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Upregulation of Interleukin-2 Among Hypertensive Subjects in Bayelsa State, Nigeria

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ABSTRACT

Hypertension is a killer disease that increases with age. Several inflammatory biomarkers have been associated with hypertension. However conflicting reports exist on the relationship of interleukin-2 with hypertension. The objective of the present study was to determine the association of hypertension with interleukin-2 (IL-2) as well as the distribution of blood pressure among adults in Bayelsa State, Nigeria. Randomly selected 270 adults were screened for hypertension using Andon blood pressure monitor and real time PCR was used to quantify IL-2 gene expression. A structured questionnaire was used to collect sociodemographic information. A normal systolic and diastolic blood pressure distribution was found. Mean systolic and diastolic blood pressure were 123.69±22.65 and 77.60±13.53 mmHg respectively. The prevalence of hypertension was 47.4% (isolated systolic hypertension, 10.4%; isolated diastolic hypertension, 4.4%; co-occurring systolic and diastolic hypertension, 32.6%). Exactly 71.09% of hypertensive subjects were unaware that they were hypertensive. Age related increase in hypertension was found. Compared with normal control, a significant upregulation in IL-2 gene expression was found among hypertensive subjects ($p < 0.05$). In conclusion, the present study showed that a high prevalence of hypertension and low hypertensive status awareness exist in rural areas in Bayelsa State, Nigeria. It also showed that hypertension is associated with a significant upregulation in IL-2 gene expression. Hypertension interventions to rural areas and the use of IL-2 as a target biomarker for novel antihypertensive drug design is implicated in the present study.

Keywords: [Hypertension, interleukin-2, rural areas, Bayelsa State, Nigeria]

INTRODUCTION

Hypertension is a silent killer and a non-communicable disease that is associated with heart problems, kidney diseases, hardening of the arteries, eye damage, stroke, high morbidity and mortality. However, it is the most important modifiable risk factor for death resulting from cardiovascular diseases [1]. The prevalence of hypertension varies across different geographical regions, populations, age groups, occupations, behavioural patterns, gender and socioeconomic groups [1 - 2]. Worldwide, cases of hypertension among adults aged 30-79 years is estimated at 1.28 billion with two-thirds living in low-income and middle-income countries [1].

IL-2 is a cytokine (a type of protein) that modifies biological response. It stimulates the production and functions of T cell and natural killer cells, which are major parts of the immune system. The expression of IL-2 in a plethora of cell types across the immune system, orchestrates cellular interactions that shape the nature and magnitude of immune responses [3 – 4]. The role of IL-2 in cancer research has been studied extensively where it is used as immunotherapy for cancer with the brand

name Proleukin and Aldeleukin [5 -6]. In literature, most studies on the relationship between hypertension and IL-2 used the rat model of hypertension and conflicting results exist [3, 7].

Despite improvement in phytomedicine research for the treatment of hypertension and other communicable diseases [8 – 10] and the availability of antihypertensive clinical medications [11], yet hypertension still remains a public health problem. Research on inflammatory biomarkers as potent target for antihypertensive drug design might provide an alternative to the existing antimalarial drug. Information on the association of IL-2 with hypertension among human subjects is limited in literature. Therefore the aim of the present study was to examine the pattern of blood pressure distribution among adults in Bayelsa State as well as the association of blood pressure with inflammation in terms of interleukin-2 gene expression.

MATERIALS & METHODS

Study location

Bayelsa State, Nigeria was the study location. Bayelsa State is located on latitude 6.0699^0 E and longitude 4.7719^0 N. The State is situated in the core of Niger Delta, Nigeria and it is a riverine and estuarine setting, with bodies of water preventing the development of significant road infrastructure especially in rural areas. Ten rural areas in the State were randomly selected for the study [11, 12, 13].

Ethical approval and voluntary inform consent

Ethical approval for the study was obtained from Bayelsa State Ministry of Health Ethics Committee and informed voluntary consent was obtained from each participant before commencement of the study [14].

