INTRODUCTION

Coagulation, the complex process by which blood forms clots, is highly conserved throughout biology, involving both a cellular and a protein component in all mammals. Coagulation is an important part of haemostasis, wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. Platelet activation and blood coagulation, no doubt, have been shown to be mutually dependent and interactive processes.

The balance between clotting and dissolution of clots is a function of the extremely complex interactions involving all of the cellular components in the blood-arterial wall interface, especially the endothelial cells and platelets. Haemostatic dysfunction, however, arises from any alteration of this complex system, leading to pathologic thrombosis or vascular occlusion by thrombus fragments. Haemostatic dysfunction can result in increased risk of haemorrhage or thrombosis. Smoking has been shown to induce hypercoagulability and hyperthrombotic state in haemostasis, possibly by increased platelet aggregation and adhesiveness as a result of its nicotine content. About $10^{15}$-$10^{17}$ free radicals are estimated to be contained in cigarette smoke per inhalation and these are capable of oxidizing the fat components of the body. Predictors such as age, duration and average amount of cigarette sticks smoked per day, are established factors for assessing the absolute risk of developing smoke-related complications in long-term smokers. Although smokers are more likely to have acute thrombosis than stable plaques, the frequency of plaque rupture and eroded plaque that cause thrombosis is the same in smokers and non-smokers.

A study of surgically-removed plaque tissues from samples of diseased arteries revealed that plaques from smokers were more frequently complicated by thrombosis along the walls of the arteries. Long-term smoking has been shown to affect PT and APTTK, with significantly lower values in...
subjects who had smoked for 12 years or more, compared to those that had smoked for less than 10 years. The economic cost of tobacco consumption in Nigeria varies among people of low, middle and high-income earnings. Nigeria loses $591 million yearly to tobacco use in terms of health care cost and low productivity. The tobacco industry realises almost $6000 in profit for each death caused by tobacco.

Paucity of information on this area, coupled with the increase in the number of chronic smokers of both gender necessitated the present study which is aimed at evaluating the effect of long-term chronic smoking on the coagulation markers (BT, WBCT, TPC, PT, APTTK). The results of the study will substantiate the predisposition or otherwise of chronic smokers to coagulation disorders which may result to haemostatic emergencies and also the impact of long-term smoking on coagulation.

MATERIALS AND METHODS

The study was conducted at the Department of Human Physiology, College of Medicine, Enugu State University of Science and Technology (ESUT), G.R.A Enugu, Enugu state.

A total of 78 chronic smokers with mean age 41 ± 20 years who meet our criteria were studied. The subjects were grouped into four based on the duration of smoking (2-6 years, n = 28), (7-11 years, n = 23), (12-16 years, n = 18) and (17-21 years, n = 9).

All subjects gave informed consent and the study protocol was approved by the Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTTH).

Sample size was calculated using Graph pad Prism of Statmate Software version 2.0. A sample size of 50 in each group has a 90% power to detect a difference between means of 0.33 with significant difference level (alpha) of 0.05 (two-tailed).

Chronic smokers were included if they had a history of smoking 10 ± 5 cigarette sticks per day for one year and did not abstain from smoking at anytime.

Subjects having arterial hypertension, sugar in their urine (tests were done using urinalysis strip) and currently using any antioxidants were excluded. Subjects who had abstained from smoking at any time were also excluded.

Exactly 10 mls of blood was drawn from each subject under aseptic conditions, and delivered into different well-labeled test tubes for the parameters studied. Immediately after blood collection, 4.5 ml of patient’s blood were gently mixed with 0.05 ml of sodium citrate (9 parts of blood to 1 part of the anticoagulant) in Pyrex glass test tubes and centrifuged for 10-15 mins at 1500 to 3000 rpm. The plasma was immediately removed and transferred into another sets of 2-ml glass tubes and kept in plastic racks at room temperature for PT and APTTK processing. About 3.5 ml anticoagulated blood was used for TPC and the final 2 ml for WBCT and the results were compared.

Analytical method

The determination of PT was made by Quick time method (one-stage) using Plasmacann Reagent Test Kit manufactured by Quimica Clinica Aplicada S.A (QCA). Determination of Activated partial thromboplastin time with Kaolin (APTTK) was done using the Hemoscann Test Kit, manufactured by QCA S.A. Determination of BT was done by Duke’s method whereas whole blood clotting time determination was made by using Lee and White Method. Visual total platelet counts was done using Improved Neubauer Chamber.

