INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 9, Issue 1 - 2022

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2022.09.01.001

Evaluation of changes on Platelet, PT and PTTK of smokers in Okehi and its environment

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Abstract

Cigarette smoking has been shown to adversely affect coagulation parameters resulting in haemostatic complications. This study was carried out to evaluate the effects of smoking on prothrombin time, Partial thromboplastin time with kaolin and platelet of smokers in Okehi LGA of Kogi State. A total of 15 male smokers were recruited (with 40% majority in the age bracket of 20 to 29) and 10 non-smokers. Prothrombin time was estimated by the calcium-thromboplastin method, Partial thromboplastin time with kaolin was estimated using thromboplastin and kaolin reagent. The result showed that the mean values of PT, PTTK and PLATELET of smokers were significantly lower compared to non-smokers. Frequency and percentage of smoking was evaluated using the ANOVA system. A statistical difference was observed in the mean of PT and PTTK of subjects (12.47±1.77) and (39.37±2.84), as compared to control subjects (12.90±0.94) and (41.63±3.46) respectively. It is concluded that long-term smoking has significant effect on PT, PTTK and Platelet with effect in haemostatic mechanism.

Keywords: platelet, PT, PTTK, smokers

Introduction

Cigarette smoking leads to a serious health problem and includes most important avoidable causes of death worldwide (Heemskeek *et al*., 2002). Coagulation the complex process by which blood forms clot is highly conserved throughout biology, involving both a cellular and a protein component in all mammals. Today at least 20% of all cancers are estimated to be attributed to smoking, but this figure is expected to increase because of the uptake of tobacco use in low-income countries (Heemskeek *et al*., 2002).Tobacco product contain more than 50 established or identified carcinogens, and these may increase risk of cancer by causing mutation that disrupt cell cycle regulation, or through their effect on the immune system. Long-term smoking has been shown to affect PT, PTTK and PLATELETS, with significant increase in subject who had smoked for 12 years or more, compared to those that had smoked for < 10 years

(Akpotuzor *et al*., 2001). Haemostasis dysfunction, however, arises from any alteration of this complex system, leading to pathologic thrombosis or vascular occlusion by thrombus fragments. Platelet activation and blood coagulation, no Doubt, havebeen shown to be mutually dependent and interactive processes (Dacie and Lewis 2003).

Smoking has been shown to induce hypercoagulability and a hyperthrombotic state, possibly by increased platelet aggregation and adhesiveness as a result of its nicotine content (Smith and
Fischer 2003). About 10-10free 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 the average amount of cigarette stick smoked per day are established factor for assessing the absolute risk of developing smoke-related complications in long-term smokers. Haemostasis dysfunction however arises from any alteration of the complex forming system, leading to pathologic thrombosis or vascular occlusion by thrombus fragment (Burke *et al*., 1997).

The prothrombin time (PT) measures the extrinsic pathway of coagulation while partial thromboplastin time with kaolin (PPTK) is a performance indicator of the efficacy of both the "intrinsic and the common coagulation pathways. Platelets, also called thrombocytes are blood cells whose function (along with the coagulation factors) is to stop bleeding. They have no nucleus and are fragments of cytoplasm which are derived from the megakaryocytes. There have been a few studies addressing the effect of smoking on platelet. Many of the studies have not compared the data with those of non-smoking control groups. Paucity of information on this area, coupled with the increase in the number of chronic smokers of both genders necessitated the present study which is aimed at evaluating the pathological effect of smoking on coagulation markers (PT, PTTK, and platelet)(Soronnadi *et al*., 2013; Nwovu *et al.,* 2018; Ifeanyi *et al.*, 2020).

Aim: this project is aimed in looking at the effects of smoking on PT, PTTK and platelet of smokers in Okehi community, Kogi State.

Materials and Methods

Study design

The study is a cross sectional study.The study was conducted in Okehi community in Kogi State. Okehi is a city in Kogi State of Nigeria. The local government has population of 222,262, based 2006 population (Weisser *et al*., 2008).

Ethical consideration

Ethical approval is sought out at the Federal Medical Center Lokoja, Kogi state.

Sampling

Community sensitization

The study will be carried out in Okehi community. The king (Ohinoyi) will be informed about the study and permission will be sought from the Ohinoyi of the community. The villagers will be sensitizes about the purpose of the research through the village announcer.

Inclusion criteria

They are as follows:

1. Male and female that had been smoking for at least the past six months
2. People the smokes at least three stick of cigarette per day.

Exclusion criteria

They are as follows:

1. People that smokes less than three sticks of cigarette per day and with signs of illness.
2. Those that are not willing

Sample size calculation

A sample is a representative subgroup of the population that meets the researchers' criteria. A minimum size will be obtained by using sample size calculation for proportion (formula as shown below);

$n=Z^2 (p \times q)/d^2$

n= desired sample size
Z= standard normal deviate (1.96) that corresponds to 95% confidence level.
p= prevalence 54% which is the prevalence of smoking obtained from the south-western Nigeria (Soronnadi *et al*., 2013).
q= 1-p
d= margin of allowable error (0.05) which is the alpha level at 5%

Where:

Z= 1.96
p= 1.2; =1'2/100 = 0.012
q= 1-p; =1-0.012 = 0.988
d= 0.05

So therefore, the sample size population is calculated as thus:

$$N= (1.96)^2 \times \frac{[(0.012 \times (1- 0.012)]}{(0.05)^2}$$

$$= \frac{3.84x0.012x0.988}{0.0025}$$

$$= \frac{0.0455}{0.0025}$$

$$= 18.21$$

The sample size is approximated to 50 due to errors that may occur

Sample collection and materials

Sodium citrate bottle and EDTA bottle will be used to collect blood sample. 5ml of Blood sample is collected randomly into each bottle and processed immediately. Sodium citrate bottle is used for PT and PTTK while EDTA bottle is used for platelet count. Sample collected into sodium citrate container was centrifuged at 1500g for 5min. to get clear plasma.

