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RESEARCH ARTICLE

AN ASSESSMENT OF AUTOIMMUNITY IN ARTHRITIS PATIENTS

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ABSTRACT: Objectives: The goal of this study is to estimate autoimmune biomarkers that characterize the development and severity of arthritis, but probably normalize following successful therapy. **Materials and methods**: In this study a total of 109 subjects were used out of which treated and untreated arthritics were 48 and 44 respectively, the remaining 17 were healthy individuals which were used as control. Samples were collected from patients attending Rheumatology and Orthopedic clinic of Federal Teaching Hospital Ido-ekiti, Ekiti State Nigeria. Antinuclear antibody was estimated using Enzyme Linked Immunosorbent Assay (ELISA) while Lupus Erythematosus cells were ascertained microscopically using Leishman staining technique. All parameters were assessed in treated and untreated arthritic patients relative to healthy subjects. Body mass index was also calculated. Statistical analysis was done using SPSS. **Results**: Body mass index and Antinuclear antibodies were significantly higher in treated and untreated arthritics compared to control ($P<0.05$). When treated and untreated arthritics were compared, Body mass index and Antinuclear antibody were found to be significantly higher in untreated arthritics ($P<0.05$). Antinuclear antibody and Age correlated directly in untreated arthritics. Lupus Erythematosus cell prevalence was found to be higher in untreated arthritics having a percentage Lupus Erythematosus test positivity of 6.8% compared to the 2.1% seen in treated arthritics. **Conclusion**: It was found that Autoimmunity in arthritics can be significantly lowered through treatment with Arthritic drugs, diets, life style modifications over a period of time. The study suggests that Antinuclear antibody and Lupus Erythematosus estimations could be adopted as markers of diagnosis, prognosis and monitoring of arthritis.

Keywords: autoimmune biomarkers, ELISA, arthritics, Lupus Erythematosus

INTRODUCTION:

Arthritis means inflammation or swelling of one or more joints [1]. Arthritis occurs when the joints in the body are inflamed or there is a breakdown of cartilage in the joints. When cartilage breaks down in a joint, the bones rub together.

According to the National Centre for Disease Control and Prevention (CDC) [2] in 2015, arthritis is the leading cause of disability in people 15 years of age and older. Globally, it imposes a huge financial burden through wage loss along with the cost of medications [3].

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Arthritis describes more than 100 conditions that affect the joints, tissues around the joint, and other connective tissues. The factors that trigger arthritis include possible cartilage damage, shortage of the synovial fluid and infections [4]. Specific symptoms vary depending on the type of arthritis but usually include joint pain and stiffness. Arthritis can also occur when the immune system, which normally protects the body from infection, attacks the body's tissues as seen in autoimmune arthritis. Most outbreaks are seen for Osteoarthritis, Rheumatoid arthritis and Gout arthritis, whereas remaining others are less frequent [5]. Cartilage tissue covers the bone surfaces to prevent from direct rubbing against each other thereby smoothening limb movement without causing pain or bone erosion due to friction. The cavity in the joint is filled with synovial fluid produced by the cells from the synovial membrane which is aligned with the ligaments within the joint cavity [6]. Therefore, cartilage is the cushion that protect the jointts from pressure thereby making movements smooth. When cartilage breaks down in a joint, the bones rub together, pain, swelling, and stiffness then occur [7] Autoimmunity on the other hand can be defined as a specific immune effector response against self-components, causing harm on the host [8]. The self-damaging response is inflammatory in nature, but other effector mechanisms such as complement activation by autoantibodies bound to self-structures, can also be the major cause of pathology [9]. Autoimmune disease occurs when the antigens of an organism are attacked by the autoantibodies as a result of disturbed self-tolerance on a multifactorial basis involving inflammatory pathogens, genetic background, altered receptors or radiation [10]. Generally, this relationship leads to humoral or cell-mediated immune reactions against one or more of the body's self-structures [11]. While many autoimmune diseases are rare, collectively these diseases afflict millions of patients [12].