Study population and sample size determination

The study population comprised all adults in the selected rural areas in Sagbama and Ekeremor LGA, in Bayelsa State, Nigeria. The sample size was calculated using the Cochran formula below as presented by Charan *et al*., [15].

$$n_0 = \frac{Z^2pq}{e^2}$$

Where n_0 is the sample size; e is the desired level of precision (i.e. the margin of error), 0.05; P is the expected prevalence or proportion, which was estimated from a previous study as 0.22 [16]; q is 1 – P which is 0.78; Z-value was found in a Z table, Z-value at 95% confidence interval was 1.96 (two-tailed test).

$n_0 = ((1.96)^2\ (0.22)\ (0.78))\ /\ (0.05)^2 = 264$

Assuming a non-response rate of 2% the sample size was made up to 270. A multistage random sampling technique was used to select 270 individuals from ten villages (rural areas) in Bayelsa state [17].

Study design

A descriptive cross-sectional study design was employed [17]. The study participants were grouped according to the American Heart Association hypertension guidelines [18]. Normal = systolic BP < 120 / diastolic BP < 80; Pre-hypertension = systolic BP 120 -129 / diastolic BP < 80; Stage I hypertension = systolic BP 130 - 139 and / diastolic BP 80 - 89 mmHg. Stage II hypertension = systolic BP ≥ 140 mmHg and / diastolic BP ≥ 90 mmHg [18].

Blood pressure measurement and sociodemographic variable collection

Blood pressure was measured following the manufacturer`s protocol for Andon automatic blood pressure monitor (Model: KD-595; Andon Health Co., LTD. Tianjin 300190, China). Blood pressure values were recorded from an average of 3 consecutive measurements with a standard deviation lower than 10. A structured interviewer-administered questionnaire was used to obtain socio-demographic and COVID-19

vaccination information from the participants [16].

Blood sample collection and total RNA extraction

Venipuncture was used to collect 2 ml of blood from each study participants. Collected blood **s**amples were transported on ice to the laboratory for total RNA extraction [19]. Total RNA was extracted from whole blood samples within 24 hours of collection following the manufacturer`s protocol for total RNA minikit (Geneaid, New Taipei city, Taiwan). Briefly, 300 µl of whole blood was mixed with 1 ml of RBC lyses buffer by inversion, followed by incubation on ice for 10 minutes and vortexing twice during incubation. Centrifugation for 5 minutes at 3000xg was carried out followed by decantation to obtain the pellets. Four hundred microliter of RB buffer and 4 µl β-mecarptoethanol were added to the pellet and the pellet was re-suspended by pipetting followed by incubation at room temperature for 5 minutes with vigorous shaking. The reaction mixture was centrifuged at 16000xg for 1 minute in an RB spin column and the flow through was discarded. Exactly 400 µl of W1 buffer was added to the RB spin column followed by centrifugation at 16000xg for 30 seconds. The process was repeated twice using 600 µl of wash buffer mixed with ethanol. The RB spin column was centrifuged at 16,000xg for 3 minutes to dry the column matrix and 50 µl of RNase-free water was added into the center of the matrix of the dried RB spin column. The RB spin column matrix was left to stand for 1 minute to ensure that the RNase water was well absorbed. It was then centrifuged at 16,000xg for a minute in order to elute the purified RNA.

Complementary DNA (cDNA) synthesis

The extracted RNA was reverse transcribed into complementary DNA (cDNA) using Fire Script cDNA synthesis kit (Solis BioDyne, Tartu Estonia) following the manufacturer`s instruction. Briefly, 5 µl of Solisbiodyne Firescript cDNA synthesis kit (containing 0.5 µl of Oligo(dT) primer (G), 0.5 µl random hexamer primer (H), 0.5 µl dNTP mix (I), I µl reverse transcriptase (RT), 2 µl of 10x reaction buffer (J), and 0.5 µl riboGrip RNase inhibitor) was mixed with 15 µl of RNA template in a 0.2 ml PCR tube. Spin, vortex, spin of all reagents and RNA template was carried out before dispensing. After dispensing, spin, vortex, spin of the reaction mixture was carried out before loading unto the thermal cycler. The conventional cDNA PCR run was as follows: Primer annealing at 25^0C for 10, reverse transcription at 48^0C for 15 min, inactivation at 85^0C for 5 min and hold at 4^0C.

