



(RESEARCH ARTICLE)



Prevalence of systemic lupus erythematosus amongst child-bearing female patients attending University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria

Asuzu-Samuel Henrietta Ogadimma ^{1,*} and Oluwatayo, Beatrice Olatundun ²

¹ *Biomedical Technology Option, School of Science Laboratory Technology, University of Port Harcourt, Choba, Rivers State, Nigeria.*

² *Department of Haematology & Blood Group Serology, Federal College of Veterinary & Medical Laboratory Technology, Vom, Plateau State, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2021, 14(03), 090–097

Publication history: Received on 21 January 2021; revised on 27 February 2021; accepted on 02 March 2021

Article DOI: <https://doi.org/10.30574/gscbps.2021.14.3.0052>

Abstract

Systemic lupus erythematosus (SLE), a chronic and progressive multisystem autoimmune disorder is chiefly mediated by immune complexes in which the body's immune system produce antibody (mostly antinuclear antibody) against normal cells and organs leading to inflammatory injuries. Several studies on this disease have been done in different parts of the world but very little exist in Nigeria, particularly in Port Harcourt. The study aim to determine the prevalence of systemic lupus erythematosus in Child-bearing female patients between the age ranges of 15 – 45 years, attending University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. Ethical approval was sought for and obtained from the Ethical committee of the University of Port Harcourt Teaching Hospital. A total sample size of 207 was used. 5ml of blood samples were collected with 5ml syringe and needle into non-heparinized bottle from the antecubital fossa of recruited study group between 8am and 11am each day and was taken to the laboratory for analysis. De-fibrination method was used with the aid of centrifuge and microscope for detection of LE cell. Using an SLE latex reagent, the serum was analyzed for the presence of antinuclear antibody after centrifuging for 10mins. The SLE test kit used contained positive and negative controls to which the results were compared. Results gotten were all negative for the test group. This indicates a zero (0) prevalence level of systemic lupus erythematosus amongst child-bearing female patients attending the University of Port Harcourt Teaching Hospital.

Keywords: Syetemic; Lupus erythematosus; Immune; Antibody; Inflammation

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, potentially fatal multisystem inflammatory disorder that can be difficult to diagnose, Edworthy [1]. It is a multisystem autoimmune disorder Ramnik [2] in which auto antibodies to nuclear proteins are produced, including anti-DNA antibodies and immune complexes causing tissue damage, Cheesbrough [3]. SLE is also a progressive multisystem autoimmune disorder chiefly mediated by immune complexes in which the body's immune system produce antibody against normal cells and organs leading to inflammatory injuries, Wahren-Herlenius et. al. [4]. In a normal self, the immune system produce proteins called antibodies that protect the body against pathogens (antigens) such as viruses and bacteria, but in systemic lupus erythematosus, the antibodies present; most commonly anti-nuclear antibodies which are found in nearly all cases of systemic lupus erythematosus; usually greater than 90% Earl and Allison [5], are rather directed against a person's own proteins; and the subsequent consequence of the attack of these antibodies on the body's own protein is

* Corresponding author: Asuzu-Samuel Henrietta Ogadimma

Biomedical Technology Option, School of Science Laboratory Technology, University of Port Harcourt, Choba, Rivers State, Nigeria.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.

inflammation. The disease has no single diagnostic marker; instead, it is identified through a combination of clinical and laboratory criteria, Petri [6]. A lupus erythematosus cell (LE cell) is a neutrophil which contains a basophilic, opaque, homogeneous mass in its cytoplasm. The nucleus is located towards the periphery and appears crescent shaped around the ingested mass. Systemic lupus erythematosus (SLE) is an autoimmune disease that affects five to six million people worldwide. Systemic lupus erythematosus usually comes with a broad spectrum of clinical presentations, encompassing almost all organs and tissues, ranging from minor cutaneous involvement to severe major organ damage. Autoantibodies are generated and directed against multiple organs, including the heart, brain, lungs, kidney, joints, blood and skin and as a result of this multisystem effect, it is considered as a potential diagnosis in all patients who present with multi-organ system disease, Mok et al. [7]. The diagnosis of SLE can be a long and slow process due to the manifestation of a diverse set of symptoms, which include psychological, cardiovascular, musculoskeletal, and nephrology complications, Paul and Frank [8]. Although people with the disease may have many different symptoms, some of the most common ones include extreme fatigue, painful or swollen joints (arthritis), unexplained fever, malaise, typical facial 'butterfly' skin rashes, cytopenias, central nervous system complications, cardio-pulmonary, and kidney problems. Although the age of onset of systemic lupus erythematosus is between the 15 years and 45 years, the disorder can occur in childhood or in individual older than 45 years, Ramnik [2].

