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Evaluation of Blood Levels of Prothrombin Time, Activated Partial Thromboplastin Time, D-Dimer and TNF-A in Adult Male Cigarette Smokers in Nnewi Metropolis

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Abstract:

Cigarette smoking is the most common method of tobacco consumption. WHO has estimated that cigarette smoking will kill about ten million people by 2030, if its trend persists. This study aimed to evaluate the PT, APTT, D-dimer and TNF-α levels of male cigarette smokers in Nnewi. Sixty-seven smokers and sixty-seven non-smokers aged 18-60 years were enrolled in the study. Five millitres of blood were withdrawn from the participants, 3mls was dispensed into 0.3ml of trisodium citrate for analysis of PT and APTT, which was properly mixed, spun and the plasma separated for immediate analysis. The remaining blood was dispensed into a plain tube, spun and serum separated for D-dimer and TNF-α analysis. Quick one stage and Proctor and Rapaport methods were employed for PT and APTT analysis respectively while ELISA method was used for D-dimer and TNF-α assessments. SPSS version 23 was used to analyse data obtained, Students'-t test was done and P value $\leq$ 0.05 was considered significant at 95% confidence interval. Results revealed a significant shortened PT (seconds) (9.24±2.81) and APTT (seconds) (29.94±5.88) of smokers when compared with the controls (12.60±1.56 and 33.19±4.78, P= 0.000 and 0.001) respectively, while a significant increase was seen in D-dimer (ng/ml) (159.84±28.82) and TNF-α (pg/ml) (14.07±7.08) levels of smokers when compared to the controls (117.03±19.29 and 3.65±1.62, P=0.000 and 0.000) respectively. TNF-α showed a weak positive significant increase (r=0.276, P=0.024) when average number of sticks smoked per day were correlated with the studied parameters while PT (r=-0.272, P=0.026) and APTT (r=-0.264, P=0.031) showed a weak negative significant decrease, TNF-α(r=0.654, P=0.000) revealed a strong positive significant increase when correlated with the duration of cigarette smoking. Conclusively, it can be elucidated that cigarette smoking has both coagulatory and inflammatory effects on smokers. It's recommended that the Nigerian government should ban audios and visuals that encourage smoking and make the product less easily available.

Keywords: Cigarette smoking, Coagulation, Inflammatory, PT, APTT, D-dimer, TNF- α

Introduction:

Smoking is a practice in which a substance, most commonly tobacco or cannabis is burned and the smoke tasted or inhaled. The most common method of smoking today is through cigarette (Shiffman and Robert, 2007). A cigarette is a narrow cylinder containing a combustible material, typically tobacco which is rolled into a thin paper for smoking, the resultant smoke is orally inhaled. It has been estimated that cigarette smoking will kill about ten million people by the year 2030, if the trend of smoking persists (World Health Organization,2005). Cigarette smoking is one of the greatest contributors to preventable illness and premature death (Mather and Loncar, 2006) and a global important risk factor for many non-communicable diseases. Long term smoking has been shown to increase the ability of blood to coagulate (Smith and Fischer, 2001; Akpotuzor *et al;* 2009) and potentiate inflammation (Lee *et al.,* 2012). Blood coagulation is a cascade of enzymatic reactions that lead to the conversion of fibrinogen to fibrin (Moake, 2021). Any haemostatic dysfunction, arising from alteration of this complex system, leads to serious pathologic

thrombosis, vascular occlusion by thrombus fragments or bleeding. (Mesele *et al*; 2004, Furie and Furie, 2008). Inflammation is a part of the defense mechanism usually by the immune system to remove or eliminate harmful irritants/antigens in the body and subsequent healing (Fritsch and Abreu, 2019; Michels da Silva *et'al*; 2019; Zhang *et al*; 2019) which could be acute or chronic. The presence of inflammation often leads to higher levels of inflammatory markers. Prothrombin time (PT) is a measure of the integrity of the extrinsic and final common pathway of the coagulation cascade (Tripodi *et al; 2007*). Activated Partial Thromboplastin Time (APTT) is a screening test for evaluating the overall integrity of the intrinsic and common coagulation pathway (Hoffman and Monroe, 2005). D-dimer is a fibrin degradation product present in the blood after degradation of a blood clot (Weitz *et al.,* 2017). TNF-alpha (TNF-α) is a cytokine produced by several cell types (predominantly macrophages) and plays a key role in pathological conditions such as infections, lesions, inflammation, and tumour development (Ruder *et'al*; 2019).

