UNIVERSITY OF GHANA

GEOGRAPHICAL DIFFERENCES OF ANTINUCLEAR ANTIBODIES AND THE PREDISPOSITION OF AUTOIMMUNE DISEASE AMONG ADULTS FROM FOUR REGIONS OF GHANA

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DEDICATION

This thesis work is dedicated to my parents, Mr. Samuel Kwame Sarpong and Mrs. Elizabeth Sarpong for their endless care and support for my education.

DECLARATION

I, BAFFOUR KYEI SARPONG of the Department of Medical Biochemistry of the University of Ghana School of Biomedical and Allied Health Sciences, do hereby declare that, with exception of the quoted articles and references, this project work was duly carried out by me and the results obtained herein are true reflection of the work done under the supervision of **DR. BARTHOLOMEW DZUDZOR** of the Department of Medical Biochemistry and **DR. DZIFA DEY** of the Department of Medicine and Therapeutics, University of Ghana School of Medicine and Dentistry.

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ABBREVIATION

AD……Autoimmune disease

ANA……. Antinuclear antigen

ANF……. Antinuclear Factor

CD21……Clusters of differentiation 21

Chrom……Anti-Chromatin antibody

CLIA……. Chemiluminescent Assay

CTD……. Connective tissue diseases

DFS70……...Dense fine speckled 70

DNA……Deoxyribonucleic acid

dsDNA……..Anti-double stranded deoxyribonucleic acid antibody

ELISA……Enzyme linked Immunosorbent assay

ENA……...Extractable Nuclear antigen

HLA…….Human Leukocyte antigen

HSV1……...Herpes Simplex Virus 1

IIF…….Indirect Immunofluorescence test

IL……..Interleukin

Jo1…….Anti-Jo1 antibody

kDa……...…KiloDalton

MCTD……...Mixed connective tissue disorder

MDSS……. Medical decision support software

PM…….Polymyositis

PNAR……...Peanut agglutinin receptor

POR……..Pooled odd's ratio

PPV…….Positive predictive value

PTPN22……Protein tyrosine phosphatase, non-receptor type 22

RIA……...Radio immunosorbent assay

RiboP…….Anti-Ribosomal P antibody

RNP……...Anti-Ribonucleoprotein antibody

RNPA……Anti-Ribonucleoprotein A antibody

RNP68……..Anti-Ribonucleoprotein 68 antibody

Scl70……..Anti-scleroderma 70 antibody

SLE……Systemic lupus erythematosus

Sm…….Anti-Smith antibody

SmRNP……...Anti-Smith/Ribonucleoprotein antibody

SS……....Sjogrens syndrome

SSA……. Anti-Sjogrens syndrome related antigen A antibody

SSA52…….Anti-Sjogrens syndrome related antigen A 52kDa antibody

SSA60……..Anti-Sjogrens syndrome related antigen A 60kDa antibody

t-RNA……transfer Ribonucleic acid

ABSTRACT

Introduction: Despite being thought to be a group of rare disorders, the incidence of autoimmune conditions like Type 1 Diabetes Mellitus, Systemic Lupus Erythematosus, Rheumatoid arthritis and autoimmune thyroiditis are on the rise in Ghana. Autoimmune disorders result from a breakdown of immunologic tolerance leading to an immune reaction against self-autoantigens. Antinuclear antibodies are a group of autoantibodies that mediate the pathogenesis of several autoimmune conditions especially SLE and has been relevant biomarkers in the diagnosis of several connective tissue disorders. Recent studies have demonstrated the presence of antinuclear antibodies in the sera of the healthy population and in the sera of patients with autoimmune disorders, many years before the onset of the disease. Research on the susceptibility and potential of autoimmunity in the Ghanaian population is lacking. This study determined the prevalence, types, demographic and ethno-geographic correlates of antinuclear antibodies among healthy Ghanaian adults.

Methods: Sera from 370 (133 males and 237 females, 1:1.8 male/female ratio) healthy Ghanaians from four regions and aged 18 years and above were tested for the presence of different antinuclear antibodies. The presence of anti-DFS70 was detected by chemiluminescent immunoassay and anti-dsDNA, chromatin, Ribosomal-P, Ro /SSA, SSA 52, SSA60, La / SSB, centromere B, anti-Sm, SmRNP, RNP 68, RNP A, Scl-70, and Jo1 were detected by multiplex immunoassay using Bio-Rad Bioplex 2200 system. The health status and demographics of each participant was determined through the administration of a structured questionnaire.

Results: The total prevalence of antinuclear antibodies (ANA positive) in the study participants is 14.21% which falls within the prevalence range estimated for other healthy populations. There was

no significant difference in the positivity of antinuclear antibodies between both sexes, 14.84% and 12.61% (p-value=0.555) for male and female respectively. Most of the autoantibodies tested negative among majority of the study participants.

There was no significant difference between age and the positivity for specific antinuclear antibodies tested (p-value 0.073-1) but there was a significant difference in ANA positivity with age (p-value=0.015). This conforms to the well-known hypothesis that autoantibody production increases with increasing age due to reduced self-regulatory mechanisms. There were significant ethnical and geographical differences in the prevalence of anti-dsDNA (p-values=0.048 and 0.013 respectively) with detection in only Ewes and Northerners (1.87% and 5.62% respectively) and the Upper West and Volta Regions (6.25% and 2.83% respectively). ANA positivity did not correlate with ethno-geographic distribution.

Conclusion: It can be concluded that there is a high prevalence of antinuclear antibodies among healthy Ghanaian adults and that seems to be influenced mainly by age and sex. ANA positive frequency does not have a strong ethnic variability. Anti-dsDNA exhibit ethno -geographic variability.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Autoimmune diseases are a diverse group of chronic diseases initiated by the loss of immunological tolerance to self-antigens (Janeway et al, 2001a). Autoimmune disease can be organ specific like myasthenia gravis, Hashimoto's thyroiditis, and type 1 Diabetes mellitus or systemic illnesses such as Rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus.

Virtually all autoimmune diseases are associated with circulating autoantibodies that bind to self-antigens and initiate an immune reaction that may eventually lead to cellular and tissue injury. There are more than 80 different autoimmune diseases (Selgrade, Cooper, Germolec & Heindel, 1999) that affect hundreds of millions of people around the world. Incidence rates vary among individual autoimmune diseases. Women account for about 75% of the estimated 23.5 million people in America afflicted by autoimmune diseases, this constitute some of the leading causes of death and disability in women below 65 years of age (Sirois & Touger-decker,2014 .)

For over 50 years, the serological detection of autoantibodies has played a major role in the classification and diagnosis of autoimmune conditions. Most autoantibodies can be used as biomarkers for disease activity and have prognostic relevance (Shapira et al, 2012). Several studies have demonstrated that asymptomatic people may carry autoantibodies for many years before the manifestation of the disease. The detection of serum autoantibodies may have a good predictive significance (Scofield, 2004a).

Antibodies to double stranded Deoxyribonucleic acid (dsDNA), Ribonucleoprotein (RNP), Sjogren's syndrome related antigen A (SSA), Sjogren's syndrome related antigen B (SSB), Smith (Sm) and cardiolipin can appear in the serum 7-10 years before the diagnosis of systemic lupus erythematosus (Shapira et al, 2012). They have a positive predictive value of between 94% and 100% (Arbuckle et al, 2003). Serological detection of autoantibodies in the initial stages of autoimmune diseases with a good predictive capacity of autoimmune diseases will play an important role in disease prevention. Intervention in the preclinical phase may modify the risk with development of autoimmune diseases(Deane, Norris, & Holers, 2011) (Symposia, 2009). The predictive utility of several autoantibodies for the development of autoimmune diseases is limited as there is scarce data on the prevalence of autoantibodies in the healthy population at different ethno-geographic locations(Shapira et al, 2012).

Antinuclear antibodies are found in about 25% of the general population, with significantly elevated levels at a prevalence of 2.5% (Wandstrat et al., 2006). Numerous studies have demonstrated consistency among racially and ethnically distinct study populations with the use of different methods of ANA determination. The presence of this auto reactivity in the healthy human population may suggest that the production of antinuclear antibodies may be part of the normal immune reaction. Majority of individuals with positive ANA may not have an autoimmune condition and may not develop the condition in the future (Davidson Anne; Diamond Betty;, 2001). Systemic lupus erythematosus (SLE) has been closely linked to ANA positivity and is rare, affecting about 1 to 1.5 per 1000 persons (0.1 to 0.15%) in the United States of America. Moreover, the titre value of an antinuclear antibody is more relevant in the diagnosis of SLE than only the positivity of the antibody (Q. Z. Li et al, 2011).

1.2 PROBLEM STATEMENT

Death and disability from autoimmune diseases among young and middle aged people especially women, has increased over the past decade worldwide (Thomas, Griffiths, Smeeth, Rooney, & Hall, 2010). The overall cumulative global prevalence of all Autoimmune diseases as a class is 5.0%,with a prevalence of 3.0% for males and 7.1% for females (Hayter & Cook, 2012). Autoimmune diseases are becoming increasingly prevalent in Ghana and majority of individuals with the disease experienced poor quality of life since the cost of health care is high. Almost every week, about five people are admitted in Korle-Bu Teaching Hospital, Ghana for management of complications of systemic lupus erythematosus. Autoimmune diseases are not curable and can be debilitating, with relapsing and remitting episodes. This poses a huge health burden and economic strain on the limited resources in Ghana. Autoimmune diseases are therefore of public health importance especially in Sub-Saharan Africa as it is known that people of African descent tend to have more severe disease. Very few studies have been undertaken to determine the risks of developing autoimmune diseases among individuals especially among black population with ethnic diversity. It is imperative that some attention is given to autoimmunity research in Ghana, because of the growing prevalence and incidence of autoimmune diseases.

1.3 JUSTIFICATION/ RATIONALE

Autoimmune diseases are usually preceded by a long preclinical stage that can only be identified by the presence of autoantibodies implicated for that disease. Apparently healthy individuals may develop autoantibodies many years before the manifestation of autoimmune diseases with a good predictive value (Lleo, Invernizzi, Gao, Podda, & Gershwin, 2010) (Shoenfeld, Selmi, Zimlichman, & Gershwin, 2008) (Scofield, 2004a). Such identification may allow for

immunological modulation as a way of prevention or perhaps monitoring more carefully for life threatening complications.

1.4 RESEARCH HYPOTHESIS

The prevalence of antinuclear antibodies is high among healthy Ghanaian adults and ethno-geographic distribution varies within the population.

1.5 AIM

To determine the prevalence of antinuclear antibodies among healthy Ghanaian adults, and the ethno-geographic diversity of the antibodies.

1.6 SPECIFIC OBJECTIVES

- To determine the prevalence of antinuclear antibodies among healthy Ghanaian Adults.
- To determine geographical distribution of antinuclear antibodies among healthy Ghanaian Adults.
- To determine the relationship between the socio-demographics of participants and antinuclear antibodies among healthy Ghanaian Adults.

CHAPTER TWO

LITERATURE REVIEW

2.1 IMMUNE RESPONSE AND MECHANISM OF AUTOIMMUNITY

The main function of our immune system is to protect us from infections and foreign substances in the surroundings. Organisms with varied spectrum of pathogenic mechanisms threatens the host and the immune system employs sophisticated processes to protect, control and eliminate these pathogenic organisms. Structural features of pathogens are used predominantly in the distinction of pathogens from host antigens and this differentiation is essential to enable the host to eliminate the pathogen without damage to its own tissues (Chaplin, 2006).

Our immune system uses several processes to eliminate microbes and other particles it encounters, and it is very important for the immune response to prevent destructive mechanisms against its own tissues; a process known as self- tolerance (Chaplin, 2010). Many parts of the innate and adaptive immune system employs mechanisms to avoid reaction to self-antigens. Autoimmune diseases result from failure of self-tolerance and involve a number of mechanisms (Vojdani, 2014).

2.1.1 Molecular Mimicry: A foreign antigen of pathogen inside a host may have similarity in structure with the host antigen. Antibodies produced against that foreign antigen may cross react with host antigens, resulting in a heightened immune response and subsequent damage to host tissues. Rheumatic fever is partly due to molecular mimicry following antibodies to *Streptococcus pyogenes* which cross react to cardiac and other tissue antigens (Cunningham, 2014). Molecular mimicry is one key mechanism involved in the pathogenesis of autoimmune disorders like Graves' disease, spondyloarthropathies, and Diabetes mellitus. Molecular mimicry may explain how viruses initiate and regulate autoimmune reactions leading to tissue damage (Cusick, Libbey, &

Fujinami, 2012). Work by Fujinami and colleagues 2012, obtained antibodies in mouse to herpes simplex 1 (HSV-1) and measles virus from B-cell clones secreting antibodies. The antibodies reacted to the proteins of measles and HSV-1 viruses and the intermediate filaments of normal mouse cells. This demonstrated a relationship between the host and viral antigens (Cusick et al., 2012).