Evaluation of IL-2 gene expression

5x HOT FIREPol Evagreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) was used for interleukin-2 gene expression analysis following the manufacturer`s protocol. The reaction was carried out in an Applied Biosystem StepOne Plus real-time polymerase chain reaction system (Thermo Fisher Scientific, California, USA) using custom designed primers synthesized by Genewiz (Genewiz, South Plainfield, New Jersey). Amplification cycle (Ct) was normalized by the mRNA expression of the endogenous control, Glyceraldehyde-3-phosphate dehydrogenase **(**GAPDH). Primer sequences for IL-2 gene expression were: Forward-5`AAGAATCCCAAACTAACCAGGAT3` and reverse-5`TCT AGA CAT GAA GAT GTT TCA GTT CTC3` and GAPDH Forward-5`GTC TCC TCT GAC TTC AA3` and reverse-5`ACC ACC CTG TTG CTG TA3`. The cycling protocol were as follows: Initial activation at 95^0C for 12 mins, followed by 40 cycles of denaturation at 95^0C for 15 s, annealing at 53^0C for 30 s, elongation at 72^0C for 30 s. The reaction mixture contained 4 µl of 5x Evagreen Supermix (Solisbiodyne), 0.4 µl of forward primer (10 µM), 0.4 µl of reverse primer (10 µM), 2 µl of cDNA template and 13.2 µl of sterile water to make a total volume of 20

µl. The assay was carried out in duplicates using water as negative control.

STATISTICAL ANALYSIS

SPSS software version 24 was employed for data entry and analysis. Prevalence was expressed as percentage. Relative quantification of IL-2 gene expression was calculated using $2^{-\Delta\Delta CT}$ method and was expressed as normalized mean fold change ± standard error of mean (SEM) relative to control [20]. T-test was used to compare the mean fold change of groups for significant difference in IL-2 gene expression. Significant level was set at $p < 0.05$.

RESULTS

Characteristics of the study population

The characteristics of the study population are presented in Table 1. Overall, 270 adults participated in the study. Age related increase in blood pressure was found.

Table 1 Characteristics of the study population

Variable	N (%)
Age (Years)	
18 – 25	24 (8.9)
26 – 35	35 (13.0)
36 – 45	97 (35.9)
46 – 55	67 (24.8)
≥ 56	47 (17.4)
Sex	
Male	151 (55.9)
Female	119 (44.1)
Marital status	
Single	128 (47.4)
Married	134 (49.6)
Divorced	8 (3.0)
N = number of subjects	

Systolic blood pressure distribution of adults in rural areas in Bayelsa State, Nigeria

Figure 1 shows the systolic blood pressure distribution of adults in rural areas in Bayelsa State, Nigeria. The systolic blood pressure distribution displayed a bell-shape curve typical of a normal distribution with mean, mode, median and range of 123.69±22.65 mmHg, 140.00 mmHg, 121.00 mmHg, and 127.00 mmHg (78 – 205 mmHg) respectively.