A marker, as defined by the Food and Drug Administration (FDA) of the United States, is any characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [13]. Some examples of Autoimmune biomarkers include Antinuclear Antibody, Rheumatoid factor, C-reactive Protein, Erythrocyte Sedimentation Rate, Lupus Erythematosus cell and Anti-cardiolipin. Antinuclear antibodies (ANA) mostly target specific antigens in the nuclear part of the cell. The most recognized clinically useful antigens are RNA–protein or DNA-protein complexes [14]. ANAs are found in many disorders, as well as some healthy individuals. They are usually subdivided according to their specificity, and each subset has different propensities for specific disorders [15]. Autoantibody screening is useful in the diagnosis of autoimmune diseases and monitoring levels helps to predict the progression of the disease [16].

LE cell was described as a mature polymorphonuclear leukocytes which had phagocytosed the liberated nuclear material of another leukocyte [17]. As athritis have been associated with inflammation and autoimmunity, this study is aimed at evaluating the levels of Antinuclear antibodies and LE cell positivity in arthritis patients

MATERIALS AND METHODS:

STUDY AREA

The study area which is Ido- Ekiti is located in Ido-Osi Local Government Area of Ekiti State, Nigeria. with latitude 7.8431^{o} N, and longitude 5.1823^{o} E.

STUDY DESIGN

This study involved the use of a case-control sampling method. Stratification was by age and therapy. A total of 109 subjects were investigated 17 apparently healthy subjects were used as control subjects, 44 were newly diagnosed arthritic subjects while the remaining 48 were arthritic subjects on therapy. Each participant received written and

verbal explanations about the nature of the study before signing an informed consent form.

INCLUSION CRITERIA

Subjects recently diagnosed of the disease without treatment yet and those that have been receiving treatment for a while were included. Age limit of 35 years and above. For control samples, healthy participants were non-athletes, non-smokers and non-alcoholics at age 18 years or more without the history of arthritis

EXCLUSION CRITERIA

Subjects with respiratory and cardiac diseases and conditions associated with neurological symptoms were excluded from both groups. In addition, subjects who have renal diseases, hepatic disorders, those on nonselective β blockers and presence of malignancy were also excluded.

ETHICAL CLEARANCE

Ethical approval was sought for, from federal teaching hospital, ido-Ekiti, Ekiti state.

SAMPLE COLLECTION

Sample required: Whole blood (defibrinated) and Serum

Venous blood sample of About ten milliliters(10ml) was collected from the cubital fossa using 22G needle and syringe. 6ml of whole blood was placed into a sterile universal container with glass beads and processed for the detection of LE cell. The remaining 4ml was placed in a plain bottle and allowed to clot, then centrifuged. The supernatant serum was placed into a plane bottle (non-anticoagulated bottle) and was stored at 4^0C for up to seven days before it was assayed.

GROUPING

Treated arthritics are those that have been diagnosed of having arthritis and have been on treatment for at least 2months.

Untreated arthritics are those that have just been newly diagnosed of having arthritis and have not been on treatment

Controls are those that are healthy subjects without arthritis.

METHODS OF DETERMINATION OF PARAMETERS

a. Body Mass Index

The height and weight of each subject was measured using stadiometer to which a weighing scale (ZT-120 health scale) was attached [18]. Measurements were taken with patients standing erect, wearing light clothing and putting on no footwear. Height was measured to the nearest 0.01meter (m) and weight to the nearest 0.5kilogram (kg) [19]. The body mass index was calculated using the formula: **BMI= Weight (kg)/ Height (m^2)**

b. LE cell was determined using Rotary bead method

Principle: L.E. Cell using Rotary bead method

Leukocytes are broken down in vitro allowing the abnormal plasma protein to react on the altered nuclear material. Agitation enhances the nuclear deterioration and phagocytoses. Leishman-stained slides are prepared and examined for the peculiar "L.E." cell.

c. Antinuclear antibody was estimated using ELISA based method

Specific anti ANA polyclonal antibodies pre-coated onto the microwell plates and the enzyme labelled antibody and a serum containing native antigen is

mixed, resulting in the native antigen and the antibodies to form a sandwich complex. After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation. The addition of substrate and stop solution results in a yellow-colored complex. The density of color produced is proportional to the concentration of ANA present in the sample captured in the plate. The concentration of ANA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Statistical Analysis

Results obtained were subjected to statistical analysis using Statistical Package for Social Sciences version 23 (SPSS 23). All parameters were expressed as Mean ± SD. Values were statistically significant or otherwise at $p<.05$. Results were illustrated with the aid of tables and charts wherever they are necessary.

