dsDNA antibodies are absent. Thus anti-nucleosome antibodies test can be a better tool for the diagnosis of SLE. Anti-nucleosome antibodies have a stronger and significant correlation with SLEDAI score than other traditional disease activity markers. Therefore, it can be a useful and additional disease activity marker to other laboratory tests that can help in the assessment SLE disease activity.

ABSTRAK

ANTIBODI ANTI-NUKLEOSOM SEBAGAI PETANDA AKTIVITI DALAM PENYAKIT SYSTEMI LUPUS ERYTHEMATOSUS

Pengukuran paras antibodi anti-nukleosom dalam pesakit SLE telahpun diselidik pada peringkat klinikal dan ianya membuktikan bahawa kehadiran antibodi ini berkaitan dengan aktiviti penyakit itu sendiri. Tujuan utama kajian ini adalah untuk mengukur paras antibodi anti-nukleosom pada pesakit SLE dan mengesan sensitiviti serta spesifisiti antibodi anti-nukleosom dalam diagnosis SLE. Selain itu, penyelidikan di antara hubungan paras antibodi, aktiviti penyakit dan petanda aktiviti penyakit lain (antibodi anti-dsDNA, C3, C4 dan CRP) juga turut dibanding dan diperukurkan.

Dalam kajian secara rambang, semua pesakit dipilih dari HUSM, ini termasuklah mereka yang hadir di klinik pakar perubatan dan yang telah dimasukkan ke wad. Seramai 90 pesakit SLE telah dipilih dalam kajian ini, 45 adalah pesakit yang berhubung dengan penyakit tisu penghubung (seperti, rheumatoid arthritis, penyakit tisu penghubung campuran, scleroderma, sindrom Raynaud dan sindrom Sjögren) dan 90 individu normal yang bertindak sebagai kawalan. Mereka ini telah diuji dengan ujian-ujian antibodi anti-nukleosom dengan ELISA, anti-dsDNA dan ANA dengan IF, C3, C4 dan CRP menggunakan teknik immonoturbidometry. Aktiviti penyakit SLE telah dikaji dengan menggunkan skor (SLEDAI). Seseorang yang dikatakan mempunyai SLE aktif apabila paras SLEDAI melebihi 5.0.

Daripada 90 pesakit SLE, seramai 52% pesakit mempunyai antibodi anti-nukleosom yang positif manakala 7% pesakit daripada penyakit tisu penghubung yang lain. Konsentrasi median antibodi anti-nukleosom adalah berbeza diantara pesakit SLE dan penyakit tisu penghubung yang lain. Antibodi anti-dsDNA adalah 37% positif pada pesakit SLE, manakala 8% lagi positif pada penyakit tisu penghubung yang lain. Tidak seorangpun individu normal positif kepada antibodi anti-dsDNA. ANA didapati positif dalam 81% pesakit SLE,61% pada penyakit tisu penghubung dan 7% pada kawalan normal. Antibodi anti-nukleosom mempunyai sensitiviti sebangak 52%, sementara antibodi anti-dsDNA mempunyai sensitiviti sebanyak 37%. Spesifisiti antibodi anti-nukleosom dan anti-dsDNA adalah 98% dan 97% . ANA mempunyai sensitiviti dan ketepatan sebanyak 81% dan 75%. Antibodi anti-nukleosom adalah positif iaitu sebanyak 98% pada pesakit SLE yang aktif, sementara antibodi ini positif iaitu sebanyak 14% pada pesakit SLE yang tidak aktif. Konsentrasi median antibodi anti-nukleosom adalah berbeza diantara pesakit SLE aktif dan tidak aktif, sementara antibodi anti-dsDNA telah ditemui positif sejumlah 61% daripada pesakit SLE aktif dan 16% pesakit SLE yang tidak aktif. Antibodi anti-nukleosom mempunyai perkaitan yang kuat dengan antibodi anti-dsDNA melalui skor SLEDAI. Konsentrasi median C3 dan C4 adalah berbeza diantana pesakit SLE yang aktif dengan yang tidak aktif, dan ini menunjukkan perkaitan terhadap skor SLEDAI, sementara itu, konsentrasi median CRP tidak menunjukkan sebarang berbezaan diantara pesakit SLE aktif dan yang tidak aktif.

Kesimpulannya, ujian antibodi anti-nukleosom telah berkembang dan ini akan membantu dalam diagnosis pesakit SLE. Antibodi anti-nukleosom mempunyai sensitiviti yang tinggi dan ketepatan dalam diagnosis SLE, terutama apabila ketiadaan antibodi anti-dsDNA.