2.1.2 T-cell Bypass: Normal immune response involves B cells activation by T-cells. Activated B-cells differentiate into plasma B- cells and plasma B-cells produce antibodies in large quantities (Janeway et al, 2001b). B-cell activation by T-cells is crucial in the determination of specificity of the antibodies produced against a foreign body. In certain instances, like infection with a super antigen producing organism, there is a direct polyclonal activation of B-cells leading to the production of large amounts of less specific antibodies capable of reacting with the host antigens (Pozzi et al., 2015). There could be direct activation of T-cells by binding to the beta subunit of the T-cell receptor in a non-specific fashion or the bypass of tolerant T-cell and activation of competent B-cells (Höllsberg, 1999).

2.1.3 T-cell/B-cell Discordance: In a normal immune reaction, there is reaction of T-cell and B-cell to the same antigen. However, in 1987 Lanzavecchia and Rosnek demonstrated that B-cells can recognize IgGFc and recruit help from any T-cell stimulated by an antigen that has been co-endocytosed by the B-cell, leading to non-specific immunoglobulin production. In Coeliac disease, B-cells that recognize transglutamine are helped by T cells that can recognize gliadin (Vergani & Mieli-vergani, 1993).

2.1.4 Idiotype cross-reaction: Idiotypes are a set of antigenic epitopes found on the antigen binding region (Fab) of an immunoglobulin (Sela-Culang, Kunik, & Ofran, 2013). Autoimmunity

can arise as a result of cross reaction between the idiotype on an antiviral antibody and a host cell receptor for the virus. The host cell receptor bears structural resemblance to the virus and the antiviral antibody reacts with the host cell (Cusick et al., 2012).

2.1.5 Aberrant B-cell receptor mediated feedback: One feature of autoimmune diseases is that it is related to a small group of antigens which are involved in signaling roles in the immune response, example: DNA, Ro, La, C1q, Con A receptor, IgGFc, and Peanut agglutinin receptor (Campden, 2000). Spontaneous autoimmunity can therefore result when there is binding of antibody to certain antigens leading to abnormal signals to parent B cells through membrane bound ligands. These ligands include the Toll-like receptors 7 and 9 (can bind DNA and nucleoproteins), IgG cell receptors, CD21 (binds complement C3d) and PNAR (Edwards & Cambridge, 2006). Indirect aberrant activation of B-cells is linked with production of autoantibodies to hormone binding elements and acetylcholine receptor. This is one of the basis for self-perpetuating autoreactive B-cells. Autoreactive B-cells survive because of the bypass of the T-cell pathway and feedback signals through the B-cell receptor (Edwards & Cambridge, 2006)

2.1.6 Cytokine dysregulation: T helper 1/T helper 2 (Th1/Th2) cytokine balance was studied in the pathogenesis of autoimmune diseases, and the two T subsets cross react with each other (Mitsias et al., 2002). Cytokines produced by Th1 cells and macrophages such as interleukin 1, IL-6, IL-12, Tumor necrosis factor α and interferon gamma mediate T helper 1 responses. IL-4, IL-5, IL-13 mediate T helper 2 responses. Autoimmune diseases can be classified into events that are mainly driven by T helper 1 cell if the major processes are cell mediated and T helper 2 cell driven if the main events are humoral or immune complex formation (Moudgil & Choubey, 2011).

2.1.7 Epitope drift/Epitope spreading: This occurs when the immune system targets other epitopes instead of the primary epitope (structurally different from the primary epitope) of the

antigen leading to production of numerous nonspecific antibodies capable of cross reacting with other antigens including host antigens(Srinivasan, Houghton, & Wolchok, 2002).

2.1.8 Dendritic cell apoptosis: Dendritic cells are antigen presenting cells that present antigens to active lymphocytes. Dendritic cells defective in apoptosis can lead to continuous and inappropriate lymphocyte activation and lead to a decline in self-tolerance (Sallusto et al., 2002).

2.2 RISK FACTORS FOR AUTOIMMUNE DISEASES

There are many factors that predispose individuals to autoimmunity and autoimmune diseases. These factors may be modifiable or non-modifiable. Below is a discussion of some common risk factors to the development of autoimmune diseases.

2.2.1 Genetic factors: Certain individuals are susceptible genetically to the development of autoimmune diseases. There are basically three genes implicated in autoimmunity related to T cell receptors, Immunoglobulins and the Major Histocompatibility Complex (MHC) (Matzaraki, Kumar, Wijmenga, & Zhernakova, 2017). PTPN 22 has been associated with Systemic lupus erythematosus, Type 1 Diabetes, Rheumatoid Arthritis, and Graves' disease, Hashimoto's thyroiditis, Addison's disease, Myasthenia gravis and Vitiligo most recently (Svensson et al., 2011).

2.2.2 Sex: Women are predominantly affected with autoimmune diseases, approximately 78% of total cases. It is well known that the basic immune reaction differs between women and men. Men develop intense inflammatory response to infection, trauma and vaccination whereas women have increased antibody production, leading to increase death among men and protection against infection in women (Fairweather, Frisancho-Kiss, & Rose, 2008).

2.2.3 Immunodeficiency: There is a paradoxical relationship between immunodeficiency diseases and autoimmunity (Petrovsky, 1994). Immunodeficient patients may not necessarily develop autoimmunity and not all patients with autoimmunity are immunocompromised. Inherited deficiencies of the complement system have a high incidence of systemic lupus erythematosus (SLE), glomerulonephritis, and vasculitis (Grammatikos & Tsokos, 2012). Mechanisms have been proposed to explain the associations between autoimmunity and immunodeficiency. Defects in the immune system may result in a failure to exclude microbial antigens, resulting in chronic immunologic activation and autoimmune symptoms. Genetic factors such as common HLA alleles has been known to predispose individuals to immunodeficiency and autoimmunity. Defects within one component of the immune system may alter the way a pathogen induces an immune response and lead to an inflammatory response directed at self-antigens (Sleasman, 1996).

2.2.4 Environmental factors: Genetics is certainly a factor in the pathogenesis of autoimmunity, but since it normally takes quite a long time for the human genetic pattern to change and to register on a worldwide scale, increasingly the attention of research has been focused on the environmental factors of a rapidly changing and evolving civilization. New forms of technology, industries, inventions, chemicals and drugs, foods and diets are constantly and rapidly being introduced in this fast-paced ever-changing world (Vojdani, 2014). However, exposure to toxic chemicals, toxins, infections and dietary components trigger immune system through different molecular mechanisms to produce an autoimmune state (Jo, 2016). Environmental predis constitute about 70% of autoimmune diseases (Vojdani, Pollard, & Campbell, 2014).

2.3 AUTOIMMUNE DISEASES

One main role of the immune system is to discriminate self-antigens from non-self-antigens. The development of tolerance is a highly regulated process, and to maintain it, the immune system must be able to recognize self-reactive lymphocytes as they mature. The production of autoantibodies is due to failure of tolerance. Although autoantibodies are an important serological feature of autoimmune diseases, they are not pathognomonic(Lleo et al., 2010). Autoimmune disease is a pathological condition that results from an immune response mediated by auto-antibodies or self-reactive T lymphocytes to antigens that are normally present in the body. The auto-antibodies and self-reactive T lymphocytes may exist in a person's blood without causing an immune response or disease condition (Romagnani, 2006). Insight into molecular biology has proposed that three types of evidence can be used to establish that a human disease is autoimmune in origin. They include direct evidence from transfer of pathogenic antibody or pathogenic T cells; indirect evidence based on reproduction of the autoimmune disease in experimental animals; and circumstantial evidence from clinical clues(Noel R. Rose & Bona, 1993).Autoimmune diseases damages tissues and alters the function and growth of organs systems (Janeway et al, 2001b).

Systemic Lupus erythematosus(SLE): It is a clinically heterogenous disease, autoimmune in origin and involves the presence of autoantibodies against nuclear antigens (Vojdani et al., 2014). SLE affects many organs including the joints, skin, nervous system and kidneys (O'Neill & Cervera, 2010). The diagnosis of SLE involves the clinical findings in relation to the skin, joints, kidneys and nervous system and serological detection of parameters such as antinuclear antibodies, especially dsDNA (Kuhn et al., 2015).

Sjogren's syndrome: Sjogren's syndrome is characterized by lymphocytic infiltration of exocrine glands mainly lacrimal and salivary glands, resulting in oral dryness, parotid enlargement, ocular

dryness, dry skin, and dryness of the upper respiratory tract (Fox & Fox, 2016). Other extraglandular features include Raynaud's phenomenon, fatigue, hepatomegaly and peripheral neuropathy (Mavragani & Moutsopoulos, 2014). The most common autoantibodies in Sjogren's syndrome are directed towards antigens Ro/SSA and La/SSB (Fayyaz, Kurien, & Scofield, 2016).

Mixed Connective Tissue disease: Mixed Connective tissue disease is an autoimmune disorder with features of several different connective tissue diseases like rheumatoid arthritis, systemic lupus erythematosus, polymyositis, and systemic sclerosis (Ungprasert et al., 2016). It is associated with high titer of anti-RNP (Sharp, Irvin, Tan, Gould, & Holman, 1972).

Scleroderma: Scleroderma is a chronic multisystem disease characterized by skin fibrosis, immunological abnormalities, vascular injury and accumulation of extracellular matrix proteins (V. et al., 2013). Antinuclear antibodies associated with Scleroderma include Anti-Scl-70, Anti-centromere, Anti-RNP antibodies (Adnan, 2008).

2.4 PREVALENCE AND INCIDENCE OF AUTOIMMUNE DISEASES

In the United States, there is an increased risk of Systemic lupus erythematosus (SLE) among reproductive age women of African-Americans descent (Lim et al., 2014). However, in other populations, a different pattern is seen, with the highest age-specific incidence rates occurring in women after age 40 years. Systemic lupus erythematosus is about 2-4X more common in non-white populations across the world and more severe in children, men and late-onset Lupus. Patients with SLE now have a higher than 90% survival rate at 5 years. Ethnic minorities have less favorable outcome, and is possibly linked to socioeconomic status(Pons-Estel, Alarcon, Scofield, Reinlib, & Cooper, 2010).Prevalence of Type 1 Diabetes ranged from 3.5 per 100,000 persons in

Mozambique, to 12 per 100,000 persons in Zambia. Recorded incidence ranged from 1.5 per 100,000 persons per year in Tanzania to 2.1 per 100,000 persons per year in Ethiopia (Hall, Victoria; Thomsen, Reimar W.; Henriksen, Ole; and Lohse, 2011). In a retrospective study at the Korle-Bu Teaching Hospital in Ghana over a two-year period, there were 51 cases of Systemic lupus Erythematosus on admission over a mean period of 26.12 days with a mortality rate of 43.1% (n=22 deaths) (Dzifa et al., 2018).

2.5 ANTINUCLEAR ANTIBODIES

Antinuclear antibodies are autoantibodies that bind to contents of cell's nucleus .There are many subtypes of ANAs such as anti-Ro antibodies, anti-La antibodies, anti-Sm antibodies, anti-nRNP antibodies, anti-Scl-70 antibodies, anti-dsDNA antibodies, anti-histone antibodies, antibodies to nuclear pore complexes, anti-centromere antibodies and anti-sp100 antibodies (Reeves, Xu, Zhuang, Li, & Yang, 2011). Each of the subtypes of antibody binds to different proteins or protein complexes within the nucleus. ANA are detected in many conditions, infections, cancer and autoimmunity, with different levels of prevalence depending on the condition. This allows the use of antinuclear antibodies in the diagnosis of some autoimmune disorders, including systemic lupus erythematosus, Sjögren's syndrome (Cervera et al., 2000), scleroderma(Barnett & McNeilage, 1993),mixed connective tissue disease (Burdt MA, 1999) polymyositis, dermatomyositis, autoimmune hepatitis (Krawitt, 2014) and drug induced lupus (Kavanaugh, Tomar, Reveille, Solomon, & Homburger, 2000a).

2.5.1Antinuclear antibodies and autoimmune diseases

The presence of antinuclear antibodies in the sera of patients with suspected autoimmune disease constitute one of the diagnostic criteria for autoimmune diseases (Kumar et al., 2009). Table 1 shows autoantibodies implicated in specific disease conditions.

Table 2.1 Common autoantibodies implicated in autoimmune diseases (Harel M, 2006).

Disease	Autoantibodies
Sjogren's syndrome	Anti-SSB (La)
	Anti-SSA (Ro)
SLE	Anti-dsDNA
	Anti-Sm
	Anti-SSA
	Anti-SSB
	Anti-RNP
	Anti-RiboP
	Anti-Chrom
Autoimmune Hepatitis	ANA
Scleroderma	Anti-Scl70
	Anti-Cent_B
Idiopathic Inflammatory myopathies	Anti-Jo1

2.5.2 Antinuclear antibodies and cancer

Serologic findings such as antinuclear antibodies (ANA), usually found in systemic rheumatic diseases, have been known for decades to occur in patients with various cancers (Leirisalo-repo, 1990) (Burnham, 1972). These and other early reports on ANAs in cancer sera (Fernandez-madrid, Karvonen, Kraut, Czelusniak, & Ager, 1996) (Zhao et al., 2013)and the remarkable work responsible for the well-recognized value of autoantibodies for the diagnosis of autoimmune diseases (ADs) such as systemic lupus erythematosus (SLE), scleroderma, and dermatomyositis (DM)/polymyositis (PM) (Eng M. Tan, 1989) (Alspaugh & Maddison, 1976) suggested that autoantibodies could also be potential diagnostic and prognostic biomarkers for cancer.

