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ABSTRACT

Aims: Evaluation of lipoprotein profile, atherogenic ratios and haemorheological parameters as indicators and significant adjuncts for predicting atherosclerosis in an apparently healthy population was investigated.

Methodology: A total of 300 subjects comprising 169 (56.3%) males and 131 (43.7%) females within the age range of 18-45 were recruited into the study. Haemorheological parameters such as haemoglobin, haematocrit, erythrocyte sedimentation rate, platelets, fibrinogen, whole blood viscosity and plasma viscosity were determined using standard methodologies. Fasting lipid profile parameters were assessed using standard procedures. Results: Results of the lipoprotein determinations were used to calculate the atherogenic indices namely Atherogenic Index of Plasma, and Castelli Risk Index I and II. Analysis of variance and student t-test were used to compare the means of the parameters. ANOVA analysis of the means of the haemorheological parameters showed no significant difference (p>0.05) in the means of the haemorheological parameters between the age classes except for fibrinogen (p<0.05). However, significant differences (p<0.05) in means of the lipoproteins were observed between the age classes except for triglycerides. Comparison of the means of the haemorheological parameters between the male and female subjects showed significant difference (p<0.05) for all parameters except platelets whereas no significant difference (p>0.05) was observed in the means of the lipoproteins levels and the atherogenic indices between the male and female subjects. Conclusion: Analysis of the haemodynamic parameters, the lipoprotein profiles and the atherogenic risk of the population for predisposition and development of cardiovascular disease events and atherosclerosis in the absence of risk factors such as cigarette smoking, hypertension, and diabetes mellitus did not reveal risk in the subjects.

KEYWORDS: Atherogenic index, haemorheology, cardiovascular disease, lipoproteins, Castelli risk index.

INTRODUCTION

Cardiovascular disease (CVD) is the cause of one third of deaths worldwide and it is predicted that this status will progress because the risk factors of CVD are increasing by the day.\[1]\ The most important risk factors for CVD consist of dyslipidemia, hypertension, obesity, physical inactivity, poor diet and smoking.\[1,3]\ However, blood lipids and lipoprotein profile have been reported to be the major risk factors and predictor for CVD.\[4,5]\ Dyslipidaemia has been described as a condition whereby elevated plasma concentration of lipid triglyceride (TG) and total cholesterol (TC) and their blood transporting lipoproteins; HDL-Cholesterol, LDL-Cholesterol and VLDL-Cholesterol) occurs in the plasma of an individual.\[6-8]\ Atherogenic Index of Plasma (AIP) has been shown to be a strong marker of CVD with the potential to predict the risk of atherosclerosis and coronary heart disease.\[8-11]\ The true relationship between protective and atherogenic lipoprotein and its association with the size of pre- and anti- atherogenic lipoprotein particle is usually reflected by the atherogenic index of plasma (AIP).\[12]\ AIP is calculated according to the formula, log (TG/HDL-C).\[5,11,13]\ It has been suggested that an AIP value of under 0.11 is associated with low risk of CVD; the values between 0.11 to 0.21 and upper than 0.21 are associated with intermediate and increased risks, respectively.\[12,14]\ The total/high-density lipoprotein (HDL) cholesterol ratio, known as the Castelli risk index is an indicator of vascular risk, and the predictive value is greater than the isolated lipoprotein parameters. An increase in total cholesterol concentration, and specifically LDL cholesterol, is indicates atherogenicity, whereas reduced HDL cholesterol concentration is correlated with numerous risk factors, including the components of the metabolic syndrome and is considered an independent
risk factor.[15] The total/HDL cholesterol ratio has high discriminatory as well as great predictive capacity for coronary heart disease. The obesity epidemic is a global public health problem. Blood flow through the cardiovascular system help in maintaining the haemostasis conditions of the human body. Acute cardiac events may be caused by an insufficient blood supply due to reduced vessel lumen or altered mechanical properties of blood including increased blood viscosity and red blood cell (RBC) aggregation, and decreased RBC deformability. A number of studies have demonstrated that increased haematocrit, RBC aggregation and blood viscosity are important risk factors of cardiovascular disorders.[16,17] Wintrobe[18] reported that during the reproductive period of female life, there is a significant difference in haematocrit levels in male and female blood. Accordingly, haematocrit was reported to be equal to 47 ± 5% for adult males and 42 ± 5% for females resulting in lower blood viscosity in women during the reproductive period. The role of blood viscosity in the development of atherosclerosis and of coronary heart disease has been commented reported.[19] Clinical observations suggested that patients with debilitating diseases, with lower than normal haemoglobin (red cell) content, have a lower incidence of overt atherosclerotic vascular disease than those with higher values for this parameter.[20] Since the high circulating red cell mass is known to maintain high viscosity, it could be postulated that this might play a role in the development of atherosclerosis and/or atherosclerotic vascular disease. In Nigeria, mortality arising from strokes and related coronary disease is high and is still rising. In this study, we evaluated the clinical utility of adopting the use of some haemorheological parameters, lipoprotein profile, Atherogenic Index of plasma and Castelli risk indices as indicators and significant adjuncts for predicting atherosclerosis and identify the cardiovascular risk status of an apparently healthy population.