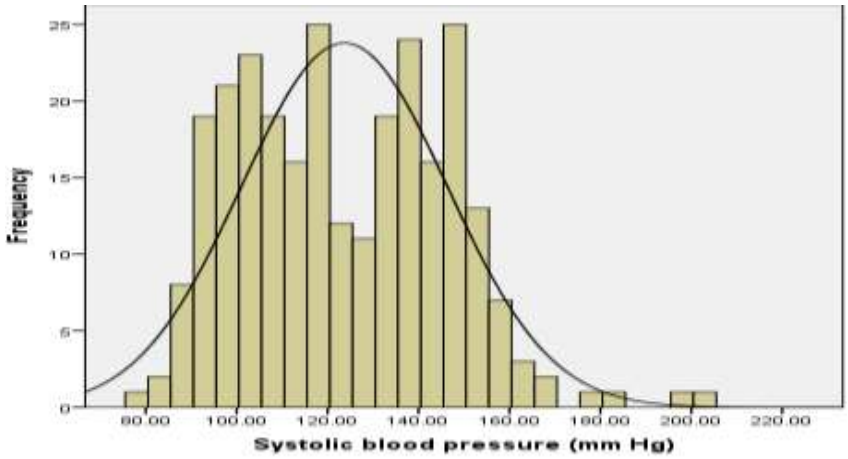

Figure 1 Systolic blood pressure distribution of adults in rural areas in Bayelsa State, Nigeria

Diastolic blood pressure distribution of adults in rural areas in Bayelsa State, Nigeria

Figure 2 below shows the diastolic blood pressure distribution of adults in rural areas in Bayelsa State, Nigeria. Diastolic blood pressure distribution displayed a bell-shape curve typical of a normal distribution. The mean, mode, median and range were 77.60 ± 13.53 mmHg, 90 mmHg, 76 mmHg and 108 mmHg (42 – 150 mmHg) respectively.

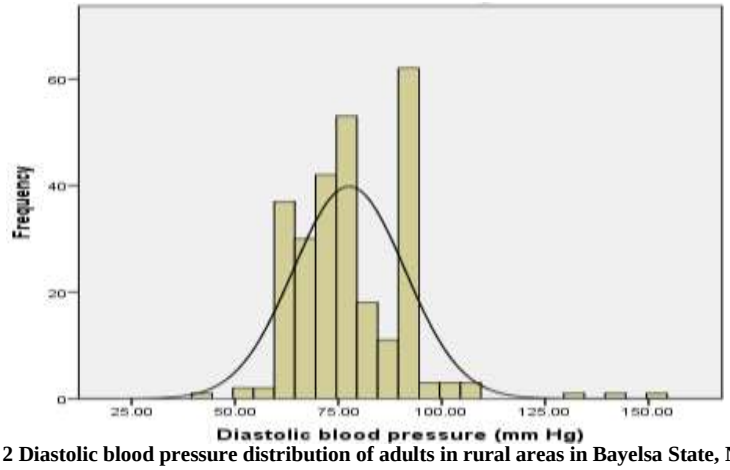

Figure 2 Diastolic blood pressure distribution of adults in rural areas in Bayelsa State, Nigeria

Prevalence of hypertension among adults in rural areas in Bayelsa State, Nigeria

Presented in Figure 3 is the prevalence of hypertension among adults in rural areas in Bayelsa State, Nigeria. A high prevalence of hypertension (47.4%) was found.

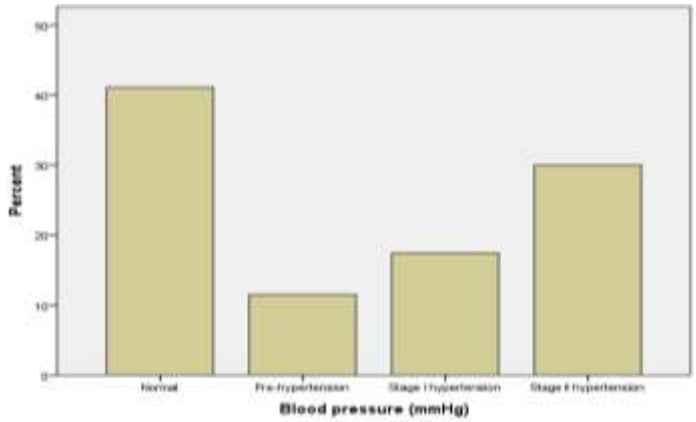

Figure 3 Prevalence of hypertension among adults in rural areas in Bayelsa State, Nigeria Normal BP: < 120 / < 80; Pre-hypertension: 120 -129 / < 80; Stage I hypertension: systolic BP 130 - 139 and / or diastolic BP 80 - 89 mmHg; Stage II hypertension: Systolic BP ≥ 140 mmHg and / or diastolic BP ≥ 90 mmHg; BP = Blood pressure.