2.5.3 Antinuclear antibodies among the healthy population

Naturally occurring autoantibodies are frequently found in the serum of normal humans and are important in clearing cellular debris induced by inflammation or physical damage (Cells, 2007) (Fairweather & Root, 2015).However, autoantibodies may induce damage by binding self-antigens and activating the complement cascade, resulting in direct cytotoxicity or an immune complex (IC)-associated pathology. The number of different autoantibodies present in an individual is a good predictor of the risk of developing an autoimmune disease. For example, estimates based on first degree relatives show that the likelihood of a child developing type 1 diabetes within 5 years is 10% in the presence of one autoantibody, 30% for two autoantibodies, and 60 to 80% if three autoantibodies are present (N R Rose, 1989). Thus, the risk for developing an autoimmune disease increases as the number of autoantibodies increases, and the number of autoantibodies increases as we age, regardless of sex (Fairweather et al., 2008).

In medical practice, it is quite frequent to report positive ANA tests in the general population, and the interpretation is perplexing because no definite cause of this result is evident when the

individual does not have a systemic autoimmune disease. It has been hypothesized that the frequency of autoantibodies increases with age. However, Li and colleagues, (Q. Z. Li et al., 2011) found that age was not related to ANA positivity in healthy subjects who were negative for past or current autoimmune disorders. It has been proposed that humans may be predisposed to autoimmunity (Rosenblum, Remedios, & Abbas, 2015).

The detection of autoantibodies in the serum of individuals plays an important role in the diagnosis and categorization of autoimmune diseases (Konstantinov, Tzamaloukas, & Rubin, 2013). Some autoantibodies can be used as prognostic markers and also determine disease activity. There is currently limited data on prevalence of autoantibodies among healthy populations in different geographical locations. Asymptomatic people may have autoantibodies in their serum for years before they develop an autoimmune disease. This observation has become evident in recent years (Scofield, 2004b). The detection of these autoantibodies have a strong predictive value. Autoantibodies like dsDNA, Sm, Ro(SSA), RNP, and La (SSB) which commonly occurs in systemic lupus erythematosus may precede the disease by 7-10 years. There is a positive predictive value of 94-100% (Shapira et al., 2012).

Shapira and colleagues studied the autoantibodies profile of about 557 healthy volunteers from six different centers (Mexico, Columbia, Netherlands, Italy, Israel and Papua New Guinea). Sera were tested for the presence of antinuclear antibodies (ANA) and autoantibodies associated with thrombophilia, vasculitis, and gastrointestinal (GI) disease. Most of the antibodies were negative in all the six geographic areas with no ethno-geographic variability. Antinuclear antibodies had the most variation among the groups. The frequency of ANA positive sera was significant, about 45%, 38%, 26% and 12% in the Columbia, Papua New Guinea, Mexican and European participants respectively (Shapira et al., 2012). Measurement of antinuclear antibodies should be

related not only to the use of reliable tests to allow for early and reliable diagnosis of autoimmune diseases (Bragazzi et al., 2017) , but also with the analysis of personal and family history, as well as social lifestyle including dietary habits. ANA are commonly found in up to 20% of healthy subjects, but significantly elevated levels are observed in 2.5% (Wandstrat et al., 2006) of the general population. Such prevalence is comparable between populations of different ethnicity and race (Q. Z. Li et al., 2011).

Positive ANA are more often detected in women and thus female gender is a risk factor for significant ANA positivity (Wandstrat et al., 2006). This fact is in accordance with a study in a Brazilian healthy population, which showed nearly two-fold higher prevalence of ANA in females as compared to males (Fernandez et al., 2003). In a study among 124 healthy migrant Ghanaian and Nigerian African population in Italy, the prevalence of ANA was found to be 27.4%, which is high for a healthy population (Cainelli, Betterle, & Vento, 2004). 115 out of the 130 patients with SLE (88 percent), at least one SLE autoantibody tested was present before the diagnosis (up to 9.4 years earlier; mean, 3.3 years). Antinuclear antibodies were present in 78 percent (at a dilution of 1:120 or more), anti–double- stranded DNA antibodies in 55 percent, anti-Ro antibodies in 47 percent, anti-La anti- bodies in 34 percent, anti-Sm antibodies in 32 percent, anti–nuclear ribonucleoprotein antibodies in 26 percent, and antiphospholipid antibodies in 18 percent. Antinuclear, anti-Ro, and anti-La antibodies were present earlier than anti-Sm and anti–nuclear ribonucleoprotein antibodies (a mean of 3.4 years before the diagnosis vs. 1.2 years, P = 0.005) (Arbuckle et al., 2003).

Prevalence of ANA among the study population was 13.8% (95%CI) in a cross sectional study of 4754 individuals in the National health and nutrition examination survey in the United States. ANA were significantly higher (17.8% female and 9.6% male, P<0.001) in females than males

and increased with age (P=0.01). It was therefore estimated that among 32.2 million people (95% CI, 28.5 to 36.1 million), 21.5 million females (95% CI, between 18.7 to 24.3 million) and 10.8 million males (95% CI between 8.6 to 13.1 million) had ANA in the United states from 1999 to 2004 The prevalence of ANA in the 50 to 59 year and more than 70 years were significant (P<0.03) than in other age groups. ANA prevalence was higher in non- Hispanic blacks than in other ethnic groups. There was no variation in ANA prevalence with education and family income (Satoh et al., 2013).

2.5.4 Correlation between age, sex and ANA positivity

The National health and nutrition examination survey 1999- 2004 determined in a cross sectional study of 4754 participants that ANA increase with age (P=0.01) and higher in females than in males (P< 0.001). ANA prevalence was found to be significantly higher in the 50 to 59 year and greater than 70-year age groups than the younger age groups (P< 0.03)(Satoh et al., 2013). In a survey done among 124 (58 women and 66 men) African immigrants (Ghanaians and Nigerians) in Italy, there was no preponderance of ANA in the female immigrant population tested. Among the 34 participants who tested positive for ANA, 17(13.7%) were men and 17(13.7%) were women, demonstrating no sex predominance in ANA positivity (Cainelli et al., 2004).

2.5.5 Ethnic and Geographic distribution of Antinuclear Antibodies

Several studies have demonstrated significant ethnic differences in antinuclear antibodies, with the possibility of a genetic predisposition to the development of these antibodies (Reveille, 2003). There is no study on the ethnic differences in antinuclear antibodies in Ghana and Africa. In the NHANES study (1999-2004), the ANA prevalence was found to be higher in non-Hispanic Blacks than other ethnic groups and this is consistent with increased prevalence of SLE and lupus antibodies like anti-Sm and anti-RNP among non-Hispanic blacks however, there was no

significant differences in ANA prevalence among racial/ethnic groups (Mexican American, Non-Hispanic white and Non-Hispanic black) (Satoh et al., 2012). In a study among 624 SLE patients at the University College London Hospitals, Afro-Caribbeans had the highest prevalence of anti-Smith, anti-Ro and anti-La compared to South Asians and East Asians (Morais & Isenberg, 2017). Analysis of sera samples from 557 healthy individuals from six different countries for the presence of antinuclear antibodies showed significant gradient in ANA positivity among the groups; 38% Kitavans, 45% Columbians, 26% Mexicans, 12% Italians, 12% Dutch and 11% Israelis. There were geographical differences in the prevalence of anti-dsDNA, SmRNP, Ro/SSA, La/SSB, Scl 70 and Chromatin (Shapira et al, 2012).

2.5.6 Antinuclear antigens subtypes

ANAs are found in many disorders, as well as some healthy individuals. These disorders include: systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren's syndrome, scleroderma, polymyositis, dermatomyositis, primary biliary cirrhosis, drug induced lupus, autoimmune hepatitis, multiple sclerosis, discoid lupus, thyroid disease, antiphospholipid syndrome, juvenile idiopathic arthritis, psoriatic arthritis, juvenile dermatomycosis, idiopathic thrombocytopenic purpura, infection and cancer. These antibodies can be subdivided according to their specificity, and each subset has different propensities for specific disorders (Malleson, Mackinnon, Sailer-Hoeck, & Spencer, 2010) (Kavanaugh, Tomar, Reveille, Solomon, & Homburger, 2000b)**.**

2.5.7 Extractable nuclear Antigens (ENA)

Extractable nuclear antigens are also targets in people with autoimmune disorders. They are termed ENA because they can be extracted from the cell nucleus with saline (Kavanaugh et al., 2000b) (Damoiseaux & Cohen Tervaert, 2006). ENAs consists of non-histone proteins and

ribonucleoproteins which were named after the donor who provided the prototype serum (Sm, Ro, La, Jo), or the name of the disease condition in which the antibodies were found (SS-A, SS-B, Scl-70) (Wenzel et al, 2001). Antibodies against extractable nuclear antigens are a group of antibodies that are used to screen for mixed connective tissue disease (MCTD), systemic lupus erythematosus and Sjogren's syndrome. They are commonly composed of six antibodies: anti-Sm (for SLE), anti-La (for Sjogren's syndrome), anti- RNP (for MCTD), anti-Ro (for Sjogren's syndrome), anti-Scl 70 (for Scleroderma), and anti-Jo1 (for dermatomyositis)

2.5.8 DFS70 (Dense Fine Speckled 70)

The dense fine speckled 70 (DFS70) antigen (also known as the lens epithelium derived growth factor - LEDGFp75) is a highly conserved DNA-binding protein, contributing to cellular protection against oxidative DNA damage (Shinohara, Singh, & Fatma, 2002) (Singh, Ohguro, Chylack, & Shinohara, 2016). The protein is present in cell nuclei of all organs and tissues and may be overexpressed or altered during inflammation, thus stimulating autoantibody responses (Ganapathy, Daniels, & Casiano, 2003).

DFS70 pattern is found at a high frequency by indirect immunofluorescence assay on HEp-2 cells. With this method the presence of DFS70 antibodies is suspected when fine speckles uniformly distributed in the nucleus, sparing the nucleoli, are present in interphase cells, and staining of the chromatin region is observed in metaphase cells. Currently, the clinical significance of this finding is not clear as the presence of anti-DFS70 antibodies has been described in patients with diverse chronic inflammatory conditions (Ochs et al., 2000) but also in healthy subjects (Watanabe et al., 2004).

There is a negative association between anti-DFS70 and AARD (ANA associated rheumatic diseases) (Conrad, Röber, Rudolph, & Mahler, 2015). The prevalence of anti-DFS70 is extremely

low in ANA associated rheumatic diseases patients compared to healthy persons. Isolated anti-DFS70 are found in about 1% of AARD but detectable in 2-22% of healthy people (Conrad, Röber, Andrade, & Mahler, 2017). Five hundred and ninety-seven healthy individuals working in a hospital (142 men, 455 women) had a serological analysis for antinuclear antibody and DFS70 antibodies by indirect immunofluorescence with HEp-2 cells as a substrate and by immunoblotting using DFS70 recombinant protein and whole HeLa cell extract (Watanabe et al., 2004). ANAs were present in 20% of all individuals by IIF. Nine percent of subjects were ANA positive at a serum dilution of 1:40, 4.0% at 1:80, 5.5% at 1:160, 1.0% at 1:320, and 0.3% at 1:640. There were 64 anti-DFS70 antibody-positive individuals. Surprisingly, constituting 11% of the whole population and 54% of the ANA-positive population. The percentage of female anti-DFS70 antibody-positive subjects (86%; 55 of 64 subjects) was higher than the percentage of female anti-DFS70 antibody-negative subjects (75%; 398 of 533 subjects) ($P < 0.05$). The prevalence of anti-DFS70 antibody-positive sera decreased with increasing age ($P = 0.0017$) (Watanabe et al., 2004).

2.5.9 Anti-dsDNA (Anti-double stranded DNA antibodies)

Anti-double stranded DNA antibodies are a group of antinuclear antibodies with double stranded DNA as the target antigen. Enzyme-linked immunosorbent assay (ELISA) and Immunofluorescence are the blood tests that are usually done to detect anti-dsDNA in the laboratory. They are of relevant diagnostic value in SLE and lupus nephritis (Kavanaugh et al., 2000b) (Rekvig, 2008). Although the exact mechanism of the generation of dsDNA antibodies is still unknown, it is likely that extracellular DNA is one cause of an immune response against dsDNA. There is a great deal of evidence supporting the idea that dead or dying cells are one major source of this extracellular DNA (Su & Pisetsky, 2009). Antibodies to DNA (anti-DNA antibodies) are the serological hallmark of SLE and clinically have diagnostic and prognostic significance

(Rahman & Isenberg, 2008). Anti-dsDNA antibodies are mainly specific for systemic lupus erythematosus, with close to 100% specificity. Higher titres of anti-dsDNA antibodies strongly suggests SLE and lower titres may be found in healthy individuals (Conti et al., 2015). Despite the high specificity, anti-dsDNA have a sensitivity of about 25-85%. Therefore, the presence of anti-dsDNA suggests SLE but absence of the antibodies does not rule out the disease (Kavanaugh et al., 2000).