MATERIALS AND METHODS

Subjects’ Selection
A total of 300 apparently healthy young men and women within the age range of 18-45 years who were residing in Port Harcourt were recruited into this study. Out of the 300 subjects, 169 (56.3%) were males while the remaining 131 (43.7%) were females

Ethical Approval
Ethical approval was obtained from the Ethics Committee of Rivers State Health Management Board and Rivers State University of Science and Technology Health Services Department, Port Harcourt, Nigeria and informed consent was obtained from all participants that were involved in this study.

Inclusion and Exclusion Criteria
Participants included in the study were apparently healthy adult male and female subjects attending the University Health Services Department Out-patient Clinic. Excluded from the study were pregnant women, subjects who admitted to having history of bleeding or clotting disorders, hypertensive and diabetic subjects, smokers and subjects who admitted to a family history of cardiovascular disease. Also, subjects who were less than 18 years of age and those that declined from participating in the study were excluded.

Sample Collection
The subjects’ vein were selected and tourniquet was tied round the arm, the skin area was disinfected with methylated spirit and 10ml syringe and 21G needle size was used to draw blood from the ante cubital vein. About 4ml of blood was put into lithium heparin bottle, 3ml put into ethylenediamine tetra-acetic acid (EDTA) bottle while 3.8ml was put into sodium citrate bottle. All the bottles were labeled, capped and gently the blood was mixed with the anticoagulants.

Estimation of Haemoglobin Concentration
This test was done using the modified azide methaemoglobin reaction method with Hb 201® HemoCue Analyzer, model No. HemoCue AB, Sweden. The erythrocytes are haemolyzed to release the hemoglobin. The haemoglobin is converted to methaemoglobin and then combined with azide to form azidemethaemoglobin which was measured at two wavelengths in order to compensate for turbidity by the analyzer.

Determination of Haematocrit (Hct)
The microhaematocrit method involving the use of the microhaematocrit centrifuge and microhaematocrit reader was used.[21] Haematocrit (Hct) levels reflect the proportion of blood occupied by red blood cells (RBCs). When a well-mixed blood specimen in a capillary tube is centrifuged by the microhaematocrit centrifuge, the centrifuge provides a centrifugal force of 12000g and a 5minutes centrifugation results in a constant packed cell in the capillary tube referred to as haematocrit (Hct).

Platelets Count
Platelets count was done using the visual cell counts method by using the improved Neubaeur counting chamber. Diluted blood was passed under a cover glass on a counting chamber and the chamber was placed in position on the microscope with the cover glass on it. The cover glass rest upon the two outer platforms of the chamber producing a clearance between itself and the rulings on the central platforms of the chamber. The clearance produced is referred to as the depth of the counting chamber.[21]
Determination of Erythrocyte Sedimentation Rate (ESR)
The method for measuring the ESR is based on that of Westergren, recommended by the International Council for Standardization in Haematology (ICSH). The erythrocyte sedimentation rate (ESR) measures the degree of red blood cells settling during a specified time of 1 hour. The red blood cells in diluted blood in an open-ended glass when mounted vertically on a stand, descend in the tube, and displace an equal volume of plasma upward, which shows the downward progress of other settling blood elements. 