Statistical analysis

Graph pad prism software (Statmate) version 2.0 and SPSS version 20.0 were used for the statistical analysis and the test of significance was calculated using paired Student’s t-test. Results were presented as mean ± standard error of mean (mean value ± SEM) and $P < 0.05$ was considered significant.

RESULTS

Table 1 shows the distribution of chronic smokers of different durations of smoking (in years), whereas Table 2 shows the demographic profile of chronic smokers studied. Figure 1 shows the relationship between BT and duration of smoking whereas Figure 2 shows the relationship between WBCT and duration of smoking. There was a negative relationship, which implies that increase in duration leads to a reduction in BT and WBCT, with the greatest effect observed in the 12-16 and 17-21 years, respectively ($P < 0.05$).

Figure 3 shows a positive relationship between TPC and duration of smoking, implying that increase in the duration of smoking leads to increase in the TPC. When the subjects were grouped according to the number of years they indulged in smoking, there was no perfect pattern, but the greatest effect was observed in 17-21 years and 2-6 years, respectively.

Table 1: Chronic smoker: Duration distribution

| Duration of smoking (in years) | Chronic smokers n = 78 (100%) |
|------------------------------|--------------------------------|
| 2-6                          | n=28 (35.9)                    |
| 7-11                         | n=23 (29.5)                    |
| 12-16                        | n=18 (23.1)                    |
| 17-21                        | n=9 (11.5)                     |
Figures 4 and 5 show the effect of long-term smoking on PT and APTTK, respectively, with both figures showing negative relationship. Hence increase in duration of smoking leads to a decrease in both PT and APTTK, with the greatest negative effect observed in the 12-16 years and 17-21 years of smoking, respectively.

**DISCUSSION**

The effect of duration of smoking on coagulation parameters was observed in this present study. The results showed that there was correlation between the two variables (duration of smoking and the coagulation parameters) in all the different durations of smoking. The BT in the different durations showed a negative correlation coefficient in all the groups (2-6 years, \( r = -0.22, P > 0.05 \); (7-11 years, \( r = -0.43, P > 0.05 \)); (12-16 years, \( r = -0.61, P < 0.05 \)) and (17-21 years, \( r = -0.74, P < 0.05 \)). Increase in duration of smoking was correlated with decrease in the BT in these chronic smokers, with the strongest negative effect on 12-16-years and 17-21-years durations.

A shortened whole blood clotting time resulting from increasing platelet aggregation in chronic smokers has also been reported.\(^{10,11}\) The correlation coefficient computed on the effect of duration smoked against whole blood clotting time, showed negative correlation in all the durations (2-6 years, \( r = -0.20, P > 0.05 \); (7-11 years, \( r = -0.40, P < 0.05 \)); (12-16 years, \( r = -0.58, P < 0.05 \)) and (17-21 years, \( r = -0.67, P < 0.05 \)). A unit increase in duration of smoking causes a unit decrease in the whole blood clotting time in these chronic smokers. The strongest negative effect were also observed in 12-16-years and 17-21-years durations (\( P < 0.05 \)).

Smoking increases activation of platelets by 100 times, which can lead to a significant increase in blood clots.\(^{12}\) The total platelet counts revealed positive correlation coefficients with the duration of smoking in the chronic smokers in all the durations (2-6 years, \( r = -0.44, P < 0.05 \); (7-11 years, \( r = -0.47, P < 0.05 \); (12-16 years, \( r = -0.52, P < 0.05 \)) and (17-21 years, \( r = -0.69, P < 0.05 \)). Those who smoked for 12-17 years and 17-21 years showed a stronger positive correlation when compared to the others. The positive correlation implied that unit increase in duration of smoking increases the total platelet counts in these smokers.

A negative correlation coefficient (\( r \)) was observed on the effect of duration smoked on prothrombin time (PT) and activated partial thromboplastin time with kaolin (APTTK) respectively in the different durations smoked. The correlation coefficients for PT in the different durations were (2-6years, \( r = -0.54, P < 0.05 \); (7-11years, \( r = -0.50, P < 0.05 \)) and (12-16years, \( r = -0.89, P < 0.05 \)). In both coagulation markers, stronger negative correlation was observed in subjects who have smoked for 12-16-years and 17-21-years durations.

