Oxidative Stress and Lipid Profile among Hypertensive Patients at a Tertiary Centre in Kano, Northwest, Nigeria
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ABSTRACT
Background: Hypertension as a global public health challenge is a major risk factor for cardiovascular (CVD) and coronary heart diseases (CHD) because of its chronic sequelae. It is accompanied by dyslipidemia and oxidative stress leading to increase in lipid peroxidation. This study aimed to measure the fasting serum lipid profile and malondialdehyde (MDA) and determine the atherogenic index as well as the cardiovascular risk ratio among hypertensive patients in Kano, Nigeria. Patients and Methods: Two hundred subjects (100 hypertensive patients vs. 100 normotensive controls) were recruited for the study. The fasting serum lipid profile and MDA were assayed using routine laboratory methods. Lipid ratios that predict and identify an individual’s increased risk for cardiovascular diseases were then determined from the results of the profile. Results: The serum total cholesterol (7.0±0.5 vs 4.1±0.4 mmol/L), triglycerides (2.9±0.2 vs 2.0±0.3 mmol/L/L), LDL cholesterol (3.8±0.4 vs 2.6±0.4 mmol/L), VLDL cholesterol (3.0±0.2 vs 2.1±0.2 mmol/L) and MDA (TBARS) (9×10^-5±1.4×10^-5 vs 3×10^-6±0.9×10^-6 mol/l) were significantly (p<0.05) increase in hypertensive patients compared to normotensive controls. HDL cholesterol was significantly higher (p<0.05) in normotensive controls compared to hypertensive patients (31.4±8 vs 23.9±6 mg/dl). A statistically significant (p<0.05) positive correlation was observed between LDL cholesterol and MDA only. Both the atherogenic index (AI) ratio and the CardioRisk ratio were significantly higher in Hypertensives than Normal controls (10.4 vs 4.1; 11.7 vs 5.1 respectively). Conclusion: This study demonstrated an increased occurrence of atherogenic lipid profile and oxidative stress among hypertensive patients. It further showed a strong correlation between dyslipidaemia and oxidative stress. Therapeutic lifestyle changes and use of statins should be considered an integral part of the treatment for hypertensive patients in Nigeria.

Key words: Dyslipidemia, Oxidative Stress, Atherogenic Index

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Introduction
Cardiovascular disease (CVD) is the leading cause of disability and death worldwide, and a great majority of CVDs are associated with dyslipidemia. Hypertension, the leading
cause of mortality in the world, is also a potentially-treatable risk factor for stroke, myocardial infarction, cardiac failure, peripheral vascular disease, aortic dissection, atrial fibrillation, and end-stage renal disease.\(^2\) The risk of hypertension increases with age, sedentary lifestyle, high body mass index (BMI), hyperlipidemia and increase in energy, fat and sodium intake.\(^3\) About 80% of hypertensive patients have co-morbidities such as obesity, glucose intolerance, abnormalities in lipid metabolism, among others.\(^4\)

The reactive oxygen species (ROS) family comprises many molecules that have divergent effects on cellular function. Importantly, many of these actions are associated with oxidative stress, leading to pathological changes observed in cardiovascular diseases.\(^5\) According to Schramm and Matusik\(^6\) oxidative stress is no longer seen as a simple imbalance between the production and elimination of ROS, but also as a dysfunction of enzymes involved in the production of ROS. Oxidative stress has gained attention as one of the fundamental mechanisms responsible for the development of hypertension.\(^5\) ROS have an important role in the homeostasis of the vascular wall; hence, they could be part of the mechanism that leads to hypertension,\(^5\) essential hypertension is associated with greater than normal lipoperoxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of essential hypertension or in arterial damage related to essential hypertension.

Disturbance of the lipid profile as observed in hyperlipoproteinemia and hypercholesterolemia was also associated with increased susceptibility to lipid peroxidation by promoting generation of more free radicals and increased incidence of atherosclerosis.\(^7\)\(^-\)\(^9\) The major risk factor for the development of CVD is dyslipidemia, which may be primarily correlated with high blood pressure (hypertension), diabetes and obesity. Shen\(^10\) defines dyslipidemia as elevated plasma levels of TGS, TChol, LDL-C, VLDL-C and a decrease level of HDL-C.

