ISSN No. 2456-4400
Int J Med Lab Res 2021, 6(3):35-43

IJMLR International Journal of Medical Laboratory Research

RESEARCH ARTICLE

AN ASSESSMENT OF ANTINUCLEAR ANTIBODIES AND LE CELL POSITIVITY IN ESSENTIAL HYPERTENSIVE SUBJECTS

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Received: 28 Sep, 2021/Revision: 06 Oct, 2021 /Accepted: 09 Nov, 2021

ABSTRACT: Objectives: This study is designed to assess and ascertain the serum level of antinuclear antibodies (ANA) concentration and lupus erythematosus (LE) positivity in treated and untreated hypertensive patients relative to non-hyprtensive apparently healthy individuals.

Methods: This study investigated a total number of 116 subjects age between 18-70 years comprising 54 hypertensives on known anti HBP medications, 35 hypertensives not on anti HBP medications and 27 apparently healthy individuals. Blood samples were collected from patients attending the General Out-patient Department of Federal Teaching Hospital Ido-ekiti, Ekiti State Nigeria. Blood pressure was taken from the non-dominant amusing appropriate cuff size and mercury sphygmomanometer, while, blood sample was Anti-nuclear antibodies was measured using ELISA while LE cells was ascertained with Leishman-stained microscopy of defibrinated blood. Statistical analysis was done using SPSS, all values were significant at $p<0.05$.

Results: Both systolic and diastolic blood pressure were observed to be significantly higher in both treated and untreated essential hypertensives compared with controls ($P<0.0001$). Serum ANA levels were also found to be significantly higher in untreated hypertensives compared with both treated hypertensives and controls ($P<0.0001$). Diastolic blood pressure correlated directly with systolic blood pressure and ANA levels in untreated hypertensives ($r=0.556$) ($r=0.318$) respectively but inversely with BMI in treated hypertensives. LE cell positivity was found to be higher in untreated hypertensives having a percentage LE test positivity of 2.9% as compared to the percentage in treated hypertensives where 1.9% were positive.

Conclusion: It was deduced that serum elevated ANA levels as well as LE positivity are also characteristics of essential hypertension. It was also found out that the possibility of LE positivity and ANA levels in essential hypertensives increases with weight, age and being of the female gender.

KEYWORD: Essential hypertension; Antinuclear antibodies, LE cell.

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INTRODUCTION:

Hypertension (HTN or HT), also known as high blood pressure (HBP) is a long-term medical condition characterized by persistently elevated blood pressure in the arteries above the normal arterial pressure [1] leading to heart failure, coronary artery disease, stroke, arterial fibrillation, peripheral vascular disease, vision loss, kidney failure and death [1,2]. HBP can be classified as either primary (essential) high blood pressure or secondary high blood pressure. A normal blood pressure as classified by the Joint National Committee 7 (JNC7), is a systolic BP less than 120 mmHg and diastolic BP less than 80 mm Hg. Hypertension is defined as systolic BP level of greater than 140 mmHg and/or diastolic BP level greater than 90 mmHg. However, values between 120–139 mmHg and 80–89 mmHg of systolic and diastolic values respectively are termed as "pre-hypertension" [3].

Moreover, over 90% of HBP cases are of primary origin, and can be traced to nonspecific lifestyle and genetic factors [4]. Some lifestyle risk factors for HTN include; excess salt intake, excess body weight, smoking and alcohol use. The remaining 5-10% of cases are classified as secondary HBP, and can be traced to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills [4]. The etiology of hypertension is multifactorial [5]. Apart from genetic factors, several behavioral and socio-economic factors can also put an individual at risk thus, lifestyle modification is an important feature in the prevention and management of hypertension [6].