Co-occurrence of systolic and diastolic hypertension among adults in rural areas in Bayelsa State, Nigeria

As shown in Figure 4, the co-occurrence of both systolic and diastolic hypertension was very high in the present study. Only a few had either isolated systolic hypertension or isolated diastolic hypertension.

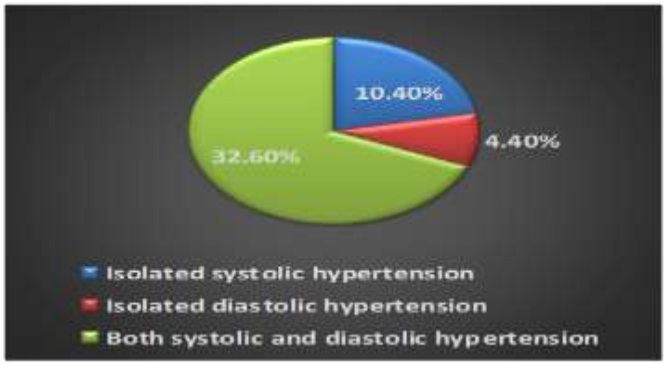

Figure 4 Co-occurrence of systolic and diastolic hypertension among hypertensive adults in rural areas in Bayelsa State, Nigeria

Awareness of hypertensive status in rural areas in Bayelsa State, Nigeria

As shown in Table 2, most of the study participants with high blood pressure were not aware that they were hypertensive.

Table 2 Level of awareness of hypertensive status among hypertensive subjects in rural areas in Bayelsa State, Nigeria

Status	N (%)
Aware	37 (28.91)
Not aware	91 (71.09)
Total	128 (100)
N = number of subjects	

Upregulation of interleukin-2 among hypertensive subjects in rural areas in Bayelsa State, Nigeria

The gene expression of IL-2 among hypertensive subjects in rural areas in Bayelsa State, Nigeria is presented Figure 5. Compared with the normal blood pressure group**,** a significant upregulation in interleukin-2 gene expression was found ($p < 0.05$) among hypertensive subjects ($p < 0.05$).

