PREVALENCE OF SYSTEMIC LUPUS ERYTHEMATOSUS AND ASSOCIATED RISK FACTORS IN PATIENTS WITH SUSPECTED AUTOIMMUNE DISEASES IN SELECTED HOSPITALS IN ZARIA, NIGERIA

BY

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DECLARATION

I declare that the work in this dissertation entitled '**Prevalence of Systemic Lupus Erythematosus and Associated Risk Factors in Patients with Suspected Autoimmune Diseases in Selected Hospitals in Zaria, Nigeria'** has been carried out by me in the Department of Microbiology, Ahmadu Bello University, Zaria under the supervision of Dr. E. E. Ella and Prof. Maryam Aminu. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this work was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This dissertation entitled '**Prevalence of Systemic Lupus Erythematosus and Associated Risk Factors in Patients with Suspected Autoimmune Diseases in Selected Hospitals in Zaria, Nigeria'** by Rashida, Idris KABIR meets the regulations governing the award of the degree of Master of Science (M.Sc) in Microbiology, Faculty of Life Sciences of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Systemic lupus erythematosus is a chronic autoimmune connective tissue disorder, with a heterogeneous presentation thataffects many organs of the body. Though it is a common disorder among the Nigerian population,it is just beginning to receive attention.This study was carried out to determine the prevalence of systemic lupus erythematosus and its association with antinuclear antibody and extractable nuclear antigen among patients attending selected hospitals in Zaria Kaduna State, Nigeria. A total of 300 blood samples were collected and assayed for systemic lupuserythematosususing latex test kit, while Antinuclear Antibody and Extractable Nuclear Antigenwere analysedby enzyme-linked immunosorbent assay.A structured questionnaire wasused to obtain data on socio-demographic and risk factors of systemic lupus erythematosusfrom the study participants. A sero-prevalence of 21% (63/300) was found among the study population. Theprevalence was higher in female patients(21.8%:52/239) than in male patients(18%:11/61) though not statistically significant (p=0.011).The highest prevalence was recorded among patients above 60 years (36.7%:11/30) and lowest in patients below 20 years (12.8%:5/39). Factors found to be statistically associated with systemic lupus erythematosus were hospital location (p=0.011), marital status (p=0.038), loss of weight (p=0.005), photosensitivity (p=0.042) and seizures (p=0.014). History of blood transfusion (p=0.037) was found to be a risk factor (OR=1.998)for systemic lupus erythematosusand antinuclear antibody was found to be associated with systemic lupus erythematosus (p=0.007). The result of the extractable nuclear antigens assay showed Scl-70, Jo-1, Sm/RNP and SSB as the most common antigens while Sm and SSA antigens were not detected.The high prevalence of systemic lupus erythematosusobtained in this study necessitates the need to enlighten the population on systemic lupus erythematosus and its diagnostic importance. Antinuclear antibody assay can also be included as a diagnostic tool for systemic lupus erythematosus.

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ABBREVIATIONS

ABUMC	Ahmadu Bello University Medical Centre
AD	Autoimmune disease
ANA	Antinuclear antibody
CREST	Calcinosis, Raynaud's, Esophageal Dysmotility, Sclerodactyly and Telangiectasis
CTD	Connective tissue disease
DIL	Drug-induced lupus
DILE	Drug-induced lupus erythematosus
DMARD	Disease modifying anti-rheumatic drugs
DNA	Deoxyribonucleic acid
dsDNA	Double stranded deoxyribonucleic acid
ssDNA	Single stranded deoxyribonucleic acid
EBV	Epstein Barr Virus
ELISA	Enzyme linked immunosorbent assay
ENA	Extractable nuclear antigen
HGSGH	HajiyaGamboSawaba General Hospital
IgG	Immunoglobulin G
IFN	Interferon
IFN α	Interferon alpha
MCTD	Mixed connective tissue disease
MIBA	Major Ibrahim Bello Abdullahi Hospital
PHCS	Primary Health Care,Samaru
RA	Rheumatoid arthritis
RBC's	Red blood cells
RD's	Rheumatic diseases
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
SLE	Systemic lupus erythematosus
SS	Sjogren's syndrome

Sm	Smith antigen
UV	Ultraviolet light

CHAPTER ONE

1.0 INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune connective tissue disorder, with a heterogeneous presentation in terms of clinical symptoms (Ben-Menachem, 2010; Tahernia *et al*., 2016).The name 'lupus erythematosus' refers to the facial skin rashes commonly found in SLE patients. It was the 13th century physician Rogerius that first used the term 'lupus' (Greek for wolf) erythematosus (Latin for red) to describe these facial lesions resembling a wolf bite. However, the facial rash is only one of many signs and symptoms of SLE and as the disease was further characterized in the beginning of the 20th century, the word "systemic" was added to describe the disseminated form of lupus erythematosus that can affect almost every organ of the body (Hochberg, 1991).

Systemic lupus erythematosus is a prototypic systemic autoimmune disease of unknown causewith a wide array of signs and symptoms (Sánchez-Guerrero *et al*., 2007; Feldman *et al*., 2013) that isprimarily a disease of young women (Graham *et al*., 2004; Dall'Era, 2013). It is characterized by the production of antibodies to components of the cell nucleus (Dall'Era, 2013). The main cause of SLE is still unknown. However, numerous studies indicate that lupus isas a result of interaction between environmental, immunologicaland genetic factors. These interactions breakdownbody tolerance towards endogenous antigens and causeabnormal immunologic response to healthy tissue, resultingin tissue damage (Tahernia *et al*., 2016).

People with a family history of SLE or other autoimmune diseases are at slightly higher risk for developing SLE. This risk increases as the number of first-degree family members with SLE increases (Dall'Era, 2013).Systemic lupus erythematosus is a chronic systemic inflammatory disease that affects many organ systems which includes skin, joints, kidneys, lungs and heart

(Jayapal, 2007). However, pathologic changes in the kidney are the most common cause of morbidity and mortality (Tan *et al*., 1982). The course of the disease is characterized by periods of remission and relapse (flares) (Lahita, 1995). Flares is a characteristic of SLE that progressively results in deterioration of the patient's health and the flares are often associated with triggers, such as viral infections which could also trigger unabated production of interferon-α in SLE patients (Pascual *et al.,* 2006).

Systemic lupus erythematosus is characterised by a myriad of immune system aberrations that involve B cells, T cells, and cells of the monocytic lineage, resulting in polyclonal B cell activation, increased numbers of antibody producing cells, hypergammaglobulinaemia, autoantibody production, and immune complex formation (Mok and Lau, 2003). It appears that excessive and uncontrolled T cell help in the differentiation and activation of autoantibody forming B cells is probably a final common pathway (Mok and Lau, 2003).Females are affected far more than males (4 to12 females for every male) with SLE. Blacks (Hispanics, Asians, and Native Americans) are affected more than whites (Dall'Era, 2013).

Systemic lupus erythematosus is reported to be more common in African-Americans, African-Caribbeans, and Asians, than in Caucasians (Petri, 2002; Danchenko *et al*., 2006; Pons-Estel *et al*., 2010). It occurs from infancy to old age, with peak in the childbearing years for females (Dall'Era, 2013). The age at diagnosis is younger in African-Americans than in Caucasians (Petri, 2002) with a peak age of onset between 15 and 40 years (Ben-Menachem, 2010). Cases may present anytime in childhood through advanced age, at which time female-to-male ratio are approximately 2:1 (Gaubitz, 2006). Males and patients with advanced age of onset tend to have more severe disease and poorer prognosis (Doria *et al*., 2006; Kasitanon *et al*., 2006). The mean age of presentation of SLE as previously reported is between the age range of 31-

50years(Adelowo and Oguntona, 2009; Budhoo *et al*., 2013; Ella *et al*., 2016). Systemic lupus erythematosus is listed as a contributing cause of death (Sacks *et al*., 2002). The causes of death are mainly due to active disease, organ failure (e.g., kidneys), infection, or cardiovascular disease from accelerated atherosclerosis (Sacks *et al*., 2002).

In several studies conducted, possible risk factors of SLE include, gender, age, pregnancy, viral infections, smoking, alcohol consumption, blood transfusion, exposure to ultraviolet radiation and family history of autoimmune diseases (Bengtsson *et al*., 2002; Kiyohara *et al*., 2012; Takvorian *et al*., 2014; Ella *et al*., 2016). Patients with SLE often tested positive for rheumatoid arthritis (RA) and it affects the middle aged compared to other age group (Ella and Agibilly, 2015).

The disease starts with a preclinical phase characterized by autoantibodies common to other systemic autoimmune diseases and proceeds with a more disease-specific clinically overt autoimmune phase (Bertsias *et al.*,2013). The most common autoantibody found in the serum of SLE patients is the autoantibody to double stranded DNA (dsDNA). Patients with SLE produce autoantibodies to a number of self-antigens such as DNA, histones, RBCs, platelets, leukocytes and clotting factors (Jayapal, 2007).

The criteria for identification and clinical diagnosis of SLE includes presence of malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis (pleuritis, pericarditis), renal disorder, neurological disorder (seizures, psychosis), haematological disorder (haemolytic anaemia, leucopenia, lymphopenia, and thrombocytopenia), immunologic disorder, and antinuclear antibody production or elevated antinuclear antibody levels (Tan *et al*., 1982). The

production of antinuclear antibodies (ANA) is one of the defining features of SLE (Revees *et al*., 2013).

Antinuclear antibodies are a diverse group of antibodies, often directed at large cellular complexes containing protein and nucleic acid components that react with components of deoxyribonucleic acid (DNA)-protein or ribonucleic acid (RNA)-protein complexes (van Venrooij and Maini, 1994). The production of these antinuclear antibodies, which are generally, high-affinity immunoglobulin G (IgG) antibodies andT-cell dependent, are driven by the host autoantigen (Reichlin and Harley, 1997). The persistence of its autoreactivity in the human population suggests that ANAs may be an important component of the normal immune response. Most individuals with a positive ANA do not have an autoimmune disease and most also are unlikely to develop one (Davidson and Diamond, 2001). Furthermore, the disease that is most closely linked to ANA positivity, SLE, is relatively rare, affecting no more than 1 to 1.5 per 1,000 persons (0.1 to 0.15%) in the United States (Chakravarty *et al*., 2007).

Because antibodies against extractable nuclear antigen (ENA) have a partial marker function for the individual diseases, the isolated detection of these antibodies with the ENA -6-profile allows serological differentiation of these diseases(Orton *et al*., 2004). Autoimmune disease detection protocol starts with determination of ANA. Positive ANA test leads to further investigation of ENA (Orton *et al*., 2004).

Extractable nuclear antigens refer to nucleoproteins that can be extracted from the cell nucleus with saline. They include Ro (SS-A), La (SS-B), RNP, Sm, Scl-70 and Jo-1 (Kavanaugh *et al*., 2000; Aziz and Faisal, 2004). Extractable nuclear antigen is a well-established group of autoantigens that have pathological value in Sjögren's syndrome, mixed connective tissue

disease, sclerodema and systemic lupus erythematosus (Hamburger *et al*., 1997). Reactivity with these extractable nuclear antigens is more disease specific and may also provide clinically useful prognostic information (Kavanaugh *et al*., 2000). The ENA complex consists of nuclear antigens hidden from the immune system in intact cells. However, during cell death via apoptosis this structure is exposed to the immune system, triggering specific reactions in susceptible patients (Ohlsson *et al*., 2002).

1.1 Statement of Research Problem

In spite of the numerous studies and report on the epidemiology of SLE around the world, there have been few and limited studies and reported cases on its occurrence in Nigeria. Due to lack of effective routine diagnosis for its detection in Nigeria, few records have been kept on the incidence of SLE. The disease has not been considered in the Nigerian population and as such the expected routine diagnosis has not been put in place to possibly identify and manage cases that may be present. This has led to poor diagnosis and management of patients with the disease in the country. However, emerging reports have indicated that the disease is present in the study area though has received little attention (Ella and Agibilly, 2015; Ella *et al*., 2016).

