Long-term smoking results in haemostatic dysfunction in chronic smokers

Soronnadi Clara Ngozi, Neboh Emeka Ernest[1]

Department of Physiology, [1]Medical Biochemistry, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria

ABSTRACT

Background: Smoking has been known to cause endothelial dysfunction and bronchial carcinoma and duration of smoking has been implicated in the effects of smoking on regular smokers. This study evaluated the effects of long-term smoking on some coagulation markers in chronic smokers. **Materials and Methods:** A total of 78 chronic smokers (age, 41 ± 20 years) where grouped according to duration of time they have smoked (2-6 years, 7-11 years, 12-16 years and 17-21 years), and included in the study. Bleeding time (BT), whole-blood clotting time (WBCT), total platelet count (TPC), prothrombin time (PT) and activated partial thromboplastin time with kaolin (APTTK) were estimated in the subjects using standard operative procedures. **Statistical Analysis used:** 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:** There was an inverse correlation between the durations of smoking and BT, WBCT, PT and APTTK coagulation markers and a linear correlation between the different durations and TPC, in the chronic smokers. The strongest effects was in the 12-16 years and 17-21 years duration ($P < 0.05$). **Conclusion:** The study revealed that long-term chronic cigarette-smoking can lead to haemostatic dysfunction in chronic smokers. Smoking should be generally discouraged as it could have far-reaching medical implications on this group of subjects, especially in bleeding emergency cases.

Key words: Chronic smoking, clotting factors, haemostatic dysfunction, long-term smoking

Address for correspondence:
Mr. Neboh Emeka Ernest,
Department of Medical Biochemistry, College of Medicine,
Enugu State University of Science and Technology (ESUT),
Enugu State, Nigeria.
E-mail: emmyneboh@yahoo.com

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.[1] 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.[2] Platelet activation and blood coagulation, no doubt, have been shown to be mutually dependent and interactive processes.[1]

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.[3] Haemostatic dysfunction can result in increased risk of haemorrhage or thrombosis.[4] 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.[5] 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.[5] 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.[6] 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.[3]

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.[7] 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.[8] The economic cost of tobacco consumption in Nigeria varies among people of low, middle and high-income earnings.[9] 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.[9]

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.[2] Visual total platelet counts was done using Improved Neubauer Chamber.[2]

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)

Table 2: Demographic profile of chronic smokers

	N = 78	(%) 100
Ethnicity		
Igbo	78	100
Age		
21-25	22	28.2
26-30	14	18.0
31-35	11	14.1
36-40	9	11.5
41-45	9	11.5
46-50	8	10.3
>50	5	6.4
Gender		
Males	78	100
Marital Status		
Single	35	44.9
Married	43	55.1
Occupation		
Medical Students	21	26.8
Civil Servants	28	35.9
Self-employed/business	29	37.2
Religion		
Christianity	78	100

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

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 (17-21years, $r = -0.74$, $P < 0.05$). 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 ($P < 0.05$).
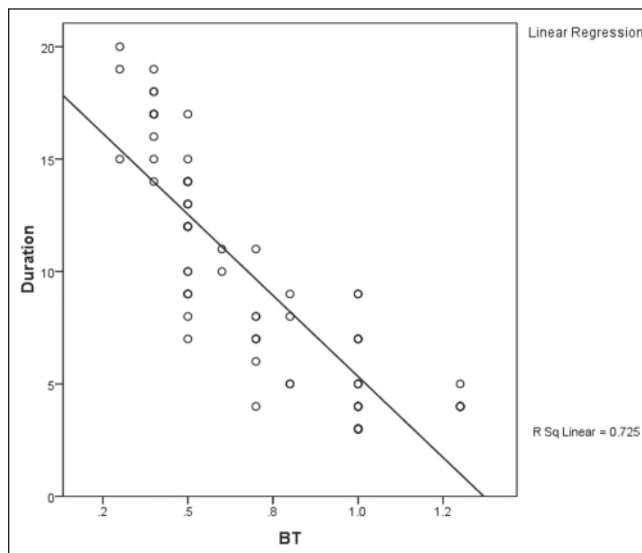
Smoking increases activation of platelets by100 times, which can lead to a significant increase in blood clots.[12] The total platelet counts revealed positive correlation coefficients with the duration of smoking in the chronic smokers in all the durations (2-6 years, $r = -0.44$, $P < 0.05$); (7-11 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, $r = -0.50$, $P < 0.05$); (12-16years, $r = -0.61$, $P < 0.05$) and (17-21years, $r = -0.89$, $P < 0.05$), whereas for APTTK, the correlation coefficient in the age groups were (2-6 years, $r = -0.40$, $P > 0.05$); (7-11 years, $r = -0.59$, $P < 0.05$); (12-16 years, $r = -0.69$, $P < 0.05$) and (17-21 years, $r = -0.91$, $P < 0.05$). In both coagulation markers, stronger negative correlation was observed in subjects who have smoked for 12-16years and 17-21years, respectively.