Determination of Whole Blood Viscosity (WBV)
The modified needle and syringe method of Reid and Ugwu was used. When anticoagulated blood is withdrawn into a syringe with the plunger, the blood cannot drop except pushed by the plunge. But in this method for the determination of blood viscosity, the plunger is removed for a flow without force. As the whole blood interacts with the wall of the syringe, it influences the flow and blood starts to drop. The relative viscosity time was recorded compared to flow-time of distilled water at the same temperature in seconds.

Relative whole blood viscosity was calculated by the following equation:

\[ \text{RBV} = \frac{t_{\text{blood}}}{t_{\text{water}}} \]

Where \( t_{\text{blood}} \) is the time of flow of 2ml of whole blood, and \( t_{\text{water}} \) is the time of flow of 2ml of distilled water which was used as standard.

Determination of Plasma Viscosity
Plasma Viscosity (PV) were carried out by a modification of the method of Reid and Ugwu (1987). For the plasma viscosity, a part of the whole blood was centrifuged in a stopped sterile clean bottle to obtain clean and clear plasma. It was centrifuged for 5 mins at 3000g. The result was calculated in the same way as that of the whole blood viscosity.

Relative Plasma viscosity was calculated by the following equation:

\[ \text{RPV} = \frac{t_{\text{plasma}}}{t_{\text{water}}} \]

Where \( t_{\text{plasma}} \) is the time of flow of 2ml of plasma, and \( t_{\text{water}} \) is the time of flow of 2ml of distilled water which was used as standard.

Determination of Plasma Fibrinogen
Fibrinogen was estimated using the modified method of Claus. The modified Clauss method involves the use of the fibrinogen reagent kit, manufactured by Technoclone, Vinenna, Austria. The determination of fibrinogen with throbmin clotting time is based on the method originally described by Clauss; in the presence of an excess of thrombin, fibrinogen is transformed into fibrin and clot formation time is inversely proportional to the concentration of fibrinogen in the sample plasma.

Determination of Plasma Total Cholesterol
The enzymatic procedure for total cholesterol determination in serum based upon the Trinder method as modified by the Centers for Disease Control and Prevention was used. The method is popularly known as the enzymatic endpoint method. Cholesterol esterases (CHE) hydrolyzes the cholesterol esters into free cholesterol. Cholesterol oxidase (CHOD) oxidizes the cholesterol into cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide reacts with a mixture of 4-aminoantipyrine and phenol in the presence of peroxidase enzyme (POD) and converts the reactants into a red quinoneimine dye. The absorbance of the quinoneimine is directly proportional to the cholesterol concentration when measured at 520nm.

Determination of Plasma High Density Lipoprotein - Cholesterol
The method of Lopes-Virella et al. for the determination of high-density cholesterol in plasma was employed. Low density lipoproteins and very low density lipoproteins (LDL and VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined at 520nm.

Enzymatic Determination of Plasma Triglycerides
The colorimetric method of Tietz was employed. Lipase hydrolyses triglycerides sequentially to di and monoglycerides and finally to glycerol. Glycerol kinase (GK) using ATP as phosphate source converts glycerol liberated to glycerol-3-phosphate (G-3-Phosphate). Glycerol-3-phosphate oxidase (GPO) oxidizes Glycerol-3-phosphate and forms dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidize 4-aminoantipyrine and TOOS (N-ethyl-N-sulphohydroxy propyl-m-toluidine) to a purple coloured complex. The absorbance of the coloured complex is measured at 520 nm and is proportional to triglyceride concentration in the sample.

