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Original Article

Evaluation of Serum Creatine Kinase and Lactate Dehydrogenase Activities in Hypertensive Patients Attending General Hospital Ilorin, Kwara State, Nigeria

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ARTICLE INFO	ABSTRACT
 Corresponding Author*:* *Busari, Akeem Olayinka* *Department of Medical Laboratory Science, Faculty of Health Sciences, Al-Hikmah University Ilorin, Kwara State, Nigeria.* *Phone Number: +2348077609212* *Email: busakeem@yahoo.com* *Zip code: 240212*	The main factor contributing to morbidity and mortality is hypertension. Although blood pressure is a complicated feature, studies have shown that the activity of adenosine triphosphate (ATP)-generating enzymes like creatine kinase (CK) and lactate dehydrogenase (LDH) is a major predictor of hypertension. As a result, this study evaluated the activities of CK and LDH activities in hypertensive patients attending the General Hospital Ilorin, Kwara State. One hundred and sixty-one (161) hypertensive patients and thirty-nine (39) normotensive subjects were recruited into a case-control study of 200 participants. Body mass index (BMI) was estimated from each participant's measured weight and height, while the blood pressure was determined from the measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) using conventional mercury sphygmomanometer. A 5 mls sample of blood was taken from each subject, and the extracted serum was analyzed using an enzymatic-kinetic method to quantify the CK and LDH activities. Results showed that hypertensive patients had significantly at $p < 0.05$ higher serum CK and LDH activities in hypertensive compared to apparently healthy normotensives. Thus, this study observed significantly increase CK and LDH activities in hypertensive patients.

Introduction

Hypertension, commonly referred to as high blood pressure or arterial hypertension, is a chronic medical disorder when the blood pressure in the arteries is increased (William and Shiel, 2018). It is typically understood as the arterial blood pressure at which the risk of developing a long-term cardiovascular disease doubles (Rastogi *et al*., 2004). The normal blood

pressure at rest is between (60 and 90) mmHg diastolic and (100 to 140) mmHg systolic (ACC, 2017). Blood pressure drops as we sleep and quickly rises shortly before we wake, which is when the risk of cardiovascular events is at its maximum. As a result, hypertension is defined as a persistent systolic/diastolic blood pressure reading of 140/90 mmHg or higher (James *et al*., 2013). The main factor in preventable mortality and morbidity worldwide is hypertension, a key risk factor for cardiovascular diseases (CVD) (Schmieder, 2010).

According to estimations, there will be 31.1% of adults worldwide (1.39 billion) with hypertension by 2023, with low- and middle-income countries having a larger incidence of the condition (28.5%; 1.04 billion people) than high-income nations (349 million people) (Katherine *et al*., 2020). The estimated prevalence of hypertension in Nigeria is 15%, and it has become a significant health issue with significant socioeconomic implications. It is the most prevalent single risk factor for cardiovascular-associated illnesses, including stroke, congestive heart failure, chronic renal disease, and coronary artery disease, as well as related events and fatalities (Lim *et al*., 2012). Because of ignorance and poverty, the illness is underdiagnosed and undertreated. Complications like long-term disability and early death as a result. Additionally, it was said to be the most potent and developing risk factor for heart failure and stroke in sub-Saharan Africa (Onwuchekwa *et al*., 2012).

Many of the body's tissues and organs contain CK and LDH, which are primarily used to locate and assess the degree of tissue damage as well as track the progression of various conditions. The heart-specific isoenzyme utilized to diagnose acute myocardial infarction is CK, specifically CK-MB (Al-Hadi and Fox, 2009). LDH is a versatile tetrameric intracellular cytoplasmic enzyme that is present in all major organ systems (Garg et al., 2019). Serum LDH levels have been found to be higher in sodium fluoride-induced hypertensive rats, indicating the development of oxidative stress, renal damage, and cardiac damage following exposure (Oyagbemi *et al*., 2017). Early diagnosis of this enzyme's dysfunctional activity reduces the risk of hypertension-related heart problems such as stroke, heart failure, heart attack, and kidney failure, all of which can shorten life expectancy. Unfortunately, the majority of Nigerian healthcare facilities have given little attention to the laboratory evaluation of cardiac biomarkers in hypertension subjects for severity and its progression.