To the best of the authors’ knowledge, a study of the association of oxidative stress and lipid profile of hypertensive patients in this part of the country has not been conducted. Thus, a preliminary study will provide insight into this and assist in the prediction of the outcome of these diseases. A better understanding of the correlation between blood lipids as well as the level of oxidative stress may provide an insight into the mechanism(s) by which hypertension is associated with increased risk of coronary artery diseases. This study was aimed at preliminarily, evaluating the association of lipid profile, oxidative stress marker and atherogenic indices among hypertensive patients.

**Patients and Methods**

A total of 200 study participants were recruited using a structured, self-administered, pre-tested questionnaire to obtain information on age, sex, anthropometric indices and treatment. They were grouped as; Group I: 100 hypertensive patients; Group II: Age and Sex matched 100 normotensive controls. Group I subjects were known hypertensive patients attending follow-up clinic in Aminu Kano Teaching Hospital while the controls were recruited from voluntary blood donors at the donor clinic and healthy volunteers. Both groups were recruited consecutively until the desired sample size was obtained.
To determine the sample size; Fisher’s formula was used
\[ n = \frac{Z^2 \times P \times (1-P)}{d^2} \]
Where; 
- \( n \): the desired sample size
- \( Z \): 95% confidence interval or 1.96
- \( d \): degree of precision usually set at 0.05

Prevalence of dyslipidaemic hypertension = 15%

The sample size determined was 195.84 which was rounded up to 200 (100 study group and 100 controls).

Adults (>18 years) on treatment for hypertension were recruited for this study while those excluded include those with self/family history of hyperlipidaemia/statin treatment; history of thyroid, liver or chronic kidney disease; drug treatment with steroids, contraceptive pills, \( \beta \)-blockers and diuretics; history of diabetes mellitus, smoking and alcohol intake as well as pregnant and lactating mothers.

Approval to carry out this study was obtained from the ethical committee of Aminu Kano Teaching Hospital. Informed written consent was obtained from all participants in the study after a verbal explanation has been made to each participant. All data from participants was regarded as confidential. The provision of the Helsinki declaration was respected at every step of the study.

After obtaining a written consent, a total of 5 cm\(^3\) blood was withdrawn aseptically from the antecubital veins from each patient, the sample was centrifuged at 3000 rpm for 10 minutes to separate serum and RBC's respectively, the separated serum was collected for further analysis in polythene tube.

Malondialdehyde (MDA) was determined using the method described by Ohkawa et al. and Modified by Grotto et al. The Thiobarbituric acid (TBA) assay is the simplest and most popular method for quantifying lipid peroxidation in biological samples. The assay works on the reaction of TBA with MDA to produce a pink colored MDA-(TBA) Schiff base adduct.

Lipid profile parameters (serum triacylglycerol, cholesterol, HDL and LDL cholesterol) were determined using enzymatic commercial test kits obtained from Randox Company. The atherogenic indices were calculated as follow:

- Castelli index (Cholesterol/HDL) and the LDL/HDL ratios
- Cardio Risk Ratio (CRR) = TCh / HDL-C
- Atherogenic Coefficient (AC) = (TCh – HDL-C) / HDL-C

The results obtained were expressed as mean ± SD. The data were subjected to one-way analysis of variance (ANOVA), Student’s t test was employed for comparison of the group means. Pearson’s correlation coefficient analysis was used to observe correlation between TGS, cholesterol, HDL cholesterol and LDL cholesterol with MDA (TBARS) levels in hypertensive patients. The results were accepted to be statistically significant when \( p \) value was less than 0.05.
Results

Table 1: Age, Sex and BMI of Hypertensive patients and Controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total</th>
<th>Hypertensives</th>
<th>Controls</th>
<th>p value</th>
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<td></td>
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<tr>
<td>Female</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Age (±SD)</td>
<td>46.8 ± 13.2</td>
<td>41.3 ± 12.6</td>
<td>0.0029</td>
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<tr>
<td>BMI (±SD)</td>
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<tr>
<td>Male</td>
<td>24.8 ± 3.7</td>
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<td></td>
</tr>
<tr>
<td>Female</td>
<td>26.3 ± 6.2</td>
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<td>Widowed</td>
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</table>