This immunological self-intolerance is regarded as an early hallmark of systemic lupus erythematosus and it has become clear that this is due to a complex process involving a variety of molecules and cells, Hahn [9]. Accurate diagnosis of systemic lupus erythematosus is important because treatment can reduce disease related morbidity, McGrath, Martinez-Osuna, and Lee; Dammacco et. al.; Carneiro and Sato; Alvarez-Nemegyei et. al.; Johnson et. al. [10,11,12,13,14] and mortality.

Genetic, viral, and environmental factors are thought to be involved in the development of systemic lupus erythematosus, Cheesbrough [3]. There is a striking gender disparity in the burden of systemic lupus erythematosus as it is reported to be markedly more prevalent in women (nine times) than in men (9:1), having its incidence highest in pre-menopausal women, with increasing incidence after puberty, Danchenko et. al. [15]. It is also more common in those of African-American or Caribbean descent, with a global disease rate varying from 20 to 70 per 100,000, Lisnevskaja et al. [16].

The condition is said to be uncommon in most African tropical countries. It is found in Southeast Asia, in people of African origin living in the USA and in other countries. In the United States, systemic lupus erythematosus is reported to be more common in women (particularly black women) than in white men, Lawrence et al. [17]. Young women are most commonly affected, Cheesbrough [3]. While the etiology of the disease is still not fully understood, the varied penetrance and lack of concordance of the disease amongst genetically identical twins suggest that both susceptible genetic profiles and environmental triggers are likely instrumental in inducing the onset of the disease and disease flare, Sherer et al. [18].

2. Material and methods

Ethical approval was first obtained from the University of Port Harcourt Ethical Committee before the commencement of the study. Informed consent was sought and obtained from each patients prior to collection of the blood samples. 5mls of blood were collected from each of the female patients between 8:00 and 11:00 am each day. While in the sitting position, the tourniquet was applied on the upper arm above the cubital fossa. The usual precaution of selecting an easily accessible vein in the anti cubital fossa and applying the minimum of venous stasis was observed and venous blood was drawn by venipuncture into glass bottles which contained glass beads. All analysis was carried out within 3hrs of blood collection each day. Four milliliters of venous blood were collected in a bottle which contains few glass beads. Screening test for SLE was done by De-fibrination method which involved mixing blood for 15 minutes by shaking until a fibrin clot was formed. The blood was incubated at room temperature and mixed intermittently for 2 hours. The blood sample was aspirated into a narrow bore test tube and centrifuged at 1200 rpm for 20 minutes to obtain the buffy coat layer between the plasma and the red cells. The capillary tubes were then broken with caution between a layer of tissue and this was smeared on a glass slide. The 2 smears made from buffy coat layer were fixed and stained by Leishman stain. Each slide was mounted and examined under $\times 100$ oil immersion objective for LE cells. Qualitative and Semi-Qualitative method of analysis with SLE Latex reagent (Teco Diagnosis 2012) was further carried out. In Qualitative test, all reagents and serum samples were brought to room temperature. Each series of test sera were tested with positive and negative control. 0.045 ml test serum was dropped with separate disposable pipettes onto the glass slide circle. 0.045 ml of SLE Latex reagent was dropped to each circle that contained specimen on the slide and mixed with the paddle end of the pipette. (Different paddle end was used to mix each test serum or control to avoid contamination). Slide was gently tilted and rotated by hand for one minute. The preparation was observed for macroscopic clumping using the indirect oblique light source and the reaction of the test serum was compared to the

SLE positive and negative control sera, within 1 minute. While in the Semi-Qualitative test, 6 test tubes (12 × 75 mm) were labeled for each test serum titrated. 0.2 ml of physiological saline was added to each test tube. 0.2 ml of undiluted test serum was added to tube no.1. Serially, two-fold dilutions was made by mixing contents of tube no. 1 with a pipette and transferred 0.2 ml to tube no. 2 and this was repeated up to no. 6 tubes and the last discarded, the dilutions ranged from 1:2 to 1:64. The rest of the procedure was carried out just like in steps of the qualitative test above. The results were interpreted such that agglutination showed positive results while negative results were shown as smooth milky suspension.