Table 2: Demographic profile of chronic smokers

| Ethnicity   | \( N = 78 \) | (\%) 100 |
|-------------|-------------|---------|
| Igbo        | 78          | 100     |

| Age         | (\%) 100 |
|-------------|---------|
| 21-25       | 22      | 28.2    |
| 26-30       | 14      | 18.0    |
| 31-35       | 11      | 14.1    |
| 36-40       | 9       | 12.5    |
| 41-45       | 9       | 12.5    |
| 46-50       | 8       | 10.3    |
| >50         | 5       | 6.4     |

| Gender      | (\%) 100 |
|-------------|---------|
| Males       | 78      | 100     |

| Marital Status | (\%) 100 |
|----------------|---------|
| Single         | 35      | 44.9    |
| Married        | 43      | 55.1    |

| Occupation     | (\%) 100 |
|----------------|---------|
| Medical Students | 21    | 26.8    |
| Civil Servants  | 28      | 35.9    |
| Self-employed/business | 29 | 37.2    |

| Religion       | (\%) 100 |
|----------------|---------|
| Christianity    | 78      | 100     |

Figure 1: The effect of long-term smoking (years) on bleeding time (mins)

The higher negative correlation coefficient (\( r \)) values observed in the BT, WBCT, PT and APTTK and higher positive correlation coefficient (\( r \)) value observed in TPC was seen in those that smoked for a longer duration of

---

**Table 2: Demographic profile of chronic smokers**

| Ethnicity   | \( N = 78 \) | (\%) 100 |
|-------------|-------------|---------|
| Igbo        | 78          | 100     |

| Age         | (\%) 100 |
|-------------|---------|
| 21-25       | 22      | 28.2    |
| 26-30       | 14      | 18.0    |
| 31-35       | 11      | 14.1    |
| 36-40       | 9       | 12.5    |
| 41-45       | 9       | 12.5    |
| 46-50       | 8       | 10.3    |
| >50         | 5       | 6.4     |

| Gender      | (\%) 100 |
|-------------|---------|
| Males       | 78      | 100     |

| Marital Status | (\%) 100 |
|----------------|---------|
| Single         | 35      | 44.9    |
| Married        | 43      | 55.1    |

| Occupation     | (\%) 100 |
|----------------|---------|
| Medical Students | 21    | 26.8    |
| Civil Servants  | 28      | 35.9    |
| Self-employed/business | 29 | 37.2    |

| Religion       | (\%) 100 |
|----------------|---------|
| Christianity    | 78      | 100     |
between 12 and 16 years and 17 and 21 years than those that smoked for a shorter duration of between 2 and 6 and 7 and 11 years. Doll and Peto,\textsuperscript{13} in a study on the effect of duration on inflammatory indices in male chronic smokers aged 40-79 years, reported duration as the most important predictor tool rather than intensity of smoking. As duration smoked increases, the smoking effect increases. Peto\textsuperscript{14} reviewed the study and collaborated their early work. Lubin and Caporaso\textsuperscript{6} reported same thing. A study done in Calabar, Cross-River State, Nigeria reported significant lower PT and APTTK in those that has smoked 11 years and above than those the smoked 10 years and less,\textsuperscript{8} this is also in agreement with the present study although the present study assessed more coagulation markers in the study subjects.

Takajo \textit{et al.}\textsuperscript{15} reported that each cigarette smoked per day increases mean plasma fibrinogen by 0.35 g/l, whereas significant lower values ($P < 0.05$) on PT and APTTK of smokers when compared with the non-smokers was recorded by Akpotuzor \textit{et al.}\textsuperscript{8}

The perceived benefits of smoking to some individuals, which include boldness, pleasure, aiding digestion, prevention of vomiting and soothing of depression\textsuperscript{16} cannot be compared to the dangers of smoking as shown...
by the present study. The higher levels of fibrinogen in chronic smokers may promote cardiovascular disease by affecting blood viscosity, platelet aggregation and general fibrin formation,\textsuperscript{11} and although fibrinogen levels were not measured in our study subjects, a strong indicator for elevated fibrinogen levels in the chronic smokers can be seen in the recorded short time observed in the coagulation parameters (required for fibrinogen to be converted to fibrin clot by thrombin) in our study. The lack of stringent measures by governments of most developed countries, coupled with the westernization of these nations (including Nigeria) has led to an especially high risk.\textsuperscript{12} Smoking should be generally discouraged while oral vitamin C supplementation should be encouraged in these smokers as it has been shown to reverse the haemostatic dysfunction caused by smoking.\textsuperscript{4} Although the present study shows that the most affected subjects are those who have smoked for ≥12 years, some of the coagulation markers (TPC and PT) were significantly affected in subjects who have smoked for as little as 2 years.