Autoimmunity can be generally defined as a specific immune effect or response against self-components thereby inflicting harm on the host [7]. Autoimmune diseases occur when antigens of an organism are attacked by the autoantibodies as a result of disturbed self-tolerance on a multifactorial basis which involves inflammatory pathogens, genetic background, altered receptors or radiation [8]. Not quite long, significant progress was made toward demystifying the part played by specific T cell subgroups including T helper 17 (Th17) cells [9], T regulatory cells [10], and cytotoxic T cells (CD8+) [11] in blood pressure regulation. As a result of these research, a knowledge concerning the involvement of T cells and adaptive immunity in hypertension has emerged. This school of thought theorizes that a natural stressor like angiotensin II or long term high salt can causes local injury, resulting in the release of neoantigens that initiate an adaptive immune response and contribute to sustained hypertension [12,13]. Antinuclear antibodies (ANAs, also known as antinuclear factor) are autoantibodies that bind to contents of the cell nucleus. In normal individuals, the immune system will produce antibodies to foreign proteins (antigens) but not to human proteins (autoantigens). In certain individuals, antibodies to human antigens are produced atypically [14]. 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-centriomere antibodies and anti-sp100 antibodies. Each of these antibodies subtypes binds to different proteins or protein complexes within the nucleus [15]. ANAs are found in many disorders, as well as some healthy individuals. These antibodies can be subdivided according to their specificity, and each subset has different propensities for specific disorders [16].

Lupus Erythematosus cell (le cell). Systemic Lupus Erythematosus (SLE) is a connective tissue disease (CTD) [17]. LE cells have earlier been described as mature polymorphonuclear leukocytes bone marrow of a patient with SLE [18]. The LE cell test facilitated the diagnostic procedure. However, its intense and wide use during the following years disclosed that the LE cell is not only peculiar to LE-patients [19,20]. Now there is a body of evidence that a deregulation of apoptosis (programmed cell death) plays a role in the pathogenesis of autoimmune diseases such as LE [21,

[22]. Owing to this background knowledge of essential hypertension in relation to the immune system, this research aim to assess and ascertain the serum level of antinuclear antibodies (ANA) and lupus erythematosus (LE) positivity in treated and untreated hypertensive subjects relative to control.

MATERIALS AND METHODS:

Study Area

This cross-sectional study was carried out at tertiary health facility (Federal Teaching Hospital, Ido-ekiti-FTH-IDO) in Ekiti State, Nigeria. Ekiti State is located on latitude 7.8431oN, and longitude 5.1823oE. FTH-IDO serves as a referral center for primary and secondary public and private healthcare facilities in Ekiti State. Ido-ekiti is a densely populated Yoruba community comprising civil servants, students, businessmen, professionals and semi-skilled workers as the main population. FTH-IDO

Study Design/ Subjects/ Ethical Consideration

This descriptive cross-sectional study comprises of a total of 89 test subjects stratified based on age, gender and type of therapy/management at FTH-IDO. Each participant received written and verbal explanations about the nature of the study before signing an informed consent form however, random sampling technique was employed to recruit subjects into the study. 35 newly diagnosed HTN subjects currently receiving no medication and 54 HTN subjects on therapy were recruited from the Hospital general outpatient department (GOPD). Control subjects include 27 apparently healthy individuals recruited from among the hospital staff. Exclusion criteria for test subjects include pregnancy, obesity, diabetes mellitus, chronic kidney disease, arthritis and other disease condition. In addition, control subjects were, apparently healthy individuals without family history of HTN, non-athletes, non-smokers and non-alcoholics. Ethical approval was obtained from the research ethics review committee of the FTH-IDO, Ekiti in accordance to Helenski declaration.

Sample collection

Sample required: Whole blood (defibrinated blood) and Serum

Venous blood sample of About 10ml was collected from the cubital fossa using 22G needle and syringe and dispensed into an anticoagulated bottle by the phlebotomist at the phlebotomy section. 5ml of whole blood was placed into a universal container. The remaining 5ml was centrifuged. The serum gotten was placed into a plane bottle (non-anticoagulated bottle) and was stored at 4C for up to seven days before it was assayed.

Anthropometric data which included body weight and height were obtained using bathroom scales and height was obtained using a height gauge respectively. Blood pressure was taken from the non-dominant arm using appropriate cuff size and mercury sphygmomanometer. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were the first and the fifth koroktoff sounds respectively. Venous blood sample of about 5ml was collected from the cubital fossa using a needle and syringe and dispensed into a lithium heparin bottle. The blood was centrifuged at 3000rpm for 5 minutes to separate the serum from cells. The samples were stored at temperatures of -20 degree Celsius for a maximum of 21 days before assayed.