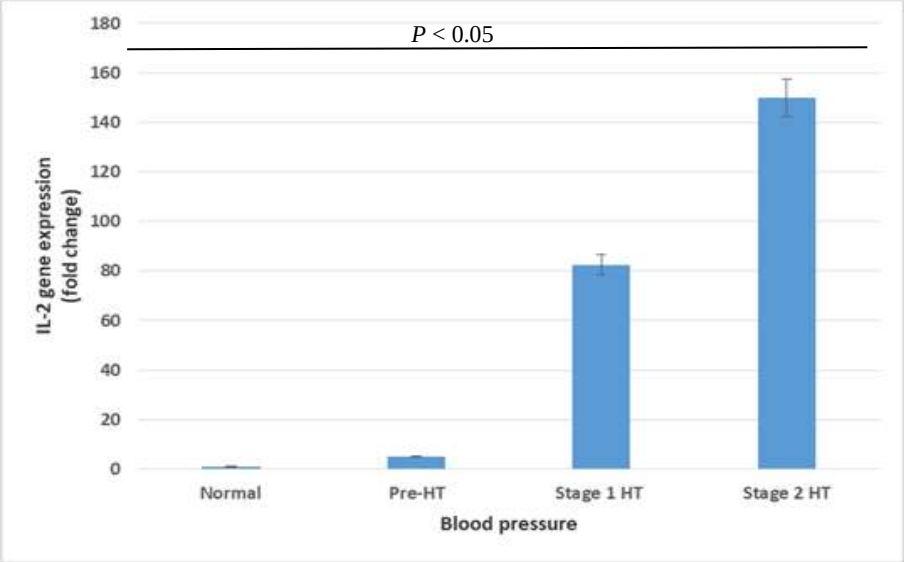

Figure 5 IL-2 gene expression among hypertensive subjects in rural areas in Bayelsa State, Nigeria Each bar represents mean fold change ± standard error of mean. Difference between each bar is statistically significant ($p < 0.05$). HT = Hypertension

DISCUSSION

Hypertension is a major modifiable risk factor for cardiovascular diseases, this makes it an important area for public health attention l21]. Research during the pandemic hypertension was associated with the risk of COVID-19 morbidity and mortality [10] and a worldwide rising trend in the prevalence of hypertension has been reported by the Non-communicable disease (NCD) Risk Factor Collaboration group [22]. The long term efficacy of COVID-19 vaccine among hypertensive subgroups has not been fully established. The present study examined the pattern of blood pressure distribution among adults in Bayelsa State and the long term effect of COVID-19 vaccine in terms of IL-2 gene expression among hypertensive subjects.

A higher prevalence of hypertension (41.9%) was found in the present study compared with a prevalence of 23.3% found in a previous study carried out by Banigbe *et al.*, [2] among men in Benue State, North Central Nigeria. Also, a lower prevalence of hypertension was found in separate studies carried out in Gondar, Northwest Ethiopia and Urban Varanasi respectively [24, 23] compared with the result of this study. Furthermore, many of the hypertensive subjects were not aware that they were hypertensive resulting in a high level of unawareness of hypertensive status in the present study. World Health Organization (WHO) had earlier noted that an estimated

46% of adults with hypertension are unaware that they have the condition [1]. Sudden hospitalization and complications reported among hypertensive subjects has been attributed to late detection of hypertensive status [1]. Age related increase in blood pressure was found in the present study. This is in agreement with the findings of previous studies carried out by Akilew *et al*., [23] and Solomon *et al*., [24] and re-emphasis the importance of hypertension management with increasing age.

The prevalence of isolated systolic hypertension and isolated diastolic hypertension was very low in the present study, compared with the high prevalence of co-occurring systolic and diastolic hypertension. The importance of taking both systolic and diastolic blood pressure measurement into consideration in the management of hypertension was reemphasized in the present study. Isolated systolic blood pressure is when systolic blood pressure is higher than 130 mmHg but diastolic blood pressure is under 80 mmHg, while isolated diastolic hypertension is when systolic blood pressure is lower than 130 mmHg but diastolic blood pressure is higher than mmHg [25]. A previous study demonstrated that both systolic and diastolic hypertension independently influences the risk of having cardiovascular diseases [25 - 26].

The present study showed that IL-2 plays a vital role in hypertension. A significant upregulation in the expression of the inflammatory IL-2 gene was found among hypertensive subjects in the present study compared with normal group. A previous study also found an upregulation of interleukin-2 gene expression among diabetes subjects [27]. This suggests a relationship between IL-2 and metabolic diseases. This increase in IL-2 gene expression among hypertensive subjects might provide a potent target for an alternative antihypertensive drug development. Researches in relation to hypertension and IL-2 is sparse in literature. Thus the present study has helped to fill the gap in research.

CONCLUSION

The present study found a high prevalence of hypertension among adults in rural areas in Bayelsa State, Nigeria. This was coupled with a low level of awareness of hypertensive status. Furthermore, the present study showed that hypertension is associated with a significant upregulation in IL-2 gene expression. Direction of hypertension interventions to rural areas is recommended. Also the potential of IL-2 to serve as a target and biomarker for novel antihypertensive drug design is implicated in the present study. The present study is novel, further large scale study to replicate the findings of the present study in other geographical locations is recommended

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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