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The Impact of Chronic Smoking on the Intrinsic and Extrinsic Coagulation Pathways of Smokers in Enugu, South-East Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author CNS designed the study, wrote the protocol and wrote the first draft of the manuscript. Author FOU managed the literature searches. Authors OO and OIO did the statistical analysis. Author LMN managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Many studies have linked smoking with cardiovascular disease, but the components and the mechanisms responsible are unclear. Smoking has been reported to enhance platelet aggregation and adhesiveness, probably via nicotine. The study is aimed at ascertaining which coagulation

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pathway is mostly affected in chronic smokers in Enugu, South-east Nigeria. The study comprised of 200 subjects (100 chronic smokers and 100 non-smokers as controls). The chronic smokers had mean age of 40±19 years, whereas the control had mean age of 41±20 years. Exactly 4.5mls of blood was drawn and gently mixed with 0.5ml of sodium citrate anticoagulant in a ratio of 9 parts of blood to 1 part of the anticoagulant and used for the assay. Prothrombin time (PT) and activated partial thromboplastin time with kaolin (APTTK) were analyzed using standard operating procedures with Plasmascann® kit reagent and Hemoscann® test kit from Quimica Clinica Aplicada S.A (QCA) respectively. The statistical analysis was done using Graph pad prism software of Statmate. The result showed statistical significant decrease ($P<0.05$) in PT and APTTK in the smokers compared to the age-matched controls. A linear regression was used to show that chronic smoking affects the intrinsic pathway more than the extrinsic pathway ($p<0.05$). The study showed that chronic smoking affects coagulation pathways generally, most especially the intrinsic pathway.

Keywords: Smoking; blood coagulation; impact; health risk; pathways.

ABBREVIATIONS

PT : Prothrombin Time
APTTK : Activated Partial Thromboplastin Time with Kaolin
QCA : Quimica Clinica Aplicada
GATS : Global Adult Tobacco Survey
COPD : Chronic Obstructive Pulmonary Disease

1. INTRODUCTION

The coagulation cascade of secondary haemostasis has two pathways' which lead to fibrin formation. These are the contact activation pathway (formerly known as the intrinsic pathway), and the tissue factor pathway (formerly known as the extrinsic pathway). Smoking has been shown to increase the ability of blood to coagulate [1,2]. Disorders of increase in coagulation can lead obstructive clotting called thrombosis in the cardiovascular system [3]. There are vast numbers of literatures linking smoking and hyper-coagulation with cardiovascular disease, but the components and the mechanisms responsible are unclear. Smoking has been shown to increase the ability of blood to coagulate, and according to some reports smoking enhances platelet aggregation and adhesiveness, probably via nicotine [4,5,6]. The Global Adult Tobacco Survey (GATS) Nigeria release a statistics that showed an increase in the population of smoking in country. In 2012, Nigeria conducted GATS and is the first country in the African region to do so with 7.3% of men, 0.4% of women, and 3.9% overall (6.4 million adults) currently smoked tobacco [7]. The south-eastern part of the country in which Enugu has the highest prevalence of tobacco use of 9.1% [7]. Considering the data release by GATS, there has been few or no literature on health effects of tobacco smoking from the region.

Evaluation of the impact of chronic smoking on the coagulation pathways is a very vital issue in assessing the health implication of hyper-coagulation state in the genesis of cardiovascular disorder in a population with growing number of smoker. Its importance therefore cannot be down played especially its impact on the health which had been mention above.

This study therefore is aimed at determining the impact of chronic smoking on the coagulation of smoker vis-à-vis the intrinsic and extrinsic pathways. The result will help us to know the health burden of hyper-coagulablity in smokers and enable us to establish a database for health policies formulation.

We are not aware of any study of this nature from this environment with the national and state's population of smokers of 6.4 million and 0.3million adults respectively [7]. It is hope that this will add to the bank of knowledge available on the health effect of smoking and the findings of this study could form the template for intervention strategies in helping reduce the mortality and morbidity relating to chronic smoking.

2. METHODS

2.1 Study Population

The study was conducted in Enugu State. The subjects recruited for the study were chronic smokers with mean age 40±19 years. And we defined chronic smokers as subjects with history of smoking of 10±5 cigarette sticks per day for at

least two year. Age-matched controls with no history of smoking in the last five years were also recruited. Subjects having arterial hypertension, glycosuria (tests were done using urinalysis strips) and currently using any antioxidants were excluded from the study.

2.2 Study Protocol

All subjects gave a verbal or written informed consent and the study protocol was approved by the Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Park Lane G.R.A. Enugu, Nigeria. Questionnaires were used to extract some useful data required in this study. Subjects were made up of Undergraduate students of tertiary institutions in Enugu City, footballers of a club based in the Enugu (Sunshine Football Club of Enugu), and some residents of city who volunteered to be part of the study. Seminars and health talks were conducted to create the awareness and the conviction needed for the subjects' participation in the research. Also incentives like lunch and drinks were given to the subjects to ensure their total commitment to this work.

2.3 Specimen Collection and Processing

The subjects came to the laboratory between 7.30 and 10 am. Pressure was applied using tourniquet and sterilization of the upper arm was done using with swab. A 21-gauge butterfly needle of 5mls syringe was inserted by a clean puncture into an antecubital fossa vein, and 4.5 mls of blood was drawn. The blood collected was gently mixed with 0.5 ml of sodium citrate (that is 9 parts of blood to 1 part of the anticoagulant) in Pyrex made glass test tubes of 6mls volume capacity. The samples above were centrifuged for 10-15 mins at 1500 to 3000 rpm in bucket centrifuge. The plasma was immediately removed and transferred into another sets of plain 2 mls Pyrex made glass and kept in plastic racks at room temperature for PT and APTTK processing.

