www.ajbrui.org

Afr. J. Biomed. Res. Vol. 25 (September, 2022); 395 - 402

Research Article

Buccal Mucosal Smears Cytomorphology among Active and Passive Cigarette Smokers in Abeokuta City, Ogun State, Nigeria

Aigbogun D.O.[1], Eze G.I.[2] and *Ajileye A.B.[3]

[1]*Histology Unit, Department of Anatomic Pathology, Federal Medical Centre, Abeokuta, Ogun State.*
[2]*Department of Anatomy, College of Medical Sciences, University of Benin, Benin City, Edo State.*
[3]*Department of Biomedical Laboratory Science, College of Medicine, University of Ibadan, Ibadan, Oyo State.*

ABSTRACT

Cigarette smoke is a well-known main risk factor for cancer related early death. Exposure to cigarette smoke by both active and passive smokers increases the incidence of infections and causes atypical changes. This study aimed at examining the cytomorphological features of buccal mucosal smears of active, passive cigarette smokers and non-smokers. About 290 subjects were recruited for this study, of which 220 were active cigarette smokers, 20 were passive cigarette smokers and 50 were non-smokers. Sterile Ayre's spatulas were used to collect buccal mucosa samples from each subject and smeared immediately on 3 clean frosted slides, which were fixed immediately with 95% Methanol for at least 30 minutes before they were stained using Papanicolaou, Giemsa and Heamatoxylin & Eosin techniques respectively. Stained smears were viewed with the microscope to examine their cytomorphological features. Cytological features such as pleomorphism, hyperchromatism, micronuclei, fungal infection and increased nuclei-cytoplasmic ratio were observed in increased severity in the buccal mucosa smears of active cigarette smokers when compared with the Passive cigarette smokers and the non-smokers. Oral exfoliative cytology is a non-invasive, low-cost test that should be advocated in cigarette smokers for early detection of any precancerous diagnosis.

Keywords: *Buccal mucosa, cigarette, giemsa, papanicolaou, pleomorphism.*

*Author for correspondence: Email: *ayobless05@gmail.com; Tel: +2348030445624*

Received: January 2022 Accepted: May 2022

DOI: 10.4314/ajbr.v25i3.16

INTRODUCTION

Oral mucosa cytology is part of non-gynaecological specimens that are less frequently handled in our routine cytology laboratories. Cytological examination of buccal smear specimen is simple, non-invasive and cost effective (Bancroft and Marilyn, 2007). Oral cancer is preceded by precancerous changes, such as leukoplakia and erythroplakia years before invasive stage in patients. Prompt action taken at this precancerous stage may reverse the cellular changes. Even though oral mucosal is an easy accessible site for examination, patients of oral cancers are presented at late stage leading to poor prognosis. Hence, it is very important to have techniques that can pick early precancerous changes before its development into carcinoma. Exfoliative cytology is a non-invasive procedure that involves examining buccal mucosal cells under a microscope. It could be useful for detecting and intervening in persons with oral cancer at an initial stage. Several studies have shown that the cytomorphometrical data acquired by exfoliative cytology can be used as an early predictor of premalignant oral mucosa lesions (Parmar *et al.*, 2020).

Consumption of tobacco (cigarettes) is the leading cause of mouth cancer (Bardi, 2012). The use of exfoliative cytology to detect cytomorphological alterations in the buccal mucosa of smokers could aid in the diagnosis of early premalignant changes and thereby reduce morbidity in mouth cancer patients. Cigarette smoking is a well-known main risk factor for cancer-related early death. Infections and abnormal alterations are more likely in both active and passive cigarette smokers (Alberg *et al.*, 2013). Cigarette smoking is one of the world's major causes of preventable morbidity and mortality, and it has been linked to cancer in all regions of the body (Breland *et al.*, 2014). About 443,000 people have died as a result of diseases linked to cigarette smoking. This also includes those that are affected secondarily such as babies born prematurely due to prenatal maternal smoking and victims of second hand exposure to tobacco carcinogen (Arcavi and Benowitz, 2004). Chemicals that cause cancer in cigarette smoke can become concentrated in the buccal cavity

which eventually has negative effects on the lining of the oral buccal mucosa, which can also increase the chances of developing oral cancer than the non-smokers (Doll *et al.*, 2004). The risk increases based on the numbers of cigarette sticks an individual smokes in a day and the number of years an individual has been smoking.

