Relative plasma viscosity and fibrinogen levels of male cigarette smokers resident in Calabar, Nigeria

Effa Faith Assam, Agu Chidozie Elochkwu*, Offor Jeremiah Sunday and Ofutet Emmanuel Oleba

Department of Medical Laboratory Science, University of Calabar, P.M.B. 1115 Calabar, Cross River State

Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, P.M.B. 1115 Calabar, Cross River State

ABSTRACT

Cigarette smoking leads to serious health problems and remains the most important avoidable causes of death worldwide. Hemorheology, a measure of rheological properties of blood, is often correlated with cerebral blood flow and cardiac output; an increased blood viscosity may increase the risk of thrombosis or thromboembolic events. This study aims to evaluate the levels of fibrinogen and relative plasma viscosity in male smokers and to study the association among duration of smoking, age and number of sticks smoked per day with these parameters. A total of 189 apparently healthy subjects were enrolled in this study; 106 male smokers and 83 male non-smokers. Male smokers were group into mild and heavy smokers based on the number of stick smoked per day; mild smoker (1-19 stick per day) and heavy smokers (>20 sticks per day). Whole blood was dispensed into a trisodium citrate bottle, RPV was determined using simple syringe method, fibrinogen was determined using Clauss method. The mean values of RPV and fibrinogen for the male smokers were significantly higher than those of the non-smokers (P<0.05); an significant negative correlation was observed between fibrinogen levels and age among the male smokers (P<0.05). Smokers in the age range of 15-30 years recorded significantly higher mean fibrinogen levels compared to those in the age range of 31-55 years (P<0.05). Results from the present study reveal that smokers recorded higher mean values of fibrinogen and RPV and thus maybe at an increased risk of developing atherothrombotic diseases.

Keywords: fibrinogen, cigarette smoking, hemostasis, relative plasma viscosity, rheology

INTRODUCTION

Cigarette smoking is a major public health problem globally and in Nigeria. Cigarette smoke contains a complex mixture of about four hundred (4000) chemicals some of which are toxic and at least sixty (60) causes cancer [1]. In view of this complexity, cigarette smoke has multiple diverse effects on human health. The negative effects and high mortality associated with it has caused several works to be done in the health sector and beyond[2]. The prevalence of cigarette smoking varies across the globe with the highest prevalence in developing countries. According to World Health Organization WHO (2008) out of the 1.22 billion smokers, one (1) billion of them live in developing countries[3].
The study of hemorheology has been of great interest in the fields of biomedical engineering and medical research for many years. Hemorheology plays an important role in atherosclerosis[4]. Hemorheological properties of blood include whole blood viscosity, plasma viscosity, hematocrit, RBC definability and aggregation, and fibrinogen concentration in plasma[5]. Although a number of parameters such as pressure, lumen diameter, whole blood viscosity, compliance of vessels, peripheral vascular resistance are well-known physiological parameters that affect the blood flow, the whole blood viscosity is also an important key physiological parameter. However, its significance has not been fully appreciated yet [4].

Activation of the coagulation cascade converts soluble fibrinogen to insoluble fibrin, which polymerizes to produce, along with platelets, the hemostatic clot [6]. Whereas the normal activation of the coagulation cascade is essential for life, inappropriate activation may result in thrombosis and complications that arise from the formation of fibrin clots[7]. Indeed pathologically induced thrombogenesis is associated with adverse cardiovascular events, thromboembolism in chronic obstructive pulmonary disease, and vascular complications in autoimmune diseases [8].

**MATERIALS AND METHODS**

**Selection of subjects**
A total number of 106 healthy adult male cigarette smokers resident in Calabar metropolis were enrolled for the study. The cigarette smokers that participated in the study are those who have smoked 100 cigarette sticks during their life time and who currently smoke everyday or some days. Only male between the ages range of 15 to 55 were enrolled. Eight three (83) apparently healthy adult males who are non smokers (active and passive) were used as control subjects in this research.

A structured questionnaire was used to get data on each individual enrolled in the study, detailing his family history, age, occupation, drinking habit, smoking habit and whether or not on medication that may affect test results.