Materials and Methods
Study Area
The study was conducted at General Hospital Ilorin, which is situated at 8.4799^0N and 4.5320^0E in the Ilorin West Local Government of Kwara State, Nigeria. Every week on Tuesdays and Thursdays, the hospital's cardiology clinic attends to more than 200 hypertensive patients. Additionally, the hospital runs a standard phlebotomy unit and a Medical Laboratory department where services are provided in line with standard operating procedures by licensed phlebotomists and Medical Laboratory professionals who collect and process clinical samples.

Study Population
The study's case subjects were newly diagnosed and follow-up hypertension patients at General Hospital Ilorin, whereas the controls were the hospital's employees who appeared to be apparently healthy normotensive.

Study Design
This study is a case-control study among hypertensive patients attending General Hospital Ilorin and apparently healthy control subjects.

Ethical Approval
Ethical approval was obtained from the ethical review committee of Kwara State Ministry of Health and General Hospital Ilorin with Reference numbers MOH/KS/EU/777/552 & GHI/ADM/134/VOL.II/389 respectively prior to the commencement of the study. Both verbal and written informed consent was obtained from each participant before their inclusion in the research study.

Sample Size Determination
The formula described by Shiv *et al*. (2020) was used to determine the sample size;

$$n = \frac{Z^2\,pq}{d^2}$$

Where;
n = Minimum sample size
Z = Critical value and standard value for the corresponding level of confidence (at 95% CI or 5% level of significance (type-I error) it is 1.96
p = prevalence of hypertension in Ilorin from previous study = 11.9% = 0.119 (Abu-Saeed *et al.,* 2014)
q = 1-p
d = Margin of error or precision = 0.05
Therefore,

$$n = \frac{(1.96)^2 \times 0.119 \times (1\text{-}0.119)}{(0.05)^2}$$

n = 161.40
n = 161

The minimum sample size = 161.
A total of 200 participants comprising 161 hypertensive patients and 39 normotensive apparently healthy controls were recruited in the study.

Inclusion Criteria
i. Hypertensive patients aged (18 to 60) years attending General Hospital Ilorin
ii. Apparently healthy normotensive subjects as control.
iii. Participants that gave informed consent.

Exclusion Criteria
i. Participants with other health issues such as diabetes, sickle cell patients, pregnant women, etc.
ii. Those that decline the informed consent.
iii. Participants not up to the age of 18.

Sampling Technique
A convenient random sampling technique was adopted to recruit the research participants. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a Standard Mercury Sphygmomanometer (Hosseininasab *et al.,* 2014). The participants' body weight (in kilogrammes) and height (in meters) were measured by a weighing balance (Bivins and Marland, 2016) and standard stadiometer (Ahmed *et al.,* 1990) respectively. Body mass index (BMI) was calculated by dividing the weight (kg) measured by the square of height (meters) of each participant.

Blood Sample Collection
A five (5) millimeter of venous blood sample was collected from the antecubital fossa of the study participants and was dispensed into a plain vacutainer tube. After the clotting of the blood sample, it was spun at 3000rpm for 5 minutes. The serum was separated, dispensed into a plain tube, and estimated for CK and LDH activities.

Laboratory Analysis
Quantitative Estimation of Creatine Kinase Activity
The CK activity was determined using an enzymatic-kinetic based method (Brewster *et al*., 2006)

Principle
The procedure involves the measurement of CK activity in the presence of an antibody to CK-MB monomer. The antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB, while not affecting the B subunit activity of CK-MB and CK-BB. Then the CK method was used to quantitatively determine CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by two, measured at 340 nm in a spectrophotometer.

Quantitative Estimation of Lactate Dehydrogenase Activity
LDH activity was determined using the enzymatic-kinetic-based method (Schumann *et al*., 2002).

