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Platelet Count, Platelet Indices and some Clotting Parameters among Smokeless Tobacco (Snuff) Consumers in Cross River State, Nigeria

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Abstract

Snuff is a smokeless tobacco made from ground or pulverized tobacco leaves that contain alkaloid nicotine which is an oxidant. This study assessed the Platelet Count (PC), Platelet Indices, Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT) and Thrombin time (TT) of sixty-five (65) Smokeless Tobacco (Snuff) consumers in Ugep, Yakurr Local Government Area Cross River State and sixty-five non-consumers (control subjects). One hundred and thirty participants were enrolled into this study including 65 snuff consumers (subjects) and 65 non-snuff consumers (controls). Ugep is a town and largest unit in Yakurr LGA of Cross River State with a population of 200,276. It is known as the largest village in West Africa. The local language is Lokaa language, majority of the locals are traditional worshippers and mainly yam farmers. The study lasted for a period of four months.The PC and platelet indices [plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW)]was assessed using the automated haematology analyzer Smart FI (Include Country of Manufacture) and PT, APTT and TT was assessed using Quick's One-Stage technique ((Include name of reagent manufacturer and country of manufacture). The results show a significant drop in the PC and plateletcrit (139.8 X 10^9/L and 0.11%) of snuff consumers when compared with control subject (197.1 X 10^9/L and 0.24%). The mean value of PT, APTT and TT (15.2, 41.9 and 14.5 seconds respectively) of snuff consumers was seen to be significantly prolonged than that of the non-consumers (12.2, 36.1 and 12.7 seconds respectively). We observed a significant difference in the PT parameters of Snuff consumers based on the duration of consumption. In this study, age, gender and smoking additional substances (Snuff & Gin, Snuff, Gin & Cannabis, Snuff, Gin & Cigarette, Snuff, Cannabis & Cigarette) seems to have no effect on the parameters. The reduction in PC and PCT may result in thrombocytopenia and affect the vascular integrity, alongside the slight prolongation of the PT, APTT and TT values and also the duration of use acting as a contributing factor. The study revealed that long-term consumption of smokeless tobacco (snuff) can predispose these individuals to increased risk of developing bleeding and coagulation disorders.

Keywords: Smokeless Tobacco (Snuff), Platelet count, Plateletcrit, Ugep (Yakurr), Prothrombin time

Introduction

Basic tests for coagulation studies are often performed with no specific diagnosis in mind and in the absence of any clinical indication of a haemostatic disorder. Platelets play a major role in blood clotting and thus are important components of haemostasis. Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT) and Thrombin Time (TT) test are screening tests for studying clotting disorders. Prothrombin Time (PT) is a blood test that measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract (Thromboplastin). It indicates the overall efficiency of the extrinsic clotting system. The normal value for Prothrombin Time depends on the thromboplastin and, the exact technique whether visual or instrumental end point reading is used. Partial Thromboplastin Time (PTT) or Activated Partial Thromboplastin Time (APTT) is a blood test that measure the clotting time of plasma after the activation of contact factors and addition of phospholipid and calcium chloride ($CaCl_2$) without addition of tissue thromboplastin. This test indicates the overall efficiency of the intrinsic pathway. Thrombin Time (TT), also known as Thrombin Clotting Time (TCT) is a blood test that measures the time it takes for a clot to form in plasma sample containing anticoagulant after an excess of thrombin has been added. They are all expressed as the mean of the duplicate clotting times in seconds for the control and the test plasma (Lewis *et al.,* 2010).

Tobacco is a product prepared from the leaves of tobacco plant. It is of the genus Nicotiana and of the Solanaceae (nightshade) family. There are more than 70 known species of tobacco with the chief commercial crop being the *Nicotiana tabacum.* Tobacco contains Alkaloid nicotine which is a stimulant and other compounds such as germacrene and anabasine and other harmala alkaloids with nicotine being the most active agent. Tobacco is mostly consumed in the form of smoking, chewing, snuffing, or dipping tobacco. One of the forms of smokeless tobacco is snuff (Gilman and Xun, 2004; World Health Organization, 2008).

