Lipid Concentration And The Extent Of Their Peroxidation In Nigerian Hypertensives
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Citation

Abstract
Plasma total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, Phospholipids and malonydialdehyde were determined in normotensives, hypertensives on anti – hypertensive drugs and hypertensives not on any anti hypertensive drugs aged between 30 – 60 years. Plasma samples were used. Result were statistically significant in all parameters studied at the level of (p < 0.001) in normotensives, hypertensives on drugs and hypertensives not on drugs except in phospholipids where there was no statistical difference. Relationship between blood pressure and lipid concentration were established.

The product of lipid peroxidation, malonydialdehyde was statistically significant in hypertensives on anti-hypertensive drugs and hypertensives not on any anti-hypertensive drugs when compared to normotensive control at (p<0.001) indicating free radical generation as a result of oxidative stress. These finding indicates that there are high lipid and lipoprotein concentration in hypertensives.

The Research work was done at The Federal Medical Centre Asaba and The University of Benin.

INTRODUCTION
Hypertension has emerged as a significant public health problem in most developing countries of the world . Uncontrolled hypertension remains a major threat to global health especially in developing countries such as Nigeria, where cardiovascular diseases mainly resulting from hypertension are still responsible for significant rates of death and disability .

The major risk factors for cardiovascular disorder is associated with plasma cholesterol concentration and fractions known as low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol, whereas the fraction known as high density lipoprotein (HDL) cholesterol appears to have a protective role . Also peroxidation of these lipids by free radicals is believed to be one of the important causes of cell membrane destruction and could be implicated in the pathogenesis of cardiovascular disorder .

This study is aimed at evaluating the lipids most commonly implicated in hypertensives; assessing the lipid levels in hypertensives and normotensives and to find out if there is any variation in the lipid concentrations of hypertensive patients compared to normotensive patients and also to investigate lipid peroxidation assessed by plasma malonydialdehyde level in hypertensive patients attending the cardiovascular clinic at Federal Medical Centre Asaba.

MATERIALS AND METHODS
SUBJECTS FOR THE STUDY
130 subjects composed of 30 hypertensives not on any drug therapy, 50 hypertensives on drug therapy and 50 normotensives attending cardiology clinic at Federal Medical Centre Asaba were assessed. The normotensives, apparently healthy subjects who were not suffering from any known disease at the time of this study have their systolic / diastolic blood pressure below 130/80 mmHg. The hypertensives, were newly diagnosed and were not on any anti hypertensive drug have their systolic / diastolic blood pressure above 130/80 mmHg. The hypertensives on drugs also have blood pressure above 130/ 80mmHg and above.

Fasting blood were collected from the subject with a 10ml syringe after a 12 hour fast overnight prior to venepuncture.
PREPARATION OF PLASMA
The blood specimen collected into lithium heparin containers were centrifuged within one hour of collection for 10 minutes using a Gallenkamp centrifuge. The plasma was separated from the red cell using a Pasteur pipette into containers containing no anticoagulant and stored frozen prior to analysis.

ESTIMATION OF PLASMA TOTAL CHOLESTEROL
The enzymatic colorimetric method by Tinder was used.

REAGENTS
4-Aminoantipyrine 0.5 mM, Phenol 6mM, 3,5-dichlorophenol 0.2mM, Cholesterol esterase ≥ 500kµ/l, Cholesterol oxidase ≥ 300kµ/l, Peroxidase ≥ 1200kµ/l, Phosphate buffer (pH 6.7) 70mM.

PRINCIPLE
Figure 1

PROCEDURE
To 1000ul of reagent in tubes meant for samples, standard and blank, 10µl of samples, standard and distilled water were added respectively. The content was mixed and incubated for 5mins at 37 °C. The absorbance of the samples and standard was measured against reagent blank at 546nm.

CALCULATION
Figure 2

ESTIMATIONS OF HDL-CHOLESTEROL
REAGENTS
HDL-cholesterol precipitant (Phosphotungstic 4.8%, Magnesium chloride 3M)

PRINCIPLE
Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of acid phosphotungstic in the presence of magnesium ions. After centrifugation the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant is determined.