Maka, antibodi anti-nukleosom merupakan ujian alternatif yang boleh digunakan dalam diagnosis SLE. Antibodi anti-nukleosom mempunyai perkaitan yang kuat dan hubungan melalui skor SLEDAI berbanding dengan petanda aktiviti penyakit tradisional yang lain. Oleh itu, ianya boleh digunakan petanda aktiviti penyakit bagi tujuan ujian makmal dalam membantu mengenalpasti aktiviti penyakit SLE.

CHAPTER ONE

INTRODUCTION

INTRODUCTION

1. Autoimmunity and autoimmune diseases

Autoimmunity is defined as a condition when an individual's immune system begins reacts adversely against its own tissues. The primary cause is a loss of tolerance to one's own tissue antigens. Normally, the immune system is able to distinguish self tissue antigens from foreign non-self tissue antigens. Hence, the essential mechanism of autoimmunity is the failure of the immune system to recognize its own tissues as self and to react to these as foreign antigens (Brostoff and Howell, 1992).

Many factors participate in the generation of autoimmunity. Helper T cells control the immune response to protein antigens: defects in this cell population may lead to high affinity autoantibody production reactivity to self antigens. These phenomena are often linked to certain major histocompatibility complex (MHC) loci leading to the production of autoimmunity and various immunologic alterations may consequently lead to autoimmune diseases (Blackman *et al.,* 1990).

Autoimmune diseases affect 5-7% of the population, and can be either organ-specific or non-organ specific (systemic autoimmune disease). In organ–specific autoimmune disease, such as autoimmune thyroiditis, the immune response is directed to a target antigen unique to a single tissue or organ, so that the autoimmune destruction could be largely limited to that organ.

In non-organ specific autoimmune disease, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis, the response is directed towards a broad range of target antigens and involves a number of organs and tissues (Brostoff and Howell, 1992). Non-organ specific autoimmune diseases, which also are called connective tissue or rheumatic diseases have clinical importance and they represent a major group of autoimmune diseases. The diffuse connective tissue diseases include SLE, rheumatoid arthritis, Sjögren's syndrome, scleroderma, Raynaud's syndrome, mixed connective tissue disease (MCTD) and polymyositis (Alarcon *et al.,* 1991).

Each of the connective tissue diseases has its own pattern of clinical features, time course, location of lesions, auto-antibody reactivity and immunopathologic mechanisms. There is considerable overlap of pathologic features within the connective tissue disease group and a given patient may have features of several of the connective tissue diseases (overlap syndromes) (Tan, 1989). Many connective tissue diseases share common signs and symptoms, which frequently makes the diagnosis of a specific connective tissue disease difficult. Current clinical practice relies heavily on serologic testing for the prompt and accurate diagnosis of connective tissue diseases (table 1.1). Serologic testing is used to support the medical history and physical examination and in some cases to monitor disease activity (Barland and Lipstein, 1996).

Table 1.1 Autoimmune diseases and respective autoantibodies

Disease	Autoantibodies directed against:
SLE	Nuclear antigens, DNA, Sm
Scleroderma	Nuclear antigens, Scl 1
Sjögren's syndrome	Nuclear antigens, SS-A, SS-B
Raynaud's syndrome	Extractable nuclear antigens (RNP)
MCTD	Extractable nuclear antigens (RNP)
Rheumatoid arthritis	Fc portion of IgG (rheumatoid factor)

Cited from the textbook of autoimmune diseases, page: 301 (Deodhar, 1990)

A major clinical problem is distinguishing SLE from other connective tissue diseases. Multisystem involvement is a common feature of all theses conditions, and all frequently present with a mixture of systemic symptoms (Barland and Lipstein, 1996). Historically, laboratory tests have been developed to diagnose SLE, whereby anti-nucleosome antibodies stand as the most recent diagnostic criteria.

1.1. Systemic lupus erythematosus

Systemic Lupus Erythematosus (SLE) is one of the many autoimmune disorders. It is a chronic, often life long, autoimmune disease characterized by inflammation and damage of various body tissues including joints, skin, kidneys, heart, lungs, blood vessels and brain. People with the disease may present with many different symptoms and some of the most common symptoms at onset are extreme fatigue, painful or swollen joints, fever, skin rashes and renal manifestations (NIAMS, 2002).

1.1.1. Epidemiology

Primarily SLE is a disease of young women of childbearing age between 15 and 40 years old and female to male ratio is about 9: 1 during this period. However the onset of disease can range from infancy to old age. SLE affects approximately 1 in 2000 individuals in the general population, although the prevalence varies with race, ethnicity and the socioeconomic status (Ward *et al.,* 1995). Estimation of the incidence of SLE in United States varies; it is reported that it was 124 cases per 100 000 (Hochberg *et al.,* 1995) while in another study the incidence in the United States and continental Europe ranged from 14.6 to 68/100,000 (Bongu *et al.,* 2002). However, some reports indicate that because of improved diagnostic measures, the incidence of SLE is increasing. For example, in a study covering a span of greater than 40 years found that the pooled incidence of SLE had more than tripled from 1.51/100,000 during 1950-1979 to 5.56 /100,000 during 1980-1992 (Uramoto *et al.,* 1999).