3. Results

Agglutination on the test kits gives a positive test result for each of the blood sample tested. All data obtained from the questionnaire and laboratory diagnosis carried out and results reported



Figure 1 Blood film of buffy coat of de-fibrinated cell

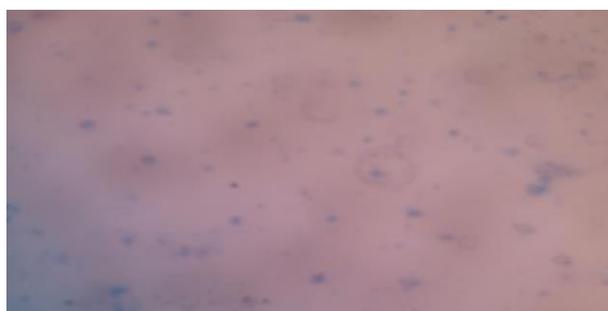


Figure 2 De-fibrinated blood film buffy coat.

Table 1 Results of the screening test for Systemic Lupus Erythematosus

STUDY GROUPS	TEST	RESULT
1-19	-	-
20-39	-	-
40-59	-	-
60-79	-	-
80-99	-	-
100-119	-	-
120-139	-	-
140-159	-	-
160-179	-	-
180-199	-	-
200-207	-	-

Table 2 Study Results of Test Kit Method for SLE in 207 of Child-bearing female patient samples.

STUDY GROUPS	POSITIVE CONTROL	NEGATIVE CONTROL	TEST RESULT
1-19	+	-	-
20-39	+	-	-
40-59	+	-	-
60-79	+	-	-
80-99	+	-	-
100-119	+	-	-
120-139	+	-	-
140-159	+	-	-
160-179	+	-	-
180-199	+	-	-
200-207	+	-	-

3.1. Mean age of patients

In order to obtain the mean age of the patients (15 – 45) years of age, a frequency distribution table was constructed using a class limit of three (3)

Table 3 Age frequency distribution table of the subjects.

CLASS INTERVAL (YEARS)	FREQUENCY (f)	x	Fx
15-18	5(2%)	17	85
18- 21	8(4%)	20	160
21- 24	15(7%)	23	345
24-27	45(22%)	26	1170
27-30	41(20%)	29	1189
30 - 33	36(17%)	32	1152
33-36	22(11)	35	770
36 - 39	12(6)	38	456
39 - 42	14(7)	41	574
42 - 45	9(4)	44	396
	207		6297

Arithmetic Mean (A.M) is given by the formula below:

$$A.M = \frac{\sum fx}{f}$$

Since $\sum fx = 6297$

$$A.M = \frac{6297}{207}$$

∴ A.M = 30.4

Therefore, the mean age of the patients involved in the research is approximately 30 years.

Table 4 Frequency distribution table for no of children of subjects.

NO OF CHILDREN	FREQUENCY (f)
0	11(5%)
1	14 (7%)
3	38 (18%)
4	63 (30%)
5	44 (21%)
6	37 (18%)
	207

Table 5 Occupation distribution of the child-bearing female patients.

OCCUPATION	FREQUENCY (f)
House Wife	19 (9%)
Private Establishment	42 (20%)
Civil Servant	59 (29%)
Self Employed	74 (36%)
Unemployed (single)	13 (6%)
	207

Table 6 Some Haematological Parameters amongst child-bearing Patients

Parameters	Test sample	Control sample	P- Value
PCV (%)	25 ± 6.4	38± 8.2	p<0.05
Total WBC (×10 ⁹ /L)	8.3 ± 5.3	5.5 ± 2.3	p<0.05
Differential WBC (%)			
Neutrophil	30.77 ± 8.74	55.6 ± 7.87	p<0.05
Eosinophil	2.0 ± 1.21	5.4± 1.50	p<0.05
Lymphocyte	51.77 ± 6.59	30.5± 7.08	p<0.05
Monocyte	14.56 ± 5.57	6.7 ± 2.30	p<0.05
Basophil	1.86 ± 1.08	1.7 ± 1.2	p>0.05
Platelets (×10 ⁹ /l)	143.0 ± 36.28	171.72 ± 23.6	p<0.05
ESR (mm/H)	13.6 ± 4.3	4.7 ± 2.8	p<0.05

All values are expressed as mean ± S.D, n = 207

The result was analyzed using student t-test. There was marked increase in ESR, Total White cell and Lymphocyte counts while PCV, Neutrophil, Lymphocyte and platelets were low when compared with the control.