2.5.10 Chrom (Anti-Chromatin antibodies)

Anti-chromatin autoantibodies were one of the first autoantibodies ever detected since they make up the majority of antibodies causing LE Cell formation (Mulay et al., 2016). Anti-chromatin autoantibodies have had many names over the last few decades: LE cell factor; anti-nucleosome; anti-deoxyribonucleoprotein (DNP); and anti-(H2A-H2B-DNA). These autoantibodies are found in approximately 75% of people with systemic lupus erythematosus and up to 100% of people with drug-induced lupus. They are also found in 20-50% of patients with autoimmune hepatitis type I (lupoid hepatitis). Anti-chromatin are not generally found in any other disease, thus showing very good sensitivity and specificity for patients with lupus, drug-induced lupus and lupoid hepatitis (Burlingame, 2002).

2.5.11 Ribo P (Anti-Ribosomal P antibodies)

Autoantibodies to ribosomal P proteins (anti-P antibodies) were recognised several years ago as a distinct group of autoantibodies directed against the P0, P1, and P2 proteins located on the larger (60 S) subunit of the eukaryotic ribosomes (B. K. B. Elkon, Parnassa, & Foster, 1985) (Francoeur, Peebles, Heckman, & Lee, 1985). Sera containing anti-P antibodies react preferentially with a common epitope consisting of 22 amino acid residues and located at the carboxyl-terminal end of all P proteins (K. Elkon et al., 1986). These autoantibodies are predominantly found in sera of

patients with systemic lupus erythematosus (SLE) and have been correlated with neuropsychiatric lupus in some but not all studies (Nagai, Arinuma, Yanagida, Yamamoto, & Hirohata, 2005). The frequency of anti-P antibodies in SLE population is generally low and can vary from 6% to 36% in different ethnic groups (Arnett, Reveille, Moutsopoulos, Georgescu, & Elkon, 1996).

2.5.12 Anti-SSA autoantibodies (Anti-Sjogren's syndrome related antigen A or anti-Ro): are antinuclear antibodies that are linked to numerous autoimmune disorders such as systemic lupus erythematosus, subacute cutaneous lupus erythematosus, primary biliary cirrhosis and neonatal lupus and Sjogren (Franceschini.F, 2004). Anti-Ro /SSA can be detected in 70-100% of patients with Sjogrens's Syndrome, and the presence of this antibody is used in the diagnosis and classification of Sjogren's syndrome and its pathological significance still remains unclear. (Yoshimi, Ueda, Ozato, & Ishigatsubo, 2012).

2.5.13 SS_A52 and SS_A60

Two types of anti-Ro/SSA antibodies have been identified. They are specific for two different Ro/SSA antigens of 60 kDa and 52 kDa (Heinlen et al., 2011) derived from the RNP complex. Anti-SSA-60 kDa antibodies (aSSA60) are linked to certain disorders such as SS, SLE, neonatal lupus and congenital heart block (Franceschini & Cavazzana, 2005) (Tsuzaka, 1994). Clinically, the presence of aSSA52 has been reported in a wide variety of diseases. The spectrum of autoimmune disorders associated with aSSA52 antibodies is broader than that associated with aSSA60 antibodies, and includes inflammatory myositis(Rutjes et al., 1997) primary biliary cirrhosis (Granito, 2007) and SSc (Rutjes et al., 1997) (Granito, 2007).

2.5.14 SS_B (Sjogren's syndrome Antigen B)/Anti-La antibody

SS-B/La is an extractable nuclear antigen (ENA) composed of a 48-kD protein combined with RNA species. SS-B/La antibodies are found primarily in patients with Sjogren syndrome or lupus

erythematosus (LE), where they occur with frequencies of approximately 60% and 15%, respectively (Robert et al, 2013). SS-B/La antibodies occur only infrequently in the absence of SS-A/Ro antibodies.

2.5.15 Cent_B (Anti centromere antibody)

Anticentromere antibodies (ACA) have been described in a variety of presumed autoimmune diseases including CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia) (Moroi, Peebles, Fritzler, Steigerwald, & Tan, 1980), diffuse systemic sclerosis (E M Tan et al., 1980) primary biliary cirrhosis (Bernstein, Callender, Neuberger, Hughes, & Williams, 1982), as well as healthy subjects (Terao et al., 2014).

2.5.16 Sm

Sm antigen is a non-histone nuclear protein composed of several polypeptides of differing molecular weights. They include B (26 kD), B'(27 k D), and D (13 kD). The principle reactivity has been shown to reside in the B, B', and D polypeptides (James, Mamula, & Harley, 2008). Anti-Sm autoantibodies were described originally as precipitating autoantibodies in sera of patients with Systemic Lupus Erythematosus (E M Tan & Kunkel, 1966). Anti-Sm antibodies are also usually accompanied by antinuclear ribonucleoprotein (nRNP) antibodies (Yasuma et al., 1990). Autoantibodies to Sm antigen have been observed in 15 to 30% of SLE sera as a diagnostic marker (Eng M. Tan, 1982).

2.5.17 SnRNP

Small nuclear ribonucleoprotein particle (snRNP) is a target of autoreactive B cells and T cells in several rheumatic diseases including systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD)(Kattah, Kattah, & Utz, 2010a).

2.5.18 RNP, RNP_68 and RNP_A

RNP (also called nRNP and U1RNP) is a small nuclear ribonucleoprotein that contains 3 protein autoantigens (called A, C, and 68 kD) (Kattah, Kattah, & Utz, 2010b). Sera that contain RNP antibodies react predominately with the A and 68-kD autoantigens. Antibodies to RNP occur in patients with lupus erythematosus (LE) and in patients with other connective tissue diseases, notably mixed connective tissue disease (MCTD) (Didier et al., 2018). MCTD is characterized by high levels of RNP antibodies without detectable Sm or double-stranded DNA (dsDNA) antibodies. MCTD resembles LE but is not accompanied by renal involvement (Robert et al, 2013).

2.5.19 Scl_70 (scleroderma-associated autoantigen of 70 kDa)

Anti-Scl-70 (also called anti-topoisomerase I after the type I topoisomerase target)(Guldner et al., 1986) Scl 70 (topoisomerase 1) is a 100-kD nuclear and nucleolar enzyme. Scl 70 antibodies are considered to be specific for scleroderma (systemic sclerosis) and are found in up to 60% of patients with this connective tissue disease. Scl 70 antibodies are more common in patients with extensive cutaneous involvement and interstitial pulmonary fibrosis, and are considered a poor prognostic sign(Robert et al, 2013).

2.5.20 Jo_1 (histidyl-tRNA synthetase protein)

The Jo-1 antigen is histidyl-transfer ribonucleic acid (t-RNA) synthetase (Kagen, 2009). This enzyme is partially responsible for attaching t-RNA to their cognate rRNA (Freist, Verhey, Rühlmann, Gauss, & Arnez, 1999). The Jo-1 antigen migrates as a 53 kD protein on SDS-PAGE. Anti-Jo-1 autoantibodies were originally described as precipitating autoantibodies in sera of patients with polymyositis (Satoh, Tanaka, Ceribelli, Calise, & Chan, 2017). It was later realized that the anti-Jo-1 antibodies were specific for patients with polymyositis (Mileti, Strek, Niewold, Curran, & Sweiss, 2009). The target for the anti-Jo-1 antibodies was one of a family of distinct

cellular enzymes, the aminoacyl t-RNA synthetases (Targoff & Reichlin, 1987). The presence of autoantibodies against the Jo-1 antigen has been reported in up to 23% of polymyositis patients by immunodiffusion (Nishikai & Reichlin, 1980). Anti-Jo-1 antibodies are almost completely specific for myositis, being more common in polymyositis than dermato-myositis (Reichlin et al, 1984). The anti-Jo-1 response appears to be self-antigen driven, having a broad spectrotype over time undergoing isotype switching. Anti-Jo-1 antibodies also inhibit the function of human histidyl tRNA synthetase more than they do from other species.

CHAPTER THREE

METHODOLOGY

3.1 RESEARCH DESIGN

A cross sectional community based survey was conducted from February to April, 2017. The health status and demographics of study participants was determined through the administration of a well-structured questionnaire. Venous blood (2 to 3mls) was drawn from each participant to prepare samples for serology and these were stored at -20 ℃ until use.

3.2 RECRUITMENT OF STUDY PARTICIPANTS

Study participants were recruited consecutively. A total of 370 participants were studied. They were made up of healthy adults selected randomly from different communities: Greater Accra Region (Korle Gonno and Amasaman), Upper West (Wa and Kaleo), Eastern region (Gyankama and Koforidua), and Volta region (Akatsi). These four regions were conveniently selected because of the distinct ethnic distribution of Ghanaians in these locations. The participants were enrolled after the study objectives were explained to them in English or a local dialect where necessary and a written consent was given. Each participant was interviewed to complete a standard questionnaire at the time of recruitment.

3.3 SAMPLE SIZE

The estimated prevalence of antinuclear antibodies in the general population is 13.8% (Satoh et al., 2013)

Using the relation $N= Z^2 \times P (1-P)/d^2$

Where N= minimum sample size, p= prevalence and d=absolute error or precision, and z= standard normal variate = 1.96 at 5% type one error

The minimum sample size for this research was calculated as follows:

$$N=\frac{1.96^2 X 0.138 X 0.862}{0.0025}$$

N=185

3.4 STUDY SITES AND SAMPLE COLLECTION

The study was conducted in four randomly selected regions in Ghana: Greater Accra, Volta Region, Eastern Region, and the Upper West Regions.

Greater Accra: The study was conducted in two districts in this region, the Ga West Municipal Assembly and Ablekuma South Sub-Metropolitan district. Participants were selected from two communities, Korle-Gonno in the Ablekuma South sub-metropolitan district and Amasaman, the Capital of the Ga West Municipal Assembly. Korle-Gonno is a coastal community in Accra, located to the west of Korle Lagoon and the densely populated districts of James Town and Usher Town. It is also one of the most rapidly and radically changing Ga communities. The AMA Town and Country Planning Department defines is borders as follows: Guggisberg Avenue (north), St. Mary's Secondary School (east), Old Winneba Road (south) and Eduardo Mohlana Road (west). The major landmarks include the St. Mary's Secondary School, the Korle Lagoon and Korle-Bu Teaching Hospital, one of Ghana's most important teaching hospitals and tertiary care centers (Arguello, 2010).Total population is 27,826 (Kareem & Samba, 2016).

Amasaman is the capital of the Ga West Municipal Assembly. The Municipality lies within latitude 5°35° North, 5°29' North and longitude 0°10' West and 0°24' West. It shares common boundaries with Ga East and Accra Metropolitan Assembly to the East, Akwapem South to the North and Ga South and Ga Central to the South. It occupies a land area of approximately 284.08 sq km with about 412 communities. The total population of the Municipality is 219,788 of which 49.0 percent are males while 51.0percent are females(Ghana Statistical Service, 2010b).

Volta Region: Akatsi is the capital of the Akatsi South District. The District is located between latitudes 60S 70N and longitudes 00W 10E and shares boundaries with Keta Municipal to the South, Ketu North to the East, to the West by South Tongu District and to the North by Akatsi North District. Its total land area is about 536 square kilometers. The population of Akatsi South District, according to the 2010 Population and Housing Census, is 98,684 representing 4.6 percent of the total population of the Volta Region (Ghana Statistical Service, 2010a).

Upper West Region: Kaleo: Kaleo is located in the Nadowli-Kaleo district in the Upper West region of Ghana. It lies in the centre of the Upper West between latitude 10^0 and 11^130^1 North and longitude 3^110^1 and 2^110 West. The district has a population of approximately 61,561(Ghana Statistical Service, 2014a).

Wa: Wa is a town and is the capital of Wa Municipal District and the Upper West Region of north Ghana. Wa has a 2012 settlement population of 102,446. Features of the town include several mosques, the Wa-Na Palace, a museum and a nearby hippopotamus sanctuary. The geography of Wa is notable for the dramatic monadnock Ombo Mountain which is located around Kaleo and visible from much of the Wa town. Other notable towns around Wa wa region include Naaha and Ga.The town serves as a transportation hub for the Upper West region, with major roads

leading north to Hamile, and northeast to Tumu and the Upper East Region. There is also a small airport, the Wa Airport(Ghana Statistical Service, 2014b).

Eastern Region: Gyankama is located in the Akuapim South Municipal District of Ghana. The Akwapim South District is located at the south eastern part of the Eastern Region of Ghana between latitudes 5.450N and 5.580N, and Longitudes 0.0W and covers a land area of about 224.13 kilometres square. The district is predominantly Akwapims, who are part of the Akan ethnic group. The Akwapim South District recorded a total of 37,501 persons in the 2010 Population and Housing Census, comprising of 19,327 females representing 51.5 percent and 18, 174 males (48.5%)(Ghana Statistical Service, 2014c).

Koforidua: Koforidua is the capital of the New Juaben Municipal District and the Eastern region of Ghana. Koforidua has a settlement central city population of 127,334 people in 2012 and is dominated by Akans(Ghana Statistical Service (GSS), 2013).

3.5 INCLUSION CRITERIA

Participants who were found to be apparently healthy with no family history of autoimmune disease and above age 18 were recruited. The volunteers consented to the use of their information and samples for the research.