Methods of determination of parameters

Body mass index: Height and weight of each subject was measured using a stadiometer to which a weighing scale (ZT-120 Health scale) was attached. Measurements were taken with patients standing erect, wearing light clothing and putting on no footwear/headgear. Height was measured to the nearest 0.01 meter (m) and weight to the nearest

0.5kilogram (kg). The body mass index was calculated using the formula: BMI= Weight (kg)/ Height (m2)

Blood Pressure (Systolic and Diastolic) readings were taken from the non-dominant arm using a digital sphygmomanometer (Omros, Japan) according to manufacturer's guidelines. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were displayed digitally.

LE Cell was demonstrated using Rotary bead method. Leukocytes are broken down in vitro allowing the abnormal plasma protein to react on the altered nuclear material. Incubation enhances the nuclear deterioration and phagocytosis. Slides are prepared and examined for the peculiar "L.E." cell

Antinuclear antibodies were estimated using ELISA based kit: Microtiter plate was precoated with antibodies. Sample, calibrator standards and HRP-conjugate antibody are added to microwell. Incubation brings about equilibrium and all unbound antibodies are washed away, the addition of chromogen solution A and B turns the solution blue, the effect of the acid present in the stop solution changes the color from blue to yellow and the intensity of the color is measured spectrophotometrically at 450nm using a ELISA microplate reader. In order to measure the concentration of ANA in the sample, this ANA ELISA Kit includes a set of calibration standards. The calibration standards were assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus ANA concentration. 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 at $p<0.05$. Results were also illustrated with the aid of tables and charts wherever they are necessary.

RESULTS:

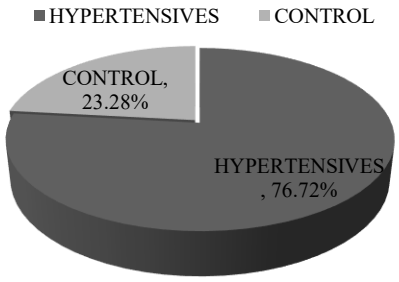


Figure 1. Distribution of all subjects under examination

Distribution of all subjects under examination (Figure 1)

A total of One Hundred and sixteen (116) subjects comprising of eighty-nine (89) hypertensive individuals as test subjects and twenty-seven (27) apparently healthy individuals with no history of hypertension and any other diseases as control subjects.

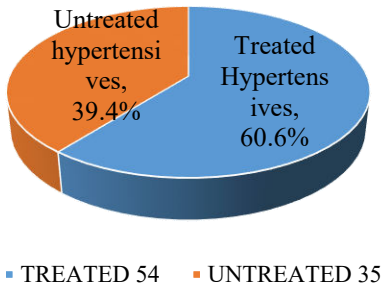


Figure 2. Distribution of all hypertensives according to treatment status

Distribution of all hypertensive according to treatment status (Figure 2)

A total of eighty-nine (89) hypertensive subjects, thirty-five (35) subjects were untreated and fifty-four (54) subjects were on treatment.

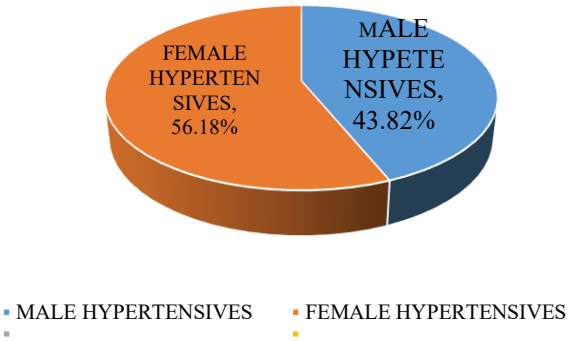


Figure 3. Distribution of all hypertensives according to gender

Distribution of all hypertensives according to gender (Figure 3)

A total of eighty-nine (89) hypertensive subjects; thirty-nine (39) of which are male subject and fifty (50) subjects were female. This finding shows that the incidence of hypertension is higher among females than in males.

Table1: mean ± SD in treated and untreated subjects compared with control for all parameters (BMI, ANA, SBP, DBP).

GROUP (n)	TREATED (n=54)	UNTREATED (n=35)	CONTROL (n=27)
BMI (kg/m2)	24.4± 5.95[c]	29.19 ± 2.67	23.4 ± 3.15[b]
Antinuclear antibody (U/L)	8.57±8.34[c]	22.57±3.76	6.35±2.65[b]
SBP (mm hg)	130.36 ± 9.91[c]	152.10 ± 5.13	121.09 ± 5.05[ab]
DBP(mmhg)	81.79 ± 5.11[c]	94.55 ± 6.67	76.09 ± 3.99[ab]

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

Table 1. Mean ± SD, t-value and p-value in treated subjects compared with control for all parameters (BMI, ANA, SBP, DBP).