Materials and Methods

This study was conducted in Nnewi, Anambra State, southeastern Nigeria. The study enrolled 134 apparently healthy male participants residing in Nnewi aged 18-60 years. The participants were grouped into two; 67 cigarette smokers, and 67 non-smokers (controls). Male cigarette smokers and non-smokers aged 18-60 years who gave their consent were included in the study. Participants who are below 18 and above 60 years of age, on oral anticoagulant, have had recent blood transfusion in the last 3 months, smoked other substances, as well as those who refuse to give their consent were excluded from this study. Consent of the participants was sought and obtained before commencement of the research. They were informed of the protocol and objectives of the study and absolute confidentiality of their results. The participants were neither paid nor charged for participation. Ethical approval for this study was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital. Under aseptic conditions, 5mls of blood was withdrawn from the antecubital fossa of the participants. Three milliliters (3mls) was dispensed into 0.3ml of trisodium citrate for analysis of PT and APTT, The anticoagulated blood was properly mixed, spun and the plasma separated for immediate analysis. The remaining 2mls of blood was dispensed into a plain tube, which was centrifuged, and serum separated, subsequently kept frozen for D-dimer and TNF-α analysis. Quick one stage and Proctor and Rapaport methods as described in Dacie and Lewis Practical Haematology (2017) were employed for PT and APTT analysis respectively while ELISA method was used to assess D-dimer and TNF-α. All analysis was carried out based on the manufacturer's instructions. Data obtained was organized and subjected to appropriate statistical analysis using statistical package for social sciences (SPSS), version 23 and presented as mean ± standard deviation. Students't -test was done, P value ≤ 0.05 was considered significant at 95% confidence interval. Correlation was performed using the Pearson's correlation coefficient.

Results

Table 1: Levels of PT, APTT, D-dimer and TNF-alpha in cigarette smokers and non-smokers (mean±SD)

Parameters	cigarette smokers (n=67)	Non-smokers (n=67)	t-test	p-value
PT(seconds)	9.24±2.81	12.60±1.56	-8.542	<0.001*
APTT(seconds)	29.94±5.88	33.19±4.78	-3.506	0.001*
D-dimer (ng/ml)	159.84±28.82	117.03±19.29	10.103	<0.001*
TNF-alpha (pg/ml)	14.07±7.08	3.65±1.62	11.747	<0.001*

* = statistically significant difference

Table 2: Levels of the studied parameters in cigarette smokers based on the average number of sticks smoked per day (mean±SD)

No. of cigarette sticks/day	PT(seconds)	APTT (seconds)	D-dimer(ng/ml)	TNF-alpha(pg/ml)
<5 (A) n=18	10.08±2.96	30.12±7.93	156.67±30.02	11.44±6.39
5-10 (B) n=18	9.02±2.72	29.70±4.62	156.52±25.84	13.26±7.82
>10 (C)n=31	9.10±2.86	30.07±6.08	163.87±31.07	15.79±6.44
f-value	0.635	0.034	0.524	1.933
p-value	0.533	0.967	0.595	0.153
A vs B	0.877	1.000	1.000	1.000
A vs C	0.951	1.000	1.000	0.555
B vs C	1.000	1.000	1.000	0.218

*= statistically significant difference
n= number of participants

Table 4: Correlation of period of cigarette smoking with PT, APTT, D-dimer and TNF-alpha in cigarette smokers.

Parameters	r	p-value
Period of smoking vs PT	-0.272	0.026*
Period of smoking vs APTT	-0.264	0.031*
Period of smoking vs D-dimer	-0.231	0.060
Period of smoking vs TNF-alpha	0.654	<0.001*

* = statistically significant difference

Table 5: Correlation of average number of cigarette sticks smoked per day with PT, APTT, D-dimer and TNF-alpha in cigarette smokers.

Parameters	r	p-value
No. of sticks/day vs PT	-0.083	0.504
No. of sticks/day vs APTT	0.004	0.972
No. of sticks/day vs D-dimer	0.178	0.149
No. of sticks/day vs TNF-alpha	0.276	0.024*

*=statistically significant

Discussion

The mean PT and APTT were significantly shortened in cigarette smokers when compared to non-smokers in this study which is consistent with previous studies (Akpotuzor *et al*., 2009; Isah *et al*., 2015; Elkhalifa, 2018).

However, this finding contradicts an earlier study that reported a prolonged PT and APTT in cigarette smokers relative to non-smokers (Okeke and Ekeanumba, 2017) and other studies that reported no significant difference in the PT among cigarette smokers and non-smokers (Al-Dahr, 2010; Metta *et al*., 2015).

The precise mechanisms responsible for the effect of cigarette smoking on the haemostatic function is thought to be due to the exacerbation of platelet adhesiveness and aggregation, decrease in plasminogen activation and increase in fibrinogen levels (Ambrose and Barua 2004; Kashiwagi *et al*; 2017).