Table 2: Lipid profile and Malondialdehyde Level of Hypertensive and Normotensive subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hypertensive (n=100)</th>
<th>Normotensive (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerides (mg/dl)</td>
<td>258.38±56.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>175.35±108.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>271.29±59.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159.96±62.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dl)</td>
<td>23.87±11.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.43±9.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dl)</td>
<td>146.42±48.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.02±54.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>117.35±25.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.75±49.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (TBARS) (mol/l)</td>
<td>9.98×10&lt;sup&gt;-5&lt;/sup&gt;±1.36×10&lt;sup&gt;-5&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00×10&lt;sup&gt;-6&lt;/sup&gt;±2.89×10&lt;sup&gt;-6&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Artherogenic coefficient (AC)</td>
<td>10.37</td>
<td>4.09</td>
</tr>
<tr>
<td>CardioRisk Ratio (CRR)</td>
<td>11.37</td>
<td>5.09</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. Values across a row bearing different superscript are statistically significant at p<0.05.

The lipid profile determination of hypertensive and normotensive controls showed that, the mean serum TAG was significantly higher in hypertensive patients (258.38±56.32) compared to normotensive patients (175.35±108.61) at p<0.05. Serum cholesterol was extremely higher in the case group compared to the control group p<0.05, LDL was also significantly higher in
hypertensive patients than normotensive controls (p<0.05), however HDL cholesterol was significantly lower in hypertensive patients (23.87±11.69) than normotensive controls (31.43±9.80). Serum mean serum MDA level was significantly (p<0.05) higher in hypertensive patients (9.98×10^{-5}) than normotensive controls (3.00×10^{-6}). The atherogenic coefficient (AC) in hypertensives and non-hypertensives were 10.37 and 4.09 respectively while Cardio risk ratios (CRR) were found to be 11.37 and 5.09 for hypertensive patients and normotensive controls respectively.

Table 3: Association of MDA with Lipid profile parameters of hypertensive patients.

<table>
<thead>
<tr>
<th>Correlation parameters</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerides and MDA</td>
<td>0.2659a</td>
</tr>
<tr>
<td>Cholesterol and MDA</td>
<td>0.4402b</td>
</tr>
<tr>
<td>HDL Cholesterol and MDA</td>
<td>0.0147a</td>
</tr>
<tr>
<td>LDL Cholesterol and MDA</td>
<td>0.5305c</td>
</tr>
</tbody>
</table>

r = Pearson correlation coefficient. r value(s) with superscript: a=considered not significant, b=considered not quite significant, c=considered significant.

The correlations coefficient (r) revealed that there was moderate positive correlation between the LDL Cholesterol in hypertensive patients and the MDA (r=0.5305) which was statistically significant p<0.05. In addition, there was a positive association between total cholesterol and MDA (r=0.4402) though not statistically significant (p>0.05). TGS and HDL cholesterol showed weak and very weak correlation with MDA (r=0.2659 and 0.01472) respectively which was not statistically significant p>0.05, as shown in the table 3.

Discussion

Hypertension is globally documented as a major risk factor for CVD, stroke, diabetes, and renal diseases. Most of the hypertensive patients have co-morbidities such as obesity, glucose intolerance, abnormalities in lipid metabolism, among others. Lipid profile could provide a good platform for establishing individuals’ risk of CVDs.

The increased levels of serum LDL-cholesterol are atherogenic, while increased HDL-cholesterol is regarded as cardioprotective. Increased TGs serum concentrations have also been recognized as a risk factor for cardiovascular disease. In this study, the lipid profile; total cholesterol (T-chol), triglycerides (TGS), LDL and VLDL were higher in hypertensive patients in comparison with normotensive individuals. These findings were completely in agreement with previous studies of Raksha and Nandini as well as Choudhury et al.