CONCLUSIONS

The present study shows that long-term smoking induces haemostatic dysfunction resulting from chronic cigarette smoking. Although the subjects that were mostly affected where those who have smoked for 12 years and above, PT and TPC where altered even in those who have smoked for only 2 years. This shows that long-term smokers are not the only ones at risk of bleeding or coagulation disorder, but smokers in general, hence smoking should be strictly discouraged. Oral vitamin C supplementation should also be recommended in these subjects.

LIMITATIONS OF THE STUDY

The study limitations were evident in the difficulty in recruitment of the subjects, since most people were not willing to get pricked by a needle during sample collection, just for a research study. The subjects that eventually accepted to be part of the study, did so after incentives such as lunch and drinks were offered them with much persuasion and this adversely affected the sample size of the study.

REFERENCES

1. Heemskeek JW, Bevers EM, Lindhout T. Platelet activation and blood coagulation. Thromb Haemost 2002;88:186-93.
2. Dacie JV, Lewis SM. Reference ranges and normal values. In: Practical Haematology. 9th edition. Churchill Livingstone, U.S.A; 2003. p. 339-89.
3. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 1997;336:1276-82.
4. Soronnadi CN, Anyaehie BU, Iyare EE, Neboh EE, Odiegwu CN, Odurukwe O. Oral supplementation of vitamin C reverses haemostatic dysfunction in chronic smokers. Biomed Res Int 2013;24:458-62.
5. Smith CJ, Fischer TH. Particulate and vapor phase constituents of cigarette mainstream smoke and risk of myocardial infarction. Atherosclerosis 2003;158:257-67.
6. Lubin JH, Caporaso NE. Cigarette smoking and lung cancer: Modeling total exposure and intensity. Cancer Epidemiol Biomarkers Prev 2006;15:517-23.
7. Spagnoli LG, Mauriello A, Palmieri G, Santeusiano G, Amante A, Taurino M. Relationships between risk factors and morphological patterns of human carotid atherosclerotic plaques: A multivariate discriminant analysis. Atherosclerosis 1994;108:39-60.
8. Akpotuzor JO, Agwunobi LE, Inyama MA. Prothrombin Time (Pt) and Partial Thromboplastin Time with Kaolin (Pttk) of cigarette smokers in Calabar, Cross river State, Nigeria. Adv Med Dent Sci 2001;3:17-20.
9. Available from: http://www.tobaccoctrl.ng
10. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: Part I: Evolving concepts. J Am Coll Cardiol 1992;1992:48:937-54.
11. Vyssoulis GP, Karpanou EA, Kyvelou SG, Adamopoulos DN. The effect of smoking on Inflammation, prothrombotic state and endothelial dysfunction in patients with essential hypertension. HBP Card Med Prev 2009;7:47-53.
12. Willerson JT, Hillis LD, Winniford M, Buja LM. Speculation regarding mechanisms reposable for acute ischemic heart disease syndromes. J Am Coll Cardiol 1998;8:245-50.
13. Doll R, Peto R Cigarette smoking and bronchial carcinoma: Dose and time relationships among regular smokers and lifelong non-smokers. J Epidemiol Community Health 1978;32:303-13.
14. Peto R, Doll R, Cigarette smoking and bronchial carcinoma: Dose and time relationships among regular smokers and lifelong non-smokers. J Epidemiol Community Health 1978;32:303-13.
15. Takajo Y, Ikeda H, Haramaki N, Murohara T, Imaizumi T. Augmented oxidative stress of platelets in chronic smokers: Mechanisms of impaired platelet-derived nitric oxide bioactivity and augmented platelet aggregability. J Am Coll Cardiol 2001;38:1320-7.
16. Abiodun OA, Olu-Abioudun OO, Oluwole FA. The pattern of smoking among commercial motocyclists in a semi-urban town in Nigeria. Appl J. Hygiene 2012;1:8-14.
17. Mackay J, Croftont J. Tobacco and the developing world. Br Med Bull 1996;52:206-21.

How to cite this article: Ngozi SC, Ernest NE. Long-term smoking results in haemostatic dysfunction in chronic smokers. Niger Med J 2014;55:121-5.

Source of Support: Nil, Conflict of Interest: None declared.