PROCEDURE
Into centrifuged tubes, 500µl of sample and 100µl of HDL-cholesterol precipitant was added, mixed and allowed to stand for 10mins at room temperature. The tubes were centrifuged at 4,000rpm.100µl of supernatant were assayed for cholesterol estimation with the procedure given above for total cholesterol estimation.

CALCULATION
Figure 3

ESTIMATIONS OF LDL-CHOLESTEROL
REAGENT
Polyvinyl sulphate 0.7g/l EDTA Na2 5.0mM Polyethylene glycol monomethyl ether 170g/l

PRINCIPLE
LDL-cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene glycol monomethyl ether.

PROCEDURE
To 0.1ml of precipitant solution in a centrifuge tube 0.2ml of sample was added. The mixture was then mixed and allowed to stand at room temperature. The mixture was then centrifuge at 2,000g for 15mins and the supernatant cholesterol concentration was determined using enzymatic colorimetric method.

Calculations
LDL-cholesterol (mg/dl) =Total cholesterol (mg/dl) – 1.5 x supernatant cholesterol (mg/dl)

ESTIMATION OF TRIGLYCERIDE IN PLASMA
The enzymatic colorimetric method by McGowan was used.

REAGENT
Pipes buffer pH 6.8 50mM, 4-chloro-phenol 4.2 mM, Magnesium aspartate 40 mM, 4-aminophenzone 0.35mM.
ATP 2mM, lipase ≥9000 µ/l, Glycerol-Kinase ≥ 500µ/l, Glycerol-3-phosphate oxidase ≥ 200µ/l, peroxidase ≥ 500µ/l.

**PRINCIPLE**

**Figure 4**

\[ \text{Triacylglycerides} + \text{H}_2\text{O} \xrightarrow{\text{lipases}} \text{Glycerol} + \text{fatty acids} \]

\[ \text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP} \]

\[ \text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{glycerol phosphate oxidase}} \text{Glycerol-phosphate} \]

\[ \text{Dihydroxyacetone phosphate} + \text{H}_2\text{O}_2 \xrightarrow{\text{peroxidase}} \text{H}_2\text{O} + \text{4-Aminophenazone} + \text{4-Chlorophenol} \]

**PROCEDURE**

To 100µl of reagent in tubes meant for samples, standard and blank, 10µl of samples, standard and distilled water was added respectively. The content were mixed and incubated for 5mins at 37ºc. The absorbance of the samples and standard was measured against reagent blank at 546nm.

**CALCULATION**

**Figure 5**

\[ \text{Triglycerides Concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc of triglyceride in standard} \]

**DETERMINATION OF MALONDIALDEHYDE, A PRODUCT OF LIPID PEROXIDATION**

Lipid peroxidation end product was determined according to a modification of Buege and Aust.

**REAGENTS**

- 15% w/v Trichloroacetic acid
- 0.25 mol/l Hydrochloric acid
- 0.375 W/v Thiobarbituric acid

**PRINCIPLE**

Malondialdehyde (MDA), a product of Lipid peroxidation when heated with thiobarbituric acid under acid condition form a pink colored complex which has a maximum absorbance at 532nm.

**PROCEDURE**

One volume of plasma was mixed thoroughly with two volumes of stock solution of 15% trichloroacetic acid, 0.375% thiobarbituric acid and 0.25 mol/l hydrochloric acid. The mixture was heated for 30mins in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 1000g for 10 minutes. The absorbance of the sample was determined at 535nm and the concentration was calculated using the extinction coefficient, 1.56 X 10⁻³ m⁻¹ cm⁻¹.

**ESTIMATION OF PLASMA PHOSPHOLIPIDS**

**REAGENT**

1. Chromogenic reagent

   Solution i) 16g Ammonium molybdate was dissolved in 120ml distilled water.

   Solution ii) 80ml of solution i was added to 40ml conc HCl and 10ml of mercury. The solution was shaken constantly for 30 minutes and filtered.

   Solution iii) 200ml conc H₂SO₄ was added to the remaining solution i above cooled and added to the filtered solution ii. The chromogenic solution was obtained by adding 45ml methanol, 5ml chloroform and 20 ml distilled water.