3.6 EXCLUSION CRITERIA

The following persons were excluded from the study: under age 18 years, persons with known chronic and acute medical illness, and persons with family history of autoimmune diseases.

3.7 ETHICAL CONSIDERATION

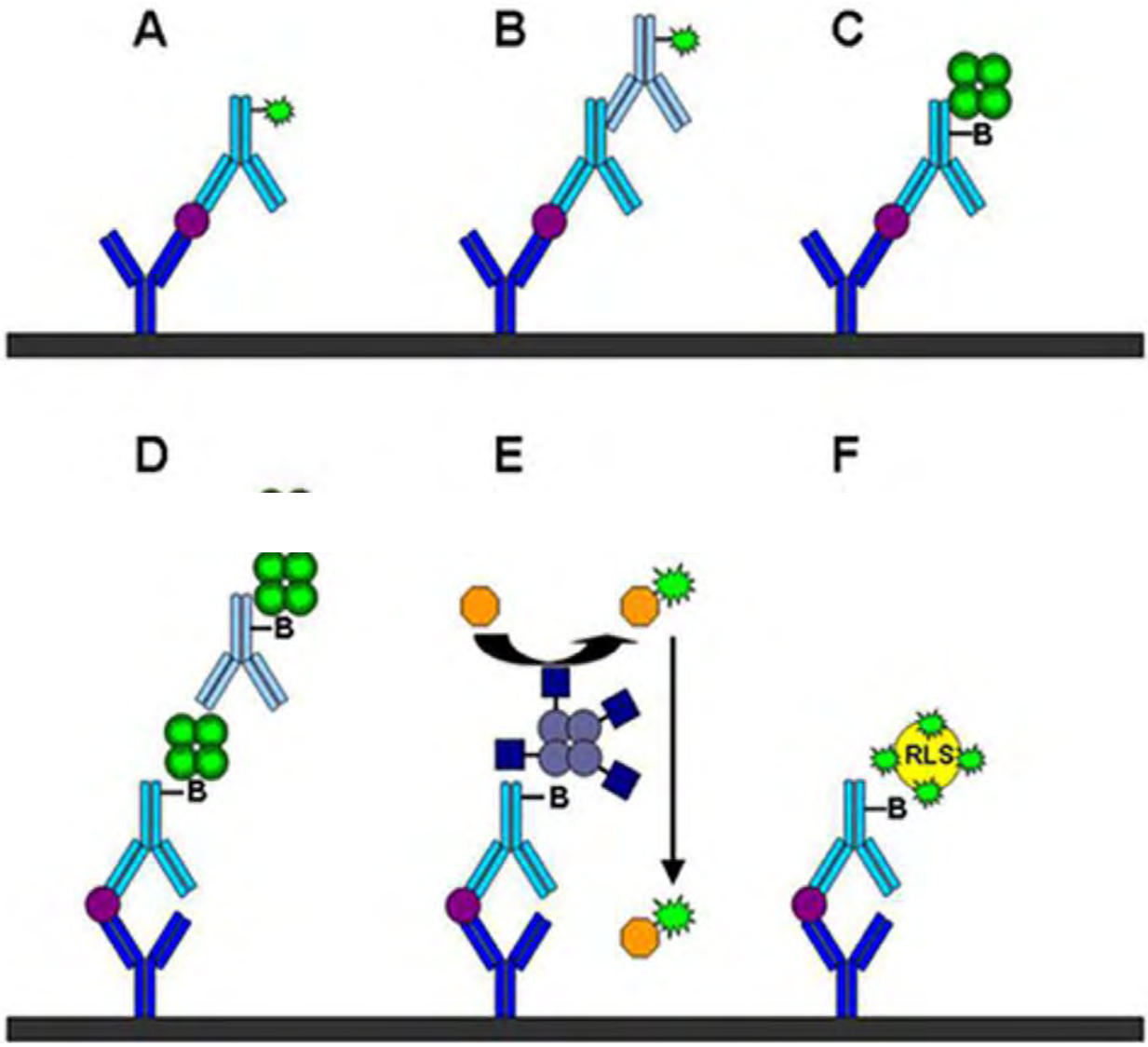
Ethical Clearance was obtained from the Ethical and Protocol Review committee of the College of Health Sciences, University of Ghana with Protocol Identification number CHS-Et/M.4-P3.1/2016-2017, before blood samples were collected from volunteers who had consented to partake in the study.

3.8 MULTIPLEX ASSAY

The autoantibodies (anti-dsDNA, chromatin, Ribosomal-P, Ro /SSA, Ro / SSA 52, Ro / SSA60, La / SSB, centromere B, anti-Sm, SmRNP, RNP 68, RNP A, Scl-70, Jo1 were detected utilizing fully automated multiplexed platform (Bio-Rad Bioplex 2200 system, Bio-Rad Laboratories Hercules, CA USA). Bio-Rad Bioplex 2200 system is a multiplex flow immunoassay that can detect and identify multiple antibodies to different antigens in a single incubation. The sera of the subjects (5µl) were incubated with a sample diluent and a reagent containing distinctly colored bead sets (coated with different antigens) (Figure 3.1 A-C), created by the use of two fluorescent dyes at distinct ratios. After incubation and a wash cycle, an anti-human IgG or IgM antibodies conjugate with phycoerythrin were added (Figure D-F). The beads were passed through the detector that identified the fluorence intensity; specific lasers detected both bead dyes and tagged detection antibodies as seen in Figure 3.2. The quantity of the antibodies "captured from antigen" were determined by the fluorescence of an anti-human IgG or IgM-phycoerythrin-labeled conjugate. Furthermore, the relative fluorescence intensity (RFI) was normalized to an antibody index (AI), which is a qualitative numeric result. Samples with results exceeding 120 arbitrary units (AU)/ml were considered positive samples. Samples below 100 AU/ml were considered negative. All samples with results between

100 and 120 AU/ml were considered equivocal (Avaniss-Aghajani, Berzon, & Sarkissian, 2007).

Figure 3.1 showing a schematic representation of the Multiplex Immunoassay reaction.



A, fluorescent bound detection antibodies. B, fluorescent labeled compounds-tertiary antibodies. C, biotinylated detection antibodies with streptavidin-phycoerythrin conjugate. D, streptavidin phycoerythrin conjugate staining amplified with biotinylated anti-streptavidin-phycoerythrin antibodies. E, streptavidin linked horseradish peroxidase linked to a species-specific tertiary

antibody activates chemiluminescent substrates or generates different flourophores. F, resonance light-scattering colloid gold particles coated with an antibiotin antibody (Nielsen & Geierstanger, 2004).

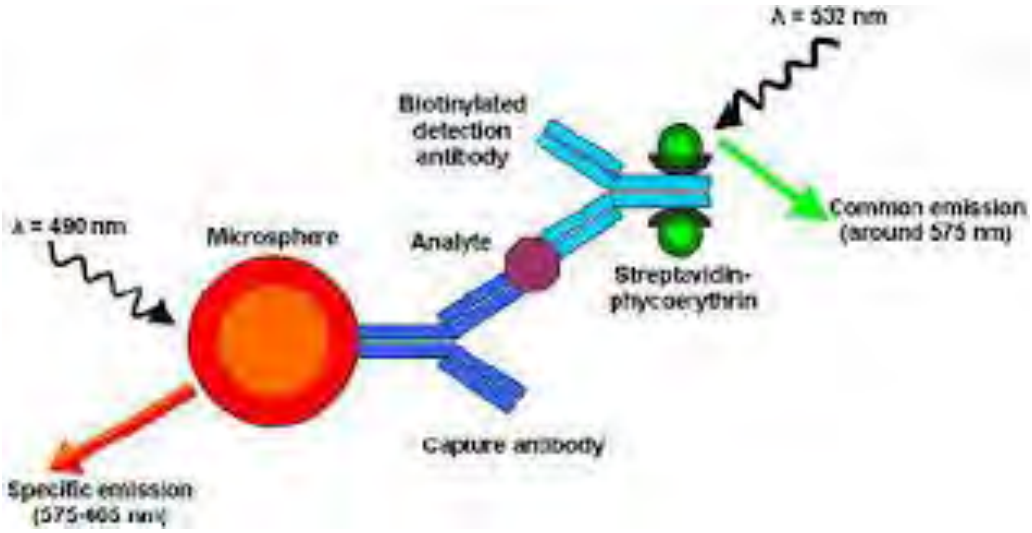


Figure 3.2. Schematic representation of an isolated reaction of a MBIA with the reagents used in this technique. The capture monoclonal antibody is coupled to the bead (microsphere). After binding of the analyte a second biotinylated antibody is added. After addition of a streptavidin phycoerythrin conjugate, dyes embedded in the beads and phycoerythrin are excited (wavy lines) and both compounds give two types of light emission (Barrios et al, 2007).

3.7.3 CHEMILUMINESCENT IMMUNOASSAY: TESTING OF DFS 70 ANTIBODIES

The anti DFS70 antibodies were tested using Chemiluminescent immunoassay (CLIA, Bio Flash INOVA). Specifically, the methods used paramagnetic beads coated with the different antigens. After incubation with patients' sera and washing bound antibodies, were identified by an anti-IgG-IgM linked to an isoluminol derivative. Thus, the starter reagents are added and a flash chemiluminescence reaction is generated. The light signal is measured in a photomultiplier, indicating the presence of IgM or IgG antibodies. In our study we had incubated the sample of the patients with the paramagnetic beads (coated

with antigens munched above, then the beads were washed to remove unbound antibodies. Immediately after this washing step, tracer was added and the beads were again incubated. After the incubation, the beads were washed again. Finally, the chemiluminescent reaction takes place, by adding peroxide solutions. The light produced from the reaction was measured as Relative Light Units (RLUs) optical system as demonstrated in figure 3.3. The RLUs were proportional to the concentration of isoluminol conjugate bound to the human IgG or IgM antibodies and results were reported in chemiluminescent units or CUs per ml. A value of ≥ 20 chemiluminescent units was considered positive (Lucas, Chang, Merien, & Elisa, 2018).

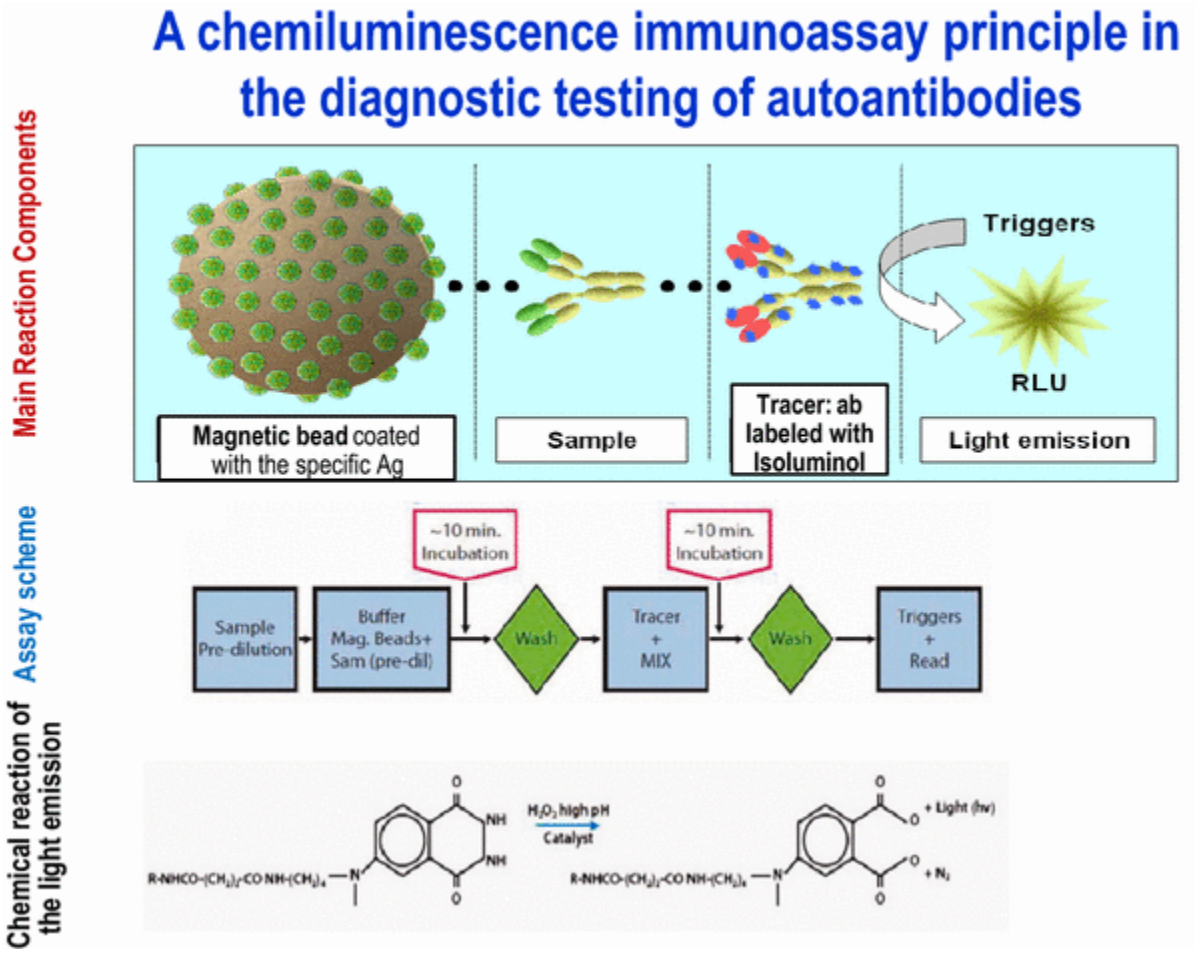


Figure 3.3. Illustration of BIOFLASH system, a widely used CLIA in the field of autoimmunity(Cinquanta, Fontana, & Bizzaro, 2017).