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
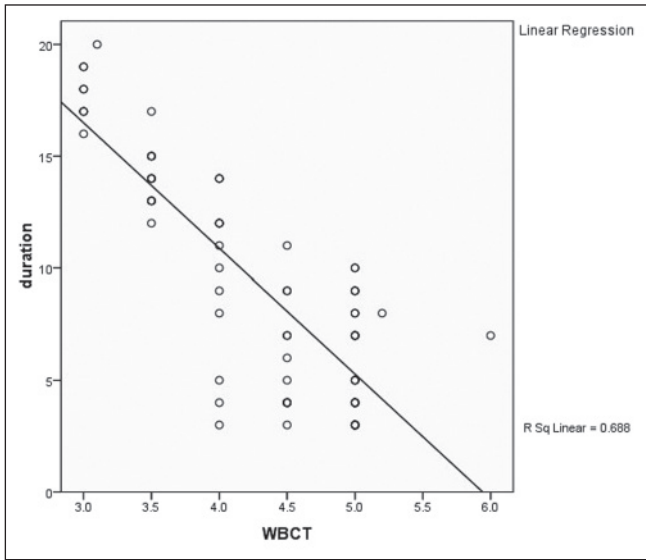
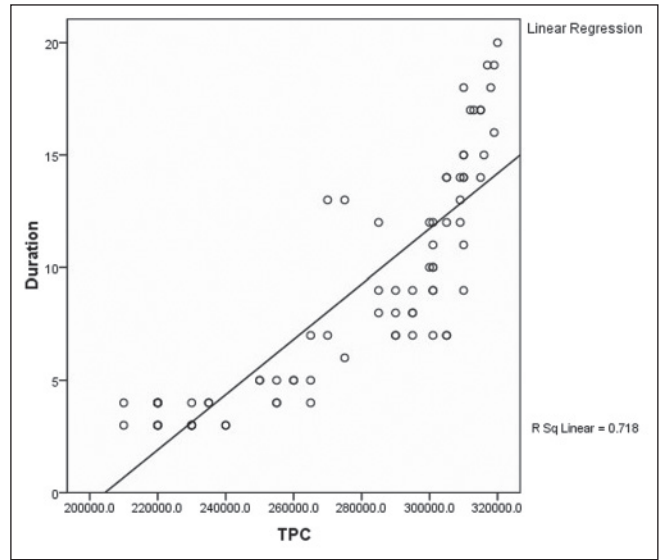**Figure 2:** The effect of long-term smoking (years) on whole blood clotting time (mins)

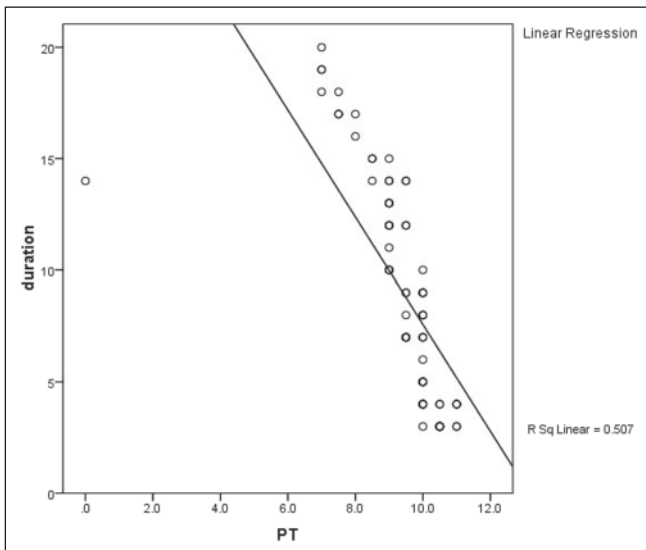**Figure 3:** The effect of long-term smoking (years) on total platelet counts (mm³)

Figure 4: The effect of long-term smoking (years) on prothrombin test (secs)
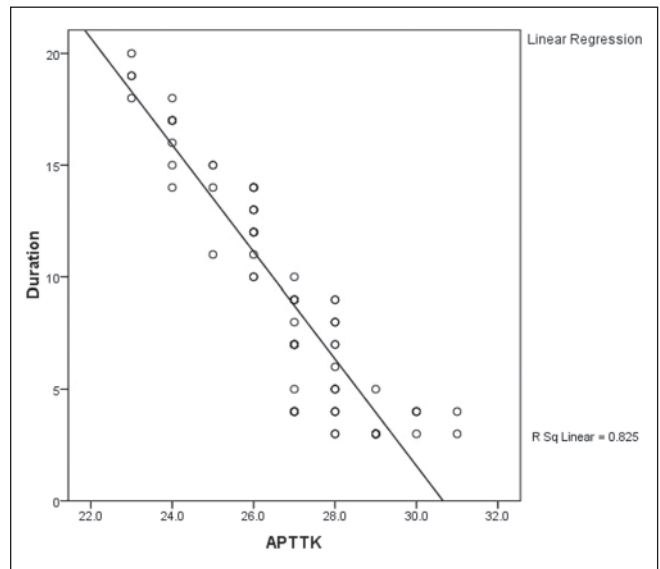

Figure 5: The effect of long-term smoking (years) on activated partial thrombin test with kaolin (secs)

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,[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[14] reviewed the study and collaborated their early work. Lubin and Caporaso[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,[8] this is also in agreement with the present study although the

present study assessed more coagulation markers in the study subjects.

Takajo et al.,[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 et al.[8]

The perceived benefits of smoking to some individuals, which include boldness, pleasure, aiding digestion, prevention of vomiting and soothing of depression[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,[15] 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.[17] 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.[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 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, Santeusanio 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;46:937-54.
11. Vyssoulis GP, Karpanou EA, Kyvelou SG, Adamopoulos DN. The effect of smoking on Inflammation, prothrombiotic state and endothelial dysfunction in patients with essential hypertension. HBP Cardio Prev 2009;7:47-53.
12. Willerson JT, Hillis LD, Winniford M, Buja LM. Speculation regarding mechanisms reposible 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. Influence of dose and duration of smoking on lung cancer rates. IARC Sci Publ 1986;74:23-33.
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-Abiodun 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.