RESULTS:

Distribution of examined subjects

A combined total of One hundred and nine (109) subjects; Ninety-two (92) subjects were arthritic and seventeen (17) subjects that does not have history of arthritis were used as control samples. **Figure.1**

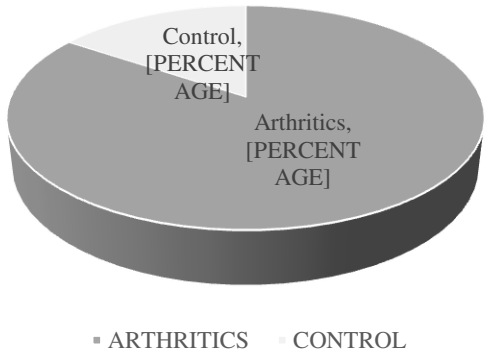
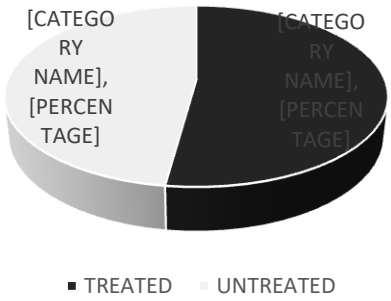


Figure 1: Distribution of examined subjects

Distribution of arthritics according to treatment

A combined total of Ninety-two (92) arthritics subjects; forty-four (44) subjects were untreated and forty-eight (48) subjects were on treatment. **Figure.2**

Figure 2: Distribution of arthritis patients according



to treatment

Distribution of arthritics according to gender

A total of Ninety-two (92) arthritics were examined; Twelve (12) of which are male subjects and Eighty (80) subjects were female. This finding shows that the incidence of arthritis is higher in female than in males. **Figure.3**

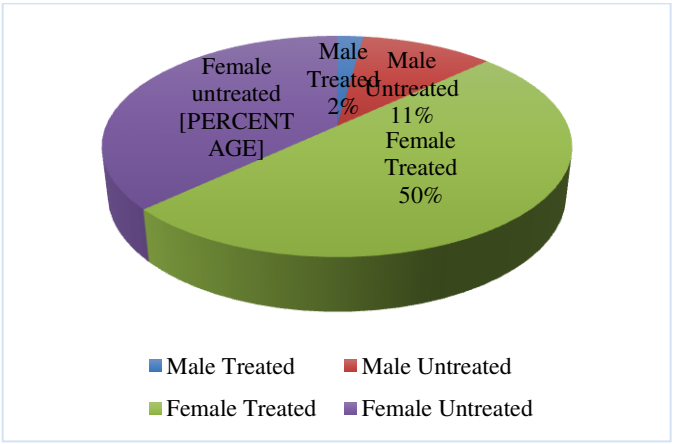


Figure 3: Distribution of arthritics according to gender

Mean ± SD, t-value and p-value in treated and untreated subjects compared with control for all parameters

Both BMI and ANA were significantly higher in both treated and untreated arthritis subjects compared with control, and also when untreated arthritis were compared with treated arthritis. **Table.1**

Table 1: Mean ± SD, t and p-values, when all parameters in treated and untreated arthritics were compared with control.

GROUP (n)	TREATED (n=48)	UNTREATED(n=44)	CONTROL (n=17)
BMI (kg/m^2)	31.23± 11.70^{ac}	21.50 ± 1.86^{b}	23.13 ± 3.52
Antinuclear antibody (U/L)	10.71±4.94^{ac}	13.49±6.36^{b}	6.35±2.77

a= significant at P<0.05 when treated arthritics were compared with control
b= significant at P<0.05 when untreated arthritics were compared with control
c= significant at P<0.05 when treated were compared with untreated arthritics

LE Cell characterization of treated and untreated arthritics

A total of ninety-two (92) arthritics were examined; 48 were treated out of which one (1) was positive and 47 were negative. The percentage of treated arthritics that were positive for the LE test was 2.1%. On the other hand, out of the 44 untreated arthritics, three (3) were also positive while 41 were negative resulting in a percentage LE positivity of 6.8%. It appears treatment lowers the possibility of LE positivity in arthritis subjects. **Table. 2**

Table 2. LE cell positivity in treated and untreated arthritis subjects

Group	LE positive cases/group total	% LE Positivity
Treated arthritics	1/48	2.1
Untreated arthritics	3/44	6.8
Control	0/17	0