Principle
LDH is an oxidoreductase enzyme that catalysis the interconversion of lactate and pyruvate. When disease or injury affects tissue containing LDH, the cells release LDH into the bloodstream where it is identified in a higher-than-normal level. Kinetic determination of lactate dehydrogenase is according to the following reaction.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^+$$

Results
All measured data were presented as mean $\pm$ standard deviation and an independent t-test was used to compare the mean differences in the variables between the hypertensive patients and apparently healthy controls.

Table 1: Socio-Demographic Distribution of the Study Population

	Hypertensive patients	Normotensive control
Gender		
No. of Male	33 (20.5%)	9 (23.1%)
No. of Female	128 (79.5%)	30 (76.9%)
BMI		
Underweight (<18.5) Kg/m^2	1 (0.6%)	0 (0%)
Normoweight (18.5-<25) Kg/m^2	54 (33.5%)	16 (41.0%)
Overweight (25.0-30.0) Kg/m^2	59 (36.6%)	16 (41.0%)
Obesity (>30) Kg/m^2	47 (29.2%)	7 (17.9%)

SBP: Systolic blood pressure, DBP: Diastolic Blood Pressure, BMI: Body Mass Index

Table 1 showed the socio-demographic description of the study population. A total of 200 subjects comprising of 161 hypertensive and 39 normotensive participants were recruited for this study. The study was dominated by 128 females (79.50%) while 33 (20.90%) were male hypertensive patients. The hypertensive group was populated with overweight and obese participants comprising 59 (36.6%), followed by normoweight of 54 (33.5%), Obesity was 47 (29.2%) and underweight was the least with 1 (0.6%) participant. All the 39 controls were normotensive with both normoweight and overweight having the same frequency 16 (14.1%) each and only 7 (17.9%) were obese.

Table 2: BMI Comparison between Hypertensive Patients and Normotensive Control

Body Mass Index	Hypertensive patients	Normotensive control	p-value
Underweight ($<18kg/m^2$)	17.5±0.10	0	
Normoweight (18.5-24.99kg/m²)	26.07±17.40	24.5±14.70	0.000*
Overweight (25.0-30.0kg/m²)	30.10±27.35	27.5±12.09	0.000*
Obese ($>30kg/m^2$)	35.30±13.5	30.58±19.67	0.000*

Values were expressed as Mean ± SD. The level of significance was considered at p<0.05.

Table 2 showed the computed mean difference of BMI between the Hypertensive patients and normotensive controls. The mean value of BMI among the hypertensive was found to be $17.5\pm0.10kg/m^2$ underweight and 26.07±17.40 kg/m² normoweight which was significantly higher when compared to normotensive normoweight $24.5\pm14.70kg/m^2$ at p<0.05 level of significance. Also, the BMI of hypertensives overweight 30.10±21.35 kg/m² was higher compared to normotensive overweight of 27.5±12.09 while obese hypertensive was 35.30±13.35 kg/m² compared to 30.58±19.697 kg/m² of the normotensive.

Table 3: Comparison of Anthropometric Indices of the Study Participants

Blood pressure	Hypertensive patients	Normotensive Control	p-value
SBP (mm/Hg)	193.34±46.80	110.10±13.17	0.000*
DBP (mm/Hg)	106.82±17.91	74.92±12.61	0.000*
Weight (kg)	78.85±13.78	74.41±11.76	0.000*
Height (m)	169.21±6.86	168.51±6.64	0.000*

SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, BMI-Body Mass Index, * significant at p<0.05

There is a significant difference in Systolic Blood Pressure, Diastolic Blood Pressure, weight, and height in the two study groups with a statistically significant increase in mean of Systolic Blood Pressure, Diastolic Blood Pressure, weight, and height among hypertensive patients compared to the normotensive control group. The mean value for Systolic Blood Pressure, Diastolic Blood Pressure, weight, and height for the hypertensive group was found to be 193.34±46.80, 106.82±17.91, 78.85±13.78, and 169.21±6.86 respectively, while for the normotensive group was 110.10±13.17, 74.92±12.61, 74.41±11.76 and 168.51±6.64 respectively.