2.4 Analytical Method

The determination of PT was by Quick time method (one-stage) and a Plasmascann reagent test kit manufactured by Quimica Clinica Aplicada S.A (QCA) was used. The method was according to the manufacturer's instructions. Determination Activated Partial Thrombin Time with Kaolin (APTTK) was done using the Hemoscann test kit manufactured by Quimica Clinica Aplicada S.A (QCA), also according to the manufacturer's instructions.

2.5 Statistical Analysis

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). The mean and standard errors of mean (mean value ± SEM) of the data were tabulated for each group. The data was analyzed with Statistical Package for Social Sciences (SPSS PC. version 20.0; SPSS Inc., Chicago, III., USA), and the test of significance was done using Paired Samples T-test. Differences between the groups were considered statistically significant at $p < 0.05$.

3. RESULTS

A total of two hundred (200) subjects (one hundred chronic smokers and one hundred non-smokers) were studied. The chronic smokers who met our criteria had mean age of 40±19 years, whereas the control had mean age of 41±20 years. The mean ± SD of PT and APTTK for the chronic smokers in the present study is (9.8±0.06 and 27.00±0.17 secs) respectively while the values for the control is (12.70±0.08 and 33.04±0.12 secs) respectively. The range of PT and APTTK for the test subjects is (9.86–9.74 secs) and (27.17–26.83 secs) respectively while that of the control is (12.78–12.62 secs) and (33.16-32.92 secs) respectively (Table 1).

Table 1. Baseline coagulation parameters of the subjects

Parameters	Normal values	Control results (n=100)	Chronic smokers (pre-sample results) (n=100)
Prothrombin time (secs)	11-16 seconds	12.70±0.08	9.8±0.06 *
Activated partial thrombin time with kaolin (secs)	30-40 seconds	33.04±0.12	27.00±0.17 *

** = Statistically significant (P<0.05)*

There were significant decreases (P<0.05) in both the PT and APTTK in the chronic smokers compared to the control (Table 1). A negative sign was observed in the coefficient of variation of smoking with ages as constant which implies an inverse relationship between smoking age and the two pathways. A unit increase in smoking reduces APTTK values by 0.22 seconds and PT by 0.70 seconds. The R^2 of APTTK=0.90, and PT= 0.80 which implies that smoking affects intrinsic pathway (APTTK) by 90% and extrinsic pathway (PT) by 80% (p<0.05) (Table 2). The demographic profile of the smokers is shown on Table 3.

Table 2. Effect of chronic smoking on the two coagulation pathways

	Coefficient of variation	Std error	R^2
Constant (age)	127.62		
APTTK	-3.60	0.22	0.90
PT	-8.40	0.70	0.80

Table 3. Demographic profile of the test subjects

	N=78	(%) 100
Ethnicity		
Igbo	78	100
Gender		
Males	78	100
Females	0	0
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		
Christian	78	100
Islam	0	0
Others	0	0

4. DISCUSSION

Coagulation is a complex process by which blood forms clots. Coagulation is highly conserved throughout biology and in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is therefore the best understood [8]. Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called primary heamostasis. Secondary heamostasis occurs simultaneously: proteins in the blood plasma, called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug [9]. The coagulation cascade of secondary heamostasis has two pathways' which lead to fibrin formation. These are the contact activation pathway (formerly known as the intrinsic pathway), and the tissue factor pathway (formerly known as the extrinsic pathway).

The coagulation factors circulate as inactive zymogens and the coagulation cascade is classically divided into three pathways. The tissue factor and contact activation pathways both activate the "final common pathway" of factor X, thrombin and fibrin [10].

In this present study, it was observed that chronic smoking affects the intrinsic pathway more than the extrinsic pathway; however, no subsequent literatures have been found to correlate with this report yet. Pretorius et al in their study showed that smoking causes the fibrin network to have a netlike appearance in some areas, as well as areas where thick plaques are present [11]. They argue that even in occasional smokers, fibrin, in the presence of thrombin, forms thickened areas that might be the cause of a thrombotic event such as stroke [11].

Smoking is the single greatest cause of preventable death globally [12]. Cigarette smoking predispose to diseases affecting the heart, liver and lungs and major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease (COPD) (including emphysema and chronic bronchitis), and cancer (particularly lung cancer, cancers of the larynx and mouth, and pancreatic cancer). It also causes peripheral vascular disease and hypertension. The effects depend on the number of years that a person smokes and on how much the person smokes [12]. The health risk-increasing effect of smoking may be mediated through an increase in coagulation factors [11,13]. It is well known that smokers have higher fibrinogen levels [14,15,15,16] and that smoking cessation causes a rapid fall in plasma fibrinogen [15].

5. CONCLUSION

We conclude that chronic smoking affects coagulation most especially the intrinsic pathway.

There is need to continue gathering reliable, accurate data on smoking and its related health effects with the increase in the prevalence in the region. Continuous monitoring of smoking epidemic and smoking control policy achievements is critical to understanding and reversing the epidemic and ensuring success of global control measure of cigarette smoking.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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