The atherogenic ratios were calculated as follows: Atherogenic Index of Plasma (AIP) = log TG/HDL-C Castelli’s Risk Index (CRI-I) = TC/HDL-C Castelli’s Risk Index (CRI-II) = LDL-C/HDL-C
Statistical Analysis
The results were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21. Data were expressed as mean ± SD. Analysis of variance (ANOVA) was used to compare mean differences of more than two groups, whilst student t-test was used to compare the differences between two groups. Values were considered significant at p<0.05.

RESULTS
The mean and standard deviations of the haematocrit (Hct), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), platelet count (PL), whole blood viscosity (WBV), plasma viscosity (PV) and fibrinogen (Fibr) in the subjects (combined) classified by age range is shown in table 1. The mean of the haematocrit (%) ranged between 39.1±2.1 to 40.4±4.4% between the age classes of 18-24 to 40-45 years. The haemoglobin concentration also ranged between 13.0±2.2 to 13.4±3.5 g/dl between the age classes of 18-24 and 40-45years. The means of the haematocrit and haemoglobin concentration did not show any significant difference (p>0.05), (F=0.907, 1.099) respectively between the age classes.

Similarly, the means of the erythrocyte sedimentation rate (ESR) obtained from study ranged from 11.6±3.6 to 14.4±4.5 mm/hr for the age classes used in the study. The ESR value obtained from the study within each age range was higher than the stipulated reference range. However, no show significant difference (p>0.05), F=1.066 between the age classes was observed. The means of the platelet concentration in the subjects according to the age classes 181.6±23.8 to 1.93±11.5 (x10^9/L). The means were also within the stipulated reference range and were not significantly different (p>0.05), F=0.740 between the age classes. The means ±SD of the whole blood viscosity and plasma viscosity in the age classes were between 3.17±0.42 to 4.20±0.44 mPa’s and 1.23±0.04 to 2.01±0.07 mPa’s respectively for the age range of 18-24 t0 40-45years. No significant difference (p>0.05), (F=1.809, 1.494) respectively in means was also observed between the age classes. However, the mean ±SD of the fibrinogen concentration between the age classes was found to be 205.93±23.4 to 262.1±44.7 mg/L for the determined age classes. Obvious significant difference (p<0.05), F=5.783 in mean of fibrinogen was observed between the age classes in the population studied.

The mean ±SD of the lipoproteins represented in age classes showed that there was significant differences (p<0.05) in means of total cholesterol, high density lipoprotein cholesterol and low density cholesterol while no significant difference (p>0.05) was observed in the mean of triglycerides (table 2)

The means and standard deviations of the parameters in apparently healthy male and female subjects is shown in Table 3. The mean ±SD of Hct in the male subjects was 41.1±3.5% while that of the female subjects was 37.9±4.1%. A significant difference (p<0.05, F=44.21) was observed between the means. Comparison of the means of haemoglobin concentration in the male and female subjects showed haemoglobin concentration of 13.6±1.2 g/dl and 12.6±2.5 g/dl which was significantly different (p<0.05, F=29.546) respectively. The values of the erythrocyte sedimentation rate (ESR) and platelets concentration for the male and female subjects were 11.4±6.2 mm/hr and 15.9±5.7 mm/hr and 183.1±22.4 (x10^9/L) and 193.0±18.5 (x10^9/L) respectively. Obvious significant difference (p<0.05, F=32.54) in mean was observed in the means of ESR between the male and female subjects while variation in means of platelets concentration showed no significant difference (p>0.05, F=3.32).

Furthermore, the means of WBV and PV obtained from the study for the male and female subjects were 4.25±0.33 mPa’s and 2.83±0.87 mPa’s and 1.98±0.54 mPa’s and 1.76±0.87 mPa’s respectively. The means showed significant difference (p<0.05, F=7.225; 12.534) between the male and female subjects respectively. The concentration of fibrinogen in the male and female subjects were 221.9±56.77 mg/L and 251.3±77.98 mg/L respectively and significant difference (p<0.05, F=10.720) was observed between the means. Haemorheological parameters such as Hct, Hb, WBV and PV showed higher means in the males than females while the means of such parameters as ESR, platelet count and fibrinogen were higher in the females than in the males.

