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Determination of Antinuclear Antibody and Systemic Lupus Erythematosus in Selected Patients Attending University Medical Center (Sickbay) A.B.U, Zaria Nigeria

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Abstract
SLE is an auto-immune disease that is largely under-reported in most developing countries as the syndrome often resembles rheumatoid arthritis. This research was therefore carried out to determine, the level of Antinuclear, Antibody (ANA) in patients with suspected Systemic Lupus Erythematosus (SLE) in University Medical Center (Sickbay) Samaru, Zaria. Out of 100 blood samples collected, 23 samples (23%) were tested positive for SLE and 77 samples (77%) were tested negative for SLE. Then 92 out of the 100 samples were also screened for ANA, where 55 samples (59.8%) were found to be positive and 37 samples (40.2%) were tested negative for ANA. From ANA positive patients, 22 (40%) were found to be SLE positive and 33 (60%) were found to be SLE negative, while among ANA negative patients none was found to be SLE positive and 37 (100%) were found to be SLE negative. Age-range of 31-40years had the highest prevalence among the SLE patients with 7 (46.7%) positive and below 20 and above 50 years had the lowest prevalence. Among the ANA positive patients, age range of 31-40 years had the highest prevalence of 21 (85.7%) and above 50 years had the lowest 9 (34.6%) prevalence. Hepatitis patients had the highest prevalence 8 (57.1%) among the SLE positive patients. Finally, this research revealed that female patients had the highest prevalence with a total of 20 positive (33.3%) out of the 23 SLE positive patients and also female patients had the highest prevalence of 37 (66.9%) out of the 55 patients tested positive for ANA. In respect to this, females should therefore avoid doing things that will increase the risk of infections since they are at high risk of been infected. It is therefore recommended among others that ANA tests should be recommended as major diagnosis of SLE as the disease is confirmed to be the major triggering factor for ANA.
Keywords: Systemic Lupus Erythematosus, Antinuclear, Antibody (ANA), rheumatoid arthritis

1. Introduction

The first clear description of lupus erythematosus was by Biett and was reported by his student Cazenave under the term erythema centrifugum in 1833 (Hargraves 1969). In 1846 Hebra, under the name of Seborrhea Congestiva described disc-shaped patches and introduced the butterfly simile for the Malar rash (Petri, *et al.,* 2005). In 1851 Cazenave renamed erythema centrifugum, calling it lupus erythematosus and gave a classic description of discoid lupus erythematosus. In 1972 Kaposi subdivided lupus into the discoid and systemic disease with a potential fatal outcome (Janvierre and Richardson 2011). The symptoms associated with SLE vary from person to person and may be transient depending on what part of the body is affected making symptom-based diagnosis difficult because lupus mimics many other diseases (Ben-Menachem, 2010). Most people with lupus have mild disease characterized by episodes-called-flares-when signs and symptoms get worse for a while, then improve or even disappear completely for a time (Deng and Tsao 2010).

SLE is an autoimmune diseases of unknown aetiology (D'cruz, 2007) and often mistaken for other illnesses (Gimzler, 2002). There are thousands of nuclear proteins within the host that could serve as auto-antigens but the common ones that occur in SLE are mainly RNA-protein or DNA-protein complexes comprising of multiple proteins physically associated with nucleic acid. Importantly, the nucleic acid constituents of these antigens are ligands for innate immune system receptors called "Toll-like receptors" (TLRs) 3, 7, 8, and 9 localized within endosomal compartments (Kawai and Akira, 2006). In most patients, there is a marked and elevation production of autoantibodies against cellular antigens; mainly nuclear antigens, such as anti-histones (anti-HST), anti-single-stranded DNA and anti-double- stranded DNA (anti-dsDNA) antibodies (Hannahs, 1998). These are directed against a great variety of organs and tissues, the mostly involved are kidneys, bone marrow, skin, nervous system, joints, muscles, heart and gastrointestinal tract (Talaro, 2002). The association between anti-DNA antibodies and lupus is evident in the fact that a subset of antinuclear antibodies can directly cause nephritis, one of the major causes of morbidity and mortality in SLE. Thus, a variety of antinuclear antibodies can be eluted from diseased glomeruli (Mok, 2003) and in many patients, high titres of anti-DNA antibodies correlate with nephritis. Anti-dsDNA antibodies are considered the main diagnostic tool for SLE and a useful marker of disease activity; however, they are found only in 50% of SLE patients and do not always correlate with disease activity (Villarreal et al., 1997; Pisetsky, 2000). Interest in the disease has been stimulated in recent years, and improved methods of diagnosis have resulted in a significant increase in the number of cases recognized (Hahn, 2003). The study therefore was aimed at determining the prevalence and

ANA positivity rate in a cross section of patients presenting at the University Health Centre in Zaria.