**Ethics statement**
Ethical approval for the research was obtained from Cross River State Ministry of Health Research Ethic Committee Calabar, Nigeria. The subjects who participated in the study were educated on what the research was all about. Their consent was sought for and each participant signed a consent form prior to the study.

**Sample collection**
Four and half milliliters (4mls) venous blood was collected under aseptic conditions and with minimal stasis from each subject using sterile syringe and needle from the ante-cubical vein. Two milliliter of the blood sample was added into 0.5mls of 3.13% trisodium citrate for fibrinogen estimation. The samples were transported to the laboratory for analysis within one (1) hour of collection. The remaining 2.0mls of blood were dispensed into Ethylene diamine tetra acetic acid (EDTA) bottle 2mg/ml for the determination of relative plasma viscosity (RVP).

**Determination of relative plasma viscosity**
A modification of the method of Reid and Ugwu [9] was used. A 1ml syringe with a hypodermic needle (21.6 × 0.8 × 4mm) was used. Briefly, plasma or serum was drawn into the syringe, avoiding or bubbles, till the 1.0ml mark. The plunger was carefully, removed and the time taken for the entire plasma or serum to drain was noted. This was done twice for each sample and the average taken for that sample. The entire process was repeated using distilled water. The plasma viscosity is the ratio of the flow rate of plasma to that of water.

\[
\text{RPV}=\frac{\text{TPs}}{Tn (s)}
\]

Reference range: 1.47-1.86.

**Determination of fibrinogen concentration using Clauss technique[10]**

**Principle:** The determination of fibrinogen with thrombin 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.

Reference Range: 2 - 4g/l
RESULTS

Fibrinogen and relative plasma viscosity (RPV) were determined in 106 male cigarette smoker and 83 male non-smokers.

Table 1 shows the mean value of fibrinogen and relative plasma viscosity in male smokers and non-smokers which were used as control subjects. The result revealed that the mean values of fibrinogen and RPV were significantly higher in male smokers compared to the non-smokers control participants (P <0.05).

Cigarette smokers were further divided into two groups based on the number of cigarette sticks smoked per day; mild smokers, those who smoked 1-19 sticks per day and heavy smoker, those who smoked 20 sticks and above per day. Fibrinogen and relative plasma viscosity were compared between the two groups. These comparisons are shown in table 2. Results from the table reveal that no significant variation were observed in the levels of fibrinogen and relative plasma viscosity between the mild and heavy smokers (p>0.05).

Table 3 shows the comparison of fibrinogen and relative plasma viscosity in smokers based on age. Results from this table reveals that smokers between the age range of 15-30 years recorded significantly higher mean fibrinogen level compared to those in the age range of 31-55 years (P<0.05), while no significant variation was observed in the levels of relative plasma viscosity between the two age group (P>0.05).

Correlation analysis was carried out between age and fibrinogen in male smokers. Fibrinogen showed a significant negative correlation with age in the cigarette smokers (r = 0.249, P < 0.05) (Figure 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smokers (n=106)</th>
<th>Control (n=83)</th>
<th>Cal. ‘t’ value</th>
<th>P-value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE(years)</td>
<td>30.39±9.67</td>
<td>29.90±9.11</td>
<td>0.91</td>
<td>0.765</td>
<td>NS</td>
</tr>
<tr>
<td>FIBRINOGEN(g/L)</td>
<td>4.30±1.45</td>
<td>2.72±0.81</td>
<td>8.92</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>RPV</td>
<td>1.83±0.22</td>
<td>1.54±0.23</td>
<td>8.79</td>
<td>0.000</td>
<td>S</td>
</tr>
</tbody>
</table>

Table 2: Comparison of fibrinogen and relative plasma viscosity in smokers based on number of cigarette sticks smoked per day