4. Discussion

The antinuclear antibodies (ANA) also known as antinuclear factors (ANF) are unwanted molecules which bind and destroy certain structures within the nucleus. In systemic lupus erythematosus (SLE), they are produced in excess; hence their detection in the blood of patients is important for diagnosis and monitoring of the disease. However, in this part of the world, the disease is rarely known with little research work done on the subject matter, although symptoms such as malar rash are seen on the faces of some women. As a result, it is important to research on the prevalence of this disease that can wreak havoc on the health of an individual if left untreated and to know the prevalence level of the disease in this part of the world. In this study, the blood samples of two hundred and seven (207) female patients between 15– 45 years of age were screened for the presence of LE factor. Blood samples were collected from patients attending clinical investigation in the Haematology Department of the University of Port Harcourt Teaching Hospital and the laboratory investigation was done in Human Physiology Laboratory, University of Port Harcourt, Choba, Rivers state, Nigeria. The blood was tested for systemic lupus erythematosus. The result of the laboratory investigation showed no positive reaction for all the samples analyzed, indicating that systemic lupus erythematosus (SLE) was negative for all the samples involved, so also after the confirmatory test with SLE latex reagent test kits. However, all precautionary measures were followed; the positive controls were tested positive while the negative controls were tested negative throughout the research study and these were the standard to which the tests were compared to.

Standard techniques were used to determine the haematological parameters (fully automated 5-part differential haematological analyzer Model Mythic 22-CT) and the results obtained showed low packed cell volume which indicates that the patients were anaemic. It also showed low neutrophil, eosinophil and low platelets counts which could be as a result of their health condition. The total White blood cell count, the lymphocytes, erythrocyte sedimentation rate and platelets was high when compares with the normal range.

There was no Lupus cell on the blood film of the buffy coat (de-fibrinated method) cells of the child-bearing patients of the study. Both the qualitative and the semi-qualitative test methods did not show any form of agglutination in the study. From this conducted study, it is an evident that the prevalence of systemic lupus erythematosus in two hundred and seven (207) female patients aged 15-45years is zero. When compared to other previous prevalence studies conducted around Africa, it will be observed that there is a significant difference in these studies. In Ghana and Cote d Ivoire, 11 of 25 and 9 of 25 cases were recorded in a period of 6years and 11years respectively Symmons et.al. [19]. 8 of 141 cases was also recorded in Zimbabwe in 13year, Stein et.al.[20], 5.28% (66) of 1,250 cases was recorded in 6years in Lagos, Nigeria, Adelowo and Oguntona [21] and 12.5% (12) of 96 patients were recorded in five and half years (5½years) in Ile-Ife, Osun, Nigeria, Oduola et. al. [22]; whereas, this research provides a prevalence result of zero (0) case out of two hundred and seven (207) patients in this period of 3years study.

Similarly, in Vom Platue State, 0 prevalence among 200 apparently healthy female has been found (report not yet published). So also, 3 out of group of patients in Lafia were found. (report not yet published).

5. Conclusion

There are various reports on the prevalence of systemic lupus erythematosus worldwide, but just a few reports are available in Africa and precisely in Nigeria and in Port Harcourt precisely. In Jos Platueu State Nigeria, little work has been done but not published by Ekanem B. P. (2010) and the report as was seen in the study showed no positive result among 200 female samples tested. In the same year, Akpanda, E. O. in this same Jos did another study amongst antenatal women attending Vom Christian Hospital Plateau State and found no positive results amongst the subjects (not published). Therefore, this study provides information that prevalence of systemic lupus erythematosus in University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, an oil enrich state of Nigeria, is zero (0) cases out of 207 patients samples in three years of study (3years). There was no trace of lupus cell on the blood film. The haematological parameters were low in packed call volume, neutrophil, eaosinophil and platelets while total white blood cell, lymphocytes and erythrocyte sedimentation rate were high. This could be due to some other disease condition. The study shows the age, number of children of each of subjects and different occupation of the participated subject. Since the result showed zero percent prevalence, it is worthy to note the observations from the questionnaire. Age range of the child-bearing female fell within 15 to 45 years respectively. From the table, female of 24 -26 years may be said to be the peak reproductive age amongst the studied group, while female of 15 – 18 years had a minimum frequency occurrence from the study.

Compliance with ethical standards

Acknowledgments

We acknowledge the Almighty God for His love and faithfulness during this research. We also acknowledge the staff of the University of Port Teaching Hospital and for their kind support during the period of sample collection and analysis. We are so grateful to all our family and friends that stood by us, we thank you all and pray God to bless you.

Disclosure of conflict of interest

There is no conflict of interest from the both authors, hence the manuscript be published.