Methodology

Procedure for PT test

Procedure: A can tube was placed in the water bath to pre-warm for 2min. at 37^0c. 200µl of thromboplastin was added to the tube and was pre-warmed for 2min. 100µl of plasma was added into the tube and the timer was started immediately, the mixture was mixed at interval to observe for clot and when a fibrin clot is seen, the timer was stopped and the time was recorded.

Procedure for PTTK test

Procedure: A can tube was placed in the water bath to pre-warm at 37^0c for 2min. 200µl of kaolin and 100µl of plasma was added into the test tube outside the water bath and was pre-warmed for 2min. 100µ of calcium chloride (CaCl) was added to the mixture inside the water bath and the timer was started immediately. The mixture was mixed at interval to observe for clot, when a fibrin clot is seen, the timer was stopped and the time was recorded.

Procedure for platelet count

380µl of 1% ammonium oxalate solution was pipetted into a clean test tube, 20µl of whole blood collected into EDTA bottle was added to the test tube, the improved Neubeaur counting chamber was charged by moistening the grid area and placing cover slip on it firmly. The sample was remixed gently using the pipette, one of the grid areas of the chamber was filled with the mixture, the chamber was placed in a moist environment to allow the platelets to settle undisturbed for 15min. the counting chamber was then focused under the microscope using x10 to focus and x40 to count the platelets.

Results

A total of 15 smokers were enrolled into this study, after giving consent to partake voluntarily. The participants have been on smoking attitude for not less than 3 years. All the participants are male with 40% (majority) in the age bracket of 20 to 29. The details of the demographic variables are depicted in Table 1.

The platelet count, prothrombin time and thromboplastin time with kaolin of the participants, with results of ANOVA were presented in Table 2 below. The number of cigarette sticks taken per day is also reflected therein.

Table 3 shows the comparison of coagulation parameters of test and control.

Table 1: Demographic characteristics of participants

Age	**Frequency**	**Percentage**
20-29	06	40
30-39	04	26.67
40-49	03	20
50-59	01	6.67
≥60	01	6.67
Total	15	100
Gender		
Male	15	100
Female	00	00
Total	15	100

Table 2: Cigarette smoking habits and coagulation parameters of participants

Age	**Average stick per day**	**Platelet**	**PT**	**PTTK**
20-29	5.5	148	10.9	36.7
30-39	4.5	125	11.8	38.1
40-49	5.33	132	12.3	37.7
50-59	7.5	136	10.2	34.8
≥60	10.5	119	8.6	39
P-value	0.054	0.121	0.13	0.074

Table 3: comparison of coagulation parameters of Test and Control

Parameters	**Test**	**Control**	**p-value**
PT(s)	12.47±1.77	12.90±0.94	0.486
PTTK(s)	39.37±2.84	41.63±3.46	0.860
Platelet($x10^{12}$/L)	136.80±10.62	299.80±18.26	0.000

Discussion

Hypercoagulability state could be caused by cigarette smoking, potentially leading to thrombosis. Cigarette smoking also appears to play significant role in coagulation factors malfunctions. This study was conducted to know the pathological effects of smoking on prothrombin time, partial thromboplastin time with kaolin and platelet of Healthy smokers in Okehi community of Kogi State. The decrease in platelet count could be due to the nicotine-induced decrease thrombopoietic activity in smokers. The findings in this study observed that the highest number of cigarette smokers in the study was in the age range of20-29 years. However, the relationship between the five age groups of smokers (20-29), (30-39), (40-49), (50-59), and (≥60) and PT, PTTK and platelet parameters shows no significant difference ($p>0.05$); this indicates that age has no barrier as regard cigarette smoking and its effect on PT, PTTK and platelets. The result of this project work shows significantly reduced platelet count in the smokers ($136.80\pm10.62X10^{12}$/L) compared to the control ($299.80\pm18.26x10^{12}$/L),$p<0.000$). This result was not in line with the report of Takajo *et al*. (2001) where it was reported that smokers may not really have significant decrease on Platelet count.

Concerning PT result, the difference seen in result was not statistically significant when compared with the control (test 12.47±1.77, 12.90±0.94s, $p<0.486$), this is not in agreement with the results obtained by Akpotuzor *et al.* (2001), who reported that mean prothrombin time values of smokers were significantly lower when compared with the non-smokers. The difference observed in the result of PTTK values of smokers and non-smokers is not also statistically significant, (test 39.37±2.84s, 41.83±3.43s, $p<0.860$). This result is not in line with the results obtained by Akpotuzor *et al.* (2001) that the mean PTTK values of smokers were significantly lower when compared with the non-smokers.

Conclusion

The study showed that cigarette smokers tend to have lower platelet counts, shorter prothrombin time, and shorter thromboplastin time with kaolin values, when compared to non-smokers.

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	Website: www.ijcrcps.com
Quick Response Code	Subject: Medical Sciences
DOI: 10.22192/ijcrcps.2022.09.01.001	

How to cite this article:
Oke O.T, Evinavoh Maryam Osimeun, and Obeagu Emmanuel Ifeanyi. (2022). Evaluation of changes on Platelet, PT and PTTK of smokers in Okehi and its environment. Int. J. Curr. Res. Chem. Pharm. Sci. 9(1): 1-6.
DOI: http://dx.doi.org/10.22192/ijcrcps.2022.09.01.001