In a recent review of 19 studies published from 1995 to 2000, Ruiz-Irastorza *et al.,* (2001) reported an even higher incidence rate (7.3/100 000). The incidence of SLE is about 3 times higher in African American women than in white women (McCarty *et al.,* 1995 and Petri, 1998). It has been shown that Blacks are indeed more prone to develop SLE compared to Caucasians (Fessel, 1988). In the United States, morbidity and mortality appears to be greater among Hispanic than among whites (Bongue *et al.,* 2002).

SLE is also common among Asian and Chinese and an increased prevalence of SLE has been noted in people of oriental races (Frank, 1980). The highest rate is shown in African Caribbean (207/100,000), followed by Asian (48.8/100,000) and Whites (20.3/100,000) (Molokhia *et al.*, 2001 and Hopkinson *et al.,* 1994). Although SLE is not prevalent in men, male patients who develop the disease, renal, neurological, hematological and vascular involvements are usually more severe compared to women (Mok *et al.,* 1999).

1.1.2. Aetiology of SLE

The aetiology of SLE remains unknown. However, genetic predisposition, sex hormones and environmental factors may play important roles in the pathogenesis of SLE (McCarty *et al.,* 1995 and Petri, 1998).

1.1.2 (a). Genetic factors

The familial prevalence of SLE is estimated to be 10%, and siblings to SLE patients have an increase risk of developing SLE compared to the general population. The role of heredity is further supported by the concordance rate of 24-58% in monozygotic twins (Hahn, 1998).

There is evidence for an association between SLE and the major histocompatibility complex (MHC class II and III regions on chromosome 6). Furthermore, there is a strong association between autoantibody production, such as anti-SSA/Ro antibody and anti-SSB/La antibody, and the MHC class II subsets HLADR2 and DR3. Associations are also seen between SLE and complement deficiencies (C1q, C2 and C4), IL-10 promoter polymorphism, and most recently programmed cell death 1 gene (PDCD1) polymorphism (Sell and Max, 2001).

1.1.2 (b). Environmental and hormonal factors

- **Ultraviolet (UV) radiation**

Nearly three quarters of the SLE patients report photosensitivity, and UV radiation induce skin lesions in these patients. Systemic symptoms can also be induced by sunlight exposure, although the exact relationship between UV exposure and disease activity is difficult to demonstrate (Osmola *et al.*, 2004).

- **Drugs**

A number of drugs have been reported to induce SLE or drug-induced lupus (DIL). The high-risk drugs reported to induce DIL are procainamide and hydralazine, followed by quinidine, chlorpromazine, sulfasalazine and penicillamine (Osmola *et al.*, 2004).

- **Infectious agents**

Both viruses and bacteria have been suspected to be involved in the initiation of SLE. Among viruses, especially Epstein-Barr virus, cytomegalovirus and retroviruses may be linked to the development of SLE. Elevated antibody titers to several viruses have been reported in SLE patients (Cooper *et al.*, 1998).

- **Hormones**

Sex hormones play a part in the pathogenesis of SLE. An increased frequency of SLE among females is believed to be due to endogenous estrogens and androgens (Cooper *et al.*, 1998). Some studies have found that exogenous exposure to estrogen, either through oral contraceptive (Sanchez-Guerrero *et al.*, 1997) or oestrogen replacement therapy (Sanchez-Guerrero *et al.*, 1995), may increase the incidence of SLE.

- **Other environmental risk factors**

A number of additional risk factors for SLE have been described. Smoking, severe emotional/physical stress, hypertension, drug allergy, sun-reactive skin type and intake of alfalfa seeds are all reported to increase the risk of developing SLE (Shapiro *et al.*, 2004).

1.1.3. Immunopathogenesis

SLE is characterized by alterations in many different parts of the immune system, but patients may share some common characteristics; a loss of tolerance and the production of pathogenic autoantibodies with immune complex formation.

1.1.3 (a). Loss of tolerance

A fundamental process in autoimmune disease is the breakdown in immunological tolerance either centrally or peripherally. Central tolerance involves thymic deletion of self reactive cells and upregulation of T-cells with low affinity to self-MHC. The peripheral T-cell tolerance includes anergy (loss of co-stimulatory signals), deletion or suppression of reactive T-cells and ignorance of self antigens by the immune system.

Failure to become tolerant to self antigens, cross-reactivity and molecular mimicry, development of auto-antibodies that cross-react with self antigens and polyclonal stimulation of naturally occurring autoantibody producing cells may underlie the initiation and maintenance of autoantibodies secretion (Manolios and Schrieber, 1997).