Systemic lupus erythematosus has previously been rarely reported among black Africans, despite the fact that SLE occur commonly in black Americans. However, reports emanating from African countries have indicated that SLE may not be rare after all(Adelowo and Bello, 2014). Systemic lupus erythematosus might even run as severe a course as in black Americans (Adelowo and Bello, 2014). Systemic lupus erythematosus is currently the most prevalent autoimmune disease in the developed countries (Jayapal, 2007). Among rheumatic conditions, SLE had a relatively high mortality accounting for 14.5% of all rheumatic disease mortality in 1997 (Sacks *et al*., 2002).

The incidence of SLE has not received the attention it deserves in Nigeria because many people are not aware that it exists; which may be due to the fact that the symptoms mimic that of rheumatoid arthritis (RA) and therefore can easily be misdiagnosed as RA and also as a result of our dark skin which may not make the lupus rash easily visible on the patients skin.

Nigeria is a tropical country with high sunlight and sensitivity to ultraviolet light is a triggering factor in SLE resulting in photosensitivity. Consequently, individuals in Nigeria may be at risk of developing the disease especially genetically predisposed individuals such as patients with family history of SLE.

1.2 Justification for the Study

The triggers of lupus are still a puzzle, since there is no one specific cause of SLE. However, a number of unknown environmental influences such as sunlight, viruses or bacteria, hormonal changes that occurs during a woman's life time such as during puberty or pregnancy can trigger the condition in genetically predisposed people (Mok and Lau, 2003).

Nigeria is yet to adopt an effective routine programme for diagnosis of SLE as observed in many developed countries.As such, patients especially those with rheumatoid factor are not routinely screened for SLE in Nigeria.Epidemiological data on the incidence and burden of SLE in most developing countries have not been well documented.

The prevalence of SLE is on the increase; therefore there is need for more studies in developing countries that will provide data on its prevalence because most studies have been conducted in developed countries. There is also need for studies aimed at determiningrisk factors associated with the increasing prevalence of the disease. Epidemiological data on the incidence and prevalence of SLE have not been well documented in Nigeria because only few of its record

have been reported (Adelowo and Oguntona, 2009; Adelowo and Bello, 2014; Ella and Agibilly, 2015; Ella *et al*., 2016).

The presence or absence of rheumatic diseases (RDs) mainly depends on the verification of disease-related symptomatology as well as the detection of sensitive or specific antibodies in the sera of suspected patients (Salamunic, 2010).This study would provide data on the disease and some of the possible risk factors associated with the disease among the study population.

1.3 Aim of the Study

The aim of this study was to determine prevalence of systemic lupus erythematosus and associated risk factors in patients with suspected autoimmune diseases in selected hospitals in Zaria, Nigeria.

1.4 Objectives of the Study

The objectives of this study were to:

I. determine the prevalence of systemic lupus erythematosus among patients under study by analysing for SLE in serum using latex agglutination assay;
II. assess some socio-demographic and risk factors associated with systemic lupus erythematosus among the patients using structured questionnaire;
III. assay for antinuclear antibody of SLE positive patients among the study population using Enzyme-linked immunorsorbent assay (ELISA);
IV. assayfor extractable nuclear antigen profile ofantinuclear antibody positive samples using ENA profile-6 ELISA.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Overview

The immune system protects the body from harmful microbes (pathogens) such asbacteria and viruses, but it can also cause disease itself if not tightly regulated(Murphy, 2012).Keys to the ability of the immune system to distinguish self (host structures) from nonself(pathogen structures) are the pattern recognition receptors and the deletion ofpotentially self-reactive lymphocytes. In autoimmune diseases, this ability has for somereason been challenged, resulting in loss of self-tolerance (Murphy, 2012).

Autoimmune disease (ADs) is a disease caused by a breakdown of self-tolerance such that the immune system responds to self-antigens and mediates cell and tissue damage. Autoimmune diseases can be organ specific (e.g. thyroiditis or diabetes) or systemic (e.g. systemic lupus erythematosus) (Abbas *et al*., 2012).

Factors that may contribute to autoimmunity are hereditability (preferably gene variants of the human leukocyte antigen; HLA), infections and toxins. The ability of infections to cause autoimmunity is attributed to the increased general inflammation that causes tissue destruction and activation of immune cells, which in a genetically predisposed individual can result in loss of self-tolerance(Murphy, 2012). Another mechanism of infections to cause autoimmunity is molecular mimicry. Some pathogen structures are similar to structures of the host and antibodies produced to eliminate the pathogen may hence cross-react with self-structures. However, in most cases of autoimmunity a multitude of factors (both well-known and unknown) cooperates with coincidence to cause disease(Murphy, 2012).

Autoimmune diseases are a family of more than 80 chronic, and often disabling, illnesses broadly characterized by immune system dysfunction evidenced by loss of tolerance to self-antigens, presence of increased level of autoantibodies, inflammatory and mediatory cells and chronic inflammation. The family of ADs is remarkable for its complexity and similar underlying mechanisms which involves multiple organ systems (NIH, 2005).Patients often endure lifelong debilitating symptoms, loss of organ function, reduced productivity at work and high medical expenses. In women, several ADs present before or during a woman's reproductive years can have severe effects on foetal life (Carvalheiras *et al*., 2012; Ostensen *et al*., 2015), other effects are pregnancy loss in women with systemic lupus erythematosus (Clowse *et al*., 2012; Ostensen *et al*., 2015), vasculitis (Ostensen *et al*., 2015) and type 1 diabetes (McGrogan *et al*., 2014), and infertility in women with rheumatoid arthritis (Clowse *et al*., 2012).Collectively, ADs are common, affecting between five and nine percent of the population and are of considerable personal and public health burdens (NIH, 2005; Cooper *et al*., 2009).The reasons for their high prevalence, gender and ethnic disparities and rising incidence and prevalence remain unclear (NIH, 2005).

The pathogenesis of most autoimmune diseases especially systemic lupus erythematosus (SLE) has not been understood. It is suggested that if the self-tolerant lymphocytes break the state of tolerance, autoimmunity will occur (Jayapal, 2007). Autoimmunity can be mediated by self-reactive T cells or B cells or both. The autoimmune disorder may be mild, moderate, or severe and they can also be fatal (Jayapal, 2007).In systemic autoimmune diseases, the autoimmune responses are directed against self-antigens present in many organs and tissues of the body resulting in widespread tissue damage to the host (Jayapal, 2007).

2.2 Systemic Lupus Erythematosus

Systemic lupus erythematosus is the prototypic multisystem autoimmune disorder with a broad spectrum of clinical presentations encompassing almost all organs and tissues(Bertsias *et al*., 2012). The extreme heterogeneity of the disease has led some investigators to propose that SLE represents a syndrome rather than a single disease(Bertsias *et al*., 2012).

The term 'lupus' (Latin for 'wolf') was first used during the middle ages to describe erosive skin lesions evocative of a 'wolf's bite'. In 1846 the Viennese physician Ferdinand von Hebra (1816–1880) introduced the butterfly metaphor to describe the malar rash. He also used the term 'lupus erythematosus' and published the first illustrations in his *Atlas of Skin Diseases* in 1856(Bertsias *et al*., 2012). Lupus was first recognised as a systemic disease with visceral manifestations by Moriz Kaposi (Bertsias *et al*., 2012).

2.2.1 Systemic Lupus Erythematosus as a Connective Tissue Disease

Connective tissue diseases (CTD) are a group of systemic autoimmune inflammatory diseases comprising of systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), systemic sclerosis (Scl), Calcinosis, Raynaud's, Esophageal Dysmotility, Sclerodactyly and Telangiectasis (CREST) syndrome, polymyositis/dermatomyositis (PM/DM) and mixed connective tissue disease (MCTD) (Shmerling*et al*., 1996; Kumar and Clark, 2002).

Systemic lupus erythematosus is a chronic autoimmune connective tissue disorder, with a heterogeneous presentation (Ben-Menachem, 2010). Patients with CTD can present with clinical manifestations related to any organ-system of the body, often without the signs and symptoms that are classically associated with these diseases (Shmerling, 1996; Chapel *et al*., 1999; Kumar and Clark, 2002).

Systemic lupus erythematosus is a multisystem autoimmune disorder with a broad spectrum of clinical presentations (Tan, 1982).It is a prototypic systemic autoimmune disease of unknown etiology that primarily affects women of childbearing age (Graham *et al*., 2004). The development of SLE is likely influenced by a combination of genetic, environmental, and hormonal factors (Tsao, 2003). It is a chronic systemic inflammatory disease that affects many organ systems which includes skin, joints, kidneys, lungs and heart (Jayapal, 2007).

2.3 Criteria for Classification and Diagnosis of SLE

Criteria for SLE classification (Table 2.1) were developed in 1971, revised in 1982, and revised again in 1997 (Hochberg 1997). These criteria distinguished patients with the disease in question from those without the disease. The American College of Rheumatology (ACR) classification criteria were developed for clinical studies of lupus to ensure that cases reported in the literature do in fact have the disease. In addition to the wide variety of manifestations, SLE runs an unpredictable course. The dynamic nature of the disease often makes its diagnosis challenging.

Clinical features of SLE such as skin manifestations are less frequently reported in most of the studies. However, polyarthritis and polyarthralgia are common as well as serositis. Many of the Nigerian patients for instance presented with fever of unknown origin variously diagnosed as malaria or typhoid fever. Alopecia and hair loss are quite common presentations as well as neuropsychiatric symptoms (Adelowo *et al*., 2009).

Table 2.1: The American College of Rheumatology revised classification criteria for diagnosis of systemic lupus erythematosus.

CRITERIA	DEFINITION
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring occurs in older lesions
Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
Arthritis	Non-erosive arthritis involving two or more peripheral joints, characterised by tenderness, swelling or effusion
Serositis	a. Pleuritis: convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion or b. Pericarditis: documented by ECG or rub or evidence of pericardial effusion
Renal disorder	a. Persistent proteinuria >0.5 g per day or >3+ if quantitation is not performed or b. Cellular casts: may be red cell, haemoglobin, granular tubular, or mixed
Neurological disorder	a. Seizures: in the absence of off ending drugs or known metabolic derangements (eg, uraemia, acidosis, or electrolyte imbalance) or b. Psychosis: in the absence of off ending drugs or known metabolic derangements (eg, uraemia, acidosis, or electrolyte imbalance)
Haematologic disorder	a. Haemolytic anaemia with reticulocytosis, or b. Leucopenia: <4000/mm3, or c. Lymphopenia: <1500/mm3, or d. Thrombocytopenia: <100 000/mm3 in the absence of off ending drugs
Immunologic disorder	a. Anti-DNA: antibody to native DNA in abnormal titre, or b. Anti-Sm: presence of antibody to Sm nuclear antigen, or c. Positive finding of antiphospholipid antibodies based on: (1) an abnormal serum concentration of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) a false positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilisation or fluorescent treponemal antibody absorption test
Antinuclear antibody	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with 'drug-induced lupus' syndrome

Adapted from Hochberg, 1997.

2.4 Epidemiology of Systemic Lupus Erythematosus

Prevalence of SLE is estimated to be as high as 23% and 27% as reported by Ella and Agibilly, (2015) and Ella *et al*.(2016) respectively in Zaria, Nigeria. It has been said to be rare among black Africans, in contrast to its high prevalence among American blacks and Hispanics. In the report by Adelowo, SLE constituted 5.3% of a total of 1,250 cases seen in a rheumatology clinic in Nigeria (Adelowo *et al*., 2009). It has been postulated that despite the increase in frequency of SLE in the indigenous populations of East, Central and Southern Africa, SLE remains rare among West Africans (Chikanza *et al*., 1994; McGill and Oyoo, 2002). Prevalence of lupus are estimated to be as high as 51 per 100 000 people in the USA.