3.7.5 STATISTICAL ANALYSIS

Data from questionnaires were analyzed using Microsoft Excel. Results obtained were presented using appropriate charts and table. Demographic distribution of the Data were analyzed using the Chi-square test with a 95% confidence interval. Student t-test was used for the analysis of serum antibody levels. Differences between the values was considered significant at the significance level $p < 0.05$. All statistical analysis was performed using the STATA.

CHAPTER FOUR

RESULTS

4.1: Characteristics of the Study Participants

This study recruited 370 participants from four selected regions (Greater Accra, Upper West, Eastern and Volta regions) in Ghana. A third (33.59%) of the participants were from the Greater Accra region. Female were the dominating sex group (64.05%) with more than a third of them aged 18-30. Ewe (29.73%) was the predominant tribe among the studied participants. Primary (29.73%) education was the major educational qualification of the participants. Table 3 shows details of the background characteristics of the studied participants by region

Table 4.1: Distribution of background characteristics of the study participants by region

	Greater Accra n=132 (%)	Upper West n= 68 (%)	Eastern n=84 (%)	Volta n=109(%)	Total n (%)
Sex					
Male	41 (35.96)	37 (54.41)	15 (17.86)	40 (38.46)	133 (35.95)
Female	73 (64.04)	31 (45.59)	69 (82.14)	64 (61.54)	237 (64.05)
Age(years)					
18-30	21 (18.42)	51 (75.00)	9 (10.71)	51 (49.04)	132 (35.68)
31-40	30 (26.32)	9 (13.24)	14 (16.67)	10 (9.62)	63 (17.03)
41-50	28 (24.56)	7 (10.29)	14 (16.67)	9 (8.65)	58 (15.68)
51-60	18 (15.79)	1 (1.47)	25 (29.76)	19 (18.27)	63 (17.03)
>60	17 (14.91)	0 (0)	22 (26.19)	15 (14.42)	54 (14.59)
ethnic					
Akan	35 (30.70)	0 (0)	58 (69.05)	4 (3.85)	97 (26.21)
Ga	48 (42.11)	0 (0)	21 (25)	0 (0)	69 (18.65)
Ewe	6 (5.26)	0 (0)	5 (5.95)	99 (95.19)	110 (29.73)
North	25 (21.93)	68 (100)	0 (0)	1 (0.96)	94 (25.41)
(educ)					
Primary	22(19.30)	4 (5.88)	23 (27.38)	24 (23.08)	73 (19.72)
JHS	43 (37.72)	8 (11.76)	30 (35.71)	29 (27.88)	110 (29.73)
SHS	21 (18.42)	4 (5.88)	11 (13.1)	18 (17.31)	54 (14.59)
Voc/Tech	10 (8.77)	42 (61.76)	1 (1.19)	15 (14.42)	68 (18.38)
Tertiary	18 (15.79)	10 (14.71)	19 (22.62)	18 (17.31)	65 (17.57)

4.2 Distribution of the Prevalent Antibodies

Subjects who tested positive to any of the following antinuclear antibodies IgG were considered 'ANA positive': Chromatin, RNP, Sm, SmRNP, Ro/SSA, centromere, La/SSB, Ribosomal-P, Scl70, Jo-1, Anti-dsDNA.

Table 4.2 Show the distribution of the prevalent antibodies among the study participants. The prevalence of ANA was 14.21% (95% CI: 10.98 -18.19%). No RiboP antibody was found in any of the studied participants. The only Intermediate antibody found was in dsDNA at a prevalence of 0.53% (95% CI: 0.14 - 2.17%).

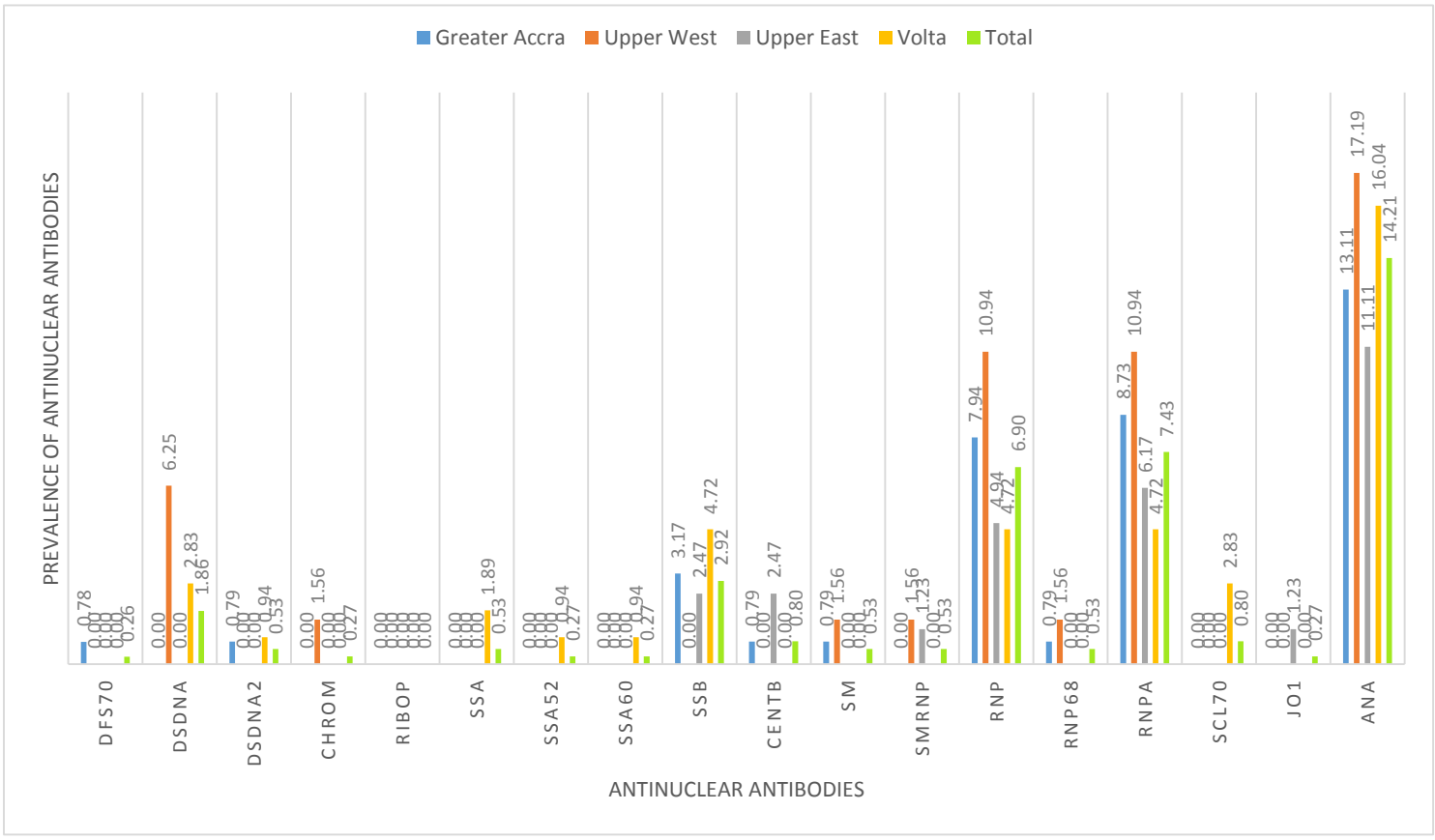
Table 4.2: Distribution of the prevalent antibodies

Antibody	Percent	95% CI
DFS70	0.26	0.04 - 1.84
dsDNA	1.86	0.91 - 3.97
dsDNA2	0.53	0.14 - 2.17
Chrom	0.27	0.04 - 1.93
RiboP	0	-
SSA	0.53	0.14 - 2.17
SSA52	0.27	0.04 - 1.93
SSA60	0.27	0.04 - 1.93
SSB	2.92	1.67 - 5.36
CentB	0.8	0.26 - 2.52
Sm	0.53	0.14 - 2.17
SmRNP	0.53	0.14 - 2.17
RNP	6.9	4.65 - 9.93
RNP68	0.53	0.14 - 2.17
RNPA	7.43	5.1 - 10.56
Scl70	0.8	0.26 - 2.52
Jo1	0.27	0.04 - 1.93
ANA	14.21	10.98 - 18.19

4.3: Distribution of antibodies by Region of Residence

The distribution of autoimmune antibodies by region is shown in Figure 4.1. The prevalence of ANA positive subjects was highest in UW (17.19%). Antibodies diagnosed in single regions were: DFS70(0.78%) in GA, Chrom (1.56%) in Upper West, and SSA (1.89%), SSA52(0.94), SSA60(0.94%), Scl70(2.83%) all in Volta. RNP, RNPA, and ANA were antibodies common to all the four regions. SSB antibodies were diagnosed in in GA, Eastern and Volta with Volta recording the highest prevalence (4.72%). dsDNA was detected in Eastern and Volta regions while the intermediate dsDNA was found in Volta and GA. Cent B antibody was found in GA and Eastern but predominant in GA than Eastern. Eastern and Upper West regions were the only places SmRNP antibodies were diagnosed but more in UW (1.56%) compared to Eastern (1.23%). Prevalence of dsDNA was significantly associated with study participants' region of residence ($p<0.05$). There was no enough statistical evidence to show an association between region and the remaining antibody prevalence.

Figure 4.1. Distribution of the prevalent antibodies by region of residence



4.4: Distribution of prevalent antibodies by sex

From Table 4.3, with the exception of RiboP antibodies which was not detected in any of the study participants, Females were found to have all of the antibodies while only SSB, CentB, RNP, RNPA antibodies were detected in males. Although none of the prevalence of antibodies was significantly associated with sex ($p>0.05$), males recorded higher prevalence in all cases where the antibodies were found in both sex. Prevalence of ANA was (14.84%) in male and (12.61%) in female participants. DFS70, Chrom, RiboP, SSA, SSA52, SSA60, Sm, SmRNP, Scl70, AND Jo1 were antibodies detected in only females. There was no statistically significant association between sex and any of the antibody prevalence ($p>0.05$)

Table 4.3: Distribution of prevalent antibodies by sex

	Sex			
	Male (n=133)	Female(n=237)	Total	p-value
DFS70	0.00	0.43	0.28	1.000§
Chrom	0.00	0.45	0.28	1.000§
dsDNA	3.08	1.34	1.98	0.355
dsDNA2	0	0.89	0.56	
RiboP	0.00	0.00	-	-
SSA	0.00	0.90	0.57	0.535§
SSA52	0.00	0.45	0.28	1.000§
SSA60	0.00	0.45	0.28	1.000§
SSB	3.85	2.23	2.82	1.000§
CentB	0.77	0.45	0.56	1.000§
Sm	0.00	0.89	0.56	0.534§
SmRNP	0.00	0.89	0.56	0.534§
RNP	8.46	4.91	6.21	1.78,0.182
RNP68	0.77	0.45	0.56	1.000§
RNPA	8.46	5.80	6.78	0.92,0.338
Scl70	0.00	1.34	0.85	0.301§
Jo1	0.00	0.45	0.28	1.000§
ANA	14.84	12.61	13.43	0.35,0.555
Overall	14.29	12.24	12.97	0.32,0.573

4.5: Distribution of Prevalent antibodies by age

The age group with the highest antibody prevalence was 18-30 years. With the exception of DFS70, dsDNA, SSA60, and Jo1, samples of all the other antibodies were detected within this age bracket. The only Intermediate dsDNA (0.81%) antibodies diagnosed was found with 31-40 and 41-50 age groups. None of the antibodies had a statistically significant association with age group ($p>0.05$) however there is statistical significance with age and ANA positivity.