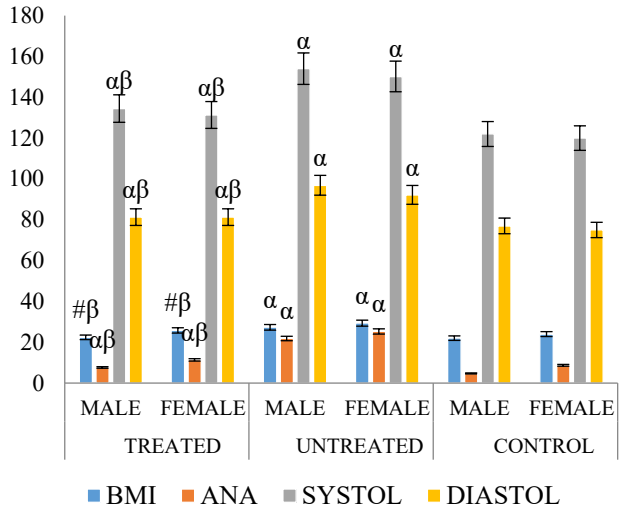
Both systolic and diastolic blood pressure were significantly higher in both treated and untreated hypertensive subjects compared with control, and also when untreated hypertensives were compared with treated hypertensives. BMI and ANA were significantly higher in untreated hypertensives compared to control and also in untreated hypertensives compared to treated hypertensives. Both (ANA and BMI) were not significantly higher in treated hypertensives compared with control.

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

Group	LE positive cases/group total	% LE Positivity
Treated hypertensives	1/35	1.9
Untreated hypertensives	1/54	2.9
Control	0/27	0

Table 2. LE Cell characterization of treated and untreated hypertensives

A total of fifty-four (54) hypertensives were examined; one (1) of which are positive and fifty-three (53) were negative. The percentage of treated hypertensives that were positive for the LE test was 1.9%. On the other hand, a total of thirty-five (35) hypertensives were examined; out of these one (1) was also positive while thirty-four (34) were negative resulting in a percentage LE positivity of 2.9%,.it appears treatment lowers the possibility of LE positivity in essential hypertension subjects.



α= significant at P<0.05 compared to control
β= significant at P<0.05 compared to untreated

Figure 4. BMI, ANA, Systolic blood pressure, Diastolic blood pressure in male and female Treated, Untreated hypertensives relative to control.

BMI, ANA, Systolic blood pressure, Diastolic blood pressure in male and female Treated, Untreated hypertensives relative to control (Figure 4)

BMI. ANA, SBP, DBP and LE cell positivity were significantly higher in both female treated and untreated hypertensives relative to their male counterparts

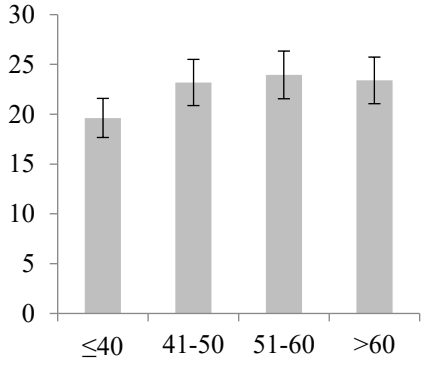


Figure 5. ANA in untreated hypertensives according to age groups.

ANA in untreated hypertensives according to age (Figure 5)

Serum ANA was seen to be highest in the 51-60 age group, its pattern of increase is fairly consistent with advancement in age.

DISCUSSION:

Hypertension, also known as high blood pressure (HBP), is a long-term medical condition characterized by persistently elevated blood pressure in arteries the above the normal arterial blood pressure [1]. Around 7.5 million deaths or 12.8% of the total of all annual deaths worldwide occur due to high blood pressure [23]. As hypertension has been theorized to be associated with autoimmunity [13], this research has been designed in addition to blood pressure (Systolic blood pressure and Diastolic blood pressure) and Body Mass Index (BMI), to assess and ascertain the serum level of Antinuclear Antibodies (ANA) and Lupus erythematosus(LE)positivity in treated and untreated hypertensives relative to control.