The incidence of lupus has nearly tripled in the last 40 years, mainly due to improved diagnosis of mild disease. Estimated incidence rates in North America, South America, and Europe range from 2 to 8 per 100 000 per year (Bertsias *et al*., 2012).

Women are affected nine times more frequently than men and African American and Latin American mestizos are affected much more frequently than Caucasians, and have higher disease morbidity (Bertsias *et al*., 2012). Mortality among black SLE has only been extensively studied among South African blacks and this is reported as being high (Budhoo *et al*., 2013).The disease appears to be more common in urban than rural areas. Sixty-five per cent of patients with SLE have disease onset between the ages of 16 and 55 years, 20% present before age 16, and 15% after the age of 55 (Bertsias *et al*., 2012). Men with lupus tend to have less photosensitivity, more serositis, and an older age at diagnosis and 1 year higher mortality compared to women. Systemic lupus erythematosus tends to be milder in the elderly with lower incidence of malar

rash, photosensitivity, purpura, alopecia, Raynaud's phenomenon, renal and central nervous system involvement, but greater prevalence of serositis, pulmonary involvement, sicca symptoms, and musculoskeletal manifestations (Bertsias *et al*., 2012).

2.5 Cause of Lupus

The exact cause of lupus is not known. A person who develops lupus probably inherits the risk from one or both parents and then develops the disease when exposed to a trigger. Triggers may include being exposed to sunlight, being ill with an infection, having surgery, being pregnant (Wallace *et al*., 2017).

2.6 Risk and Environmental Factors Associated with SLE

Over the past few decades, a number of environmental factors, such as cigarette smoke, alcohol, occupationally and non-occupationally related chemicals, ultraviolet light, hormones, infections, especially those caused by viruses, and vaccines, have been suspected to induce lupus and disease flares in a number of case series, case-control and population-based cohort studies and very few randomized controlled clinical trials (Kiyohara *et al*., 2012; Lisnevskaia *et al*., 2014; Takvorian *et al*., 2014).

Several environmental factors are considered as stimulating factors in pathogenesis of SLE. They include ultraviolet (UV) light, demethylating drugs, infections, intracellular viruses and sexual hormones, but ultraviolet light can also exacerbate SLE(Mok and Lau, 2003; Zandman-Goddard *et al*., 2012; Mak and Tay, 2014). Other factors implicated in stimulating SLE include current smoking (Costenbader *et al*., 2004), exposure to crystalline silica (Finckh *et al*., 2006), Epstein–

Barr virus seropositivity (Harley *et al*., 2006), and a protective effect of breastfeeding (Cooper *et al*., 2002). Socioeconomic factors have been associated with poorer outcomes and higher disease activity (Sutcliffe *et al*., 1999), although it remains unclear whether it plays a role in disease susceptibility or subsequent progression. Exposure to certain drugs may induce a lupus-like illness or exacerbate SLE.

2.6.1 Ultraviolet Light

Ultraviolet light increases DNA breakdown on skin and leads to subsequent production of immune complex containing nucleic acid. They also observed that men with SLE are less sensitive to light (Mok and Lau, 2003; Zandman-Goddard *et al*., 2012; Mak and Tay, 2014).

2.6.2 Epstein Barr Virus

Epstein Barr virus (EBV) enters B-cells and induces secretion of Interferon (IFN) by plasmacytoid dendritic cells, so it is considered a potential factor in pathogenesis of SLE (Bertsias *et al*., 2012).

2.6.3 Demethylating Drugs

More than 100 drugs have been proposed to cause a form of SLE, which is called drug-Induced lupus (DIL). Drug-induced lupus pathogenesis is not completely understood, but it seems genetic factors play an important role.The most important medications are procainamide andhydralazine. These drugs change gene expression in CD40 T-cells by blockingDNAmethylation and overexpression of leukocyte function dependent antigen (LFA-1); thus, they increase auto-reactivity in cells of the body (Bertsias *et al*., 2012). There are no standardized diagnostic criteria

for drug-induced lupus erythematosus (DILE), but in such cases there must have been continuous exposure to a pharmacological trigger for at least a month, with resolution after discontinuation of the drug. The clinical manifestations of drug-induced lupus erythematosus are generally milder with arthralgias and serositis being the predominant symptoms, and major organ involvement is usually absent (Vedove *et al*., 2009).

2.6.4 Hormones

Hormones are other environmental factors in stimulation of SLE. Hormones, such as estrogen and prolactin can cause autoimmune response by inducing maturation in auto-reactive B-cells with high affinity (Mok and Lau, 2003; Bertsias *et al*., 2012; Zandman-Goddard *et al*., 2012; Wahren-Herlenius and Dorner, 2013; Mak and Tay, 2014).

2.6.5 Smoking and Alcohol Consumption

Smoking and alcohol consumption have been reported to be a risk for developing SLE (Kiyohara *et al*., 2012; Takvorian *et al*., 2014). Smoking is by far the most recognized risk factor for SLE which mightcontribute to disease development via severalpathways (Afridi *et al*., 2014). Cigarette smoke contains several toll like receptors (TLR)-stimulatingcompounds, including lipopolysaccharide(a TLR-4 agonist) (Ekblom-kullberg *et al*., 2014), which can elicit an innate immune response by interacting with the HLA haplotype and changing gene expression in the joint (Klareskog *et al*., 2010).

2.6.6 Gender as a Risk Factor for SLE

Gender has consistently been shown to be a predilection factor for SLE where it is known to be predominantly a female disease (Feldman *et al*., 2013). First onset of SLE before puberty and after menopause is uncommon. The female predilection becomes less pronounced outside the reproductive age range. It is substantially more common in females of child-bearing age, where the reported female: male ratio may be as high as 8–15:1 (Lu *et al*., 2010). Pre-pubertal and post-menopausal ratios are much lower at 2–6:1 and 3–8:1, respectively (Lahita *et al*., 1983; Miller *et al*., 1983; Hochberg *et al*., 1985; Sthoeger *et al*., 1987; Kaufman *et al*., 1989; Holdcroft, 2007). This striking predominance in females probably relates to the effect of endogenous sex hormones, which have complex effects on the immune system (Lockshin, 2007; Lahita, 2008). However, a full explanation for why the disease is so uncommon in men remains elusive.Another linked debate is whether there is a particular type of lupus among males. Some others have proposed that lupus in males may take a more severe course (Tan *et al*., 2012); in particular with an increased incidence of renal disease, serositis, thromboses and discoid skin disease. These reports have been inconsistent and, like many studies of SLE, are complicated by disparities in ethnicity, duration of follow-up and selection bias(Tan *et al*., 2012).

The increased rate of SLE in females implicates hormones as essential in disease manifestations, and this influence of sex hormones is also seen in animal models of the disease. Typically, in most mouse models, females have worse outcomes, and administration of estrogens exacerbates while androgens ameliorates the disease (Mok *et al*., 1999).

2.6.7 Age of Onset of SLE

Systemic lupus erythematosus affects people of all ages from infancy to old age but it is most common in childbearing years.Women of childbearing ages are at greatest risk of developing SLE(Duarte *et al*., 2011).The peak age of onset of SLE is between 15 and 40 years (Ben-Menachem, 2010), although cases may present anytime in childhood through advanced age (Gaubitz, 2006). Males and patients with advanced age of onset tend to have more severe disease and poorer prognosis (Doria *et al*., 2006; Kasitanon *et al*., 2006). The mean age of presentation of SLE as reported by several researchers is between the age range of 31-40 years (Adelowo and Oguntona, 2009; Budhoo *et al*., 2013; Ella *et al*., 2016). Rees *et al*. (2014) also reported mean age at diagnosis as 48.9 years ± 16.9, women were significantlyyounger than men at diagnosis 48.3 years ± 16.8 vs52.1 years ± 17.2, $p<0.001$. The peak age of incidence of SLE forwomen (40–49 years) was earlier than that for men (60–69 years) and they concluded that the peak age of onset of the diagnosis is in the middle age, contrary to the generally held belief that lupus mainly targets young people(Rees *et al*., 2014).

The age at onset has an impact on SLE disease status, and infection is the main cause of death in those with late-onset lupus. Considering that the late-onset patients had simultaneously easily controllable diseases and high incidence of comorbidities, a different treatment strategy from younger patients is recommended (Feng *et al*., 2014).

2.6.8 Race and Ethnicity

Many reports from different researchers showed a significant relationship of SLE with different races and ethnic groups. Systemic lupus erythematosus is reported to be more common in African-Americans, African-Caribbeans, and Asians, than in Caucasians (Petri, 2002;

Danchenko *et al*., 2006; Pons-Estel *et al*., 2010; Rees *et al*., 2014).The age at diagnosis is younger in African-Americans than in Caucasians (Petri, 2002).Rees *et al*. (2014) also reported a statistically significant higher incidence and prevalence in people of black Caribbean ethnicity than in whites.

2.6.9 Family History of Systemic Lupus Erythematosus

Family history of SLE is associated with a clearly elevated risk of SLE and, to a much lesser degree, of rheumatoid arthritis and other autoimmune diseases (Ulff-Moller *et al*., 2017).People with a family history of SLE or other autoimmune diseases are at slightly higher risk for developing SLE. This risk increases as the number of first-degree family members with SLE increases (Dall'Era, 2013) but even so most cases are sporadic and not familial.

2.7 Pathogenesis of Systemic Lupus Erythematosus

Various factors are important in the pathogenesis of SLE, which can be divided to three major groups. All these factors cause breakdown in body's tolerance by stimulating immunologic response toward nucleic antigens. These factors are a) Environmental factors, b) Immunological factors and c) Genetic factors (Mok and Lau, 2003; Bertsias *et al*., 2012; Mak and Tay, 2014).

The aetiology of SLE includes both genetic andenvironmental components with female sex hormones strongly influencing pathogenesis. These factors lead to anirreversible break in immunological tolerance manifested byimmune responses against endogenous nuclear antigens.

The pathogenesis of SLE is complex and appears linked to autoimmunity against various native cellular components. Multiple genetic susceptibility loci have been identified in genomic studies, and specific major histocompatibility complexes are also linked to lupus (Sestak *et al*., 2005).

It is possible that these major histocompatibility complexes bind antigens in such a way that they increase the likelihood of T-cells mounting an immune response to self-antigens. Implicated susceptibility genes include *IRF 5, STAT4, ITGAM,* and several deficiencies in complement components C1q, C4, and C2 (Moser *et al*., 2009). Damage subsequently results from autoimmunity-induced inflammation or tissue deposition of immune complexes(Moser *et al*., 2009).

Autoantibodies directed against specific nuclear antigens (proteins and DNA) are central to the pathogenesis of systemic lupus erythematosus (SLE). Anti-extractable nuclear antigens (ENA) antibodies are detected to varying degrees in SLE patients. Knowledge regarding the genetic susceptibility towards developing SLE and the etiology of autoantibody development has greatly increased in recent years and it is now widely accepted that defective clearance of apoptotic bodies, viral infection, and molecular mimicry all contribute to the activation of autoreactive B cells and autoantibody production (Keogan *et al*., 2011).

Anti-ENA antibodies develop a number of years prior to disease onset and, with the exception of Anti-Ro and anti-Sm, are not directly implicated in tissue pathogenesis. The role of the IFN system in the pathophysiology of SLE is well recognized. The anti-ENA immune complexes, by virtue of their being bound to RNA, act as potent stimulators of TLR7 and help drive IFN-α production by plasmacytoid dendritic cells and promote the autoimmune cycle(Keogan *et al*., 2011). Recent research findings have suggested that a subset of ENA autoantigens, associated with the Ro/La autoantigen complex, is potentially involved in regulating IFN responses and innate immune detection mechanisms. The possibility that autoantibody generation do not only

mediate inflammation associated with the recognition of autoantigens but might also result in the inappropriate sequestering of Ro52 and Ro60(Keogan *et al*., 2011).