However, there was high level of HDL in normotensive which is in contrast with the finding of Ghooshchi et al. This may be due to differences in the dietary pattern and genetic variation of the test subjects. Indeed, the lipid profile of hypertensive patients(with mean values of total cholesterol= 271.29mg/dl, LDL cholesterol= 146.42mg/dl and HDL cholesterol= 23.87mg/dl) are
characterized with alteration in lipid patterns (dyslipidemia) and they are at more risk of developing coronary heart disease (CHD) as well cardiovascular diseases and strokes as indicated by NCEP report. The ability of lipid ratios to better predict cardiovascular disease compared with individual lipid biomarkers is of clinical importance and can be relied on to explain the association of lipid ratios with cardiovascular risk factors that are at least in part unrelated to cholesterol metabolism.

The atherogenic index showed that 95 out of the 100 subjects are at risk of developing CHD as well as CVD (with respect to atherogenic index), while for LDL/HDL cholesterol ratio, it revealed that all the subject (i.e. 100) are at risk of developing CHD as well as CVD. The lower atherogenic index and LDL/HDL ratio of the controls imply a lesser CVD risk among normotensives. Atherogenic indices are powerful indicators of the risk of heart disease the higher the value, the higher the risk of developing CVD and vice versa. The CardioRisk Ratio (CRR) and Atherogenic Coefficient (AC) were high in hypertensive patients compared to normotensive controls; this signified the increased risk of cardiovascular disease and stroke in these patients.

The total cholesterol/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the LDL/HDL cholesterol ratio are another two important components and indicators of cardiovascular risk, the predictive value of which is greater than the isolated parameters of lipid profile. The atherogenic index of (>5.0 for men and >4.5 for women) and LDL/HDL cholesterol ratio of (>3.5 for men and >3.0 for women) are regarded as risk and treatment is required.

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignacies. There is significant increase in MDA level in hypertensive subject compared to the normotensive controls. Recently, a research conducted by Dhananjay et al. and Russo et al. showed that MDA level was higher in hypertensive individuals compared to normotensive which agreed with the findings of this study.

The elevated level of MDA observed in the hypertensive group when compared with the normotensives indicated that there was an increased in lipid peroxidation and oxidative stress in the hypertensive patients that resulted in the elevation of MDA. Russo et al. showed that essential hypertension is associated with greater than normal lipoperoxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of essential hypertension or in arterial damage related to essential hypertension.

There are a many studies suggesting the importance of pressure changes on the arterial wall in the development of atherosclerosis and lipoprotein oxidation and these provide an insight into the possible mechanisms by which blood pressure elevation increases lipid peroxidation levels. Meyer et al. induced luminal pressure on the rabbit aorta in vitro and observed that this stretching increased the uptake of LDL into the arterial wall. This study may offer a possible mechanistic explanation of how slightly elevated blood pressure levels enhance the cellular oxidation process and subsequent elevation of MDA in hypertensive patients.
The relationship between HDL cholesterol and MDA in this study revealed a very weak association in the hypertensive patients, but in the case of TGS and MDA a weak association was observed which was not significant (p>0.05).
Total cholesterol and MDA, LDL and MDA showed moderate positive correlation that was significant (p<0.05) for LDL and MDA but not quite significant for total cholesterol and MDA (p>0.05) in the hypertensive patients.
This indicates that increased oxidative stress observed in most hypertensive individuals might be associated with the dyslipidemia that mostly coexists with the disease, but this correlation might not be necessarily a causation (there may or may not be a causative relationship). The significant positive correlation observed for the LDL and MDA might be due to the fact that the LDL cholesterol is more susceptible to oxidative modification by the free radical because of its high content of Poly Unsaturated Fatty Acid (PUFA).39

Conclusion
This study demonstrated a strong positive correlation between oxidative stress and LDL cholesterol level with hypertensive patients having a more atherogenic profile than apparently healthy normotensive controls and thus, at more risk of developing coronary heart disease (CHD) as well as cardiovascular diseases. Atherogenic Index can act as an adjunct that significantly adds predictive value beyond that of the individual lipid profile. Prompt treatment of dyslipidaemia is recommended to reduce the propensity for increased oxidative stress damage.

Limitation
This study was limited by the use of non-probability based sampling technique.

References