Snuff is a smokeless tobacco made from ground or pulverized tobacco leaves (Treyer *et al.,* 2010), it is inhaled or snuffed into the nasal cavity, delivering a sweet hit of nicotine and a lasting flavoured scent (especially if flavouring has been blended into tobacco). It can be processed to fine grains and packaged either in cans or pouches. Traditionally, it is sniffed or inhaled lightly after a pinch of salt is either placed onto the back surface of the hand, held pinched between the thumb and index fingers or held by a specially made snuffing device. Some addicts are also known to chew the leaves (Ugbor *et al.,* 2013). Snuff originated in America and was in common use in Europe by the 17th century. People in many regions and countries including North America, Northern Europe, India and other Asian countries and parts of Africa have a long history of using smokeless tobacco products. In Nigeria, tobacco snuff is particularly used for cultural and traditional purposes. The frequency and speed of tobacco consumption and its effect on health is directly connected to Nicotine, the most important phytochemical contained in tobacco. There are many risks from using tobacco either through smoking or consuming smokeless tobacco (snuff) some of which may include, thrombosis and weakness of the walls of blood vessels in the brain which can lead to stroke, thrombosis in the legs which may travel to the lungs. Coronary artery disease including angina and heart attack, temporary increased blood pressure after smoking, poor blood supply to the legs and problems with erection because of decreased blood flow into the penis. Consumers of smokeless tobacco (snuff) are more at increased risks of cancer of the mouth, tongue, oesophagus and pancreas. Gum problems, tooth wear and cavities, worsening high blood pressure and angina (Benowitz *et al.,* 2016). Tobacco consumption is a global public health problem affecting both developed and developing countries and is a leading cause of preventable morbidity and mortality. The aim of this current study was to assess and evaluate the adverse effect of snuff use on platelet count (PLT), and platelet indices [Plateletcrit (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW)] and some coagulation profile [Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT)] of consumers in Ugep, Yakurr Local Government Area.

Methods

The area used for this study was Ugep in Yakurr Local Government Area. Ugep is popularly known as one of the largest units that constitute Yakurr, with a population of 200,276. It is often referred to as the largest village in West Africa. The indigenes are predominantly farmers. They speak the Lokaa language which is the general language spoken in Yakurr. The people of Ugep are majorly traditional worshippers with a handful of people practicing Christianity. A cross sectional study design was used in this research study and included a total number of 65 snuff users (subjects) and 65 non- users (control group) residing in Ugep, Yakurr LGA of Cross River state. The study lasted for a period of three months and three weeks. Individuals who are snuff users and those who give their consent were included while non- snuff users and users who did not give their consent were excluded from this study. A total volume of 4.5mls of venous blood was drawn from each subject and 2ml dispensed into Potassium Ethylene Diamine Tetra Acetic (K EDTA) for platelet count and its indices while 2.25ml was collected in a sample tube containing 0.25ml of 3.13% Trisodium Citrate anticoagulant and used for some coagulation profile respectively. The samples were transported at a suitable temperature from Ugep to Calabar, where they were analyzed immediately. SPSS 20.0 was used in the analysis of the study data. Generalized data are presented in tables and figures. Student t-test, one way analysis of variance and Pearson correlation was used in the analysis of the data. An error probability of ≤ 0.05 was considered significant.

Results

The mean PLT and PCT of snuff consumers was 139.77 ± 65.71 and 0.11 ± 0.07 while that of non-snuff consumers was 197.11 ± 57.52 and 0.24 ± 0.06, which showed a significant increase in snuff consumers when compared to non-consumers (p=0.000). Mean value for MPV and PDW of snuff consumers was 10.50 ±0.98 and 39.00 ± 2.90 while that of non-snuff consumers was 10.77 ± 1.12 and 39.34 ± 2.29 (p= 0.149 and 0.470) respectively. The mean PT, APTT and TT for snuff consumers was 15.23 ± 1.37, 41.89 ± 3.12 and 14.53 ± 2.12 seconds while that of non-snuff consumers was 12.20 ± 1.31, 36.07 ± 3.46 and 12.74 ± 0.18 seconds respectively (p= 0.024, 0.004 and 0.012).

Comparison of snuff consumers was categorized based on the age ranges of 25 – 43 years (9), 44 – 62 years (35), 63 – 81 years (20) and 82 – 100 years (1). The Mean ± Standard deviation for PLT, PCT, MPV, PDW, PT, APTT and TT parameters in respect to age range showed no statistically significant difference between the parameters (p = 0.856, 0.727, 0.236, 0.740, 0.122, 0.402 and 0.874) respectively. Of the 65 subjects examined, 44 were male snuff consumers while 21 were female snuff consumers. The Mean ± Standard deviation for each haematological parameter in respect to gender showed that there was no significant difference (p= 0.700, 0.594, 0.601, 0.129, 0.773, 0.328 and 0.533) for PLT, PCT, MPV, PDW, PT, APTT and TT respectively.