2.8 Treatment of Systemic Lupus Erythematosus

There is no cure for SLE but with appropriate medication, a majority of patients may stay in remission for long periods of time(Mak *et al*., 2012). Improvements in treatment and diagnostics over the years haveprolonged the survival of SLE patients and today the 10-year survival rate is about 90% (Mak *et al*., 2012). Although there is no such thing as an SLE-specific drug, treatments are available for specific manifestations of the disease. Glucocorticoids and antimalarial agents are frequently prescribed to SLE patients(Murphy *et al*., 2013). Glucocorticoids hamper many immune functions butmay cause harmful side effects with long term use.

Diseases modifying anti-rheumatic drugs (DMARDs) are often used to minimize the need for glucocorticoids and to treat specific organ involvement (Murphy *et al*., 2013). Antimalarial agents(e.g.hydroxychloroquine) and immunemodulating drugs including antibodies targeting specific receptors (e.g.cyclosporine, azathioprine, mycophenylate mofetil, intravenous immunoglobulins and belimumab) are also recommended as combined therapy (Dall'era and Chakravarty, 2011; van Vollenhoven *et al*., 2013).

Cyclophosphamide and anti-CD20 (rituximab) are commonly used to treat the most severe manifestations of lupus(van Vollenhoven *et al*., 2013). Many of these drugs are not approved for SLE, but are commonly used when conventional therapy fails, since beneficial effects have been

shown in uncontrolled trials (van Vollenhoven *et al*., 2013). Considering the important role of IFNα in SLE, drugs targeting the IFNαpathways would appear to be a reasonable approach, yet no medicine with the primary aim to interfere with IFNαproduction or its actions is available. Of note, both antimalarials and glucocorticoids have been ascribed anti-IFNα effects (Reily *et al*., 2006; Sacre *et al*., 2012), but it remainsuncertain to what extentthis mechanism contributes to the overall drug actions (Kirou and Gkrouzman, 2013). Drugs that are designed to specifically interact with IFNα pathways are currently under evaluation and some of them seem promising with regard to desirable effects as well as side effects (Kirou and Gkrouzman, 2013; Petri *et al*., 2013).

2.9 Autoantigens Associated with Systemic Lupus Erythematosus

The autoantigens found in patients with autoimmune diseases include anti-dsDNA, anti-Sm, anti-RNP, anti-histones, anti-chromatin (antinucleosome), anti-Ro/SSA and anti-La/SSB, anti-centromere, anti-Scl-70 and antinucleolar antibodies.

2.9.1 Anti-dsDNA Antibodies

Deoxyribonucleic acid (DNA) antibodies can be against single-stranded (ssDNA; denatured) or double-stranded (dsDNA; native) DNA (Yazici, 2011). Anti-ssDNA antibodies have been separated from the kidneys of patients with proliferative nephritis and may play a role in the pathogenesis of the disease (Koffler *et al*., 1973). However, they are nonspecific and do not correlate with disease activity. On the other hand, the testing for the presence of anti-dsDNA is beneficial, as this antibody has been designated as one of the diagnostic criteria for SLE by the American College of Rheumatology (ACR) (Colglazier and Sutej, 2005; Kavanaugh and

Solomon, 2002). It has been reported to be 97.4% specific and 57.3% sensitive for SLE. It has a positive likelihood ratio (LR) of 16 (Colglazier and Sutej, 2005; Kavanaugh and Solomon, 2002) and is very rare in healthy people and in patients with other diseases (negative LR: 0.49). Anti-ssDNA is also negative in many forms of DILE. When positive, it is very useful for diagnosing SLE, but its absence does not rule out the disease. It is assayed for when there is a pre-test probability for SLE and when the generic ANA test is positive. It can give information about the disease activity (positive LR: ~4), and high levels of anti-ssDNA may be associated with the development of lupus nephritis and vasculitis (positive LR: ~1.7) (Schur, 2004). It can be detected many years before the clinical phase of the disease, and elevated levels may well predict the disease exacerbation in SLE (Eriksson *et al*., 2011). The measurements can be repeated at one-three-month intervals if the disease is active and at 6-12 month intervals if the activity is low (Kavanaugh and Solomon, 2002).

2.9.2 Anti-Sm Antibodies

Anti- Smith (Anti-Sm) and anti-Ribonucleoprotein (anti-RNP) antibodies bind to related but distinct antigens. The Smith antigens are a series of nuclear nonhistone proteins complexed with small nuclear RNAs. The complexes are called small nuclear ribonucleoprotein particles (snRNPs) and are important in the splicing of precursor messenger RNA (Schur, 2004). Anti-Sm antibodies are mostly found only in SLE patients. Although their sensitivity is not so high for SLE since they can only be detected in 25-30% of the patients, their specificity is very high, making the test very useful for confirming the diseases but not successful for ruling them out (Colglazier and Sutej, 2005).They are usually associated with high titers of ANA and cannot be used for monitoring the disease in the way that is possible for anti-dsDNA (Schur, 2004). Most

laboratories use either ELISA or hemagglutination to detect these antibodies (Houtman *et al*., 1985).

2.9.3 Anti-RNP Antibodies

The anti-RNP system binds to antigens containing only U1-RNA that are different from but related to Sm antigens(Schur, 2004). Anti-RNP antibodies are detected in 30-60% of SLE patients but are not specific for them. They are also associated with Raynaud's syndrome and a milder probability of renal involvement (Migliorini *et al*., 2005). They are useful in diagnosing mixed connective tissue disease (MCTD) with an unknown performance since the presence of the anti-U1 RNP antibody is one of the criteria for MCTD (Colglazier and Sutej, 2005). The antibodies can be found in low titers in scleroderma, and anti-U1 RNP may indicate pulmonary fibrosis and joint involvement in these connective tissue diseases (Ihn *et al*., 1999).

2.9.4 Anti-histone Antibodies

Histones are basic proteins which bind cellular DNA. Anti-histones antibodies are detectable in 95% of DILE patients and 50-70% of those with SLE. Systemic drug induced lupus erythematosus (DILE) patients usually have positive sera for ANA and anti-histone but not for anti-ds DNA and anti-ENA (Vedove *et al*., 2009). On the other hand, the positive sera for these antibodies do not necessarily mean that the patient has the disease since many patients have the antibodies and are asymptomatic (Colglazier and Sutej, 2005).

2.9.5 Anti-chromatin (anti-nucleosome) Antibody

Chromatin is the native complex of histones and DNA. Anti-chromatin (anti-nucleosome)is reported to be detected in 50-90% of SLE patients (Gòmez-Puerta *et al.,* 2006). Some of these antibody subsets are autoreactive and may take part in the nephritogenic process in SLE (Muller *et al*., 2008).

2.9.6 Anti Ro/SSA and anti La/SSB Antibodies

Anti Ro/SSA and anti La/SSB targets cellular proteins complexed with small nuclear RNAs. They are frequently seen in SLE and Sjogren's syndrome (SS) patients, (Colglazier and Sutej, 2005) and their presence are one of the criteria for the diagnosis and classification of SS (Bizzaro *et al*., 2007). These antibodies are rare in the general population and in diseases other than SS and SLE, although they can be detected in scleroderma, polymyositis, MCTD, and rheumatoid arthritis (RA). Anti Ro/SSA and anti La/SSB can also be found in patients who satisfy SLE criteria with negative ANA. (Colglazier and Sutej, 2005) Anti Ro/SSA antibodies are found in 50% of patients with SLE and are associated with photosensitivity, cutaneous vasculitis, and interstitial lung disease (Yazici, 2011). They can be detected in 40-90% of primary SS patients but not as frequently in patients with secondary SS (10-15%)(Bizzaro *et al*., 2007). In addition, they are associated with neonatal lupus and congenital heart blockage. Anti La/SSB antibodies generally show parallelism to anti Ro/SSA antibodies. They are found in 10-15% of SLE patients and 20-50% of primary SS patients. The mothers of babies born with congenital heart blockage have an 80% probability of having anti Ro/SSA and anti La/SSB antibodies, even in the absence of an RD. Only 1% of all seropositive mothers will have babies with neonatal lupus syndrome or congenital heart block (Colglazier and Sutej, 2005).

2.9.7 Anti-centromere Antibodies

Autoantibodies to the centromere proteins were first described in 1980 (Fritzler *et al*., 2011). There are three major centromere proteins: CENP-A, B, and C (Nakano *et al*., 2000)with the major target being CENP-B. These antibodies are associated with limited cutaneous systemic sclerosis and the CREST (calcinosis, Raynaud's syndrome, oesophageal dysmotility, sclerodactyly, telangiectasia) variant(Miyawaki *et al*., 2005). Compared with healthy people, the specificity for CREST is high while the sensitivity is low (99.9% versus 65%, respectively) (Colglazier and Sutej, 2005). When the antibody is found in patients assayed for Raynaud's syndrome, anti-centromere antibodies can predict the future development of scleroderma (positive LR: 3.5) (Colglazier and Sutej, 2005). However, they are more distinctive for ruling out CREST (negative LR: 0.2). They are also associated with a lower frequency of interstitial pulmonary fibrosis (Ho and Reveille, 2003). It has been reported that 5.6% of SLE patients show positive sera for anti-centromere antibodies (Nakano *et al*., 2000).

2.9.8 Anti-Scl-70 Antibodies

More than 90% of scleroderma (Scl) patients haveantinuclear antibodies (ANA) in their sera. The nucleolar ANA pattern is common in patients with diffuse scleroderma and the centromere pattern for CREST syndrome. Autoantibodies to Scl-70 target topoisomerase-I (Topo-I) (Adnan, 2008). Anti-Scl-70 is detected in about 20-40% of systemic sclerosis patients and may increase the risk for pulmonary fibrosis, diffuse cutaneous involvement, and nephropathy. Although the sensitivity is low, specificity approaches 100%. The antibodies are detected in patients with Raynaud's syndrome indicating scleroderma is almost always diagnosed since specificity is 98%

and positive LR is 10. However, the test is conclusive for ruling out the disease as the sensitivity is low (28%) and the negative LR is 0.7(Colglazier and Sutej, 2005).

2.9.9 Anti-nucleolar Antibodies

The nucleolar immunofluorescence (IF) pattern is highly specific for scleroderma. Specific antibodies leading to the formation of this pattern are anti-PM/Scl antibodies which signify the following: overlap syndromes, scleroderma/polymyositis, anti-Th/To antibodies associated with limited cutaneous types, the presence of pulmonary arterial hypertension, antiRNA polymerase I with rapidly progressive types, anti-RNA polymerase III with diffuse types with little visceral involvement, anti-U3-RNP with diffuse cutaneous types, and pulmonary artery hypertension (Reveille and Solomon, 2003).

2.10 Antinuclear Antibodies

In addition to being systemic diseases, connective tissue diseases (CTD) are associated with a wide range of autoantibodies that are directed against various components of the cell nucleus and are known as antinuclear antibodies (Swaak *et al*., 1993). The role of these antibodies in the pathogenesis of CTD is still controversial. However, their role in the diagnosis and prognosis of CTD is well established (Swaak *et al*., 1993; Hollingsworth *et al*., 1996; White and Robbins, 1987).

The production (ANAs) is one of the defining features of SLE. Description of the LE cell phenomenon by Hargraves *et al*.(1948) was the first evidence for the existence of these autoantibodies (Reeves *et al*., 2013). In clinical circumstances, the detection of ANAs is important for three reasons: specifying the diagnosis with determination of disease-specific

autoantibodies, establishing appropriate monitoring and prognosis in some rheumatic diseases (RDs), and determining the risk of developing the disease in an asymptomatic individual (Villalta *et al.*, 2007).

Antinuclear antibodies are found in varying percentages in different CTD, approaching 100% in SLE when Hep-2 cell line is used for screening. Antinuclear antibodies on their own are not diagnostic of CTD, as they can be found in other rheumatic and non-rheumatic conditions as well as in some healthy elderly people, albeit low titers. However, high ANA titers are generally strong predictor for CTD and their presence should lead to further investigation for more specific antibodies including those directed against ENA and dsDNA.

