



Some haematological variables in systemic lupus erythematosus patients Imo state Nigeria

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Abstract

The study was done to determine changes in some haematological variables in patients suffering from systemic lupus erythematosus in Imo State, Nigeria. A total of seventy (70) subjects were recruited for the study. Five (5) females were subjects suffering from systemic lupus erythematosus and sixty five (65) subjects were non-systemic lupus erythematosus females as the controls. Full blood count, erythrocyte sedimentation rate, platelet count of sufferers, and non-sufferers who served as control were determined using standard haematologic methods. t-test analysis revealed significant statistical differences in all variables ($P < 0.05$) except eosinophill in SLE ($P > 0.05$). Anaemia, leukaemia and thrombocytopenia were seen in some percentage of patients. Haematologic surveillance in systemic lupus erythematosus disease, and instituting systemic lupus erythematosus disease management programme will assist in reducing complications that may be brought about by haematologic changes and ignorance.

Keywords: Systemic Lupus erythematosus, Haematological variables, Anaemia, Leukaemia, Thrombocytopenia

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that is potentially debilitating and sometimes fatal as the immune system attacks the body's cells and tissues, resulting in inflammation and tissue damage. SLE can affect any part of the body, the heart, joints, skin, lungs, blood vessels, kidneys and nervous system. Lupus can occur at any age, but is more common in women and particularly non-caucasians in their reproductive years (Ruiz et al., 2001).

It is characterized by the production of multiple autoantibodies, typically antinuclear and anti-DNA antibodies. In some patients, autoantibodies are also produced against platelets, lymphocytes and other cellular antigens (Stevenson and Naivig, 1999).

Previous researchers reported common initial and chronic complaints as fever, malaise, myalgias and weight loss, which may equally be seen in

other diseases. Other symptom reported was arthralgia musculoskeletal. This has been reported as the most common reason patients with SLE seek medical attention. It affects small joints of the hand and wrist. Past researchers reported that as many as 20% of patients present with dermatological symptoms such as malar rash which develops after sunlight exposure (Schur, 2001). Arterial and venous thrombosis is a sequel to chronic inflammatory events triggered by autoantibodies. This has been documented to occur in the form of petechiae, purpura or ecchymoses in the skin or swollen medium sized vessels causing tender nodules, seizures, stroke or behavioural changes (Hochberg et al., 2004). There could be renal involvement, nephritic syndrome or renal failure. Painless haematuria or proteinuria may be the only presenting symptoms. This may take place in about 35% of the patients. Pericarditis, myocarditis and endocarditis can occur from nonspecific inflammation. However, these lesions can manifest as immune complexes on the mitral valve, where bacteria can accumulate lesions, even when clinically silent, are found in more than half of autopsies. Atherosclerosis tends to occur more often and to advance more rapidly in patients with SLE than in the general population. SLE patients therefore are at higher risk for myocardial infarction (Bevra and Hahn, 2003). Pluritis, pericarditis, and peritonitis are common features of SLE. Pleural effusions are usually small but can be large. The fluid often contains antinuclear antibodies (ANAS), low complement, and immune complexes. Anaemia secondary to chronic inflammation, iron deficiency and hemolysis may develop in as many as half of SLE patients. Thrombocytopenia and leucopenia may be due to SLE or side effects of pharmacologic treatment.

Patients may have an association with antiphospholipid antibody syndrome (a thrombotic disorder) where autoantibodies to phospholipids are present in the patients serum. Abnormalities associated with antiphospholipids antibody syndrome include a paradoxical prolonged prothrombin time (which usually occurs in hemorrhagic disorders) and a positive test for antiphospholipid antibodies, the combination of such finding have earned the

term "Lupus" "anticoagulant positive". Another autoantibody finding in lupus is the anticardiolipin antibody which can cause a false positive test for syphilis (Braunwald et al., 2005).

Abnormalities in T-cell signaling are associated with SLE, including deficiency in CD45 phosphatase and increased expression of CD40 ligand. Other rare manifestations include: Lupus gastroenteritis, lupus pancreatitis, lupus cystitis, autoimmune inner ear disease, sympathetic dysfunction, retinal vasculitis and systemic vasculities.

SLE is characterized by the total picture of clinical, immunological and pathological features and many of the pathological changes are non-specific and sometimes may appear exceptionally small in comparison with the extent of clinical manifestations.