2. Phospholipid standard; L- α phosphatidylcholine (1mg/ml)

3. Isopropanol

**PRINCIPLE**

This involves complexing intact phospholipids with mercury-ammonium molybdate chromogenic reagent. The absorbance was measured spectrophotometrically at 710nm.

**PROCEDURE**

0.2ml plasma was added to 3.8ml isopropanol. The mixture was centrifuged for 10 minutes at 1000g. 0.5ml of supernatant was pipetted into centrifuged tubes and evaporated to dryness at 90 ° C.0.4ml chloroform and 0.1ml chromogenic solution were added after thorough mixing, the tubes were heated at 100 ° C for 1 minute. After cooling at room temperature, the tubes were mixed and 3ml chloroform was added. At the end of 15minutes, the tubes were centrifuged at 1000g for 5minutes and the absorbance of the supernatants read at 710nm. Isopropanol was used as blank.

**CALCULATION**

**Figure 6**

\[ \text{Phospholipid Concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc of phospholipid in standard} \]

**STATISTICAL ANALYSIS**

All measures of central tendencies and dispersion were analysed using descriptive statistics in statistical packages for social sciences (SPSS) statistical package. The non-
parametric Kruskal-Wallis test was used to test for the significant difference.

**RESULTS**

The plasma lipid concentration and malondialdehyde were determined from a total of 130 subjects made up of normotensives, hypertensives on anti hypertensive drugs and hypertensives not on any anti hypertensive drugs and they were aged between 30-60 years.

**Figure 7**
Table 1: Plasma Lipid Values For Normotensives (MEAN ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Combined n=50</th>
<th>Females n=23</th>
<th>Males n=27</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPS (mmHg)</td>
<td>126.2 ± 8.11</td>
<td>134.7 ± 7.90</td>
<td>127.4 ± 8.20</td>
</tr>
<tr>
<td>BPD (mmHg)</td>
<td>80.4 ± 2.4</td>
<td>78.8 ± 2.75</td>
<td>80.7 ± 2.20</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>154.0 ± 10.74</td>
<td>155.4 ± 12.08</td>
<td>152.7 ± 8.68</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>95.4 ± 5.86</td>
<td>95.2 ± 5.09</td>
<td>95.6 ± 5.76</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>52.4 ± 7.05</td>
<td>52.4 ± 7.5</td>
<td>52.1 ± 7.4</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>82.2 ± 7.76</td>
<td>83.7 ± 8.79</td>
<td>81.9 ± 5.99</td>
</tr>
<tr>
<td>Phospholipid (gm/d)</td>
<td>165.0 ± 24.09</td>
<td>165.8 ± 25.69</td>
<td>184 ± 16.35</td>
</tr>
<tr>
<td>Malondialdehyde (umol/l)</td>
<td>4.4 ± 0.45</td>
<td>4.4 ± 0.45</td>
<td>4.4 ± 0.45</td>
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</tbody>
</table>

**Figure 8**
Table 2: Plasma Lipid Values For Hypertensives On Drugs (MEAN ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Combined n=50</th>
<th>Females n=24</th>
<th>Males n=26</th>
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</thead>
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<td>BPS (mmHg)</td>
<td>140 ± 13.00</td>
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<td>BPD (mmHg)</td>
<td>91 ± 7.10</td>
<td>90 ± 7.10</td>
<td>91 ± 7.10</td>
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<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>160 ± 10.61</td>
<td>162 ± 10.35</td>
<td>170 ± 10.84</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>111.5 ± 84.78</td>
<td>103.0 ± 82.62</td>
<td>115.5 ± 84.78</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>52.4 ± 5.05</td>
<td>54.1 ± 5.82</td>
<td>100 ± 4.89</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>107.8 ± 8.74</td>
<td>107.9 ± 8.30</td>
<td>106 ± 8.35</td>
</tr>
<tr>
<td>Phospholipid (gm/d)</td>
<td>187 ± 5.62</td>
<td>191.2 ± 5.75</td>
<td>184 ± 5.44</td>
</tr>
<tr>
<td>Malondialdehyde (umol/l)</td>
<td>5.3 ± 0.78</td>
<td>5.1 ± 0.88</td>
<td>5.5 ± 0.88</td>
</tr>
</tbody>
</table>