2.10.1 DiagnosticImportance of Antinuclear Antibody

The autoantibodies produced in SLE are directed primarily against nuclear antigens, most of which are associated with nucleic acids (DNA or RNA). This is the basis for the fluorescent ANA assay, a screening test for SLE that is positive in >95% of patients (Edworthy *et al.*, 1988). However, the specificity of a positive ANA for SLE is relatively low (Shiel and Jason, 1989). ANAs stain cells in a variety of different patterns, corresponding to reactivity with different subsets of nuclear (or cytoplasmic) antigens. Some specificityis uniquely associated with SLE. Anti-dsDNA antibodies, for example, are found in ~70% of SLE patients at some point during their disease, and are 95% specific for the diagnosis (Edworthy *et al.*, 1988). Anti-Sm antibodies are found in~10 - 25% of lupus patients' sera depending on ethnicity (Wang *et al.*, 2001), and also are virtually pathognomonic of SLE (Kurata and Tan, 1976).

Antibodies to the ribosomal P0, P1, and P2 antigens (Elkon *et al.*, 1992), proliferating cell nuclear antigen (PCNA) (Takasaki *et al.*, 1984), and RNA helicase A (Yamasaki *et al.*, 2007) are highly specific, but less sensitive, markers of the disease. These "marker" autoantibodies are highly unusual in drug-induced lupus. In contrast, anti-single-stranded (ss) DNA, chromatin/histone, nRNP, Ro-60 (SS-A), La (SS-B), Ro52, Ku, and Su (Wang *et al.*, 2001), are associated with SLE as well as other systemic autoimmune diseases, but are uncommon inhealthy individuals(Wang *et al.*, 2001). Remarkably, most of the same antibodies are associated with murine lupus: anti-dsDNA antibodies are produced by (NZB X NZW) F1 mice, anti-dsDNA, Sm, and ribosomal P by MRL mice, and anti-Sm, dsDNA, ribosomal P, and RNA helicase A by mice with pristane-induced lupus (Theofilopoulos and Dixon, 1985).

2.10.2 Autoantigens and Implications for Autoantibody Production

Although there are thousands of nuclear proteins, only a few are autoantigens in SLE. These are mainly RNA-protein or DNA-protein complexes comprised of multiple proteins physically associated with nucleic acid (Reeves *et al*., 2013). Importantly, the nucleic acid constituents of these antigens are ligands for innate immune system receptors called "Toll-like receptors" (TLRs) 3, 7, 8, and 9 localized within endosomal compartments (Kawai and Akira, 2006). Innate immune responses mediated by TLR7 (a receptor for ssRNA) and TLR9 (a receptor for unmethylated CpG motif in dsDNA) are receiving increasing attention as mediators of autoimmune inflammatory disorders, such as SLE. Toll-like receptor 7 (TLR7) recognition of U1 RNA, a component of the U1 small nuclear ribonucleoprotein (snRNP, recognized by anti-Sm/RNP autoantibodies), and TLR9 recognition of DNA, a component of chromatin (recognized by antihistone/DNA autoantibodies) is linked to the production of type I interferon (IFN-I),

which is increased in about 60% of lupus patients. The structures of some of the major nucleic-acid-containing autoantigens in lupus have been defined by immunoprecipitation studies followed by the analysis of the [^{35}S] methionine/cysteinelabeled proteins and [^{32}P]-labeled nucleic acid components of the complexes recognized by lupus autoantibodies(Reeves *et al*., 2013).

2.11 Extractable Nuclear Antigens

Extractable nuclear antigens (ENA) refer to nucleoproteins that can be extracted from the cell nucleus with saline. They include Ro (SS-A), La (SS-B), RNP, Sm, Scl-70 and Jo-1 (Aziz and Faisal, 2004). Extractable nuclear antigen is a well-established group of autoantigens that have pathological value in Sjögren's syndrome, mixed connective tissue disease, sclerodema and systemic lupus erythematosus (Hamburger *et al*., 1997). Reactivity with the antigens is more disease specific and may also provide clinically useful prognostic information (Kavanaugh *et al*., 2000). The ENA complex consists of nuclear antigens which are hidden from the immune system in intact cells. However, during cell death via apoptosis this structure is exposed to the immune system, triggering specific reactions in susceptible patients (Ohlsson *et al*., 2002).

Six of the most diagnostically useful autoantibodies include those to (SSA), which are found in 40 to 60% of patients with Sjogren's sysdrome and to 15% of SLE patients(Franceschini *et al*., 2005). Smith (Sm) antibodies are highly specific for SLE but only occur in 35% of cases (Migliorini *et al*., 2005). The ELISA monitoring of extractable antinuclear antibodies- smith antigen is usually used with the concentration value of ds-DNA to control the disease activity (Suh-Lailam *et al*.,2011).

Antibodies to ribonucleoprotein (RNP) are found in 95 to 100% of patients with mixed connective tissue disease (MCT) but are also found in up to 45% of patients with SLE (Sinico *et al.,* 2002). The presence of anti- RNP antibody alone strongly suggests a diagnosis of MCTD (Orton.,*et al.,* 2004). Scleroderma (Scl-70) autoantibodies are the specific markers of scleroderma and are found in up to 60% of patients diagnosed with this disorder (LeRoy *et al.*, 2001).

The Jo-1 autoantibody is one of a family of characterized autoantibody seen in myositis patients (Tri *et al*., 2002). In rheumatoid arthritis, antibodies to ENA have been reported in up to 16% of patients, but without an assessment of their clinical significance.

2.11.1 Autoantibodies to Extractable Nuclear Antigens (ENA)

Antibodies to extractable nuclear antigens (ENAs)—SSA, SSB, U1RNP, Sm, Scl-70, and Jo-1—are clinically important in patients with systemic rheumatic diseases (Solomon *et al*., 2002). Besides DNA and histones, autoantibodies may also target other nuclear antigens. These nuclear antigens were named ENA as originally they were extracted from the nuclei with saline (Fishbein *et al*., 1979). Autoantibody to Smith antigen (Sm) which is considered to be specific for SLE was the first anti-ENA detected in 1966 (Tan and Kunke, 1966). Thereafter further subtypes of ENA i.e. ribonucleoproteins (RNP), SSA/Ro, or SSB/La, Scl-70, Jo-1 and PM1 were more clearly identified (Asherson, 1959; Tan, 1967; Clark and Tomasi, 1969; Matticli and Reichlin, 1974; Alspaugh *et al*., 1976; Wolfe *et al*., 1977;Scopelitis *et al*., 1980; Tan *et al*., 1980). Although most of these ENA are disease specific, still a significant overlap exists.

In the last few years many other autoantibodies like topoisomerase-I (Topo-I), centromere protein (CENP)-B, RNA-polymerase I-III (RNA-pol I-III), MU, TM, Ku, Mi-2, RA33 etc. have

also been described(Miyawaki *et al*., 1978; Tan, 1989). While of scientific interest, typing of many of these antibodies has not found its way in to the clinical practice. Certain autoantibodies against cytoplasmic and cell membrane components though present are less relevant in diagnostics (Miyawaki *et al*., 1978; Tan, 1989).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1Study Area

The study was conductedin Zaria, Kaduna State, Nigeria and the hospitals from which the samples were collected includes; Major Ibrahim Bello Memorial Hospital Sabon Gari, Hajiya Gambo Sawaba Memorial Hospital Zaria City, Ahmadu Bello University Medical Services, and Primary Health Care, Samaru. Zaria is a major city in northern Kaduna State, Nigeria and is located on latitude 11º 04'N and longitude 7º 42'E. It is defined by a 15 km radius from the PZ post office and is well connected by roads and rail with other regions of the country. Zaria is the second principal town in Kaduna and a home of Ahmadu Bello University and to several other Federal institutions.

Population of Zaria is approximately 975,153 (projections from 2006 census). It is made up of two Local Government Area councils, Zaria Local Government consisting of two districts (Zaria walled city and Tudun- wada) and Sabon Gari Local Government consists of three districts; Sabon Gari, Samaru and the Government Reservation Area (GRA) (Oladimeji and Ojiboo, 2012).

3.2. Study Design

The study was hospital-based, cross-sectional and involved the use of structured questionnaire (Appendix I) and the analysis of blood samples from male and female patients with fever, joint pain, malaise and fatigue who gave consent (Appendix II).

3.3. Study Population

The study population includedmale and female presenting with: fever, joint pain, muscle pain and fatigue attending the selected hospitals in Zaria, Kaduna State.

3.4. Sample Size

Sample size used for the study was calculated using a prevalence rate of 23% as reported by Ella *et al*. (2016), using the equation below, as derived by Sarmukaddam and Gerrard (2006) at 95% confidence interval.

$n = Z^2Pq/L^2$

Where,

n = number of samples

Z = standard normal deviate at 95% confidence interval = 1.96

P = prevalence from previous study 23% (Ella *et al*., 2016) = 0.23

q = 1- P = 1 - 0.23 = 0.77

L = allowable error of 5% = 0.05

$n = 1.96^2 \times 0.23 \times 0.77/0.05^2 = 0.68034736/0.0025 = 272.138944 \sim 273$ samples

The sample size calculated was 273, which is the least number of samples that was to be used for the study therefore 300 samples were collected and used for the study.

3.5. Ethical Considerations

Ethical approval (Appendix III) was obtained from the ethical committee of the State Ministry of Health, Kaduna State.

3.6. Inclusion Criteria

Female and male patients presenting with: fever, joint pain, muscle pain and fatigue that consented were included in the study.

3.7. Exclusion Criteria

Female and male patients with no symptoms of fever, joint pain, muscle pain and fatigue and those who do not consent were excluded from the study.

3.8. Data Collection

All patients who consented were each given a structured questionnaire which was aimed at obtaining some socio-demographic data, risk factors as well as clinical information associated with SLE. Interpretation was offered where necessary.

3.9. Sample Collection and Processing

Three(3) ml of bloodwerecollected by aseptic venipuncture procedure in sterile plain containers containing no preservatives or anticoagulants from the patients under study by employing the service of authorizedmedical personnel.Samples collected weretransported in cold packs to the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria for analysis.The samples were allowed to clot at room temperature for an hour and then centrifuged at 2500rpm for three minutes to obtain the serum. The serum was then transferred into plain bottles and stored in the freezer at -17^{o}C for further assay.

3.9.1Determination of Systemic Lupus Erythematosus Status by Slide Agglutination assay

The SLE latex text kit (Diagnostic Automation, USA) is used as an aid in the diagnosis of SLE through the detection and quantitation of serum antinucleoprotein factors associated with SLE.

3.9.1.1 Principle of the assay

The principle of the test is based on the agglutination reaction between latex particles coated with deoxyribonucleoprotein when brought into contact with a serum which contains antinuclear antibodies. Agglutination indicates a positive reaction. The reaction time for this occurrence is within one minute.

3.9.1.2 Procedure of the test

All test reagents and serum specimens were brought to room temperature before use, positive and negative controls were tested with each series of test sera. Using a disposable pipette one drop (0.03 ml) of test serum was placed onto a circle on the slide (a separate disposable pipette was used for each test serum). The SLE test latex reagent was well agitated for 10 seconds prior to testing to insure that there is no aggregation of the latex particles which may occur upon standing. One drop of SLE latex reagent was added to each circle that contains specimens on the slide. The resulting mixture was spread using the paddle end of the pipette, the slide was then gently tilted and rotated by hand for one minute and observed for macroscopic clumping using the indirect oblique light source. A positive and negative control was included in each run and the reaction of the test serum was compared to the SLE positive and negative control sera and observed for agglutination not longer than one minute. Positive resultshowed agglutination while negative result is a smooth milky suspension.

3.9.2. Determination of antinuclear antibodies status by enzyme-linked immunosorbent assay (ELISA)

3.9.2.1. Principle of the Assay

The diagnostic automation ANA screen USA ELISA test system is designed to detect IgG class antibodies to a variety of common nuclear antigens in human sera. The wells of the plastic microwell strips are sensitized by passive absorption with antigen.