The principal pathological changes are seen in skin, joints, non erosive synovitis, kidneys, serous membranes pleuritis, and heart. The most typical histopathological lesions occur in the blood vessels, kidney and skin. In active SLE, vasculitis with subendothelial fibrinoid deposits may involve small arteries, arterioles, and capillaries of affected organs, kidneys, spleen, heart, lungs and rarely in the severest cases, acute necrotizing vasculitis may involve the entire vessel wall. Granular deposits of Igs and C are seen in the acute vascular lesions by fluorescence microscopy (Schur, 2001). In later stages, Derivascular fibrosis occurs. The small penicillar arteries of splenic pulp typically develop concentric pervascular laminations of fibrous tissue to reproduce typical "onion skin" which is characteristic but no longer regarded as specific for SLE. The renal glomerular are major targets of immune injury in SLE. Some 50% of SLE patients have glomerular disease shown by urinalysis, haematuria, proteinuria, cast or expressed clinically.

About 50% of patients have glomerular disease indicated by light microscopy and nearly all have glomerular abnormalities shown by immunofluorescence and electron microscopy.

In general, the basic histopathological changes in glomerular disease include one or more of the following: cellular proliferations, glomerular basement membrane thickening, leukocyte exudation, hyalinization and sclerosis.

These changes may involve virtually all glomeruli (diffuse), a minority of a part of each glomerulus segmental, (mesangial) (Hahn, 1997).

Skin lesion involving the face, trunk and extremities occur frequently in SLE and take many forms: facial erythema, such as the classical "butterfly" rash distributed over the cheek and base of the nose, urticaria, maculopapular lesions, ulceration and alopecia. The presence of liquefaction degeneration of the basal layer of the epidermis and fibrinoid change at the epidermal dermal junction is a microscopic change at the epidermal dermal junction of acute lesions and also of uninvolved "normal" skin (lupus band test).

The presence of these deposits in both involved and normal skin distinguishes SLE from other connective tissue diseases such as scleroderma and dermatomyositis and chronic discoid lupus. Apoptosis is increased in monocytes and keratinocytes. Expression of fas by B cells and T cells is increased. There are correlations between the apoptotic rates of lymphocytes and disease activity (Andrade et al., 2000).

Tingible body macrophages (TBMs) are large phagocytic cells in the germinal centres of secondary lymph nodes. They express CD68 protein. These cells normally engulf B cell which has undergone apoptosis after somatic hypermutation. In some patients with SLE, significantly fewer TBMs can be found, and these cells rarely contain materials from apoptotic B cell. Also undigested apoptotic nuclei can be found outside of TBMs. This material may present a threat to the tolerization of B cells and T cells. Dendritic cells in the germinal centre may endocytose such antigenic material and present it to T cells, activating them. Also apoptotic chromatin and nuclei may attach to the surfaces of follicular dendritic cells and make this material available for activating other B cells which may

have randomly acquired self specificity through somatic hypermutation (Gaip et al., 2006).

The prevalence of SLE varies in different countries. In France, the disease appears to be more common among immigrants from Portugal, Spain, North African and Italy than among natives. In Hawaii, the disease is more common in orientals or Polynesians than among whites. The disease has only recently been described in black Africans and appears to be more prevalent in China than in the United States. The average annual incidence in the United States has been estimated to be 27.5 per million population for white females and 75.4 per million populations for black female. The incidence of SLE among hospitalized patients was 4.6 per 100,000 per year in Baltimore and Maryland study and 1.8 per patients living in Rochester, Minnesota. The annual incidence rate is similar to that observed in Sweden, in New York and in Jefferson County, Alabama between 1955 and 1965. Incidence rate showed a steady increase beginning in early 1960, but has stabilized since then. Probably because of a greater appreciation of milder cases and improvement of diagnostic tests during the 1960s. The prevalence of SLE in a prepared health plan for 125,000 patients indicates that SLE affects approximately 1 in 1000 women in Rochester; Minnesota study which included all patients in a white population. The prevalence of definite SLE per 100,000 population in 1980 was 40.0-53.8 for women and 19.0 for men. This figure is somewhat lower than 51 per 100,000 reported for prepared health group in San Francisco from 1965-1973.