In this study, there was no significant difference in PT and APTT when compared with the average number of cigarette sticks smoked per day and at different duration of smoking periods. This finding is at variance with Okeke and Ekeanumba (2017) that reported a significantly prolonged PT and APTT in cigarette smokers who had an average of more than 10 sticks a day, relative to those of one stick per day.

Whereas this finding is consistent with Elkhalifa (2018), it contradicts the reports of Isah *et al*. (2015) and Okeke and Ekeanumba (2017) that reported statistically significant prolongation in PT and APTT with increased duration of cigarette smoking.

A weak negative statistical decrease correlation was recorded between PT, APTT and cigarette smoking duration, this implies that longer duration of cigarette smoking causes an attendant shortening in PT and APTT. Correlation of number of cigarette sticks smoked per day with PT and APTT showed no statistically significant difference.

This study reported a significantly elevated level of D-dimer in cigarette smokers relative to non-smokers. This agrees with previous studies (Danesh *et al*., 2004; Goya *et al*., 2005; Wannamethee *et al.,* 2005; Sara and Hiba, 2015). However, this finding contradicts some reports (King *et al.,* 2017; Al-tameemi *et al.,* 2022).

Since D-dimer levels have been reported as an important diagnostic marker for thrombus (Rahajuningsih, 2007; Adam *et al*., 2009) and inflammation. It is not unlikely that the elevated D-dimer levels may be due to elevation of fibrinogen in cigarette smokers which is signposted by increased fibrinolysis/fibrin removal and could potentiate vascular endothelial inflammatory state.

This study also revealed that there was no significant association between the number of cigarette sticks smoked per day, duration of smoking and D-dimer levels.

However, Sara and Hiba (2015) found a significant increase in the number of cigarette sticks smoked per day and duration of cigarette smoking when compared with the D-dimer levels of Sudanese cigarette smokers.

It is thus logical to posit that cigarette smoking may activate the release of D-dimer regardless of amount or duration of cigarette smoking.

This study reported higher levels of TNF-α in cigarette smokers relative to non-smokers. This finding is consistent with previous studies (Diez-Pina *et al*., 2009; Petrescu *et al*., 2010; Al-tameemi *et al.,* 2022) but disagrees with the report of Kuschner *et al*. (1996).

The higher TNF-α levels in cigarette smokers may suggest the presence of a smoke-induced inflammatory process and further highlights the role of this cytokine in the initiation and progression of the inflammatory process. The bacterial endotoxin present in tobacco can survive combustion as an active compound of tobacco smoke and may likely play a role in potentiating cigarette smoke-induced inflammatory reaction (Hasday *et al*., 1999; Barnes and Glantz 2007).

This study reported no significant difference in

TNF-α levels when compared with the average number of sticks that cigarette smokers consumed per day. Although this finding contradicts the reports of Zoppini *et al*. (2001) and Petrescu *et al.* (2010), it suggests that in cigarette smokers, TNF-α levels are high regardless of the number of sticks that a cigarette smoker takes.

Furthermore, there was a significant increase in TNF-α levels when compared with duration of cigarette smoking. This is consistent with the reports of Petrescu *et al.* (2010) and suggests that the smoke-induced activation of inflammation usually requires a significant duration of cigarette smoking.

A strong positive significant increase was observed in correlation of TNF-alpha and periods of cigarette smoking also, TNF-α showed a weak positive statistically significant increase when correlated with the average number of cigarette sticks smoked per day. These demonstrate that TNF-alpha keeps increasing as cigarette smoking consumption is increased.

Conclusion

In conclusion, this study shows that cigarette smoking affects both intrinsic and extrinsic pathways of the coagulation system as shown in the shortened PT and APTT, promotes thrombus formation as well as inflammation, in other words, the high levels of TNF-α and D-dimer in cigarette smokers suggest the presence of high levels of pro inflammatory and coagulatory factors.

Recommendation

The interpretation of D-dimer, cytokines and coagulation results should be done based on the smoking status of patients and this status should be established prior to surgery or any invasive procedure. There is profound need to understand the specific mechanisms by which tobacco impairs immunity as this may also provide important new therapeutic targets for the treatment of many diseases affecting cigarette smokers. Researchers should center or incorporate quantitative data on smoking status and exposure of non-smokers to environmental tobacco.

There is an urgent need for more public enlightenment on the risk of cigarette smoking via anti-smoking campaigns and the establishment of stronger surveillance systems targeted at the youths. There is need for government intervention in promoting policies that discourage cigarette smoking.

Conflict of Interest: No conflict of interest among the authors

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