Table 4.4: Distribution of the prevalent antibodies by age group

	Age groups						
	18-30	31-40	41-50	50-60	>60	Total	χ^2, p-value
DFS70	0.00	1.61	0.00	0.00	0.00	0.28	0.648§
dsDNA	4.03	0.00	0.00	1.64	1.89	1.98	0.297§
dsDNA2	0.00	1.64	1.82	0.00	0.00	0.56	
Chrom	0.81	0.00	0.00	0.00	0.00	0.28	1.000§
RiboP	0.00	0.00	0.00	0.00	0.00	0.00	-
SSA	0.81	0.00	0.00	1.64	0.00	0.57	1.000§
SSA52	0.81	0.00	0.00	0.00	0.00	0.28	1.000§
SSA60	0.00	0.00	0.00	1.64	0.00	0.28	0.649§
SSB	4.84	0.00	1.82	0.00	5.66	2.82	0.104§
CentB	0.00	1.64	1.82	0.00	0.00	0.56	0.362§
Sm	0.81	0.00	0.00	1.64	0.00	0.56	0.878§
SmRNP	0.81	0.00	0.00	1.64	0.00	0.56	0.878§
RNP	10.48	8.20	1.82	1.64	3.77	6.21	0.073§
RNP68	0.81	0.00	0.00	0.00	1.89	0.56	0.527§
RNPA	10.48	8.20	3.64	1.64	5.66	6.78	0.179§
Scl70	1.61	0.00	0.00	1.64	0.00	0.85	1.000§
Jo1	0.00	0.00	0.00	0.00	1.89	0.28	0.150§
ANA	21.95	8.33	5.66	8.20	13.21	13.43	13.21, 0.015*

P-values were based on Pearson chi-square and Fishers exact test for categorical variables, χ^2-Person Chi-square, § p-value estimate from Fisher's exact test

Table 4.5 shows the Distribution of prevalent antibodies by age group among males

From Table 4.5, with the exception of RNP68 (4.76%) and Cent B (6.67%) antibodies which were diagnosed among only males within age groups >60 and 31-40 respectively, the five remaining antibodies were diagnosed among males, dsDNA1 and SSB occurred among only males in the age groups of 18-30 and >60. No antibody was detected among males in 41-50 age group. RNP and RNPA occurred in all age groups except 41-60 age group.

Table 4.5: Distribution of prevalent antibodies by age group among males only

	Age						
	18-30	**31-40**	**41-50**	**51-60**	**>60**	**Total**	χ^2, p-value
dsDNA1	4.76	0	0	0	4.76	3.08	1
SSB	3.17	0	0	0	14.29	3.85	0.163
Cent B	0	6.67	0	0	0	0.77	0.223
RNP	11.11	13.33	0	5.88	4.76	8.46	0.711
RNP68	0	0	0	0	4.76	0.77	0.515
RNPA	11.11	13.33	0	5.88	4.76	8.46	0.711
ANA	19.35	13.33	0	5.88	19.05	14.84	0.365

P-values were based on Pearson chi-square and Fishers exact test for categorical variables,

χ^2- Person Chi-square, § p-value estimate from Fisher's exact test

Table 4.6 shows the distribution of prevalent antibodies by age group among females.

From Table 4.6, most of the antibodies were detected among females within the age group 18-30 while few of them were detected among those within the age group >60. Details of the distribution of antibodies among females by age group can be found in table 4.6.

Table 4.6: Distribution of prevalent antibodies by age group among females only

	Age						
	18-30	**31-40**	**41-50**	**51-60**	**>60**	**Total**	χ^2, p-value
DFS70	0	2.13	0	0	0	0.43	0.723
dsDNA1	3.28	0	0	2.27	0	1.34	0.642
dsDNA2	0	2.17	2.44	0	0	0.89	
Chrom	1.64	0	0	0	0	0.45	1
SSA	1.64	0	0	2.27	0	0.9	0.887
SSA52	1.64	0	0	0	0	0.45	1
SSA60	0	0	0	2.27	0	0.45	0.525
SSB	6.56	0	2.44	0	0	2.23	0.123
CentB	0	0	2.44	0	0	0.45	0.326
Sm	1.64	0	0	2.27	0	0.89	0.888
SmRNP	1.64	0	0	2.27	0	0.89	0.888
RNP	9.84	6.52	2.44	0	3.13	4.91	0.171
RNP68	1.64	0	0	0	0	0.45	1
RNPA	9.84	6.52	4.88	0	6.25	5.8	0.265
Sc170	3.28	0	0	2.27	0	1.34	0.626
Jo1	0	0	0	0	3.13	0.45	0.143
ANA	24.59	6.67	7.5	9.09	9.38	12.61	**0.025**

P-values were based on Pearson chi-square and Fishers exact test for categorical variables, χ^2- Person Chi-square, § p-value estimate from Fisher's exact test

4.6: Distribution of prevalent antibodies by Ethnicity

Table 4.7 represents the distribution of prevalent antibodies by Ethnicity

From Table 4.7 Northern tribe had the highest overall antibody prevalence of 17.02%. SSA (1.89%), SSA52 (0.93%), Scl70 (2.80%) and SSA60 (0.93%) antibodies were diagnosed among only Ewes while Cent_B (2.17%) and Jo1 (1.09%) were among only Akans. SSB, RNP and RNPA were common to all the four tribes. Only Gas 1.52%) were diagnosed of DFS70. dsDNA antibodies prevalence distribution was significantly associated with ethnicity ($p<0.05$). Table 4.7 gives details on the distribution of the distribution of antibodies prevalence by Ethnic group

Table 4.7: Distribution of prevalent antibodies by Ethnic group

	Ethnicity					
	Akan	Ga	Ewe	Northerners	Total	χ^2, p-value
DFS70	0.00	1.52	0.00	0.00	0.28	0.183§
Chrom	0.00	0.00	0.00	1.12	0.28	0.438§
dsDNA	0.00	0.00	1.87	5.62	1.98	0.048* §
dsDNA2	0.00	0.00	0.93	1.12	0.56	
RiboP	0.00	0.00	0.00	0.00	0.00	-
SSA	0.00	0.00	1.89	0.00	0.57	0.255§
SSA52	0.00	0.00	0.93	0.00	0.28	1.000§
SSA60	0.00	0.00	0.93	0.00	0.28	1.000§
SSB	4.35	1.52	3.74	1.12	2.82	0.538§
Cent_B	2.17	0.00	0.00	0.00	0.56	0.164§
Sm	0.00	1.52	0.00	1.12	0.56	0.349§
SmRNP	1.09	0.00	0.00	1.12	0.56	0.690§
RNP	6.52	3.03	3.74	11.24	6.21	0.130§
RNP68	1.09	0.00	0.00	1.12	0.56	0.690§
RNPA	7.61	4.55	3.74	11.24	6.78	4.98, 0.193
Scl70	0.00	0.00	2.80	0.00	0.85	0.119§
Jo1	1.09	0.00	0.00	0.00	0.28	0.698 §
ANA	13.19	7.81	13.21	17.98	13.43	0.352

P-values were based on Pearson chi-square and Fishers exact test for categorical variables, χ^2-Person Chi-square, § p-value estimate from Fisher's exact test

Table 4.8: Distribution of prevalent antibodies by region of residence

	Region					
	Greater Accra	Upper West	Upper East	Volta	Total	χ^2, p-value
DFS70	0.78	0.00	0.00	0.00	0.26	1.00§
dsDNA	0.00	6.25	0.00	2.83	1.86	0.013*§
dsDNA2	0.79	0.00	0.00	0.94	0.53	
Chrom	0.00	1.56	0.00	0.00	0.27	0.170§
RiboP	0.00	0.00	0.00	0.00	-	
SSA	0.00	0.00	0.00	1.89	0.53	0.229§
SSA52	0.00	0.00	0.00	0.94	0.27	0.669§
SSA60	0.00	0.00	0.00	0.94	0.27	0.668§
SSB	3.17	0.00	2.47	4.72	2.92	0.402§
CentB	0.79	0.00	2.47	0.00	0.80	0.293§
SM	0.79	1.56	0.00	0.00	0.53	0.546§
SmRNP	0.00	1.56	1.23	0.00	0.53	0.147§
RNP	7.94	10.94	4.94	4.72	6.90	3.11,0.375
RNP68	0.79	1.56	0.00	0.00	0.53	0.546§
RNPA	8.73	10.94	6.17	4.72	7.43	2.78,0.427
Scl70	0.00	0.00	0.00	2.83	0.80	0.055§
Jo1	0.00	0.00	1.23	0.00	0.27	0.385§
ANA	13.11	17.19	11.11	16.04	14.21	1.51,0.679

CHAPTER FIVE

DISCUSSION

5.1 Discussions

Early detection of autoimmune diseases is crucial because the administration of early treatment before the commencement of organ injury has a greater chance to reduce the effects of the disease (Q. Z. Li et al., 2011). This is the first research in Ghana to determine the ethno-geographical distribution of antinuclear antibodies among healthy population in Ghana. In this study, the frequencies of several Antinuclear antibodies among 370 (133 males and 237 females) healthy subjects (as shown in Table 4.1) from ethno-geographically distinct populations in Ghana were determined (. Subjects who tested positive to any of the following antinuclear antibodies IgG were considered 'ANA positive': Chromatin, RNP, Sm, SmRNP, Ro/SSA, centromere, La/SSB, Ribosomal-P, Scl70, Jo-1, Anti-dsDNA (Shapira et al, 2012).

5.2 ANA prevalence

Unsurprisingly, majority of the autoantibodies were negative across most of the subjects. There were no ethno geographic variability among most of the autoantibodies. This corroborate with findings from Shapira et al 2012. The overall frequency of Antinuclear antibodies in the study participants was 14.21% as shown in Table 4.2, which is consistent with the prevalence rate in the European group with approximately 12% but showed significant discrepancy among the Mexicans, Papua New Guinea and Columbians with 26%,38% and 45% respectively (Shapira et al, 2012). Also, the overall prevalence of 12.97% in the Ghanaian study population conforms to the findings of Wandstradt et el in 2006 that Antinuclear antibodies are commonly found in the general population in up to 20% of healthy subjects. The National Health and Nutrition

Examination survey from 1999 to 2004 among 4,754 individuals in the United States found an overall Antinuclear antibody prevalence rate of between 12.2% to 15.5%(Satoh et al., 2013), which conforms with the regional prevalence rate of between 11.1% to 17% among the Ghanaian healthy adults. In a study among 124 healthy migrant Ghanaian and Nigerian African population in Italy, the prevalence of ANA was found to be 27.4%, which is high for a healthy population(Cainelli et al., 2004). This high prevalence of ANA among Ghanaians and Nigerians in a Western country may suggest an environmental risk factor in the development of antinuclear antibodies.

5.3 Sex distribution of antinuclear antibodies

There was no significant difference in the positivity in all the Antinuclear antibodies in both sexes. However, the prevalence of all the antinuclear antibodies (except the ones with zero prevalence) were higher in males than females as shown in Table 4.3. This sharply contradicts with several studies that show significantly prevalent Antinuclear antibodies in females than males. Li et al in 2011 demonstrated that Antinuclear antibody positivity was more prevalent in females (29%) than in males (17%), and this corresponded with a relative risk of Antinuclear antibody positivity in healthy females to males of 1.21. The prevalence of Antinuclear antibody positivity in males was found to be 14.29% and 12.24% in female healthy Ghanaian subjects according to this study as shown in Table 4.3. Fernandez et al in 2003 in the determination of antinuclear antibodies in the sera of 500 Brazilian blood donors found female blood donors presented a higher risk of antinuclear antibody positivity. The National health and nutrition examination survey 1999-2004 studied 4754 individuals in a cross sectional study in the United States and found out that antinuclear antibodies are significantly higher in females than males (17.8% vs. 9.6%, $P < 0.001$)(Satoh et al., 2013). This may suggest that sex/gender may not be a major confounding

factor in the production of antinuclear antibodies in healthy Ghanaian population. In a study among 124 (58 women and 66 men) African immigrants (Ghanaians and Nigerians) in Italy, there was no preponderance of ANA in the female immigrant population tested. Among the 34 participants who tested positive for ANA, 17(13.7%) were men and 17(13.7%) were women, demonstrating no sex predominance in ANA positivity(Cainelli et al., 2004).This correlates with the findings among the healthy Ghanaian Adults. Despite males having higher prevalence the ANA detected in the Ghanaian population, majority of antinuclear antibodies were absent in males (DFS70, Chrom, RiboP, SSSA, SSA52, SSA60, Sm, SmRNP, Scl70, Jo1). However, only RiboP was absent in both males and females. All other antinuclear antibodies were present in females at varying prevalence. This may suggest that female gender may be a risk factor for the development of these autoantibodies.

5.4 Age distribution of Antinuclear antibodies

In this study, age was not correlated with positivity for all the specific antinuclear antibodies tested, but there was a significant difference in ANA positivity with age as demonstrated on Table 4.4. This conforms to the generally accepted hypothesis that senescence is associated with increased autoantibody production due to reduced self-regulatory mechanisms. Prevalence of ANA in different age groups follows a non-linear variation pattern as a result of difference in exposure to determinants that contribute the development of antinuclear antibodies in different age groups as well as intrinsic differences in the developing endocrine and immune systems or sampling bias.