SLE occurs in children and elderly but the peak age of onset of the first symptoms is between 15 and 25 years. The mean age of diagnosis is 30 years. Higher percentage is affected among males in children and elderly. Females particularly young woman have a striking susceptibility to SLE as observed in virtually all epidemiologic studies. The incidence increases early in the second decade, peaks in the third, remains high during the 45 to 64 years period and declines further thereafter. Females exceed males in clinical series in a ratio of 5: 1 and to a greater extent during the child bearing years. SLE was previously believed to be a rare disease but has

now possible to estimate the number of people with lupus. In the United States alone, an estimated 270,000 to 1.5 million people have lupus. The disease affects both females and males, though young women are diagnosed nine times more often than men. SLE occurs, with much greater severity among African American women, who suffer more severe symptoms as well as higher mortality rate.

In a study carried out by Oduola et al. (2005) on the prevalence of SLE in whites and Indian Asian immigrants in Leicester City United Kingdom, it was observed that the overall prevalence of SLE in whites of population of 152,785 was 20.2 percent. Asian had high prevalence of 69.7% for females and 31.7% for males in a population of 37,684. The overall lupus was 3.0 times more common in Asians than whites, according to the study.

Studies in blacks have shown a high prevalence of the disease in the USA and Jamaica but not in East Africa. In the Chinese, the reverse pattern holds, with the disease being less common in Chinese who have emigrated than in those who remained in their native land.

In a study by Oduola et al. (2005) on the prevalence of lupus erythematosus in Ogun State Nigeria, it was reported that LE cell positivity was on the increase mainly due to increased number of requests especially from renal patients. LE cells was also observed to be greater in females 73% than males 5,2% and occurred more in the second and third decades of life (Schur 2001).

Materials and Methods

Study area

Imo state lies in the tropical rainforest belt of South Eastern Nigeria. It is made up of 27 local Government areas and has projected population of 3.4 million people. The people enjoy two distinct seasons; the rainy season which starts in May and ends in October with annual rainfall of 222.2mm and high relative humidity of 78 percent while the dry season begins in November and ends in April with high temperature of 22.0°C. The topography of the state remains flat land around Owerri zone

and some adjoining local government areas from other zones (Orlu and Okigwe). It is surrounded by neighboring states like Abia, Anambra, River, Enugu, Akwa-Ibom and Cross River. The people accommodate immigrants from all over the world. The people of Imo state are served by 28 government owned hospitals and many private ones.

Study population

A total of seventy (70) subjects were recruited for the study. Five (5) females were subjects suffering from systemic lupus erythematosus and sixty five (65) subjects were non-systemic lupus erythematosus females as the controls. Screening of subjects was based on their clinical presentations; hospital records oral interviews and relevant laboratory Investigations. Haematological variables determined were haemoglobin level, packed cell volume, total and differential white cell count, platelet count, and erythrocyte sedimentation rate and blood picture.

Ethical consideration

Consent were obtained from the research and ethic committee of the health institution used for the study who gave approval for the research work and informal consent obtained from patients or their relations as well as nurses and physicians in charge of the wards.

Specimen collection

Blood - The subjects were made to sit comfortably. The antecubital vein of the upper arm where the blood was to be collected was sterilized with 70% alcohol soaked on a cotton wool. A rubber tourniquet was applied around the upper arm, 5.5 milliliter of blood was drawn from the vein and distributed as follows:- 1.5mls of blood was delivered into a fluoride oxalate bottle, 2.5mls into ethylene diamine tetracetic acid (EDTA) bottle, 1ml into a bottle with paper clips where necessary and 0.5mls was put in a dry tube and allowed to clot.

Demonstration of le cells:

When whole blood is mixed with paper clips and shaken properly, the cells would fracture, providing nucleoprotein for phagocytosis by the LE factor.

1ml of venous blood were put in a bottle with few paper clips, the blood was mixed by giving it a hard shake until fibrin clot were formed. This took 10 minutes. The mixture was incubated for 15 minutes at 37°C.

The mixture was then transferred into a centrifuge tube and spun for 20 minutes at 1000 (rpm) to obtain a buffy coat layer. The buffy coat layer which lies between the plasma and red cells were obtained using Pasteur pipette. Thin films were made from the buffy coat, allowed to dry and stained using Leishman stain and examined as for thin blood film.