1.1.3 (b). Cellular defect

- **T cells and B cells**

Nearly half of the SLE patients have decreased levels of circulating leukocytes, manifested as granulocytopenia and/or lymphocytopenia. The lymphocytopenia is primarily due to a decrease in T-cells and both CD4+ and CD8+ subsets seem to be affected (Horwitz *et al.*, 2002). However, the T-cells in SLE patients show signs of an *in vivo* activation and autoreactive T-cells are considered to have an important role in the disease process (Horwitz, 2001).

The hallmark of the B-cell population in SLE is hyperactivity, with an increase of proliferation rates, elevated numbers of long-lived autoreactive B-cells and differentiation to plasma cells. This results in the production of autoantibodies and polyclonal hypergammaglobulinaemia (Liossist and Tsokos, 2002).

• **Dendritic cells (DC), monocytes/macrophages and natural killer (NK) cells**

Relatively little is known about the DC population in SLE patients, but a reduced number of circulating DC and an impaired T-cell stimulatory capacity by such DC have been demonstrated.

In contrast, the number of monocytes among peripheral blood mononuclear cells (PMNC) from SLE patients is often increased, with an enhanced rate of spontaneous apoptosis (Scheinecker *et al.,* 2001). Interestingly, these monocytes may be induced to differentiate into DC by the presence of IFN-α in SLE serum. Such DC cells can function as Ag-presenting cells (APC) that may explain the T cell activation seen in SLE (Santini *et al.,* 2000).

Natural killer (NK) cells are reduced both in number and in functional capability. This might be due to cytokine abnormality or high levels of circulating immune complexes binding to their Fc receptors and altering their function. The inability to clear circulating immune complexes by the reticuloendothelial system may increase tissue deposition and inflammation. The clearance occurs mainly in the liver (mediated by C3b receptors) and spleen (mediated by Fc receptors).

In SLE there is a defective splenic Fc receptor-mediated and hepatic C3b receptor-mediated clearance of immune complexes from the circulation.

A number of cytokine abnormalities have been reported in SLE. These include decreased quantities of interleukin-1 (IL-1) and a low response and production of IL-2 by T-cells. There is an elevation of several cytokines such as IL-4, IL-5 and IL-6, which control B-cell differentiation and antibody production during active disease (Hahn, 1980).

1.1.4. Auto-antibodies and immune complexes

Auto-antibodies can be detected in healthy individuals, although these natural autoantibodies are usually of the low affinity IgM isotype. They do not undergo maturation and do not cause autoimmune diseases or tissue damage (Liossist and Tsokos, 2002).

The auto-antibodies production in SLE is thought to be of pathogenic relevance, mainly by the formation of immune complexes, and deposition in target tissues such as glomeruli, heart, skin and vessels. The deposited immune complexes then participate in inflammatory processes involving complement activation, eventually causing tissue damage. In addition, immune complexes may have other properties, such as activation of auto-antibody production (Leadbetter *et al.,* 2002) and these autoantibodies can also interfere with normal cellular functions and contribute to disease manifestations (Cho *et al.,* 2002).

1.1.5. Clinical manifestations

1.1.5 (a). Constitutional manifestations

The constitutional signs and symptoms of SLE often include fever, malaise, weight loss, symmetrical arthralgia, myalgia and headache (Greenberg, 1999).Among the most serious features of SLE are persistent fever and weight loss.

1.1.5 (b). Musculoskeletal manifestations

Over 90 percent of SLE patients have polyarthralgias or polyarthritis, which are the most common reason whereby SLE patients seek medical attention. Small joints of the hand and wrist are usually affected. Soft tissue and tendon thickening causes swelling of these joints but the effusion is small unlike in rheumatoid arthritis, in which the swelling would be pronounced and bones may be deformed. SLE arthropathy is usually not erosive or destructive to bone (Cronin, 1988).

1.1.5 (c). Mucocutaneous manifestations

More than 90 percent of patients with SLE eventually have cutaneous manifestations of the disease, including malar rash, discoid rash and alopecia. Approximately two-thirds of SLE patients have photosensitivity, defined as a skin rash due to an unusual reaction to sunlight (Laman and Provost, 1994). Skin manifestations of SLE can be acute, subacute or chronic. Some acute manifestations include bullous lesions and generalized erythema, which may or may not be photosensitive in nature (Sontheimer and Gilliam, 1992).