Levels of BMI and ANA in all groups on treatment relative to control and untreated subjects

It can be deduced from the chart that NSAIDs are the best drug for normalizing ANA levels in patients on treatment relative to untreated and control. The BMI levels were significantly higher in treated subjects. Opioid analgesics are seen to be the best drugs for normalizing LE positivity in patients on treatment relative to untreated and control subjects. **Figure. 4**

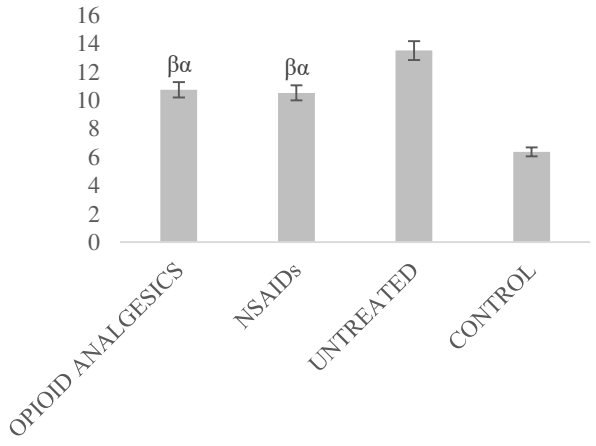


Figure 4: Levels of BMI and ANA in all groups relative to control and untreated subjects.

Levels of ANA in all groups relative to control and untreated subjects

The best drug to keep serum ANA levels under check in treated subjects is the NSAIDs relative to untreated and control subjects. However, ANA level was significantly higher in untreated subjects than control subjects. This shows that without the use of drugs, ANA levels are increased in the subjects. **Figure .5**

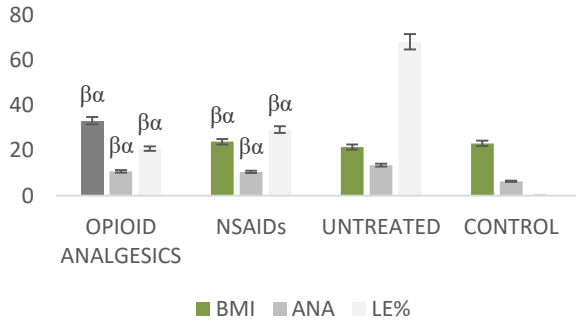


Figure 5: Levels of ANA in all groups relative to control and untreated subjects

Level of BMI and ANA in different age classifications

BMI and ANA were observed to increase with advancement in age. **Figure. 6**

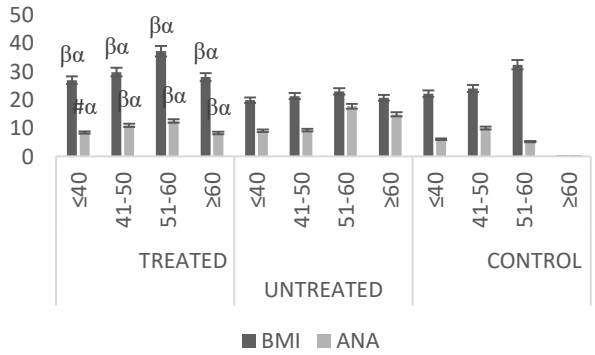


Figure 6: BMI and ANA in different age groups relative to control and untreated subjects.

Showing all parameters in males and females in all groups relative to untreated and control subjects.

BMI was significantly higher in treated male and female arthritics subjects compared to untreated and control subjects.

ANA level in treated male subjects was significantly lower than in female treated arthritic subjects compared to untreated subjects and significantly higher when compared to control subjects. ANA level for female arthritic subjects was significantly lower in treated arthritic subjects compared to untreated subjects and significantly higher when compared to control subjects. There was no significant difference in ANA level between male and female in treated arthritic subjects. **Figure. 7**