LE cells appeared as a neutrophil which has ingested nuclear material got deformed and appeared homogenous with the lobes pushed aside and surrounded the neutrophil. It stains pale mauve colour.

Haematological variables:

Haemoglobin estimation

The Cyanmethaemoglobin method was used. When anticoagulated blood is diluted in buffered solution of potassium ferricyanide and potassium cyanide, the potassium ferricyanide converts the haemoglobin to methaemoglobin which is further converted to Cyanmethaemoglobin by the action of potassium cyanide. This produces a coloured solution whose absorbance is read in a colorimeter at a wavelength of 540nm. The optical density of the mixture is directly proportional to the concentration of the Haemoglobin present in it.

Procedure: 5mls of drabkin solution were added to 0.02ml of well mixed EDTA blood using clean haemoglobin pipette. The contents were mixed properly by inversion and allowed to stand for 10 minutes for full colour development. The mixture was then transferred into cuvette and was read at 540nm, •using the Drabkin reagent as blank.

The haemoglobin values obtained were read from a standard haemoglobin calibration curve. Haemoglobin concentration (g / dl)= A of blood sample x Cone. Of Std.x D.F

A of Std. . 1000
A = Absorbance
Std. = Standard
D.F = Dilution Factor.

Haematocrit estimation:

The microhaematocrit method was used as described by Dacie and Lewis, (2001).

When anticoagulated whole blood is centrifuged in a capillary tube, there is packing of the red cells and the space occupied by the packed red cells is measured and expressed as a percentage of whole blood volume.

Procedure:

1. A plain, non-graduated capillary tube of about 7cm in length and 1 mm bore diameter was filled with a properly mixed EDTA blood by capillary action. Two capillary tubes were filled for each sample up to about 3/4 from the upper end.
2. The filled end of each tube was properly sealed using a plastic seal. The tubes were placed in the radial grooves of the microhaematocrit centrifuge, spun for 5 minutes at 12,000 revolution per minutes (rpm).
3. The value of the PCV was obtained using the microhaematocrit reader.

Total leucocyte counts

Standard method of total leucocyte count was employed, red cells were lysed when whole blood were diluted in Turk's Solution of 1% glacial acetic acid. The white cells remain intact their nuclei stained deep violet.

Procedure: To 0.38ml of Turk's solution in a glass test tube, 0.02ml of EDTA anticoagulated blood was added.

The mixture was thoroughly mixed by rotation for about 1 minute.

The chamber was cleaned and the cover slip firmly applied.

The chamber was charged and was allowed to settle for one minute for proper settling of the cells.

Leucocytes count was done in the four corners of the counting chamber using the lower power (10 x eye piece lens) and 16mm objective lens. The value obtained was multiplied by 50 to obtain the total leucocytes count (1st principle).

Differential white cell count:

These were done by visual examination of blood film on a slide by the spread technique (Cheesbrough, 2004).

Procedure: Plain microhaematocrit tubes were used to drop well mixed EDTA blood onto the surface of clean glass slides at about 1cm from the end.

Placing the slides on a smooth surface thin film of about 2cm long was made using the spreader.

Film staining:

Procedure: Prepared thin films were flooded with Leishman's stain for 2 minutes.

Equal volume of buffered distilled water (PH 6.8) was added and mixed by gentle rocking. The diluted stain was allowed to stand for 10 minutes. The films were washed with buffered solution until the appearance of a Salmon pink colour of red cells were observed.

Films were drained and dried in the air at room temperature, the back of the slide were cleaned and then set for examination.

The stained blood films were inspected microscopically using, x20, X 40, objectives for general survey and oil immersion objective (x 100). The cells were counted in a strip running the whole length of the film. The films were inspected using the longitudinally until all the cells were counted.

Erythrocyte sedimentation rate (ESR):

The Westergreen method was used as described by (Cheebrough, 2004).

When anticoagulated blood is left to stand undisturbed for 1 hour/ the red cells sediment gradually to the bottom of the tube leaving a clear

plasma on top and the distance occupied by the supernatant plasma is determined.

Procedure: The blood samples were mixed thoroughly and drawn up into a westergreen tube to a 200mm mark using teat .The blood samples were mixed in the proportion of 0.4mls of trisodium citrate to 1.0mls of EDTA blood.