2. Methodology

2.1 Study Area and Population:

The research was conducted among patients with suspected Systemic Lupus Erythemtosus (SLE) attending University Medical Center (Sickbay) ABU Samaru, Zaria. The Medical Centre serves the health needs of University Staff (majorly adults) as well as undergraduate and graduate students (who are mostly young adults). Since SLE presents with a variety of presenting features and manifestations and symptoms often vary from person to person, patients that present with fever, malaise arthralgias, myalgias, headache, loss of appetite and weight and fatigue were regarded as suspected cases and included in the study. A structured questionnaire was administered on the patients to obtain some demographic and risk factors that might be associated with the disease. Permit for the study was granted by the ethical authorities of the Hospital and informed consent was obtained from the participating patients.

2.2 Collection of Samples: A total of 100 blood samples were collected from patients with suspected SLE attending University Medical Center (Sickbay) ABU Zaria in heparinized bottles and transferred immediately to the Microbiology Laboratory, ABU. They were then centrifuged for 15 minutes at 2500rpm for 5 minutes of collection and stored at -20^{0}C.

2.3 Determination of SLE Status by Slide Agglutination Test

The test was performed using the SLE test kit (Diagnostic Automation USA) in accordance with the manufacturer's instruction. The principle of the SLE test is based on the agglutination reaction between latex particles coated with DNA being brought in contact with autoantibodies present in the patient's serum, and if the autoantibodies are present, a positive reaction is observed. The kit contains SLE latex reagent (a polystyrene latex particle coated with DNA extracted fetal calf thymus, preserved in 0.1% sodium azide), SLE positive and negative control (diluted and stabilized with buffers preserved in 0.1% sodium azide) disposable pipette and glass slide. All reagents and serum samples were brought to room temperature. Using disposable pipettes, a drop (0.045) of the positive and negative control and the patient's serum were placed on a different point on a slide or tile. A drop of the SLE latex was added on each of the specimen on the slide or tile. The end of the pipette was used to spread and mix the specimen. Different pipette was used for each sample. Then specimen was gently rocked, observing for agglutination all within one minute, the presence of agglutination indicated a positive result and its absence indicated a negative result.

2.4 Determination of ANA Status by Enzyme Linked Immunosorbent Assay ELISA

The diagnostic automation Inc. ANA screen ELISA test system is designed to detect IgG class antibodies to a variety of common nuclear antigens in human sera.

The individual components were removed from storage and allowed to warm up to room temperature (20-25^{0}C). The number of the microwells needed was determined. Six control/calibrator determinations (one blank, one negative control, three calibrators and one positive control) were allowed per run. A reagent blank was run on each assay. Then a 1:20 dilution (e.g. 10µL of serum + 200µL of sample diluent) was prepared of negative control, calibrators, positive control, and each patient specimen was added to the individual wells. A total of 100µL of sample diluent was added to well A, as a reagent blank well configuration. The plate was incubated at room temperature (20-25^{0}C) for 60 to 65 minutes. Then the microwell strips were washed 5 times. A total of 100µL of the conjugate was added to each well, including reagent blank well, at the same rate and in the same order as the specimens were added. The plate was incubated at room temperature (20-25^{0}C) for 30 to 35 minutes. The microwells were washed again and 100µL of TMB was added to each well, including reagent blank well at the same rate and in the same order as the specimens were added. The plate was then incubated at room temperature (20-25^{0}C) for 30-35 minutes. Then the reaction was stopped by adding 50µL of stop solution to each well, including reagent blank well, at the same rate and the same order as the TMB was added. After adding the stop solution, the plate was tapped several times to ensure that the samples were thoroughly mixed. The microwells reader was set to read at wavelength of 450nm and the optical density (OD) of each well against the reagent blank was measured.

3. Results

The results obtained after laboratory analysis of the samples collected shows that among the 100 samples collected and screened for SLE, 23 samples (23%) are SLE positive and 77 samples (77%) are SLE negative (fig. 1). Among SLE negative patients 33 samples (60.0%) tested positive for ANA, and all the 22 SLE positive patients screened for ANA tested positive (table 1).