References

- [1] Edworthy SM. Clinical Manifestations of Systemic Lupus Erythematosus. In: Ruddy S, Harris ED, Sledge CB, Kelley WN, eds. *Kelley's Textbook of rheumatology*. 6th ed. Philadelphia: Saunders: 2001; 1105–1119.
- [2] Ramnik, S. *Textbook of Medical Laboratory Technology; Systemic Lupus Erythematosus*. Jitendar P Vij, Jaypee Brothers Medical Publishers (P), New Delhi. 2006; 804-805.
- [3] Cheesbrough, C. *District Laboratory Practice in Tropical Countries, part 2* Press Syndicate of the University of Cambridge, United Kingdom. 2004; 295-322.
- [4] Wahren-Herlenius, M, Dörner, T. Immunopathogenic mechanisms of systemic autoimmune disease. *Lancet*. 2013; 382: 819–831.
- [5] Earl S, Allison E. *Textbook of Pediatric Rheumatology*, sixth edition. 2011; 315-343.
- [6] Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, Bruce IN, Isenberg D, Wallace DJ, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly JG, Sánchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D, Kalunian K, Costner M, Werth VP, Zoma A, Bernatsky S, Ruiz-Irastorza G, Khamashta MA, Jacobsen S, Buyon JP, Maddison P, Dooley MA, van Vollenhoven RF, Ginzler E, Stoll T, Peschken C, Jorizzo JL, Callen JP, Lim SS, Fessler BJ, Inanc M, Kamen DL, Rahman A, Steinsson K, Franks AG Jr, Sigler L, Hameed S, Fang H, Pham N, Brey R, Weisman MH, McGwin G Jr, Magder LS. Deviation and Validation of the Systemic Lupus Erythematosus International Collaborating Clinics Classification Criteria for SLE Arthritis Rheum. 2012; 64: 2677-2686.
- [7] Mok CC, Wong RW. Pregnancy in systemic lupus erythematosus. *Postgrad. Med. J.* 2002; 77: 157–65.
- [8] Paul E, Frank J. *Systemic Lupus Erythematosus: Methods and Protocols*. Humana Press. 2014; 1134.
- [9] Ohl k, Tenbrock k. Regulatory T cells in Systemic Lupus Erythematosus. *Eur J Immunol*. 2015; 45: 344-55.
- [10] McGrath H, Martinez-Osuna P, Lee FA. Ultraviolet-A1 (340–400 nm) Irradiation Therapy in Systemic Lupus Erythematosus. *Lupus*. 1996; 5: 269–274.
- [11] Dammacco F, Alberighi O, Ferraccioli G, Racanelli V, Casatta L, Bartoli E. Cyclosporine-A plus steroids versus steroids alone in the 12-month treatment of systemic lupus erythematosus. *Int J ClinLab Res*. 2000; 30: 67–73.
- [12] Carneiro JR, Sato EI. Double blind, randomized, placebo controlled clinical trial of methotrexate in systemic lupus erythematosus. *J Rheum*. 1999; 26: 1275–1279.
- [13] Alvarez-Nemegyei J, Cobarrubias-Cobos A, Escalante-Triay F, Sosa-Munoz J, Miranda JM, and Jara LJ. Bromocriptine in systemic lupus erythematosus: a double blind, randomized, placebo-controlled study. *Lupus*. 1998; 7: 414–419.
- [14] Johnson AE, Gordon C, Hobbs FD, Bacon PA. Undiagnosed Systemic Lupus Erythematosus in the Community. *Lancet*. 1996; 347: 367–369.
- [15] Danchenko N, Satia JA, Anthony MS. Epidemiology of System Lupus Erythematosus: a comparison of worldwide disease burden. *Lupus*. 2006; 15: 308–318.
- [16] Lisnevskaja L, Murphy G, Isenberg D. Systemic lupus erythematosus. *Lancet London, England*. 2014.
- [17] Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum*. 1998; 41: 778–799.

- [18] Sherer Y, Shoenfeld Y. Idiotypic network dysregulation: a common etiopathogenesis of diverse autoimmune diseases. *Appl Biochem Biotechnol*. 2000; 83: 155-62.
- [19] Symmons D. Frequency of lupus in people of African origin. *Lupus*. 1995; 4(3): 176-8.
- [20] Stein M, Davis P. Rheumatic disorders in Zimbabwe: a prospective analysis of patients attending a rheumatic diseases clinic. *Ann Rheum Dis*. 1990; 49(6): 400-2.
- [21] Adelowo O, Oguntona S. Pattern of Systemic Lupus Erythematosus among Nigerians. *J Clin Rheum*. 2009; 28(6): 699-703.
- [22] Oduola OO, Uchegbu FA, Arogundade OG, Avwioro IS, Bello, Akinjole OO. Prevalence and Pattern of Lupus Erythematosus Cell Positivity in Diseases in Ile-Ife, Nigeria. *Afr J of Biomed Res*. 2005; 8: 135.