Comparison of PLT, PCT, MPV, PDW, PT, APTT and TT of snuff consumers based on duration. Subjects who had consumed mainly snuff for 1 – 10 years were 28, 20 have been consuming it for 11 – 20 years, 8 consumed it for 21 – 30 years while 9 subjects have been consuming it for 31 – 40 years. There was a significant difference in the PT parameter based on duration of consumption of snuff (P= 0.048). The Post hoc analysis for PT parameter showed a p- value of 1.14 estimated between duration ranges of 1 – 10 years and 11 – 20 years of the PT parameter. Also, a p-value of 1.48 was estimated between duration ranges of 11 – 20 years and 21 – 30 years of PT parameter. However, there was no significant difference p >0.05 in the PLT, PCT, MPV, PDW, APTT and TT parameters with p- values of 0.958, 0.936, 0.788, 0.905, 0.243 and 0.419 respectively. Comparison of Prothrombin Time, Activated Partial Thromboplastin Time and Thrombin Time among snuff consumers was compared based on other substances consumed. Out of the 65 subjects examined, the number of those who consume only snuff was 34, 20 subjects consumed both snuff and dry gin, 4 consumed a combination of snuff, gin and cannabis, 3 consumed snuff, gin and cigarette while 4 consumed snuff, cannabis and cigarette. The Mean ± Standard deviation for each haematological parameter in respect to substances consumed also shown no significant difference (p > 0.05). Figure 1 shows the correlation between analyzed coagulation parameters. A mild positive correlation value of 0.283 was found between PT and APTT (p=0.02). A mild negative correlation value of -0.12 was found between TT and PT while a mild positive correlation value of 0.193 lies between APTT and TT.

Table 1: Platelet count, Platelet Indices, Prothrombin Time, Activated Partial Thromboplastin Time and Thrombin Time among snuff consumers and non-snuff consumers

Parameters	Snuff Consumers (n = 65)	Non-Consumers (n = 65)	T	P- value
PLT ($\times 10^9$/L)	139.77 ± 65.71	197.11 ± 57.52	- 5.016	**0.003***
PCT (%)	0.11 ± 0.07	0.24 ± 0.06	- 5.928	**0.000***
MPV (fl)	10.50 ± 0.98	10.77 ± 1.12	- 1.452	0.149
PDW (%)	39.00 ± 2.90	39.34 ± 2.29	- 0.724	0.470
PT (secs)	15.23 ± 1.37	12.20 ± 1.31	2.285	**0.024***
APTT (secs)	41.89 ± 3.12	36.07 ± 3.46	4.662	**0.004***
TT (secs)	14.53 ± 2.12	12.74 ± 0.18	3.901	**0.012***

*Values are expressed as Mean ± Standard deviation, n = Number of subjects, PLT = Platelet count, PCT = Plateletcrit, MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, TT = Thrombin Time, * = statistically significant.*

Table 2: Platelet count, Platelet Indices, Prothrombin Time, Activated Partial Thromboplastin Time and Thrombin Time among snuff consumers based on age

Parameters	Age (years)				F	P value
	25 – 43 (n = 9)	44 – 62 (n = 35)	63 – 81 (n = 20)	82 – 100 (n = 1)		
PLT ($\times 10^9$/L)	145.89 ± 51.59	163.74 ± 76.31	169.05 ± 53.13	155.00 ± 0.00	0.257	0.856
PCT (%)	0.15 ± 0.06	0.17 ± 0.08	0.18 ± 0.05	0.18 ± 0.00	0.438	0.727
MPV (fl)	9.93 ± 0.62	10.61 ± 1.12	10.52 ± 0.80	11.40 ± 0.00	1.453	0.236
PDW (%)	38.49 ± 2.29	38.89 ± 3.46	39.31 ± 2.05	41.50 ± 0.00	0.419	0.740
PT (secs)	12.89 ± 1.54	12.94 ± 1.30	12.45 ± 1.32	10.00 ± 0.00	2.007	0.122
APTT (secs)	40. 44 ± 4.13	38.34 ± 3.35	39.20 ± 3.22	38.00 ± 0.00	0.993	0.402
TT (secs)	13.69 ± 2.35	14.06 ± 1.88	14.25 ± 2.51	13.00 ± 0.00	0.231	0.874

Values are expressed as Mean ± Standard deviation, n = Number of subjects, PLT = Platelet count, PCT = Plateletcrit, MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, TT = Thrombin Time.