In respect to age, one of the samples 1(3.0%) tested positive for SLE and 9 samples (34.6%) tested

positive for ANA within the age range of > 50years. Within the age range of 21-30 years, 10 samples (30.3%) tested positive for SLE and 22 samples (71.0%) tested positive for ANA. A total of 7 samples (46.7%) tested positive for SLE and 12 samples (85.7%) tested positive for ANA within the age range of 31-40 years while 6 samples (31.6%) tested positive for SLE and 10 samples (62.5%) tested positive for ANA within the age range of 41-50 years (table 2). The prevalence of ANA and SLE were significantly associated with age ($p < 0.05$)

In respect to gender, 20 samples (33.3%) tested positive for SLE and 37 samples (66.9%) tested positive for ANA among female patients. Then 3 samples (7.5%) tested positive for SLE and 18 samples (50.0%) tested positive for ANA among male patients (table 3). There was no significantr association between ANA and gender while the prevalence of SLE was significantly higher in females ($p < 0.05$).

In respect to type of illness, 4 samples (22.2%) tested positive for SLE and 9 samples (60.0%) tested positive for ANA among hypertensive patients. 14 samples (48.3%) tested positive for SLE and 5 samples (17.2%) tested positive for ANA among diabetes patients. 2 samples (14.3%) tested positive for SLE and 9 samples (69.2%) tested positive for ANA among patients with fever. 8 samples (57.1%) tested positive for SLE and 8 samples (66.7%) tested positive for ANA among hepatitis patients. Among patients with malaria, 3 samples (18.8%) tested positive for SLE and 10 samples (66.7%) tested positive for ANA. Only 1 sample (11.1%) among typhoid patients tested positive for SLE and 5 samples (62.5%) tested positive for ANA (table 4).

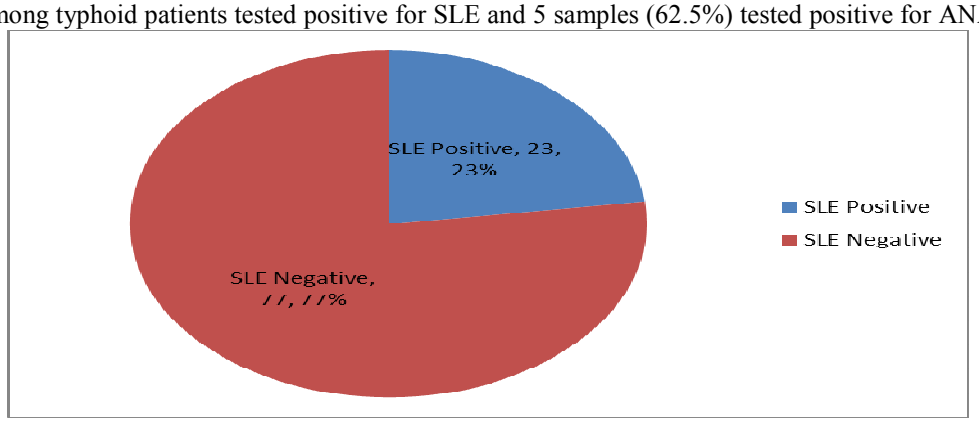

Figure 1: Prevalence of SLE in the study Population

Table 1: Prevalence of Antinuclear Antibody (ANA) in respect to Systemic Lupus Erythematosus (SLE)

ANA	N	SLE	
		No. Positive (%)	No. Negative (%)
Negative	37	0 (0.0)	37 (100)
Positive	55	22(40.0)	33 (60.0)
Total	92	22 (23.9)	70 (76.1)

X^2=19.451, df = 1, P = 0.000; ANA is significantly associated with SLE ($P < 0.05$)

Table 2: Prevalence of Antinuclear Antibody (ANA) and Systemic Lupus Erythematosus (SLE) in respect to Age

AGE	ANA*			SLE*		
	n	No. (%) Positive	No. (%) Negative	n	No. (%) Positive	No. (%) Negative
10-20years	5	2 (40.0)	3 (60.0)	5	0(0%)	5(100%)
21-30years	31	22 (71.0)	9 (29.0)	33	10(30%)	23(70%)
31-40years	14	12 (85.7)	2 (14.3)	14	6(43%)	8(57%)
41-50years	16	10 (62.5)	6 (37.5)	19	6(32%)	13(68%)
Above 50years	26	9 (34.6)	17 (65.4)	29	1(3%)	28(97%)
Total	92	56 (59.8)	37 (40.2)	100	23	77

ANA - X^2= 13.241, df = 4, P = 0.010; SLE - X^2= 16.385, df = 4, P = 0.003; * Significant association

Table 3: Prevalence of Antinuclear Antibody (ANA) and Systemic Lupus Erythematosus (SLE) in respect to Gender

Gender	ANA**			SLE*		
	n	No. (%) Positive	No. (%) Negative	n	No. (%) Positive	No. (%) Negative
Female	56	37 (66.9)	19 (33.9)	60	20 (33.3)	40 (66.7)
Male	36	18 (50.0)	18 (50.0)	40	3 (7.5)	37 (92.5)
Total	92	55 (59.8)	37 (40.2)	100	23	77