Body Mass Index (BMI) is a person's weight in kilograms divided by the square of height in meters (kg/m2). In this research, BMI in treated hypertensive subjects was found to be insignificantly higher when compared with control (p=0.44) but was significantly higher in untreated hypertensives compared with the control group (p<0.0001), furthermore, the BMI in treated hypertensives was significantly lower than that of untreated hypertensives (p<0.0001). This finding is similar to that of the study conducted by Tseng *et al*, [20]; where body mass index was seen to be significantly higher in essential hypertensives when compared to control. Also, females were observed to have a higher BMI than male hypertensives a finding which is in agreement with that of Akili *et al*. [25] where females were found to have a higher body mass index than males.

The systolic blood pressure (SBP) is the pressure exerted due to the pumping of the heart while the diastolic blood pressure is the measurement of forces per unit area as the heart relaxes to allow the blood flow into the heart [26]. Both systolic and diastolic pressure were observed to be significantly higher in both treated and untreated essential hypertensives compared with controls ($p<0.0001$) respectively, also systolic and diastolic blood pressure were significantly lower in treated hypertensives relative to untreated hypertensives ($p<0.0001$), meaning that treatment could be effective enough to bring about elevation of a severe case to a moderate one. This finding is in line with the works of Lawes *et al.* [27] where both diastolic and systolic blood pressure were seen to be significantly higher in essential hypertensives compared to controls. In this study, hypertension was seen to be more severe in males than in females showing significantly higher values in both mean systolic (134mmhg) and (131mmhg) and diastolic (83.48mmhg) and (80.36mmhg) pressure respectively between male and female essential hypertensives.

Antinuclear antibodies (ANAs) are unusual antibodies, detectable in the blood, that have the capability of binding to certain structures within the nucleus of the cells [14]. ANAs are found in patients whose immune system may be predisposed to cause inflammation against their own body tissues. Serum ANA levels in treated hypertensives was insignificantly higher compared with controls ($p=0.1821$) but was significantly higher in untreated hypertensives compared with both treated hypertensives and controls ($p<0.0001$) respectively. The not significant variation in ANA levels between the treated hypertensive subjects and controls supports the school of thought that the positive effect of treatment on hypertension and its attendant complications/ co morbidities is incontrovertible. This research agrees with the works of Gudbrandsson *et al*. [28]. where it was discovered that serum ANA levels are increased in hypertensives when compared with controls. Odewusi and Osadolor [29] had earlier observed that interleukin 18 a pro inflammatory cytokine was found to be significantly higher in essential hypertensive subjects. They also found out that interleukin 10 an anti-inflammatory cytokine was significantly lower than in controls leading to the opinion that hypertension is associated with an accelerated activation of the inflammation cascade. The finding that ANA concentrations is fairly consistent with advancement in age is in agreement with the works of Xavier *et al*. [30] where a abundance of ANA in elderly individuals was observed. Furthermore, the question of whether there is a relationship between essential hypertension and increased serum ANA levels can be boldly answered that serum ANA levels is higher in essential hypertension, even more likely when hypertension is untreated.

LE cells are described as mature polymorphonuclear white blood cells which have engulfed the liberated nuclear material of another leukocyte [18]. In this study, LE cell positivity was found to be significantly higher in untreated hypertensives having a percentage LE test positivity of 2.9% as compared to 1.9% in treated hypertensives. As a result of these findings the likelihood of autoimmunity and essential hypertension as potential co morbidities can be said to be significantly higher. Also, the effect of treatment on the alleviation of LE positivity in essential hypertensives cannot be denied.

CONCLUSION:

From this research, it was deduced that increased serum ANA levels as well as LE positivity are attributes of high blood pressure. We also found out that the possibility of LE positivity and abundance of ANA levels in essential hypertensives increases with weight, age and being of the female gender.

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Cite of article: Odewusi OO, Ebitimi C, Olaniyan OO. An assessment of antinuclear antibodies and LE cell positivity in essential hypertensive subjects. *Int J Med Lab Res.* 2021;6(3):35–43.
http://doi.org/10.35503/IJMLR.2021.6305

CONFLICT OF INTEREST: Authors declared no conflict of interest
SOURCE OF FINANCIAL SUPPORT: Nil