Table 3: Platelet count, Platelet Indices, Prothrombin Time, Activated Partial Thromboplastin Time and Thrombin Time among snuff consumers based on Gender

Parameters	Gender		t	P value
	Male consumers (n = 44)	Female consumers (n = 21)		
PLT ($\times 10^9$/L)	164.77 ± 70.98	158.57 ± 54.41	0.388	0.700
PCT (%)	0.17 ± 0.07	0.17 ± 0.06	0.537	0.594
MPV (fl)	10.55 ± 0.97	10.40 ± 1.02	0.527	0.601
PDW (%)	39.48 ± 2.08	38.01 ± 4.02	1.569	0.129
PT (secs)	12.77 ± 1.36	12.66 ± 1.43	0.289	0.773
APTT (secs)	39.18 ± 3.77	38.29 ± 2.51	0.987	0.328
TT (secs)	13.93 ± 2.22	14.29 ± 1.90	0.627	0.533

Values are expressed as Mean ± Standard deviation, n = Number of subjects, PLT = Platelet count, PCT = Plateletcrit, MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, TT = Thrombin Time.

Table 4: Platelet count, Platelet Indices, Prothrombin Time, Activated Partial Thromboplastin Time and Thrombin Time among snuff consumers based on duration

Parameters	Duration (years)				f	P value
	1 – 10	11 – 20	21 – 30	31 – 40		
PLTS ($\times 10^9$)	159.93 ± 48.25	165.60 ± 92.89	172.13 ± 59.62	157.00 ± 53.95	0.102	0.958
PCT (%)	0.17 ± 0.53	0.18 ± 0.10	0.18 ± 0.10	0.16 ± 0.06	0.140	0.936
MPV (fl)	10. 43 ± 1.06	10.69 ± 1.15	10.39 ± 0.50	10.41 ± 0.69	0.352	0.788
PDW (%)	38.69 ± 3.98	39.27 ± 1.90	39.18 ± 1.93	39.24 ± 1.17	0.186	0.905
PT (secs)	13.64 ± 1.35	11.90 ± 1.41	12.38 ± 0.92*	11.11 ± 0.93	4.295	**0.048***
APTT (secs)	38.89 ± 3.69	38.60 ± 2.91	37.50 ± 3.16	40.78 ± 3.60	1.427	0.243
TT (secs)	13.61 ± 2.13	14.10 ± 2.10	14.75 ± 1.95	14.67 ± 2.24	0.956	0.419

*Values are expressed as Mean ± Standard deviation, n = Number of subjects, PLT = Platelet count, PCT = Plateletcrit, MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, TT = Thrombin Time, * = statistically significant*
*Posthoc comparisons using Turkey's HSD. Mean difference shown. * Shows the mean difference is significant at 0.05 level between 11 – 20 and 21 – 30.*

Table 5: Platelet count, Platelet Indices, Prothrombin Time, Activated Partial Thromboplastin Time and Thrombin Time among snuff consumers based smoking additional substances

Parameters	Other Substances					F	P value
	S (n = 34)	S, G (n = 20)	S, G, CA (n = 4)	S, G, CI (n = 3)	S, CA, CI (n = 4)		
PLTS ($\times 10^9$)	168.38 ± 71.45	169.95 ± 68.82	134.50 ± 17.67	150.00 ± 40.58	117.00 ± 56.76	0.811	0.523
PCT (%)	0.18 ± 0.07	0.18 ± 0.07	0.14 ± 0.02	0.15 ± 0.05	0.13 ± 0.06	0.769	0.549
MPV (fl)	10.34 ± 0.87	10.77 ± 0.73	10.18 ± 0.69	9.93 ± 1.01	11.28 ± 2.37	0.629	0.179
PDW (%)	38.50 ± 3.35	39.59 ± 2.02	39.98 ± 2.16	37.73 ± 2.73	40.32 ± 3.16	0.919	0.459
PT (secs)	12.56±1.37	12.60±1.50	13.75±0.96	13.67±0.58	13.25±0.96	1.240	0.304
APTT (secs)	38.41±3.33	38.65±0.12	39.50±1.91	43.33±5.69	40.25±3.95	1.717	0.158
TT (secs)	13.97±2.19	14.35±2.03	13.50±2.65	13.00±3.00	14.50±1.00	0.394	0.812