3.9.2.2 Procedure of the test

The individual components were removed from storage and allowed to warm up to room temperature (20-25^{o}C). Then a 1:20 dilution (10µL of serum + 200µL of sample diluent) of negative control, calibrators, positive control, and each patient specimen were prepared and dispensed into appropriately labelled wells and 100µL of sample diluent added to well A1, as a reagent blank well configuration. The plate was incubated at room temperature (25^{o}C) for 60 minutes. Then the microwell strips werethen washed 5 times using wash buffer and blot drying on absorbent (tissue) paper to remove residual liquid not removed after washing which might affect the outcome of the test result.

A total of 100µL of the horseradish peroxidase goat anti-human IgG conjugate wasthereafter added to each well, including reagent blank well, at the same rate and in the same order as the specimens were added. This was followed by incubation at room temperature (25^{o}C) for 30 minutes. The microwells were washed again 5 times and 100µL of 3,3',5,5'-Tetramethylbenzidine (TMB) chromogenic substrate was added to each well, including reagent blank well at the same rate and in the same order as the specimens were added. The plate were once again incubated at room temperature (25^{o}C) for 30 minutes after which the reaction was stopped by addition of 50µL stop solution to each well, including reagent blank well, at the same

rate and the same order as the TMB was added. After adding the stop solution, the plate was tapped several times to ensure that the samples were thoroughly mixed. The microwells reader was set to read at wavelength of 450nm using an ELISA microwell reader and the optical density (OD) of each well against the reagent blank was measured. Positive samples turned from blue to yellow and the OD of each well against the reagent blank were measured.

3.9.3Determination of extractable nuclear antigens status using ENA-Profile 6 ELISA

The diagnostic automation ENA screenELISA test system qualitative screening assay designed to detect antibodies to extractable nuclear antigens (anti- ENA) in human sera was used for this test. When performed according to the manufacturer's instructions, the test system was capable of detecting all anti-ENAs commonly tested for, such as those against Jo-1, Sm, Sm/RNP, SSA/Ro, SSB/La, and Scl- 70.

3.9.3.1 Principle of the assay

The diagnostic automation ENA screen ELISA test system was designed to detect IgG class antibodies to a variety of common nuclear antigens in human sera. Wells of plastic microwell strips were sensitized by passive absorption with ENA antigen. The colour intensity of the solution depends upon the antibody concentration in the original test sample.

3.9.3.2. Procedure of the test

All test reagents and serum specimens were brought to room temperature before use. Dilutions of 1:20 was prepared by adding 10µL of the negative control, calibrator, positive control, and each patient serum to 200µL of sample diluent and it was mixed well. Then 100µL of each diluted control, calibrator and sample was dispensed into appropriate wells ensuring that the samples were properly mixed. Using a different pipette tip for each sample, sample diluent was added to

all wells on row A,the negative controls was added to column 1 rows B-H contains, positive controls was added to column 2rows B-H, calibrator was added to column 3 rows B-H. Subsequently, column 4 was used for test sample 1(patient serum) and the next column was used for sample 2 till column 12 for sample 9 as seen in Appendix IV. Thereafter 100µL of sample diluent was added to well A1- A12 as a reagent blank and the plates were incubated at room temperature for 30 minutes. The microwell strips were washed 5 times with the liquid from the wells vigorously shaken out. Each microwell was filled with wash buffer making sure no air bubbles were trapped in the wells repeating the washing steps for a total of 5 washes. The wash solution was shaken out from all the wells and the plates were inverted over a paper towel and tapped firmly to remove any residual wash solution from the wells.

A total of 100µL of the horseradish peroxidase goat anti-human IgG conjugate was added to each well and incubated at room temperature for 30 minutes and the microwells washed 5 times as above with washing buffer which was followed by addition of 100µL of 3,3',5,5'-Tetramethylbenzidine (TMB) chromogenic substrate, at the same rate and in the same order as the specimens were added and incubated at room temperature for 15 minutes. The reaction was stopped by adding 50µL of stop solution to each well, including the reagent blank well and the plates were tapped several times to ensure that the samples were thoroughly mixed. The plate was read within 30 minutes after the addition of the stop solution at a wavelength of 450nm with a microwell reader. Procedure laid down by the manufacturer was followed in interpreting the ENA profile-6 results obtained were sample with a concentration of ≥180 AAU/ml calibrator unit value was considered positive.

3.10Data Analysis

Data obtained from questionnaires as well as those gotten from the test analysis were analysed using the statistical package for Social Sciences (SPSS) version 17 at 0.05 level of significance and at 95% confidence interval. Relationships between variables were determined using Pearson's Chi- square analysis and the result was calculated and recorded in percentages and presented in tables and charts. All p-values ≤ 0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

Out of the 300 patients attending selected hospitals included in the study, 63(21%) were positive for SLE bythe latex agglutination assaywhile237(79%) were negative as presented in Figure 4.1.

Table 4.1 shows the result of the patients according to the hospitals sampled from with patients fromprimary health care, Samaru (PHCS) having the highest seroprevalence of 30% (6/20) while Ahmadu Bello University Medical Center (ABUMC)had the least (10%: 2/20)among all the hospitals sampled. There was a statistically significant association between SLE and the hospital location (χ^2= 11.2333, df = 3, p= 0.011).

The relationship between SLE and gender of the patient is presented in Table 4.2. Although female patients had higherseroprevalence (21.8%:52/239) compared to males(18.4%:11/61), there was no statistically significant difference observed in the seroprevalence. Even though the prevalence obtained was not statistically significant, it was observed that females are more likely to develop SLE compared to males(χ^2= 0.406, df= 1, p=0.524; OR= 1.264, 95% CI=0.614 - 2.600).

The distribution in relation to age showed that patients who were above 60 years had the highest seroprevalence (36.7%:11/30) while those below 20 years of age had the least(12.8%:5/39).There was no statistically significant difference between age and SLE (χ^2=7.177, df= 5, p=0.208) as shown in Table 4.3.

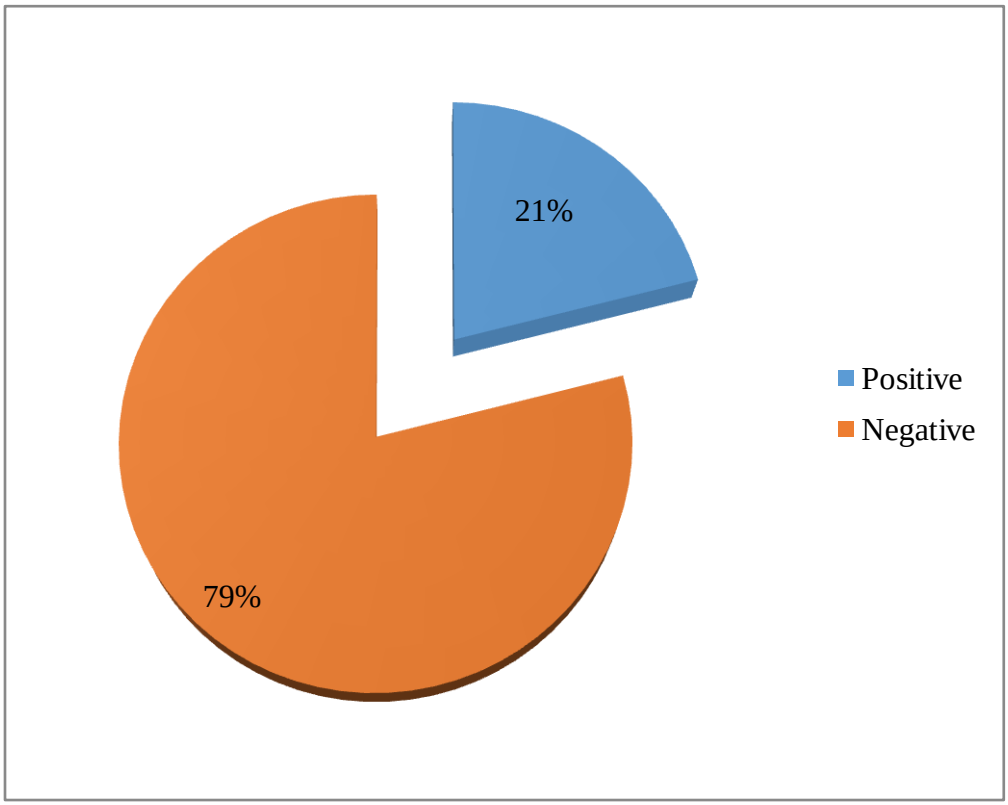


Figure 4.1: Seroprevalence of Patients withSystemic Lupus Erythematosus among the Study Population in Zaria, Nigeria

Table 4.1: Seroprevalence of Systemic Lupus Erythematosus among Patients Attending Selected Hospitals in Zaria, Nigeria

Hospital	No. examined	No. positive (%)	χ^2	*p*-value
HGSGH	115	34(29.6)	11.233	0.011
MIBA	145	21(14.5)		
ABUMC	20	2(10)		
PHCS	20	6(30)		
TOTAL	300	63(21)		

Key:
HGSGH- Hajiya Gambo Sawaba General Hospital
MIBA- Major Ibrahim Bello Abdullahi Memorial Hospital
ABUMC- Ahmadu Bello University Medical Centre
PHCS- Primary Health Care, Samaru

Table 4.2: Distribution of Systemic Lupus Erythematosus According to Sex of Patients Attending Selected Hospitals in Zaria, Nigeria

Sex	No. examined	No. positive (%)	OR(95%CI)	*p*-value
Female	239	52(21.8)	1.264(0.614 – 2.600)	0.524
Male	61	11(18)		
Total	300	63(21)		

Table 4.3: Distribution of Systemic Lupus Erythematosusin relation to Age-group of the patients studied

Age group(years)	**No. examined**	**No. positive (%)**	χ^2	***p*-value**
10-19	39	5(12.8)	7.177	0.208
20-29	125	23(18.4)		
30-39	55	11(20)		
40-49	35	9(25.7)		
50-59	16	4(25)		
≥ 60	30	11(36.7)		
Total	300	63(21)		

Table 4.4 shows results in relation to some socio-demographic factors and SLE was found to be associated with marital status in this study. Analysis of the result according toeducational status showed that patients who had tertiary education had highest seroprevalence (23%:29/126)while patients with primary education had the least(17.6%:9/51). There was no significant association between SLE and educational status of the patients (χ^2=0.706, df=3, p=0.872).

In relation with the occupation of the individuals, patients who were civil servants had the highest seroprevalence (25%:5/20)while students had the least(11.9%:5/42). There was no significant difference between SLE and occupation of the patients (χ^2=2.847, df=4, p=0.584).

The result according to marital status of the patients showed that patients who were widowed had the highest prevalence of 50% (4/8) while patients that were single had the least prevalence of 7.9% (3/38). There was statistically significant association between marital status and SLE (χ^2=8.419, df=3, p=0.038).

In relation to level of awareness of SLE, only one patient was aware of the disease and was positive for the SLE assay (χ^2=3.774, df=1, p=0.052).

Analysis of the results according to some clinical factors presented in Table 4.5 showed that a statistically significant relationship exist between SLE, loss of weight, photosensitivity and seizures. Patients who complained of loss of weight during the course of their illness had higher seroprevalence of 28.8% (36/125) compared to those who did not (15.4%:27/175). Statistically significant association exist between loss of weight and SLE (χ^2=7.858, df=1, p=0.005; OR=2.217; 95% CI=1.261 - 3.897).