The mean ±SD of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), atherogenic index of plasma (AIP), Castelli risk index 1 and 11 (CRI-1 and CRI-11) between the male and female subjects is shown in table 4. The mean ±SD of the total cholesterol in the male and female subjects were 5.12±0.91 and 5.10 1.00 mmol/L. Similarly, the means ± SD of triglycerides and high density cholesterol in the male and female subjects were 0.91±0.45 mmol/L and 0.91±0.34 mmol/L and 1.40±0.42 mmol/L and 1.43±0.39 mmol/L respectively. Computed means ± SD of the low density lipoprotein cholesterol between the male and female subjects was 3.03±0.87mmol/L and 2.96 mmol/L respectively. No significant difference (p>0.05) in means of these parameters was observed between the male and female subjects in the population studied. The means of the atherogenic ratios AIP, CRI-1 and CRI-11 in the male and female subjects were -0.187±0.05 and -0.196±0.04, 3.58±0.78 and 3.59±0.95 and 2.16±0.87 and 2.07±0.76 respectively with no significant in means being observed in the means between the male and female subjects. The p-values and t-values are indicated in table 4.
Table 1: Comparison of means ±SD of haemorheological parameters according to age group (males and females combined).

<table>
<thead>
<tr>
<th>Age-groups (years)</th>
<th>Hct (g/dl)</th>
<th>Hb (g/dl)</th>
<th>ESR (mm/hr)</th>
<th>PL (x10^9/L)</th>
<th>WBV (mPa*s)</th>
<th>PV (mPa*s)</th>
<th>Fibr (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24(77)</td>
<td>40.2±2.2</td>
<td>13.4±2.0</td>
<td>13.2±5.6</td>
<td>184.12±22.1</td>
<td>4.15±0.59</td>
<td>1.26±0.02</td>
<td>205.93±23.4</td>
</tr>
<tr>
<td>25-29(64)</td>
<td>39.3±3.2</td>
<td>13.12±3.5</td>
<td>14.4±4.5</td>
<td>193.8±11.5</td>
<td>3.17±0.42</td>
<td>1.76±0.08</td>
<td>233.6±33.6</td>
</tr>
<tr>
<td>30-34(36)</td>
<td>40.4±4.4</td>
<td>13.4±3.5</td>
<td>16.3±5.6</td>
<td>185.6±30.3</td>
<td>4.02±0.31</td>
<td>1.23±0.04</td>
<td>222.8±45.6</td>
</tr>
<tr>
<td>35-39(63)</td>
<td>39.6±1.6</td>
<td>13.0±2.6</td>
<td>14.0±6.7</td>
<td>191.2±30.7</td>
<td>4.20±0.44</td>
<td>2.04±0.07</td>
<td>252.0±22.9</td>
</tr>
<tr>
<td>40-45(60)</td>
<td>39.14±2.1</td>
<td>13.0±2.2</td>
<td>13.0±6.1</td>
<td>181.6±23.8</td>
<td>4.02±0.34</td>
<td>1.96±0.05</td>
<td>262.14±44.7</td>
</tr>
</tbody>
</table>

Note: Hct= Haematocrit, Hb = haemoglobin, ESR= erythrocyte sedimentation rate, PL= platelets, WBV=whole blood viscosity, PV= plasma viscosity and Fibr = fibrinogen, ns= not significant.

Table 2: Comparison of means of lipoprotein parameters in the subjects by age classes.