*Values are expressed as Mean ± Standard deviation, n = Number of subjects, PLT = Platelet count, PCT = Plateletcrit, MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, TT = Thrombin Time, * = statistically significant. "S = Snuff", "S, G = Snuff & Gin", "S, G, CA = Snuff, Gin & Cannabis", "S, G, CI = Snuff, Gin & Cigarette", "S, CA, CI = Snuff, Cannabis & Cigarette".*

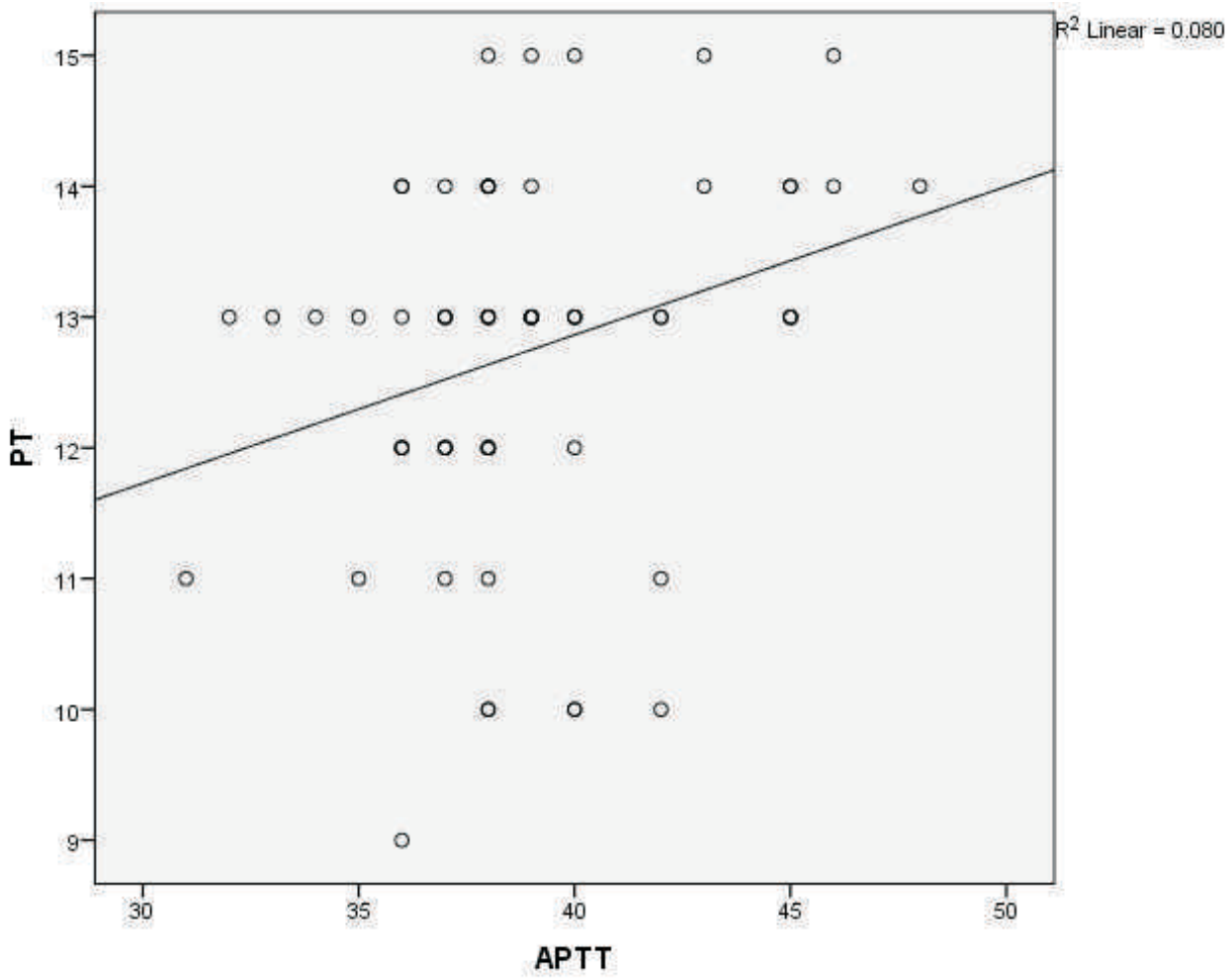

Figure 1: Correlation graph between PT versus APTT of snuff consumers in Ugep

Discussion

In this study, we observed a responsive sharp decrease in platelet count and plateletcrit among the snuff consumers compared to non-snuff consumers. Our finding agrees with the findings in previous reports (Metin *et al.,* 2004; Purushottama and Jaganmohan, 2013). The decrease in platelet count and plateletcrit (PCT) value could be attributed to an increase in the mean platelet volume (MPV) and an increased MPV indicates increased platelet diameter, which can be used as a marker of production rate and platelet activation. During activation, platelet's shapes change from biconcave discs to spherical and a pronounced pseudopod formation occurs that leads to MPV increase during platelet activation. Platelet mass is regulated to keep the plateletcrit constant. Any alteration in the platelet mass brings about a responsive change in the plateletcrit value which is evident in the result of this research work.

A significant elevation of PT, APTT and TT of snuff consumers when compared with non-consumers have been shown in table 1. Studies have revealed that oxidative stress from snuff consumption impairs the functions of the liver cell which includes the production of some coagulation factors, which could have led to the prolongation of PT, APTT and TT. Another possible reason for the prolonged PT, APTT and TT as proposed by (Zhi-De *et al.,* 2012) is that due to a possible increase in haematocrit observed in snuff (tobacco) consumers, an alteration in the anticoagulant to plasma ratio such that the excess citrate-sodium in plasma weakens the procoagulant activity of the PT and APTT reagent by reducing the availability of assay-added calcium and consequently resulting in a prolonged PT and APTT. However, this finding does not agree with that of Anjani *et al. (*2019) who reported a decreased PT and APTT in consumers of smokeless tobacco.

The effect of snuff consumption on PLT, PCT, MPV, PDW and some coagulation profiles (PT, APTT and based on age and gender is shown in table 2. We observed that there was no significant difference in