The most characteristic clinical feature of subacute cutaneous lesion is superficial, non-indurated and non-scarring photosensitivity-induced skin rash (David-Bajar *et al.*, 1992). In chronic cutaneous SLE, patients may have a discoid rash with scarring. Oral, nasal and other mucus membrane lacerations may occur (Nguyen and Foster, 1998). Raynaud's syndrome occurs in 10- 45 % of SLE patients, is the result of vasospasm and muscular damage and presents with the classical white, blue and red color changes in the fingers (Hahn, 1997). Alopecia is observed in up to 45 % of SLE patients at some time in the disease or it can occur with some therapies for SLE. Hair loss may be diffuse or patchy associated with discoid lesions (Hahn, 1998).

1.1.5 (d). Serositis

Inflammatory serositis of the pleura, peritoneum and pericardium occur in 25 % of SLE patients (Manolios and Schrieber, 1997). Patients may develop large pleural effusions, pericardial effusion or ascites. These effusions are typically inflammatory and exudative.

1.1.5 (e). Hematological manifestations

Anemia, leucopenia and thrombocytopenia are frequent manifestations of SLE and these patients often have normocytic normochromic anemia. Coombs test is frequently positive and circulating anti-erythropoietin antibodies are reported as a possible mechanism of anemia (Tzioufas *et al.,* 1997). Leucopenia with white blood cell (WBC) counts less than $4000/mm^3$ or lymphopenia with lymphocyte counts less than $1500/mm^3$ on two or more occasions are part of the diagnostic criteria of SLE (Mills, 1994).

Thrombocytopenia with a platelet counts less than 100,000/mm^3 in the absence of other causes is found in up to 25 % of SLE patients. However thrombocytopenia is often a marker of severe disease with poor prognosis (Reveille *et. al,* 1990).

1.1.5 (f). Renal involvement

The kidney is the most commonly involved organ in patients with SLE and is a major cause of mortality and morbidity. Several studies show evidence of nephritis in approximately 50-70 % of renal biopsies from SLE patients (Golbus and McCune, 1994). Several demographic, ethnic, immunologic and genetic factors have been associated with nephritis (Hopkinson *et al.,* 2000; Seligman *et al.,* 2002). The clinical manifestations of lupus nephritis that can be seen in SLE patients are asymptomatic haematuria and/or proteinuria, nephrotic syndrome or chronic renal failure. The renal involvement caused by auto-antibodies or accumulation of immune complexes containing anti-dsDNA antibodies in the kidney (Huong *et al.,* 1999). The World Health Organization (WHO) has classified lupus nephritis into six classes (Table 1.2).

Table 1.2 World Health Organization classification of lupus

Class	Histology	Prognosis
I	Normal	Excellent
II	Mesangial lupus nephritis	Good
III	Focal proliferative lupus nephritis	Moderate
IV	Diffuse proliferative glomerulonephritis	Poor
V	Membranous glomerulonephritis	Moderate
VI	Glomerulosclerosis	Poor

(Huong *et al.,* 1999) *Medicine.* **78**: 148-166.

1.1.5 (g). Central Nervous System (CNS) involvement

Neuropsychiatric involvement of patients with SLE ranges from headache to CNS vasculitis (Boumpas *et al.,* 1995). Other unusual manifestations of CNS lupus include Parkinsonism, cerebellar ataxia, pseudotumor cerebrae, hypothalamic dysfunction, aseptic meningitis, myasthenia-like syndrome, Eaton-Lambert syndrome and thrombotic thrombocytopenic purpura. Peripheral nervous system involvement of SLE is also noted in 10 % of patients and includes sensory or motor myopathies, Guillain Barre-like syndrome and mononeuritis multiplex.

The pathogenesis of neuropsychiatric SLE includes vasculitis-induced blood vessel occlusion, antibodies against brain tissue, hypercoagulable status due to anti-phospholipid antibodies (aPL) and other secondary causes such as infection, metabolic derangement and side effects of drugs that are used for treatment of SLE (Boumpas *et al.,* 1995). Recently, circulating anti-ribosomal P antibodies have been found in many lupus patients with neuropsychiatric manifestations (Yoshio *et al.,* 2005).

1.1.5 (h). Cardiac manifestations

SLE affects the heart leading to pericarditis, myocarditis, coronary artery disease and endocarditis. Pericarditis is the most common cardiac manifestation of SLE, occurring in about 20-30 % of patients and usually presents with mild anterior chest pain (Petri *et al.,* 1992). Myocarditis is suspected when SLE patients have cardiomegaly, arrhythmias, or conduction defects and they usually co-exist with pericarditis in the active phase of SLE. Sterile vegetation of the heart valves or Liebman Sacks' endocarditis is a less common manifestation of SLE (Petri *et al.,* 1992).