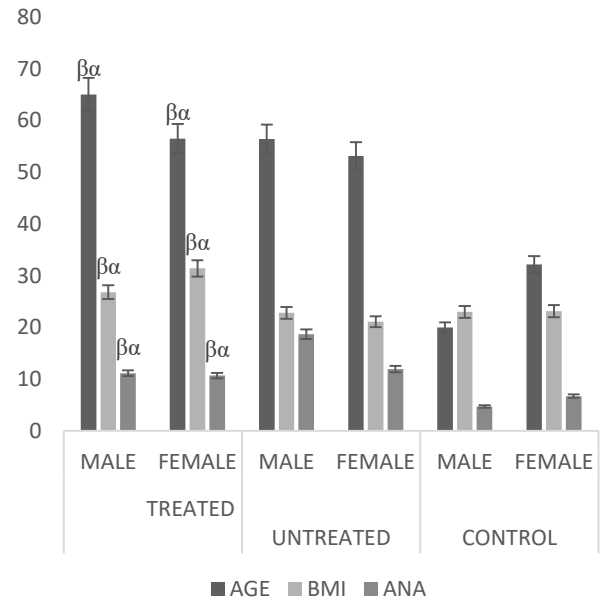


Figure 7: Age, BMI and ANA in Male and Female Treated, Untreated arthritic subject relative to control.

DISCUSION:

Arthritis is a major cause of inability and disability [1] and has been presumed to be an arthrodegenerative disease. This research therefore was designed in addition to BMI to assess and ascertain the ANA concentration and LE positivity in treated and untreated arthritics relative to control. Secondly, it was designed to assess the effect of treatment or otherwise, gender and age on all estimated parameters.

Body mass index (BMI) is a measure of weight adjusted for height, calculated as weight in kilograms divided by the square of height in meters (kg/m2) [21]. Although BMI is often considered an indicator of body fatness, it is a surrogate measure of body fat because it measures excess weight rather than excess fat [22]. In this research, BMI was significantly higher when both treated and untreated Arthritics were compared with control ($p<.05$). This finding agrees with the works [23-25] by Ajeganova *et al*. and others where there was a direct association between obesity and the activity of the disease in patients with long term Arthritis, reason being that being overweight increases the load placed on the knee joints thereby possibly elevating mechanical stress that hasten the breakdown of joint cartilage with concomitant increase in joint pain. Furthermore, BMI was significantly higher in treated compared to untreated arthritics ($p<.05$). BMI showed a pattern of increase with advancement in age. Also, females were observed to have higher BMI than males, a finding that agrees with the works of Akili *et al*. [26] where females were found to have a higher body mass index than males, possibly because females tend to

Antinuclear antibodies are specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells [27]. In this research, serum Antinuclear antibody levels was seen to be significantly higher in treated and untreated arthritics when compared to control ($p <.0001$). Also, a significantly higher serum ANA level was observed in untreated when compared to treated arthritics ($p < .0001$). This finding agrees with various works [28, 29] by of Criswell *et al.,* (2005); Zhernakova *et al*., where it was found that serum ANA levels were increased in Arthritics when compared with control. In this research, ANA was seen to be highest in the age group 51-60, while there was a steady pattern of increase with advancement in age. This finding is in line with the works of Hayashi *et al*. [30] where an abundance of ANA in elderly individuals was observed. Also, serum ANA levels were significantly higher in treated and untreated female arthritics than their male counterparts. A finding that goes along with various works [31, 32] where female dominance in ANA production suggests that hormones might play a role in this process. In this research, with respect to ANA, treatment was seen to be most effective in those treated with NSAIDs, as subjects on these drugs exhibited the lowest levels of ANA. A more critical examination of this finding shows that drug treatments have been able to bring ANA level closer to that seen in controls, the essence of therapy being to, at least close the gap in the levels of parameters seen in arthritics and bring it towards that viewed in controls.

LE cells are described as mature polymorphonuclear leukocytes which have phagocytosed the liberated nuclear material of another leukocyte [17]. In this study, it was found that there is a higher prevalence of LE cells in untreated arthritics having a percentage LE test positivity of 6.8% as compared to the percentage in treated arthritics where 2.1% were positive. This finding goes along with that of Monto *et al*. [33] where the frequency of LE cell positivity was found to be higher in rheumatoid arthritis patients. The question of whether there is any relationship between LE cell positivity and arthritis, can therefore be boldly answered in the affirmative.

CONCLUSION:

In this research, increased serum Antinuclear antibody, as well as Lupus Erythematosus positivity, are indications of Arthritis. It also found out that ANA levels and LE positivity increases with age, weight and being of the female gender.

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