Table 4: Comparison of Serum CK and LDH Activities

	Hypertensive patients	Normotensive Control	*t*-value	p-value
CK-MB	284.48±284.45	111.17±76.44	196.20	0.000*
LDH	317.96±185.92	192.94±86.17	132.12	0.000*

CK-MB-Creatine kinase (MB subunit), LDH-Lactate dehydrogenase, *t*-value for independent t-test value, * significant at p<0.05

The activities of serum CK-MB and LDH activities were considered with an independent t-test. There was a statistical increase in the mean serum of CK-MB and LDH among hypertensive patients compared to the normotensive control group. The mean value of CK-MB in the hypertensive subjects (284.48±284.45) was found to be increased compared to the mean value of the normortensive subject (111.17±76.44), while the mean value of LDH for hypertensive subjects (317.96±185.92) was also found to be significantly increased compared to the mean value of the normotensive subject (192.94±86.17).

Discussion

A total of 200 subjects comprising of 161 hypertensive and 39 normotensive participants were recruited for the study (table 1). The hypertensive group was dominated by 128 (79.50%) females while 33 (20.90%) were male participants. The majority of the hypertensives were overweight 59 (36.6%), followed by normoweight 53 (32.9) and Obese 47 (29.2%) while the least was underweighted 2 (1.2%). A statistically significant increase in SBP, DBP, and BMI was observed in hypertensive patients when compared to normotensive participants (table 2) which is in tandem with the study of Oudman *et al.* (2011) who reported the increase in mean levels of SBP, DBP, and BMI as the main cause of hypertensive treatment failure. Thus, the findings from this study suggest that obesity is an important risk factor for prehypertension and hypertension. Also, a strong indication of an association between hypertension, increase BP, and BMI.

In line with research by Brewester *et al.* (2006), who identified elevated CK-MB activities as an associated factor contributing to an elevation in blood pressure, our investigation revealed an increase in CK-MB and LDH activities in hypertensives when compared to normotensive participants (Table 3). Additionally, the results of this study are consistent with the findings of a study by Ferreira *et al.* (2014) that found that circulating CK and LDH were the primary predictors of blood pressure in a random sample of multiethnic origin led to the failure of hypertension treatment. In table 4, we found that the activities of CK and LDH were significantly higher in the hypertensive subjects compared to the normortensive subjects, which is consistent with Surankita *et al.* (2018)'s study, which also found that CK levels were significantly higher in hypertensive patients. This suggests that CK levels are a possible associated factor that contributes to the rise in blood pressure. Additionally, Johnsen *et al.* (2014)'s study showed that CK increases adjusted BP by 7.98 (3.27-12.68) mm Hg of SBP and 4.69 (1.88-7.50) mm Hg of DBP for every log of CK. By easily supplying ATP, the enzyme CK, which is part of the cellular energy system, boosts the ability of cells to satisfy the high energy needs of contractile activities and active transmembranous transport (Surankita *et al.*, 2018). Also, the study by Karamat *et al.* (2014) hypothesized higher CK and LDH activities in the black population of Sub-Saharan African descent increase hypertension risk due to an increase in pressure response. LDH is an enzyme-wide spread detectable in the cytoplasm of almost every kind of cell in the human body and is a sensitive indicator of cell injury or increased cell membrane permeability. The process of atherosclerosis begins with endothelial cell impairment and increased cell membrane permeability, resulting in the release of LDH into circulation, it was found that serum LDH gradually increased with the progress of atherosclerosis, suggesting that LDH is related to the pathogenesis of atherosclerosis (Pisoni *et al.,* 2018). The statistical t-test at (α=0.05) level of significance to explore the differences between serum CK and LDH activities among hypertensive and normotensive groups showed a statistically significant increase in serum CK and LDH activities among hypertensive participants compared to normotensive participants.

Conclusion

This study indicates that hypertensive patients attending General Hospital Ilorin have significantly higher levels of creatine kinase and lactate dehydrogenase activity than normotensive controls. According to our study's findings, CK-MB and LDH are related to hypertension, which is consistent with earlier studies.

Conflict of Interest

None

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