<table>
<thead>
<tr>
<th>Age classes (yrs)</th>
<th>TC (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>4.7±0.35</td>
<td>1.00±0.50</td>
<td>1.42±0.21</td>
<td>2.63±0.32</td>
</tr>
<tr>
<td>25-29</td>
<td>5.0±0.41</td>
<td>0.95±0.31</td>
<td>1.46±0.11</td>
<td>2.90±0.11</td>
</tr>
<tr>
<td>30-34</td>
<td>4.9±0.26</td>
<td>0.90±0.21</td>
<td>1.36±0.9</td>
<td>2.96±0.21</td>
</tr>
<tr>
<td>35-39</td>
<td>5.3±0.33</td>
<td>0.79±0.25</td>
<td>1.38±0.20</td>
<td>3.32±0.35</td>
</tr>
<tr>
<td>40-45</td>
<td>5.4±0.61</td>
<td>0.86±0.23</td>
<td>1.43±0.12</td>
<td>3.31±0.20</td>
</tr>
</tbody>
</table>

Note: TC= total cholesterol, HDL-C= high density lipoprotein cholesterol, TG= triglycerides, LDL-C= low density cholesterol.

Table 3: Comparison of means ±SD of haemorheological parameters in subjects according to gender.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Hct (%)</th>
<th>Hb (g/dl)</th>
<th>ESR (mm/hr)</th>
<th>PL (x10^9/L)</th>
<th>WBV (mPa*s)</th>
<th>PV (mPa*s)</th>
<th>Fibr (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male n=169</td>
<td>41.1±3.50</td>
<td>13.6±1.2</td>
<td>11.4±6.20</td>
<td>183.1±22.40</td>
<td>4.25±0.33</td>
<td>1.98±0.54</td>
<td>221.9±56.77</td>
</tr>
<tr>
<td>Female n=131</td>
<td>37.9±4.10</td>
<td>12.6±2.50</td>
<td>15.9±5.70</td>
<td>193.0±18.55</td>
<td>2.83±0.87</td>
<td>1.76±0.87</td>
<td>251.3±77.98</td>
</tr>
</tbody>
</table>

Note: Hct= Haematocrit, Hb = haemoglobin, ESR= erythrocyte sedimentation rate, PL= platelets, WBV=whole blood viscosity, PV= plasma viscosity and Fibr = fibrinogen, ns= not significant.

Table 4: Comparison of mean ±SD of lipoproteins and atherogenic ratios in the population.

<table>
<thead>
<tr>
<th>Sex</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>AIP</th>
<th>CRI-I</th>
<th>CRI-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5.12±0.91</td>
<td>0.91±0.45</td>
<td>1.4±0.42</td>
<td>3.03±0.87</td>
<td>-1.87±0.05</td>
<td>3.58±0.78</td>
<td>2.16±0.87</td>
</tr>
<tr>
<td>Female</td>
<td>5.10±1.00</td>
<td>0.91±0.34</td>
<td>1.43±0.39</td>
<td>2.96±0.98</td>
<td>-0.19±0.04</td>
<td>3.59±0.95</td>
<td>2.07±0.76</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk level</td>
<td>&gt;5.228</td>
<td>&gt;1.728</td>
<td>≤1.028</td>
<td>&gt;2.628</td>
<td>0.2428</td>
<td>4.0027</td>
<td>4.0027</td>
</tr>
<tr>
<td>t-value</td>
<td>1.911</td>
<td>1.312</td>
<td>2.011</td>
<td>0.907</td>
<td>0.501</td>
<td>1.601</td>
<td>1.051</td>
</tr>
</tbody>
</table>

Dyslipidaemia is defined using the National Cholesterol Education Program – Adult Treatment Panel III,[15] AIP= Atherogenic Index of Plasma, CRI-I= Castelli Risk Index-I, CRI-II= Castelli Risk Index-II.