Gentile *et al.,* (2000), found that 19 out of 91 SLE patients (20.8%) had pericardial effusion. Valvular involvement occurred in 39 patients (42.8%) whereby moderate to severe degree was more frequent in the group with aPL. Regional wall motion abnormalities were observed in 8 patients (8.8%). On rare occasions, SLE can be accompanied by coronary artery vasculitis and can result in myocardial infarction. There is an increased incidence of atherosclerotic heart disease (Urowitz, *et al.,* 2000) and this may be initiated by immune complex deposition, but certainly it is aggravated by chronic steroid therapy, which can produce hyperlipidemia and hyperglycemia.

1.1.5 (i). Pulmonary manifestations

The pulmonary manifestations of SLE include pneumonitis, pulmonary hemorrhage, pulmonary hypertension and embolism. Lupus pneumonia is a clinical dilemma that requires careful diagnosis because it must be differentiated from infectious pneumonia. Pulmonary hemorrhage from vasculitis is associated with 50-60% mortality rate and is a true medical emergency (Schwab *et al.,* 1993). Pulmonary hypertension is more commonly associated with long-term restrictive lung diseases and Raynaud's syndrome.

In a retrospective study performed by Mochizuki *et al.,* (1999), the types of pulmonary involvement found in SLE patients were pleuritis (9%), interstitial pneumonia (8%), pulmonary infarction (7%), infection (4%), pulmonary hypertension (2%), restrictive dysfunction (28%) and reduced diffusion capacity (43%).

1.1.5 (j). Gastrointestinal manifestations

Gastrointestinal symptoms in patients with SLE are relatively common and present as abdominal pain, anorexia and/or vomiting. Peritoneal inflammation is the most likely cause of gastrointestinal symptoms, but mesenteric vasculitis or pancreatitis are dangerous complications of SLE (Gladman and Urowitz, 1997).

1.1.5 (k). Ocular manifestations

Ocular involvement in SLE can cause conjunctivitis and retinal lesions can occur. The retinal lesions are vasculitis and include white exudate (cytoid bodies), hemorrhage, retinal edema and sometimes papilledema. These lesions are always associated with active SLE. Cytoid bodies occur in 10-20% of SLE patients (Nguyen and Foster, 1998).

1.1.5 (l).Secondary anti-phospholipid antibody syndrome

SLE patients have an increased incidence of secondary anti-phospholipid syndrome. This syndrome is defined as the co-occurrence of thrombotic events and the presence of autoantibodies against the negatively charged phospholipids, such as lupus anti-coagulant, or anti-cardiolipin antibodies (Sinico *et al.,* 2002). Anti-phospholipid antibodies are part of a group of antibodies that are associated with problems relating to abnormal blood clotting leading to produces such as miscarriages, deep vein thrombosis, pulmonary emboli and strokes. This syndrome occurs more frequently in patients with a high titer of IgG anticardiolipin antibodies or lupus anticoagulant.

The mechanism of this prothrombotic diathesis is uncertain, but these auto-antibodies bind to target antigens on endothelial cells, platelets or coagulation factors producing a hypercoagulable state (Asherson, 1989). These auto-antibodies are found in some people with lupus and other autoimmune diseases and occasionally in people without any known disease.

1.1.6. Laboratory tests for the diagnosis of SLE

Diagnosis of SLE can be difficult and requires knowledge and awareness of the disease and good communication with the patient. A complete, accurate medical history, physical examination and results of laboratory tests are critical for diagnosis of this disorder (NIAMS, 2002).

The LE cell test was a standard procedure to diagnose SLE. Almost for two decades an intensive research was performed to discover the specificity of antibodies that cause the LE cells phenomenon (Rekvig and Hannested, 1981). Today, laboratories have started using other tests which are more helpful in the diagnosis of SLE. One of these tests is the ANA test, commonly used to look for autoantibodies that react against components of nucleus.

In suspected SLE patients, ANA is important as a screening test and it can be done by indirect immunofluorescence method (IF) or enzyme immunosorbent assay method (ELISA) (Tan, 1989). ANA is very sensitive for SLE and elevation of ANA titer to 1:40 or more is the most sensitive according to the American College of Rheumatology (ACR) diagnostic criteria. More than 99% of SLE patients have elevated ANA titers (Tan *et al.*, 1982).

However, ANA test is not specific for SLE and the most common reason for a positive ANA test in the absence of SLE is another connective tissue disease. Diseases often associated with a positive ANA test include Sjögren syndrome, scleroderma, rheumatic disease and juvenile rheumatoid arthritis (Sinico *et. al*, 2002). Testing for antibody to anti-dsDNA and anti-Sm antibody to Smith (Sm) antigen may be helpful in patients who have a positive ANA test but do not meet the full criteria for the diagnosis of SLE.