Table 4.4: Distribution of Systemic Lupus Erythematosus in relation to some sociodemographic factors among the study population (n=300)

Variable	No Examined	No positive (%)	χ^2	*p*-value
Educational status				
Primary	51	9(17.6)	0.706	0.872
Secondary	85	17(20)		
Tertiary	38	8(21.1)		
Non-formal	126	29(23)		
Occupational status				
Student	42	5(11.9)	2.847	0.584
Civil servant	20	5(25)		
Business	42	8(19)		
Housewife	175	40(22.9)		
Artisans	21	5(23.8)		
Marital status				
Single	38	3(7.9)	8.419	0.038
Married	247	55(22.3)		
Divorced	7	1(14.3)		
Widowed	8	4(50)		
Level of awareness				
Yes	1	1(100)	3.774	0.052
No	299	62(20.7)		

Table 4.5: Distribution of Systemic Lupus Erythematosus in Relation to Clinical Information of Patients Studied (n=300)

Variable	No. examined	No. positive (%)	Odds Ratio(95%CI)	*p*-value
Loss of weight				
Yes	125	36(28.8)	2.217 (1.261 – 3.897)	0.005
No	175	27(15.4)		
Rash				
Yes	49	11(22.4)	1.108 (0.530 – 2.315)	0.785
No	251	52(20.7)		
Oral ulcer				
Yes	25	8(32)	1.882 (0.772 – 4.587)	0.158
No	275	55(20)		
Photosensitivity				
Yes	11	5(45.5)	3.319 (0.979 – 11.256)	0.042
No	289	58(20.1)		
Seizures				
Yes	21	0(0)	Undef.	0.014
No	279	63(22.6)		

The result showed that patients who had rash on their body had higherseroprevalence of 22.4% (11/49) compared to those who do not haverash(20.7%:52/251). There was no significant difference between rash and SLE (χ^2=0.074, df=1, p=0.785; OR=1.108; 95% CI=0.530 - 2.315).

Patients who had oral ulcer had higherseroprevalence of 32% (8/25) while those without oral ulcer had lower(20%:55/275). There was no statistically significant difference between SLE and oral ulcer (χ^2=1.989, df=1, p=0.158; OR=1.882; 95% CI=0.772 - 4.587).

Analysis of the result also showed that patients who had rash as a result of exposure to sunlight (photosensitive) had higherseroprevalence of 45.5% (5/11) compared to those who were not photosensitive (20.1%:58/289). There was a significant association between photosensitivity and SLE (χ^2= 4.116, df = 1, p= 0.042; OR=3.319; 95% CI=0.979 – 11.256) with antibodies to SLE likely to be detected thrice more in patients who are photosensitive.

Patients who do not experience seizures had higherseroprevalence of 22.6% (63/279) compared to those who experience seizures (0%:0/21). Significant association exist between seizure and SLE (χ^2=6.002, df=1, p=0.014).

All the possible risk factors that were considered in this study were not statistically associated with SLE except for history of blood transfusion as presented in Table 4.6. Pregnancy as a risk factorfor SLE due to hormonal changes was considered as a risk factor in this study. The result showed that women who werepregnant had higherseroprevalence of 27.5% (25/91) compared to those who were not pregnant with a prevalence of 18.2% (27/142). No significant association was seen between pregnancy and SLE (χ^2=2.820, df=1, p=0.093; OR=0.589, 95%CI=0.316 – 1.096).

Table 4.6: Distribution of Systemic Lupus Erythematosus in relation to Risk Factors assessed among the Study Population

Variable	No examined	No positive (%)	Odds ratio(95%CI)	*p*-value
Pregnancy				
Yes	148	27(18.2)	0.589 (0.316 – 1.096)	0.093
No	91	25(27.5)		
Total	239	52(21.8)		
Gestational stage				
First trimester	38	4(10.5)	-	0.335
Second trimester	93	20(21.5)		
Third trimester	17	3(17.6)		
Total	148	27(18.2)		
Smoking and Alcohol consumption				
Yes	1	0(0)	Undef.	0.606
No	299	63(21.1)		
Total	300	63(21)		
History of Blood transfusion				
Yes	54	17(31.5)	1.998 (1.035 – 3.856)	0.039
No	246	46(18.7)		
Total	300	63(21)		

Further analysis of the gestational stage of the pregnant women showed that those who were in their second trimester of pregnancy had the highestseroprevalence of 21.5% (20/93) while those in their first trimester had the least(10.5%:4/38). There was no significant association between gestational stage and SLE (χ^2=2.185, df=2, p=0.335).

Of all the 300 patients enrolled in this study, only 1 patient agreed to smoking and alcohol consumption and the patient did not have antibodies to SLE (χ^2=0.267, df=1, p=0.606).

In relation tohistory of blood transfusion, patients who had history of blood transfusion had higherseroprevalence of 31.5% (17/54) while those who never had a history ofblood transfusion had lower(18.7%:46/246). Further analysis showed that SLE was associated with history of blood transfusion. Patients who had history of blood transfusion were two times more likely to have SLE than those who had never had blood transfusionespecially in genetically predisposed individuals (χ^2=4.361, df=1, p=0.039; OR=1.998, 95%CI=1.035 – 3.856).

4.1 Antinuclear Antibody AssayResult for SLE Seropositive Samples

Of the 63 patients who had antinucleoprotein factors to SLE, 28(44%) were positive for antinuclear antibody (ANA) assay while 4(14.8%) of the SLE negative samples tested positive for ANA. Of the 90 patients tested for ANA 32(35.6%) was positive while 58(64.4%) were negative. Statistically significant association exist between SLE and ANA (χ^2=7.241, df=1, p=0.007; OR=4.6, 95%CI=1.424 – 14.856) with antibodies to SLE likely to be detected four times more in patients who have ANA as shown in Table 4.7.

Table 4.7: Relationship between Systemic Lupus Erythematosus Assay and Antinuclear Antibody Results of Patients Studied

		ANA profile			
SLE	**No. examined**	**Positive (%)**	**Negative (%)**	**OR (95%CI)**	***p*-value**
Positive	63	28(44.4)	35(55.6)	4.6(1.424 – 14.856)	0.007
Negative	27	4(14.8)	23(85.2)		
Total	90	32(35.6)	58(64.4)		

4.2 Extractable Nuclear Antigen Assay Result

The most common ENA among the study population was Scl-70 to which 6 patients tested positive (33.3%:6/18) followed by Jo-1 with 5 (27.8%:5/18). Others that occurred but very low were Sm/RNP with 2 (11.1%:2/18) and 1 for SSB (5.6%:1/18) while Sm and SSA recorded 0% as presented in Table 4.8 and Appendix V.

Table 4.8:Mean (standard deviation) concentration of Extractable Nuclear Antigens among the Patients Studied (n=18)

ENA Profiles	No. Positive (%)	Mean ± SD(AAU/ml)
Jo-1	5(27.8)	142.60 ± 87.42
Sm	0(0.0)	34.98 ± 5.31
Sm/RNP	2(11.1)	129.35 ± 108.73
SSA	0(0.0)	82.65 ± 11.10
SSB	1(5.6)	63.87 ± 25.96
Scl-70	6(33.3)	199.24 ± 222.12

Key:

ENA = Extractable nuclear antigens

SD = Standard deviation

AAU/ml = Autoantibody unit/ml

CHAPTER FIVE

5.0 DISCUSSION

In the result obtained from this study, 63 patients tested positive for SLE giving a seroprevalence of 21%. The prevalence found in this study can be considered as an evidence of presence of SLE in the population and this may be due to the fact that itusually presents with no clear cut symptoms and could be mistaken for other diseases such as rheumatoid arthritis ormalaria. In this study, all patients included had malaria symptoms.

This seroprevalence is lower than 27% by Ella and Agibilly (2015)and similar to 23% reported in Zaria by Ella *et al*. (2016).The difference in seroprevalence in this study and previous studies could be due to the fact that this study was carried out on general populationand both male and female patients irrespective of their HIV status and gender while the previous studies was carried out only in female patients and HIV positive patients respectively.Human immunodeficiency virus is known to be a predisposing factor for many diseasesincluding SLE which could have contributed to the higher prevalence recorded in the previous study, as well as its study population bias which was female patients only.

The study found the distribution of the antibody to be statistically associated with the hospital location;with Primary Health CenterSamaru (PHCS) having the highest seroprevalence compared to other hospitals.The higherseroprevalence in this hospital could be attributed to the fact that only female patients were in attendance and screened for,while all the other hospitals had both male and female patients included in the study. Systemic lupus erythematosus is known to be predominantly a female disease (Feldman *et al*. 2013), so the findings in PHCS when compared with results from the other hospitals, agreed with this statement. The predominance of

SLE among females recorded in this study, also agreed with the findings of Rees, *et al.*(2014) and Ella *et al*. (2016).

Several studies conducted in different regions of the world have identified socio-demographic variables like age, gender and environmental conditions (exposure to sunlight) as risk factors for developing SLE (Bengtsson *et al*., 2002; Kiyohara *et al*., 2012; Takvorian *et al*., 2014; Ella *et al*., 2016). Gender has been consistently shown to be a major predilection of SLE.The gender based seroprevalence showed that SLE was higher among female patients in this study compared to males though it did not reach statistical significance. It however, had an OR of 1.264 which makes it a factor for developing SLE. The increased frequency of SLE among women had been attributed in part to a postulated oestrogen hormonal effect (Chapel *et al*., 2006; Kaballo *et al*., 2009).The higher prevalence in females is in agreement with the findings of Feldman *et al*.(2013); Rees*et al.*(2014) and Ella *et al*.(2016) where higher incidence in female patients compared to male patients were obtained.

This study showed that seropositivity of SLE increased with age with highest prevalence in patients above the age of 40 years.This observation is in agreement with the findings of Rees *et al*.(2014) who reported that the peak age of diagnosis is in the middle age, contrary to the generally held belief that lupus mainly targets young people. Rees *et al*.(2014) reported peakincidence age of 50-59 years with 40-49 years in women and 60-69 years in men.

This studyalso agrees with the findings of Ella *et al*.(2016) where SLEprevalence was higher among patients between the agesof 31-50 years, confirming the fact that the disease is mostly found among individual of higher age range especially the middle aged. Patients who were below 30 years had lower prevalence among the patients studied.This also agrees with the

findings of Odhiambo *et al*.(2014). Somers*et al*. (2014) also reported incident cases of SLE with a mean age of 39.3 - 40 years.

The study did not establish any statistically significant association between educational status and SLE. However,patients with non-formal education had the highest seroprevalence while those with primary education had the least. The highest prevalence in patients with non-formal education maybe due to ignorance of the risk factors of the disease and the patients do not usually visit hospitals for treatment unless if seriously sick and beyond home management.This finding agrees with that of Odhiambo *et al*. (2014) in Kenya who reported higher prevalence among patients with non-formal education.

Occupational status was not associated with SLE in this study although civil servants had the highest seroprevalence.

In this study, there was a statistically significant association between marital status and SLE. A higher prevalence was observed in widowed and married patients while the least prevalence was observed in patients who were single. The high prevalence in the widowed patients in this study could be due to age factor and more exposure to sunlight as most of the widowed patients included in the study were above the age of 40 years. This observation in married patients could be as a result of hormonal changes during a woman's life time such as during pregnancy which could trigger the condition in genetically predisposed people (Mok and Lau, 2003). This is in agreement with previous finding in Kenya by Odhiambo *et al*. (2014)where SLE was reported to be higher among the married group of patients.

The level of awareness of SLE was very low in this study with only one patient having the knowledge of the disease. This observation showed that most of the individuals in Zaria are not

aware of the disease. The result shows that many individuals in Nigeria are not even aware of the existence of the disease nor ever heard of it before. The high prevalence in this study was accompanied by anear absolute ignorance about the disease.

The clinical manifestations found to be statistically associated with SLE were loss of weight, photosensitivity and seizures whilerash and oral ulcer a peculiar manifestation of SLE was not found to be associated with the disease. The manifestation of malar rash in blacks due to their skin coloris poor,contrary to its prominence in whites. Thus the detection of rash among the patients may be poor, resulting in wrong conclusion. Majority of the patients had arthritis/arthralgia; the most common clinical feature was photosensitivity, oral ulcer and loss of weight while the least common clinical feature was discoid rash. Malar rash was not recorded among the study population which could be due to our skin melanin.Significant association between SLE and photosensitivity and seizures confirms them as part of the criteria for diagnosis of SLE. Although the specific cause of SLE is not known, multiple factors have been associated with the development of the disease which includes genetic, racial, hormonal and environmental factors (Kaballo *et al*., 2009). A major environmental factor associated with SLE is photosensitivity.