DISCUSSION

This study examined at the possibility of establishing a relationship between haemorheological parameters and cardiovascular disease risk factors of apparently healthy population resident in Port Harcourt, Nigeria to identify the cardiovascular risk status of the population beyond the routinely done lipid profiles and haematological assessment in insufficient resource situations. The haemorheological parameters considered include haematocrit, haemoglobin, erythrocyte sedimentation rate, white blood cell count, platelets, whole plasma viscosity, plasma viscosity and fibrinogen concentration while the cardiovascular disease risk factors include total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and atherogenic indices such as atherogenic index of plasma and Castelli risk indices. Chronic anaemia has been reported to increase the risk for coronary artery disease. In the Framingham Heart...
Study, women but not men with low haematocrit at baseline reportedly showed increased cardiovascular risk during a 34 year of follow-up[31] in an over 6 year follow up, men with haemoglobin (Hb) values <13 g/dl and women with values <12 g/dl in the Atherosclerosis Risk in Communities (ARIC) project experienced a 41% increase in cardiovascular disease, when compared with men and women with higher levels of Hb.[32] In the present study the haemoglobin concentration in the male and female subjects were in the range of 12.40-14.8 g/dl and 10.5-15.1 respectively. The haemoglobin concentration was also higher in the male than in the female in agreement with the findings of Ingram.[33] It is pertinent to state that the study involved apparently healthy subjects. The results showed that there could be covert anaemia in the population which resulted in the low haemoglobin concentration in both male and female subjects. However, some reports have indicated that patients or subjects exhibiting lower than normal haemoglobin (red cell) content have a lower incidence of overt atherosclerotic vascular disease than those with higher values for these parameters.[34] Healthy males have higher haemoglobin and hematocrit levels than do healthy females. With increasing hematocrit, whole blood viscosity increases, a condition that could worsen blood flow ability hence causing atherosclerosis[35] and suggesting elevated haematocrit (Hct) as a predictor of CVD risks.[31,36] The present study recorded a range of haematocrit which is normal for the population studied. Thus, incidence of atherosclerosis if evident in this population might not be attributable to the contributions from haematocrit as a risk factor.

Blood and plasma viscosity have emerged as independent risk factors for atherothrombotic vascular disease.[37,38] Clinical manifestations of coronary and cerebrovascular disease are associated with an increased blood viscosity.[39,40] Blood viscosity is an important determinant of rate of blood flow and the greater the viscosity, the less the flow in a vessel, if all other factors are constant. Furthermore, the viscosity of normal blood is about three times as great as that of water. What makes blood so viscous is mainly the large number of suspended red cells in the blood, each of which exerts frictional tray against adjacent cells and against the wall of the blood vessel.[41] Our blood viscosity values were higher for men than for women. The high blood-viscosity values in the males may have resulted from the higher haematocrit also observed in the male subjects. This finding is in agreement with the reports of Rosenson et al.[38] and Jan et al.[40]

It has also been reported that stroke patients showed two or more elevated rheological parameters, which included whole blood viscosity, plasma viscosity, red blood cell (RBC) and platelet aggregation, red blood cell rigidity, and haematocrit and that both whole blood viscosity and plasma viscosity were significantly higher in patients with essential hypertension than in healthy ones, whereas RBC deformability was decreased.[43,44] The ESR is one of the measurements of acute phase response. It is helpful in detecting the presence of inflammation and response to treatment. It can be affected by anaemia, which may be present in inflammatory disease, and by proteins of acute phase response.[44] The ESR in the present study was higher in the female than in the male subjects and the increase was significant (p<0.05, t=32.54). The ESR was reasonably higher than the normal range for the population. The level of haematocrit has already been reported to be on the low normal which could indicate covert anaemia. However, symptoms of anaemia were rarely observed in all the subjects. Since anaemia and protein changes such as fibrinogen concentration could increase ESR, increased ESR levels as observed in this study could alter the fibrinogen-globulin ratio which has the potential to enhance rouleaux formation, a factor that has been implicated in atheroembolism.[45]

The fibrinogen concentration observed in this study was significantly (p<0.05) higher in the females compared to the males. This is also in agreement with the findings of Dapper.[46] Higher fibrinogen concentration has been reported to account for elevated erythrocyte sedimentation rates usually seen in African females.[33,36] Surprisingly, this finding of higher ESR in the females with subsequent high fibrinogen concentration also in the females was observed in this study. The association between plasma fibrinogen and cardiovascular risk does not always establish a cause-effect relationship, because plasma fibrinogen levels are related to several major lifestyle and physical characteristics known to be associated with increased risk of coronary heart disease (CHD).[47] Although the effect of plasma fibrinogen on cardiovascular risk could result profoundly from cigarette smoking or diabetes mellitus, plasma fibrinogen has emerged as a potentially confounding independent cardiovascular risk factor.[48] Since the population studied is a healthy population devoid of smoking history and diabetes mellitus and normal fibrinogen concentration was reported, the role of fibrinogen in the etiology of cardiovascular disease in the population may be poor.