Anti-dsDNA and anti-Sm antibodies, particularly in high titers, are highly specific for SLE, although their sensitivities are low. Therefore, a positive result helps to establish the diagnosis of the disease, but a negative result does not rule it out (Kavanaugh *et al.,* 2000). In addition, there are other blood tests which are helpful in diagnosis of SLE, e.g., anti-ribonuclear protein (anti-RNP) antibody, anti-Ro (SS-A) antibody and anti-La (SS-B) antibody (Hochberg, 1997). However, hypocomplementemia and antibodies against Ro (SSA), La (SSB) and to phospholipids (anticardiolipin) are detectable in SLE as well as other connective tissue diseases (Weinstein *et al.,* 1983).

Total serum hemolytic complement (CH50) and individual complement components (C3 and C4) may be low in patients with active SLE (Weinstein *et al.,* 1983). However, for the diagnostic purpose, ANA and anti-dsDNA antibodies are more useful. Therefore a combination of anti-dsDNA, C3, C4, C- reactive protein (CRP) and erythrocyte sedimentation rate (ESR) assays provide useful information for its diagnosis (Enger, 2000).

The diagnosis of systemic lupus erythematosus is based on both clinical and laboratory criteria. The criteria set by the American College of Rheumatology (ACR) are most widely used (Tan *et al.,* 1982 and Hochberg, 1997). A person is suspected to have SLE if she/he fulfills 4 out of the 11 criteria (Hochberg, 1997) (Table1.3).

Table 1.3 ACR classification criteria for SLE

Criterion	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences and sparing the nasal folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging: atrophic scarring may occur in older lesion
Photosensitivity	Skin rash as a result of unusual reaction to sunlight by history or on physical exam
Oral ulcers	Oral or nasopharyngeal ulceration observed by a physician are usually painless
Non-erosive arthritis	Involving two or more peripheral joints, characterized by tenderness, swelling or effusion
Pleuritis or pericarditis	a. Pleuritis- convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion b. Pericarditis- documented by electrocardiogram or rub or evidence of pericardial effusion
Renal disorders	a. Persistent proteinuria > 0.5 gm per day OR b. Cellular cast that may be red cells, hemoglobin, granular, tubular or mixed
Neurological disorders	a. Seizure in the absence of offending drugs or known metabolic derangement; e.g. uremia, ketoacidosis or electrolyte imbalance OR b. Psychosis in the absence of offending drugs or known metabolic derangement; e.g. uremia, ketoacidosis or electrolyte imbalance
Hematologic disorders	a. Hemolytic anemia with reticulocytosis OR b. Leucopenia less than 4.000/mm^3 OR c. Lymphopenia less than 1.500/mm^3 OR d. Thrombocytopenia less than 100.000/mm^3
Immunologic disorders	a. Anti-dsDNA: antibody to native DNA in abnormal titer OR b. Anti-Sm: presence of antibody to Sm nuclear antigen OR c. Positive finding of antiphospholipid antibodies based on: (i) an abnormal serum level of IgG or IgM anticardiolipin antibodies (ii) a positive test result for lupus anticoagulant using a standard method (iii) a false positive test result for at least 6 months and confirmed by *Treponema pallidum* inhibition test (TPI) or fluorescent treponemal antibody absorption test (FTA)
Positive ANA	An abnormal titer of anti-nuclear antibody by immunofluorescence or an equivalent assay at any point in time in the absence of drugs

(Hochberg, 1997) *Arthritis Rheum* **40**: 1725

In the last few years a number of reports have demonstrated the utility of measuring antinucleosome autoantibodies in the diagnosis of systemic lupus erythematosus (Amoura *et al.,* 1999) and in drug-induced lupus (Burlingame and Rubin, 1991). Some of the studies suggest that anti-nucleosome antibody is a more sensitive test than anti-dsDNA for SLE patients (Min *et al.,* 2002 and Cervera *et al.,* 2003). Anti-nucleosome antibodies have been reported in 40% - 80% of SLE, depending on the disease status and in 0% - 15% of other autoimmune diseases such as scleroderma, Sjögren's syndrome and rheumatoid arthritis. They are rarely found in normal blood donors or patients suffering from infectious diseases (Cacoub *et al.,* 1997 and Suer *et al.,* 2004).

1.1.7. Anti-nucleosome antibodies

Anti-nucleosome antibodies have only recently been highlighted, as they make up the majority of antibodies causing lupus erythematosus (LE) cell formation. They were given different names over the last few decades e.g., anti-histone (Fritzler and Tan, 1978), LE cells factor (Rekvig and Hannestad, 1981), anti-deoxyribonucleoprotein (DNP) (Krash *et al.,* 1982), Anti-H2A-H2B-DNA (Wallace *et al.,* 1994), anti-nucleosome (Hmida *et al.,* 2002) and anti-chromatin (Czaja *et al.,* 2003).