The tubes were allowed to stand vertically undisturbed for 60 minutes. The height of the clear plasma above the upper limit of the column of sedimented cells was read to the nearest Imm. The result expressed as mm/hour.

Platelet count:

Red cells were lysed when blood was diluted in 1% ammonium oxalate solution. The platelets remain intact. Procedure: 0.38mls of ammonium oxalate solution was mixed with 0.02mls of EDTA blood. The counting chamber was charged as in leucocyte. The four corner center square and one at the center were counted after 20 minutes of allowing the cells to settle in a wet petri dish.

$$\text{Calculation} = \frac{\text{cells counted} \times 20 \times 10^6}{0.2 \times 0.1}$$

20=dilutionfactor

0.2 = area of the chamber

0.1 dept of the chamber.

Data analysis

The data generated were analyzed statistically, using prevalence rate and student t-test to ascertain prevalence of each of the diseases and the interaction of sex and age as well as level of significant between the sufferers and non sufferers.

Results

Table 1 Haematological values in systemic lupus erythematosus females as compared with non-systemic lupus erythematosus females

Parameters	SLE Females		Non-SLE		Test statistics (T Test)
	Mean±SD		Mean ± SD		P
Haemoglobin	9.0	±1.2	12.5	±0.7	<0.05
PCV-%	27.0	±3.8	38.0	±2	<0.05
WBC x 10 ⁹ /l	2.7	±2.0	5.6	+ 1.1	<0.05
Platelet x 10 ⁹ /l	138.0	±14.8	236.0	+51.7	<0.05
ESR per hour	93.6	±19.3	9.0	±1.0	<0.05
Neutrophil %	50.6	±5.6	67.2	±5.2	<0.05
Lymphocytes %	46.8	+3.9	30.6	+3.9	<0.05
Eosinophil %	1.6	±1.3	1.2	±1.3	>0.05
Monocytes %	1.0	±1.0	0.8	±1.0	>0.05
Basophil %	—	—	—	—	—

Discussion

Based on the diagnostic criteria for SLE, 70 persons were screened and only 5 were positive for SLE giving a prevalence rate of 7.1%. This value is high and had high female gender preponderance considering the fact that SLE is not much talked about in Imo State and not many people are familiar with its clinical presentations which may be confused with some other ailments. One would still infer a low prevalence when compared with results obtained in Ogun study, 73% for females and 5.2% for males (Oduala et al.,2005) United Kingdom 20.2% Asian study 69.7% for females and 31. 7% for males in a population study Cooper and Strohla (2003). The reasons for these variations could be multifactorial: the gene predisposing one to SLE may not be common in Imo state as it is in these other places where higher prevalence were obtained. People may not be exposed to environmental trigger like UV rays, and the awareness may be lacking and symptoms may be confused with other ailments.

The most important aspect of results obtained from this study was the variations in haematologic variables determined. In SLE, significant statistical differences were not observed in monocytes, eosinophils and basophils ($P>0.05$),

but other parameters had significant statistical differences ($P>0.05$). Anaemia was observed in 60% of the subjects, having haemoglobin below 9 g/dl. Leucopenia was observed in all the subjects and more than 80% of the subjects had thrombocytopenia. The normal eosinophil counts observed could not be understood since the most important diagnostic features of SLE is skin rash which could be caused by allergy, but with this result SLE could not be allergic reaction.

Changes in other parameters may be due to renal insufficiency, infection or loss of blood due to thrombocytopenic purpura usually observed in SLE. Reduction in haemoglobin and PCV levels could be caused by haemolysis. Leucopenia may be due to drug complications as well as thrombocytopenia.

The highly elevated ESR shows the inflammatory nature of SLE and ESR has to do with acute phase protein.

Conclusion

Systemic lupus erythematosus were found in women of child bearing age .These goes on to confirm that women are more disposed to autoimmune diseases.

This finding is vital to researchers who may want to find out if there are male hormones that could be administered to the females at certain age to stop the development of autoimmune disease without much effect. It is equally interesting to note the adverse systemic lupus erythematosus had on haematologic variables determined. This is very important findings as it will help in the management of patients and in averting complications which may result from anaemia, leucopenia and thrombocytopenia.

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