the mean values of the assayed profiles. This clearly denotes that age and gender is not a determinant of any adverse effect caused by snuff on the haematological profiles studied. Moreover, it shows that every snuff consumer irrespective of age and gender stand an equal chance of having any health-related disorders of the profiles exerted by snuff. However, this finding is at variance with a previous report by Chizoba (2017) who postulated that PT and APTT were significantly prolonged in consumers aged over 30 and 40 years compared to those younger than 30 years. It should be noted that there is very limited published data about the effects snuff has on haematological profile in respect to the gender of subjects examined.

While this study was solely conducted to know the effect of snuff on platelet and some coagulation parameters, there is also a responsive need to ascertain that only snuff and no other smoking or non-smoking substances is responsible for any change in the coagulation profiles. The enrolled subjects were quizzed on if they took any other substance aside snuff. Quite a number of self-reported substances were actually drawn - Gin, Cannabis and Cigarette. The mean values of the analysed parameters is shown in Table 5. However, there was no significant difference in the set of substances complained of in respect to the parameters.

Marker such as duration is an established factor to assess absolute risk of developing smokeless tobacco-related complications in long-term snuff consumers. In this study, there was a significant difference in the coagulation parameters owing to duration. A sharp reduction in the PT value was recorded at 11 - 20 years. This finding agrees with previous works by Biljak *et al. (*2011) who reported a significantly lower PT and APTT in same age range. Posthoc comparisons were done using Turkey's HSD. There was a significant difference at 0.05 levels between 11 - 20 and 21 – 30 years. However, there was a weak positive correlation between PT and APTT parameters. This shows that an increase in the PT value in vivo will also bring about a responsive increase in the APTT value of snuff consumers.

Conclusion

This study shows a significant decrease in PLT, PCT and prolongation of PT, APTT and TT in snuff consumers. Duration of consumption and not age difference or gender was ascertained as contributing factor to the effects of tobacco consumption on the coagulation parameters. This study has shown that long-term consumers may be more predisposed to risk of bleeding or coagulation disorder. The snuff consumption status of patients should be put into consideration in interpreting haematological values in the laboratories. There is need for more public enlightenment on the effects and dangers of snuff consumption.

Conflict of Interest

The authors declare no conflict of interest.

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