In the nucleus of eukaryocytes the native complex of histone and DNA is called chromatin. It is comprised of approximately 40% DNA, 40% histones and 20% non histone proteins such as RNA and other macromolecules. The periodic arrangement of histone along with the DNA gives a "beads-on-a-string" appearance in electron micrographs (Figure 1.1).

Digesting linker DNA between the "beads" with micrococcal nuclease yield nucleosomes, each nucleosome is made up of approximately 200 bp of DNA wrapped twice around the $(H2A\text{-}H2B\text{-}H3\text{-}H4)_2$ histone octamer, with histone H1 bound on the outside (Figure 1.2). Non histone and RNA are also bound to some nucleosomes. Whole chromatin is insoluble at physiologic ionic strength and contains many nonhistone macromolecules. Since nucleosomes are only sparingly soluble, it is difficult to use either whole chromatin or nucleosomes in immunoassays. The most useful form of chromatin to be used as an antigen in ELISA is solubilized by digestion with micrococcal nuclease while H1 and nonhistone proteins are removed by extracting the chromatin with 0.5 M NaCl at neutral PH. This process yields nucleosome core particle (Figure 1.3).

Polynucleosome core particles, in which the linker DNA is not cut by the nuclease, are *called H1-stripped chromatin.* Interestingly, almost all the studies of "anti-nucleosome" and "anti-chromatin" actually used a mixture of nucleosome core particle and H1- stripped chromatin as an antigen in ELISA (Lewin, 2000).

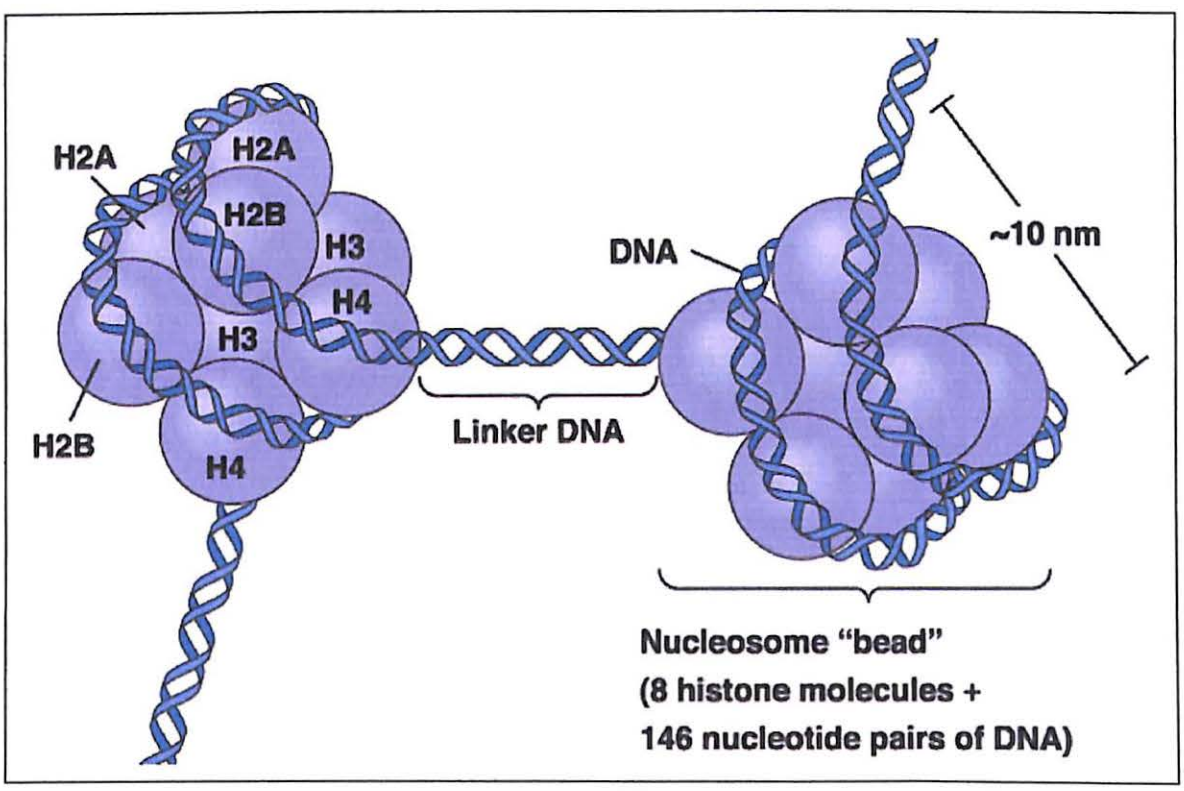

Figure 1.1 The periodic arrangement of histone along with the DNA gives a "beads-on-a-string" appearance Cited from http://www.zoology.ubc.ca/~bio463/lecture_3.htm