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mild (1-19stciks) Per day (n=75)</th>
<th>Heavy (&gt;20 sticks) Per day (n=31)</th>
<th>Cal. ‘t’ value</th>
<th>P-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE(years)</td>
<td>30.97±9.52</td>
<td>28.97±6.09</td>
<td>1.08</td>
<td>0.281</td>
<td>NS</td>
</tr>
<tr>
<td>FIBRINOGEN(g/L)</td>
<td>4.3±1.42</td>
<td>4.28±1.55</td>
<td>-0.20</td>
<td>0.843</td>
<td>NS</td>
</tr>
<tr>
<td>RPV</td>
<td>1.83±0.22</td>
<td>1.84±0.20</td>
<td>0.11</td>
<td>0.917</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3: Comparison of fibrinogen and relative plasma viscosity in male smokers based on age of the smokers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(15-30 yrs.) (n=72)</th>
<th>(31-55 yrs.) (n=34)</th>
<th>Cal. ‘t’ value</th>
<th>P-value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIBRINOGEN(g/L)</td>
<td>4.4±1.49</td>
<td>3.25±1.21</td>
<td>2.92</td>
<td>0.040</td>
<td>S</td>
</tr>
<tr>
<td>RPV</td>
<td>1.84±0.22</td>
<td>1.83±0.23</td>
<td>0.07</td>
<td>0.974</td>
<td>NS</td>
</tr>
</tbody>
</table>
FIG 1: Correlation plot of age against fibrinogen in male smokers

\[ y = -0.0328x + 5.3014 \]

\[ r = 0.196 \]

\[ p = 0.04 \]

\[ n = 106 \]

FIG 2: Comparison of fibrinogen and relative plasma viscosity in male-smokers and non-smokers

DISCUSSION

Cigarette smoking has spread widely throughout the world, from countries with low income economy to the most influential industrialized nations. The effect of smoking on human health are serious and in many cases, deadly. This study aimed at estimating the levels of fibrinogen and relative plasma viscosity in male cigarette smokers and examining the effects of the duration of smoking and number of sticks smoked per day on these parameters.
In this study, the levels of fibrinogen were significantly higher (P <0.05) in cigarette smokers compared to the non-smokers control group. This is consistent with the findings of Kamath and Lip (2003)[13], who also reported increased plasma fibrinogen levels in smokers compared to non-smokers. Plasma fibrinogen is an important component of the coagulation cascade, as well as a major determinant of blood viscosity and blood flow. Increasing evidence from epidemiological studies suggests that elevated plasma fibrinogen levels are associated with an increased risk of cardiovascular disorders, including ischaemic heart disease (IHD), stroke and other thromboembolism[11]. This increase in plasma fibrinogen levels may promote a prothrombotic or hypercoagulable state, and may in part explain the risk of stroke and thromboembolism in conditions such as atrial fibrillation (AF) that may occur in these smokers[12].

Smoking results in an inflammatory reaction, probably of the pulmonary bronchi and alveoli and the blood vessels of the lung parenchyma, as evidenced by an increase in the levels of C-reactive protein [14]. The resulting inflammation may increase the production of the cytokines, such as interleukin-6, [15] which have major roles in the regulation of synthesis in the liver of acute-phase proteins, including fibrinogen [16]. Thus increased plasma fibrinogen levels in smokers may reflect a chronic inflammatory state of the vascular wall, and may act as an intermediary in the enhanced coronary risk among smokers [17].

Previous studies have reported a decrease whole body protein synthesis with aging [18]. In line with these previous reports, we observed a significant higher mean value of fibrinogen in age group (15-30yrs) compared to age group (31-55 yrs) in the male smokers and also a significant negative correlation between age and fibrinogen concentration in male smokers (P<0.05).

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 [19]. In this study, male cigarette smokers recorded significantly higher mean value of relative plasma viscosity compared to the non-smokers (P<0.05). Thus male cigarette smokers maybe at a higher risk of developing rheological abnormalities such as atherosclerosis, cardiovascular diseases and stroke compared to the non-smokers.

A striking feature in this research is that more than 50% (72 out of 106) of the smokers enrolled fell within the age range of 15 – 30 years. This has provided information that will aid cigarette control groups to focus and target that age range. Our finding has also revealed that cigarette smoking habits starts from that age range also.

CONCLUSION

From this study, it can be concluded that cigarette intake increases plasma fibrinogen and relative plasma viscosity values and thus may predispose cigarette smokers to rheological abnormalities such as atherosclerosis, thromboembolism and stroke compared to non-smokers.

REFERENCES