Platelets play an important role in CVD, both in the pathogenesis of atherosclerosis and in the development of acute thrombotic events. Abnormal platelets, either quantitatively or qualitatively, are associated with CVD.[49,50] In cardiovascular disease, abnormal clotting occurs that can result in heart attacks or stroke. The platelet concentration reported in this study falls within the normal range indicating that the possibility of abnormal clotting which could predispose the subjects to cardiovascular disease may not exist in the population since individuals with diabetes mellitus and cigarette smoking were excluded from the population studied.

The possible role of such haemodynamic factors as blood viscosity in the development of atherosclerosis and of
coronary heart disease has been reported. Studies of whole blood viscosity and its role in cardiovascular disease has been increasing in recent years with the knowledge that it is a major determinant in the perfusion of tissues throughout the body. Changes in the plasma viscosity also has been shown to influence significantly the overall viscosity of whole blood. This study recorded higher WBV and PV in the male compared with the female, a finding that corroborates with results from other works. It is suggested that the higher viscosity of whole blood and of plasma is a contributory factor in the development of the clinical symptoms of coronary heart disease and possibly of atherosclerosis itself. The levels of whole blood viscosity and plasma viscosity recorded in this study were within the normal limit for apparently healthy populations.

Lipid profile refers to some routinely done biochemical tests to assess the atherogenic status of individuals at risk of cardiovascular disease. It includes serum triglycerides (TG), serum total cholesterol (TC) and its sub fractions like high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). The Framingham heart study over years has established the role of deranged lipid profile in the progression of CVD. Calculating certain ratios using these parameters especially in situations where LDL-C levels are below target range may increase the identification of at-risk individuals. On evaluation of lipid ratios, in the current study, we observed that Atherogenic Index of Plasma (AIP) was not significantly (p<0.05, t=0.502) higher in males as compared to females. AIP is a ratio calculated as (logTG)/HDL-C. The AIP obtained in this study was in the range of -0.196±0.04, a value considerably less than the risk cut established by Dobiasova. AIP has been recommended to be used as a significant predictor of atherosclerosis. AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high cardiovascular disease risk. As no derangement was observed in TG and HDL-C in both male and female subjects the value of AIP in the subject groups were not expected to be significantly raised, thus the population did not show predisposition to developing cardiovascular disease. The lipid profile results therefore corroborate the indications as revealed by the haemorheological parameters.

Castelli Risk Indices (CRI) is based on three important lipid profile parameters i.e. TC, LDL-C and HDL-C. CRI -I calculated as the ratio of (TC/HDL-C) and CRI-II as (LDL-C/HDL-C). In our study, no significant difference (p<0.05, 1.601) was found in the value of CRI-1 between the male and female subjects studied. Similar finding was also observed in the value of CRI-11 (p=0.05, t=1.051). Furthermore, we could not observe a significant difference in TC, TG, HDL-C and LDL-C levels between the two study groups. The computation of the ratios and the values obtained thus reinforced the position that cardiovascular disease risk within population are yet to be identified.

In conclusion, our study showed that on the basis of the haemodynamic parameters and the lipid profiles studied and risk ratios computed, development of atherosclerosis and cardiovascular events may be low in the population particularly when risk factors such as diabetes mellitus, hypertension, cigarette smoking and other cardiovascular disease risk factors are fully excluded from the subjects.

REFERENCES

12. Dobiasova M, Frohlich J, Sedov M, Cheung MC, Brown BG. Cholesterol esterification and atherogenic index of plasma correlate with