The risk factors analysed for this study showed thathistory of blood transfusion was statistically associated with the disease while pregnancy, gestational stage, smoking and alcohol consumption were not statistically associated with the disease. Pregnancy could be a possible risk factor for SLE due to hormonal changes that occur in the individuals. Data on smoking and alcohol consumption from this study was not sufficient to come up with an association with SLE because only one patient was screened and tested. Afridi *et al*. (2014) reported that smoking is by far the most recognized risk factor for SLE which mightcontribute to disease development via

severalpathways. Ekblom-kullberg *et al*. (2014) also reported that cigarette smoke contains several toll like receptors (TLR)-stimulatingcompounds, including lipopolysaccharide(a TLR-4 agonist), which can elicit an innateimmune response by interacting with the HLAhaplotype and changing gene expression in thejoint (Klareskog *et al*., 2010).In this study, it was observed that history of blood transfusion can be considered a risk factor for developing SLE. Blood screening has been targeted at HIV, hepatitis, blood grouping, and genotyping making it a risk to acquire the disease from the blood donor. This result is in agreement with other findings from southern Sweden (Bengtsson *et al*., 2002), Japan (Kiyohara *et al*., 2012) and Nigeria (Ella *et al*., 2016) that history of blood transfusion is a risk for developing SLE.

Antinuclear antibody(ANA) one of the defining features of SLE was found to be statistically associated with a p-value of 0.007 where most of the patients who tested positive for ANA were positive for SLE and ANAwas found in 44.4% of the study population.This study showed that presence of ANA is significant in the diagnosis of SLE and can be used alongside other tests to confirm SLE.Villarreal *et al*. (1997) and Pisetsky (2000) stated in their study that antinuclear antibodies have been demonstrated in only 50% of patients with SLE indicating a low specificity even though it is generally considered as one of the main diagnostic tools for SLE and a useful marker of disease activity. It was also found that some patients negative for SLE had ANA in their sera and this is because ANA could be found in several systemic autoimmune diseases and has been demonstrated in some healthy individuals (Fritzler and Salazar 1991). This observationagrees with the findings of Nashwa *et al*.(2017) where an elevated ANA titer was reported and it remains one of the diagnostic criteria for SLE even though it has low specificity and can occur in many other autoimmune diseases, some infectious diseases, malignancies and may be drug induced. In contrast to ANA, individual autoantigen antibodies tend to be more

sensitive and specific for SLE. Several studies have demonstrated that specific ANA are associated with different clinical manifestations of SLE (Nashwa *et al*., 2017).

The ENA profilein this study showed that the most common antigen among the study population were Scl-70, Jo-1, Sm/RNPand SSB while SSA and Sm were not detected. This finding agrees with the findings of Aziz and Faisal(2004). Absence of SSA and Sm antigens which are usually found in SLE patients could be due to the fact that they are not commonly detected and are only found in 25-35% of ANA positive SLE patients (Franceschini and Cavazzana, 2005; Migliorini *et al*., 2005) and only 18 patients were tested during the course of the study. This observation agrees with the findings of Agarwal *et al*. (2009) who reported that some SLE patients in their study had negative SSB, Sm and RNP antigens. The finding also agrees with that of Aganovic-Musinovic *et al*. (2012) who reported high incidence of Scl-70 (96%) and Jo-1 (83%) in his study on SLE patients. Agarwal *et al*. (2009) postulated that in SLE, clinically significant changes in disease activity do not correlate well with concentrations of anti-ENA antibodies, either within the population as a whole or on an individual basis.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

An SLE seroprevalence of 21% among the study population was obtained in this study. From the study, SLE was found to be more common among patients who were above the age of 40 years and less common in patients below the age of 30years. It was also found that SLE was more common in female patients than in male patients. Factors found to be statistically associated withSLE were hospital location (p=0.011), marital status (p=0.038), loss of weight (p=0.005), photosensitivity (p=0.042), seizures (p=0.014), history of blood transfusion (p=0.039) and the presence of antinuclear antibody (ANA) (p=0.007). History of blood transfusion was found to constitute a measure of risk for SLE. There was absolute ignorance of the disease because only 1 of the 300 patients was aware of it.

Most patients that tested positive for ANA also tested positive for SLE(44.4%)andthere was a statistically significant association between SLE and ANA (p=0.007). Therefore, ANA can be considered as one of the diagnosis and a useful marker for SLE. The antigens recorded from this study were Scl-70 (33.3%), Jo-1 (27.8%), Sm/RNP (11.1%) and SSB (5.6%) while Sm (0%) and SSA (0%) were not detected.The study showed that SLE is not rare after all in Nigeria further study should be carried out on the disease to obtain more data and possible risk factors of the disease.

6.2 RECOMMENDATION

A high seroprevalence of 21% was recorded from this study therefore, further studies on SLE and ANA profiles remains essential among Nigerian patients as these might help in predicting the clinical patterns of the disease and its prognosis.

Continual monitoring of the epidemiological data on sociodemographic and risk factors associated with the development of SLE in Nigeria will help in identification of populations at risk and development of control strategies.

There is need to carry out further studies on anti-dsDNA alongside ANA and ENA among SLE patients.

Most of the patients included in this study had persistent fever and joint pains, fatigue and malaise which necessitate the need to include SLE test as part of routine investigations with these signs.

Only one of the 300 patients was aware of the disease therefore the high ignorance of the disease calls for education on the existence of the disease and the possible risk factors.

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APPENDIX I: QUESTIONNAIRE

Department of Microbiology,
Faculty of Science,
Ahmadu Bello University, Zaria.
Research questionnaire

Topic: Determination of antinuclear antibodies and extractible nuclear antigens profile in patients with suspected systemic lupus erythematosus in some hospitals in Zaria, Nigeria.

Dear respondent, I am a postgraduate of Microbiology carrying out a research on the above topic. I seek your co-operation in the filling of this questionnaire by providing accurate information on the questions. Questionnaire would be treated with utmost confidentiality.

Instruction: please tick as appropriate and provide answers where applicable.

Hospital Name…….

Patients serial no. ……

PATIENT'S DATA

1. Age …….
2. Marital status: Single ( ) Married ( ) divorced ( ) widowed ( )
3. Level of education: Primary ( ) secondary ( ) tertiary ( ) non-formal ( )
4. Occupation ……...
5. Sex: Male ( ) Female ( )
6. Are you aware of a disease called systemic lupus erythematosus? Yes ( ) No ( )

CLINICAL INFORMATION

7. Fever: Yes ( ) No ( )
8. Fatigue: Yes ( ) No ( )
9. Muscle pain: Yes ( ) No ( )
10. Joint pain: Yes ( ) No ( )
11. Loss of weight: Yes ( ) No ( )
12. Rash: Yes ( ) No ( )
13. Oral ulcer: Yes ( ) No ( )
14. Do you have rash as a response to sunlight? Yes ( ) No ( )
15. Seizures: Yes ( ) No ( )

POSSIBLE RISK FACTORS

16. If female,are you pregnant? Yes ( ) No ( )
17. If yes, what gestational stage? First trimester ( ) Second trimester ( ) third trimester ( )
18. Do you smoke? Yes ( ) No ( )
19. Do you consume alcohol? Yes ( ) No ( )
20. Have you ever had blood transfusion? Yes ( ) No ( )

APPENDIX II: CONSENT FORM

INFORMED CONSENT FORM

My name is Kabir Rashida Idris, a student of Microbiology Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria. I am undertaking a study on thedetermination of antinuclear antibody and extractable nuclear antigens profile in patients with suspected systemic lupus erythematosus in selected hospitals in Zaria, Nigeria. This study has been reviewed and granted approval by the Ethics Committees of the Kaduna State Ministry of health. I would very much appreciate your participation in this study. This information will help the Government to plan health services and it will be strictly confidential and as well not shown to other persons. Should you have any queries, feel free to contact the Chairman of the Ethics Committee of the State. As part of the study, you will be asked some questions about you and some clinical symptoms you are presenting with during your visit to the hospital and 3ml of blood sample shall be collected from you. All the answers you give shall be confidential. Participation in the study is completely voluntary. Should you come across any question you do not want to answer, you can leave it and go on tothe next or you can stop answering questions at any time. However, I hope you will participate in the study since your information is of paramount importance.

_____ _____

Signature of respondent Date

APPENDIX III: ETHICAL APPROVAL

KADUNA STATE HOSPITALS MANAGEMENT BOARD ETHICAL APPROVAL

MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/489 17TH JANUARY, 2017

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

DETERMINATION OF ANTINUCLEAR ANTIBODIES AND EXTRACTABLE NUCLEAR ANTIGENS PROFILE PATIENTS WITH SUSPECTED SYSTEMATIC LUPUS ERYTHEMATOSUS IN SELECTED HOSPITALS IN ZARIA, NIGERIA

Name of Principal Investigator:	KABIR RASHIDA IDRIS
Address of principal Investigator:	DEPARTMENT OF MICROBIOLOGY, AHMADU BELLO UNIVERSITY, ZARIA
Date of receipt of application:	22nd DECEMBER, 2016
Date of Ethical Approval:	16th JANUARY, 2017

This is to inform you that the research described in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and given full approval by Health research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. BUTAWA NN
Secretary.
For: Chairman

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APPENDIX IV: ENA SAMPLE DISTRIBUTION

Format for the distribution of the samples on the ENA profile-6 ELISA Plates

				COLUMN	NUMBER			
Antigen :	Row:	1	2	3	4	5	-	12
Ag mixture	A	Dil	Dil	Dil	Dil	Dil	-	Dil
Jo -1	B	NC	PC	Cal	Test 1	Test 2	-	Test9
Sm	C	NC	PC	Cal	Test 1	Test 2	-	Test9
Sm /RNP	D	NC	PC	Cal	Test 1	Test 2	-	Test9
SSA	E	NC	PC	Cal	Test 1	Test 2	-	Test9
SSB	F	NC	PC	Cal	Test 1	Test 2	-	Test9
Scl-70	G	NC	PC	Cal	Test 1	Test 2	-	Test9
Control Ag	H	NC	PC	Cal	Test 1	Test 2	-	Test9

Keys:

Dil = Sample diluent

NC = Negative Control

PC = Positive Control

Cal = Calibrator

APPENDIX V: DISTRIBUTION OF ENA IN PATIENTS

Distribution of ENA-profile 6 among the patients in some hospitals in Zaria, Nigeria

Patient	SLE	ANA	Jo-1	Sm	Sm/RNP	SSA	SSB	Scl-70
M01	+	+	+	-	-	-	-	-
M02	+	+	-	-	-	-	-	+
M03	+	+	-	-	-	-	-	-
M04	+	+	-	-	-	-	-	-
M05	+	+	-	-	-	-	-	+
M06	+	+	-	-	-	-	-	-
H01	+	+	+	-	-	-	-	+
H02	+	+	-	-	-	-	-	-
H03	+	+	+	-	+	-	-	+
H04	+	+	-	-	+	-	-	+
H05	+	+	-	-	-	-	-	-
H06	+	+	-	-	-	-	-	-
H07	+	+	+	-	-	-	-	+
H08	+	+	+	-	-	-	+	-
H09	+	+	-	-	-	-	-	-
P01	+	+	-	-	-	-	-	-
P02	+	+	-	-	-	-	-	-
A01	+	+	-	-	-	-	-	-
TOTAL	18	18	5(27.8)	0(0)	2(13.3)	0(0)	1(5.6)	6(33.3)

Key

+ = Positive for the test

- = Negative for the test