ANA - X^2= 2.354, df = 1, P = 0.125; SLE - X^2= 6.362, df = 1, P = 0.012
** No significant association; * Significant association

Table 4: Prevalence of Antinuclear Antibody (ANA) and Systemic Lupus Erythematosus (SLE) in respect to Age

Type of Illness	ANA**			SLE*		
	n	No. (%) Positive	No. (%) Negative	n	No. (%) Positive	No. (%) Negative
Hypertensive	15	9 (60.0)	6 (40.0)	18	4 (22.2)	14 (77.8)
Diabetes	29	14 (48.3)	15 (51.7)	29	5 (17.2)	24 (82.8)
Fever	13	9 (69.2)	4 (30.8)	14	2 (14.3)	12 (85.7)
Hepatitis	12	8 (66.7)	4 (33.3)	14	8 (57.1)	6 (42.9)
Malaria	15	10 (66.7)	5 (33.3)	16	3 (18.8)	13 (81.2)
Typhoid	8	5 (62.5)	3 (37.5)	9	1 (11.1)	8 (88.9)
Total	92	55 (59.8)	37 (40.2)	100	23 (23.0)	77 (77.0)

ANA- X^2= 2.637, df = 5, P = 0.756; SLE - X^2= 11.246, df = 5, P = 0.047
** No significant association; * Significant association

4. Discussion

The prevalence of SLE in this study was 23% and similar to that in our earlier study (27.7%) indicating that the trend has not changed considerably as well as the predisposing factors within the community (Ella and Agibilly, 2015). SLE remains an age (P = 0.003) and gender (P = 0.012) associated autoimmune disorder, therefore in respect to age of the respondents, patients that were within the age range of 31-40 had the highest prevalence of SLE, and age range below 21 had the lowest prevalence, confirming the fact that SLE is mostly found among the patients of higher ages especially reproductive age. This association with age and gender is in agreement with the work of Ella and Agibilly, (2015). However, ANA had no association with age and gender. Antinuclear antibodies (ANAs) are unusual antibodies, detectable in the blood, that have the capability of binding to certain structures within the nucleus of the cell. ANAs are found in patients whose immune system may be predisposed to cause inflammation against their own body tissues. The most common autoimmune disease related to ANAs is known to be SLE. The antigens of an organism often regarded as self-antigens in some circumstances elicit antibody response regarded as auto-antibodies (Salamunic, 2010). This phenomenon culminate in the breakdown of the self-tolerance in which both humoral and cell mediated responses are initiated and targeted on several altered and intact host cells (Lernmark, 2001). ANA level had higher prevalence among SLE positive patients. According to researches it is well documented that SLE remains the major factor that trigger high ANA level (Yamamoto, 2003). According to previous researches almost all people with SLE had a positive ANA test result. This research demonstrated the presence of ANA as the most prevalent antibodies in the sera of patients positive for SLE, emphasising the importance of ANA in the diagnosis of SLE. Some workers demonstrated ANA activity in only 50% of patients with SLE indicating that it has a low specificity even though generally considered as the main diagnostic tool for SLE and a useful marker of disease activity (Villarreal et al., 1997; Pisetsky 2000). It was also found that some patients negative for SLE had ANA in their sera and this is because ANA are found in several systemic autoimmune diseases and have been demonstrated in some healthy patients (Fritzler and Salazar 1991). This was demonstrated by the distribution of SLE and ANA among patients presenting with varied symptoms.

Therefore ANA level is active among SLE positive patients and the incidence of SLE remains high in Zaria.

5. Conclusion

all of the patients tested positive for SLE were also tested positive for ANA with a 40% prevalence, and “P” positivity rate of ANA is significantly correlated with that identified by SLE with a P-value of 0.000 (P<0.05). This shows that SLE is confirmed to be one of the major factor that triggers the presence of ANA in patients. And 33 samples (60%) were tested positive for ANA from SLE negative patients which indicate that apart from

SLE, other autoimmune diseases may also trigger ANA. So it is advisable for patients who had ANA positive result to be tested for SLE and other autoimmune diseases as well.

Then SLE in relation to age, gender and type of illness was found to be having a significant relationship with a p-values of 0.003, 0.012 and 0.047 respectively, while that of nature of illness was not with a p-value of 0.985 (P>0.05).

Then for ANA, it was found that their relationship between gender, type of illness and nature of illness was not significant with a p-values of 0.125, 0.756 and 0.636 respectively, while only that of age was significant with a p-value of 0.010 (P<0.05)

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