**Figure 9**
Table 3: Plasma Lipid Values For Hypertensives Not On Any Anti-Hypertensive Drugs (MEAN ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Combined n=30</th>
<th>Females n=13</th>
<th>Males n=17</th>
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</thead>
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<tr>
<td>BPS (mmHg)</td>
<td>171 ± 10.63</td>
<td>170 ± 8.78</td>
<td>171 ± 11.90</td>
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<tr>
<td>BPD (mmHg)</td>
<td>104.0 ± 8.10</td>
<td>105.5 ± 8.70</td>
<td>102 ± 7.50</td>
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<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>169 ± 14.05</td>
<td>195 ± 10.81</td>
<td>166 ± 15.48</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>114 ± 29.50</td>
<td>112 ± 7.00</td>
<td>115 ± 11.10</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>46.1 ± 6.23</td>
<td>49.1 ± 3.46</td>
<td>43.2 ± 7.90</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>120.2 ± 12.75</td>
<td>123.8 ± 10.78</td>
<td>117.5 ± 13.82</td>
</tr>
<tr>
<td>Phospholipid (gm/d)</td>
<td>186 ± 7.89</td>
<td>192 ± 8.53</td>
<td>182 ± 8.11</td>
</tr>
<tr>
<td>Malondialdehyde (umol/l)</td>
<td>5.5 ± 0.71</td>
<td>5.5 ± 0.82</td>
<td>5.5 ± 0.83</td>
</tr>
</tbody>
</table>

**Figure 10**
Table 4: Statistical Comparisons (P-VALUES)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotensives and HND</th>
<th>Normotensives and HOD</th>
<th>HOD and HND</th>
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<tr>
<td>BPS (mmHg)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BPD (mmHg)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
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<tr>
<td>Phospholipid (gm/d)</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Malondialdehyde (umol/l)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**KEYS**

BPS = Systolic Blood Pressure in mmHg
BPD = Diastolic Blood Pressure in mmHg
HDL = High Density Lipoprotein Cholesterol
LDL = Low Density Lipoprotein Cholesterol
HOD = Hypertensives on Drugs
HND = Hypertensive not on Drugs
n = Number of subjects investigated

**DISCUSSION**

The result of the work has shown that there is some variation in plasma lipids in relation to sex. The total plasma cholesterol was significantly higher in females than in the males. There was a significant difference in the lipid concentration and malondialdehyde concentration between normotensives, hypertensive on hypertensive drugs and those not on any anti-hypertensive drugs.

Total cholesterol, triglycerides and LDL cholesterol were
significantly higher in hypertensives not on any drug therapy than normotensives (P<0.001). There was a significant difference in blood pressures, total cholesterol, HDL-cholesterol and LDL-cholesterol in hypertensives on antihypertensive drugs when compared to hypertensives not on any antihypertensive drugs, but triglyceride concentration increased marginally in hypertensives on antihypertensive drug than hypertensive not on any anti-hypertensive drug. The reason may be attributed to the effect of antihypertensive drug on triglycerides.

There were high levels of total cholesterol, triglycerides and LDL-cholesterol and low levels of HDL-cholesterol were observed. There was no significant difference in the phospholipids concentration (P>0.05) of normotensives compared to hypertensives.

In our study, we observed an increase in malonylaldehyde, a product of lipid concentration among the normotensives, hypertensives on anti-hypertensives and hypertensives not on any anti hypertensive drug. However there was no significant difference observed in the malonydialdehyde concentration of hypertensives on anti-hypertensive drugs compared to those not on any anti-hypertensive drugs. This may be attributed to the fact that anti-hypertensive drugs may not have any effect on lipid peroxidation.

**CONCLUSION**

The results obtained from this study indicate that lipid concentration are increased in hypertensive patients and if not controlled could lead to other cardiovascular disorders or possibly death. Regular laboratory investigations of lipid profile should be investigated along with blood pressure in hypertensives since lipid and lipoproteins are important diagnostic and predictive value in coronary heart diseases.

**CORRESPONDENCE TO**

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**References**

1. David Conen, Paul M Ridker, Julie E Buring and Robert J Glyn (2007) Risk of cardiovascular events among women with high normal blood pressure or blood pressure progression: Prospective cohort study. BMJ September 1;335(7617):432
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