In a study of the Prevalence of antinuclear antibodies in China between July 2011 and September 2013, Guo found out that antinuclear antibodies positivity correlated with age in all the age groups and there was higher prevalence in the 20 to 30 year and 40 to 50 year groups. Though there was

no significant difference in the age groups among the healthy Ghanaian adults, the prevalence of antinuclear antibodies in the 18 to 30-year group was the highest especially among females, which correlate with the peak age group in the Chinese population by Guo and colleagues. Fernandez et al in 2003 in the determination of the Prevalence of antinuclear antibodies in the serum of normal Brazilian blood donors concluded that under 40 years of age presented a tendency to smaller antinuclear antibody prevalence with no statistical significance. This conforms partially with findings from the Ghanaian healthy adults. Findings from this study correlates with a study by Li and colleagues (Q.-Z. Li et al., 2011) who found out that age was not related to positivity of antinuclear antibody in healthy subjects who were negative for current or past autoimmune diseases. These findings may suggest that in a cross-sectional analysis like this there are many reasons for Antinuclear antibody positivity. The younger age groups may in fact have abnormal immune regulation that predisposes to Systemic Lupus erythematosus-like disease while older persons may develop auto reactivity as part of normal aging immune responses that do not lead to development of pathology. In the male Ghanaian population (Table 4.5), the peak ANA prevalence occurred in the 18-30 and >60 years' age groups with no antibody detected in the 41-50 year, lower prevalence in the 51-60-year group and a peak at > 60 years. This may suggest a possible increase in autoantibody production at the younger and older/aged group in men. Also in the female Ghanaian population, the peak ANA occurred in the 18-30 and > 60 years but lower prevalence in the other age groups as shown in Table 4.6. All these findings denote an interplay between increasing age and sex (which may be influenced by sex hormones) in the production of antinuclear antibodies.

5.5 Ethnic distribution of antinuclear antibodies

From Table 4.7, there were significant ethnical differences in the prevalence of anti-dsDNA (p-value=0.048), with detection in only Ewes and Northerners (1.87% and 5.62% respectively). This compares favorably with findings from Shapira and colleagues which found anti-dsDNA with significant difference among ethno geographically distinct populations from Columbia, Papua New Guinea, Netherlands, Italy, Israel and Mexico. This may suggest that genetic factors may influence the expression of anti-dsDNA. There was no correlation between ANA positivity and ethnic groups. These results agree with findings by Li et al 2011 in the study of risk factors for ANA positivity in healthy persons that there were no significant differences in ANA positivity among African Americans and Hispanics. Also, findings from the NHANES 1999-2004 study concluded that there was no significant differences in ANA prevalence among racial/ethnic groups (Mexican American, Non-Hispanic white and Non-Hispanic black) (Satoh et al., 2013). These suggests that production of antinuclear antibodies may be influenced by the interplay of both genetic and environmental factors.

5.6 Geographic distribution of antinuclear antibodies

From Table 4.8, there was no significant difference in the geographical distribution of all the specific antinuclear antibodies except for anti-dsDNA (p-value=0.013), which goes to affirm that anti-dsDNA exhibit ethno geographic variability in the Ghanaian population. This may influence the incidence of autoimmune diseases, especially Systemic Lupus erythematosus in these ethnic and geographic distribution. Majority of the autoantibodies tested negative in most regions except for RNP and RNPA that tested positive in all the regions. This finding correlate with findings from

Shapira and colleagues in 2011 that found that most autoantibodies tested negative across most geographic regions exhibiting no geographic variability.

5.7 CONCLUSION

It can be concluded that there is a high prevalence (14.21%) of antinuclear antibodies specifically among healthy Ghanaian adults and that seems to be influenced mainly by age and gender. ANA positive frequency does not have a strong ethnicity variability. Anti-dsDNA exhibit ethno - geographic variability.

5.8 RECOMMENDATIONS

Further studies should cover all the ten regions of Ghana in order to get a wider sample size and a holistic representation of the entire Ghana population.

Future studies should consider the determination of antinuclear antibodies in patients with different autoimmune diseases and healthy controls.

Subjects with positive antinuclear antibodies should be followed up for possible future development of autoimmune diseases, in order to establish the relationship between the development of autoimmune diseases among healthy subjects with antinuclear antibodies and evaluated for possible risk factors.

5.9 LIMITATIONS OF THE STUDY

1. Few numbers in the subgroups for subgroup analysis.
2. Not all 10 regions of Ghana were involved in the study due to limited funding.

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APPENDICES

APPENDIX I

Participant Consent Form

Participant ID……

Participant Name……...

Title: **Geographical differences in antinuclear antibodies and predisposition to autoimmune diseases among Adults from four regions of Ghana.**

Principal Investigator: Baffour Kyei Sarpong

Address: P. O. Box KS 5767, Kumasi

General Information about Research

Introduction: This consent form informs you about the background, aims and the method of this study. In addition, it explains the anticipated benefits, potential risk of the study and the discomfort it may entail. Finally, it informs you of your rights regarding participating in this study.

Purpose of the research: The prevalence of autoimmune diseases is rising rapidly. Autoimmune diseases contribute to high morbidity and significant mortality around the world. Autoantibodies that mediate the pathophysiology of the disease are thought to appear in the blood of healthy people even before the clinical onset of the disease. This study seeks to determine the presence and levels

of antinuclear autoantibodies in the serum of healthy people and determine the risks associated with the autoimmune conditions.

Procedure: Samples will be taken from healthy adults by trained personnel. The samples will be stored appropriately and transported for further processing.

Possible Risks and Discomforts

This is no major risk associated with your participation in this study apart from the slight discomfort you may get from blood samples been taken by venipuncture.

Possible Benefits

Autoantibodies will be identified in the sera of healthy Ghanaian adults of different Ethno-geographical locations will be identified. This will serve as a guideline to predict the possible occurrence and risk of the different types of autoimmune diseases in various ethnicities and people located in different geographic locations in Ghana. Subjects' positive for autoantibodies will be counselled on preventive measures, followed up to determine the future development of autoimmune diseases and subsequently provide prompt management. The prevalence of autoantibodies in a geographic location can provide a causal link and risk determination in the development of autoimmune diseases.

Confidentiality

Your records will be kept in a secure location at the Department of Medical Biochemistry and Department of Medicine, Korle-Bu. All information collected during the study will be stored in a file which will not have your name on it, but a study number assigned to it. Only the research team

will have access to the names associated with the study numbers. It is likely that data obtained from tests done on you may be published in medical journals; however, your identity will not be disclosed.

Voluntary Participation and Right to Leave the Research

You have the right not to take part in the study if you do not want to, and this will not affect you any way. Your position in the community will also not be affected in any way, even if you decide not to participate in the study.

Notification of Significant New Findings

Any new finding that would be of particular help to you will be relayed to you either personal or through your district hospital.

Contacts for Additional Information

Any question related to the study can be asked now or later. For any further information, you may contact any of the following:

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APPENDIX II

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research title (name of research) has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____

Name and signature Volunteer

_____

Date

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the participant. All questions were answered and the participant has agreed to take part in the research.

_____

Name and signature of witness

_____

Date

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____

Name Signature of Person Who Obtained Consent

_____

Date

APPENDIX III

QUESTIONNAIRE

Title: **Geographical differences in antinuclear antibodies and predisposition to autoimmune diseases among Adults from four regions of Ghana**

Good morning (afternoon/evening). Thank you for agreeing to take part in this study. Our questions will cover your health, personal habits, hobbies and jobs and family history. Many of the questions will ask you to think back in time to your childhood and your teenage years. We acknowledge that there will be difficulty in recalling some things. Kindly provide the best possible answer. All answers and information provided will be confidential as required by law.

Before we begin, do you have any questions?

Date: ID: Contact No.:

1. **Demographics**

A. Name: ……..

B. Sex: Male: [ ] Female: [ ]

C. Age: 18-30 years[ ] 31-40 years [ ] 41- 50 years 51-[ ] 60 + years [ ]

D. Ethnicity: …… Tribe: …….. Nationality: …….

E. Place of Birth: …….

F. From birth through age 15, where have you lived for long? Town: ……

Region: ……..

G. Where have you lived for the past 10 years? Town: ……...

Region: ……..

H. What is your highest level of education?

Primary [ ] JHS [ ] SHS [ ] Vocational/Technical [ ] Tertiary [ ]

I. Have you heard of autoimmune diseases? Yes [ ] No [ ]

J. If yes, where did you hear about it?

a) Internet [ ]
b) Radio [ ]
c) Television [ ]
d) Newspapers [ ]
e) Social Media Platform [ ]

2. Early Environment

A. Where have you lived for long from childhood till 15 years?

a) In a city (>100,000pop.) [ ]
b) In a suburb [ ]
c) In a rural area (not on a farm) [ ]
d) In a rural area (on a farm) [ ]

B. Where have you lived for the past 15 years?

C. In a city (>100,000pop.) [ ]

D. In a suburb [ ]

E. In a rural area (not on a farm) [ ]

F. In a rural area (on a farm) [ ]

G. What has been your source of drinking water up to age 15?

a) Pipe born-water [ ]

b) Private system [ ]

c) Well water [ ]

d) Bore-hole [ ]

e) Bottled water/ sachet [ ]

f) Rain harvest [ ]

g) Others, Specify……

H. How frequent did you get sunburned as a teenager?

a) Once a year

b) Greater than once a year

c) Once every few years

3. Medical History

A. Do you have any chronic medical condition? Yes [ ] No [ ] If Yes specify……

B. Did a doctor ever say you had? How old were you when you were diagnosed? [ ]

a. Psoriasis [ ]

b. Asthma [ ]

c. Hay fever [ ]

d. Allergy to a food [ ] If Yes, specify……..

e. Eczema [ ]

f. Hives [ ]

C. Are you allergic to [ ] Age at first reaction? [ ]

a. Codeine [ ]

b. Penicillin [ ]

c. Sulfa containing drugs [ ]

d. Others, Specify……

D. Did you ever vaccinated for hepatitis B? How old were you when you vaccinated?

	more than once a year	once a year	never	don't know
a. cold	[ ]	[ ]	[ ]	[ ]
b. flu	[ ]	[ ]	[ ]	[ ]
c. cold sore on or around the mouth	[ ]	[ ]	[ ]	[ ]
d. Malaria	[ ]	[ ]	[ ]	[ ]

F. have you ever had;

	Yes	No
a. any kind of hepatitis	[ ]	[ ]
b. infectious mononucleosis	[ ]	[ ]
c. TB	[ ]	[ ]
d. Shingles	[ ]	[ ]
e. urinary tract infections	[ ]	[ ]

G. a Have been transfused with blood before? Yes [ ] No [ ] Don't Know [ ]

b. How many times have you been transfused?.....

c. Reason for transfusion? At what age?

H. Have you had any surgically implanted device for more than a month?

If yes, what was implanted …….. How old were you …….

I. Have you had a time or times of stressful situations? Yes [ ] No [ ]

If yes, how old were you? ……

Briefly tell me about one or a few of them

a. ……..

b……

c. ……..

4. Family History

Has any of your immediate relative been diagnosed of any of the following diseases?

	Mother	Father	Sibling1	Sibling2	Sibling 3	
a) SLE	[ ]	[ ]	[ ]	[ ]	[ ]	
b) Thyroid dx	[ ]	[ ]	[ ]	[ ]	[ ]	……
Specify type: …….						
c) Rheum. Arth	[ ]	[ ]	[ ]	[ ]	[ ]	…….
d) Cancer	[ ]	[ ]	[ ]	[ ]	[ ]	…….
Specify type: …….						
e) Multiple Scl	[ ]	[ ]	[ ]	[ ]	[ ]	…….
f) Diabetes Mellitus	[ ]	[ ]	[ ]	[ ]	[ ]	

Specify type: ……

g) Allergies [ ] [ ] [ ] [ ] [ ]

Specify type: ……

5. Occupational History

A. What is your current occupation? ……..

B. How long have you been in this occupation? <1year…… 1-5years……...10-15years…….>15 years

C. Did you ever work at any of these jobs?

a. Clothing, manufacturing and textile? .....Duration……...

b. Pottery, ceramics? ..... Duration

F. Grinding of rocks or stone, brick layer or a quarry, brick mason? ..... Duration……...

G. a. print making or silk screening [ ]

b. photography [ ]

c. furniture refinishing [ ]

6. Reproductive history

a) How old were you when you had your first menses? .....

b) How old were you when you turned menopausal? ……..

c) How many pregnancies have you had? .....

d) How many pregnancies have you lost? ..... Number of spontaneous miscarriage…….
Number of induced miscarriages…….

e) Have you used any contraceptive method? Yes[ ] No[ ]

f) If yes, please specify…….

g) How long did you use the above mentioned contraception?

7. Dietary

For the past 5 years, did you take any vitamins or minerals for at least 6 months in total? Yes [ ] No [ ]

If yes, did you take? Yes [ ] No [ ] Don't Know [ ]

a) Zinc? [ ]

b) Folic acid? [ ]

c) Iron? [ ]

d) Selenium [ ]

e) Vitamin A [ ]

f) Beta carotene [ ]

g) Vitamin C [ ]

h) Vitamin E [ ]

i) Multivitamin [ ]

Selenium [ ]

j) Vitamin A [ ]

k) Beta carotene [ ]

l) Vitamin C [ ]

m) Vitamin E [ ]

n) Multivitamin [ ]

o)

p) Anything else? SPECIFY: ……

8. Habits

a) Do you take alcohol? Yes[ ] No[ ] If yes specify type…….

b) How long have you used alcohol? <1 year[ ] 1-5 years[ ] 5- 10 years[ ] > 10 years[ ]

c) Do you smoke? Yes [ ] No [ ] If yes, what do you smoke? Specify……

d) How long did you smoke? <1 year……. 1-5 years……. 5- 10 years……. > 10 years……..