Cigarettes contain around 600 different components. When cigarettes are smoked, around 7000 chemicals are released, at least 69 of which are carcinogenic and some of which are deadly (Henley *et al.,* 2004). The bulk of these compounds are present in consumer products, which are always tagged with warning labels advising the public about their potentially harmful consequences (Arcavi and Benowitz, 2004). The goal of this study was to evaluate and compare the buccal mucosal smears of active and passive cigarette smokers with that of non-smokers.

MATERIALS AND METHODS

Study Area: This study was carried out among active, passive cigarette smokers and non-smokers in Abeokuta South Local Government, Ogun State, Nigeria.

Ethical clearance: Approval for this study was sought and approved by the Research and Ethics Committee of Federal Medical Centre, Abeokuta, Ogun state, Nigeria. With reference number FMCA/470/HREC/01/2019/11.

Inclusion Criteria: Subjects that fulfilled the following criteria were included in this study:-

1. Subjects (active cigarette smokers) who have been actively smoking cigarettes of any brand for at least 5 years.
2. Subjects (passive cigarette smokers) included individuals who do not smoke any brand of cigarette but stay, mingle and live with those that are actively involved in cigarette smoking.
3. Control subjects (non-smokers) were healthy males and females who have never smoked any form of tobacco, live, stay, nor mingle with individuals that are tobacco smokers.
4. Subjects recruited for this study are those that are 25years old to 55 years and above
5. Subjects that agreed to participate in the study.

Exclusion Criteria: Subjects with the following criteria were exempted from this study:-

1. Subjects (active cigarette smokers) who have not been smoking cigarettes actively for a period up to 5 years.
2. Subjects who consumed kolanut, herbal concoction, and alcohol.
3. Subjects whose ages are below 25 years old.
4. Subjects that refused participation.

Sample Size Determination: Data from World Health Organization, 2008 Shows that the incidence rate of cigarette smokers in men and women in Nigeria is 18.1%. Therefore, inthis study, sample size determination employed P (reported prevalence of cigarette smokers in Nigeria) = 0.181

Sample size for this study was determined using

$n = Z^2P(1-P)/d^2$ (Naing *et al.,* 2006).

n = Sample size; z = Confidence level at 95% (standard value of 1.96); p = Estimated prevalence (18.1%); d = Accepted error (5%); n = 1.962 x 0.181(1-0.181)/0.052

n = 228

Study Population: The minimum sample size used for this work was 290 subjects and divided into active cigarette smokers (n1 = 220), passive cigarette smokers (n2 = 20) and non-cigarette smokers (n3 = 50)

Exposed Subjects: Buccal mucosa smear samples were collected from both active (n1 = 220) and passive (n2 = 20) cigarette smokers. The active and passive cigarette smokers included both males and females with the age ranging from 25 years to 55 years and above.

Non-Exposed Subjects (Controls): The control for this work were the non-cigarette smokers (n2 = 50) and it included both males and females with the age ranging from 25years to 55years and above.

Sample Collection and preparation: Questionnaires were given to subjects to fill prior to sample collection. Those who were unable to fill the form were assisted. Subjects were given clean water to rinse their mouth; this is to remove dirt from the mouth. Disposable sterile Ayre's spatula was used in collecting (scrapping) samples from every subject at the buccal mucosa cavity. Each subject's sample was transferred to three independent clean frosted slides, where smears were produced immediately and fixed for at least 30 minutes with 95% methanol. The 3 smears made from each subject were stained with Heamatoxylin and Eosin, Papanicolaou stain and Giemsa stain respectively.[11] The slides were analyzed with the aid of a light microscope for the cytomorphological changes.

Staining Procedures for Heamatoxylin and Eosin stain (Avwioro, 2002)**:**Smears were fixed with 95% Methanol for 30 minutes. The Fixed smears were rinsed in descending grades of alcohol (90%, 80%, 70%, and 50%) and water for 10 seconds each. After which the smears were stained in Harris' Heamatoxylinfor 5 minutes and then rinsed in water. The Smears were thendifferentiated in 1% acid alcohol for 30 seconds, rinsed and blued in running tap water for 10 minutes. The Smears were stained in 1% aqueous Eosin for 2 minutes, rinsed in water and dehydrated in 70% alcohol and absolute alcohol for 10 seconds each, cleared in xylene and mounted with DPX.

Staining Procedures for Papanicolaou Stain (Avwioro, 2002)**:** Smearswere fixed with 95% Methanol for 30-minute. The Fixed smears were rinsed in descending grades of alcohol (90%, 80%, 70% and 50%) and water for 10 seconds each. The Smears were stained in Harris' Heamatoxylin for 5 minutes and then rinsed in water. The Smears were differentiated in 1% acid alcohol for 30 seconds, rinsed and blued in running tap water for 10 minutes. The Smears were rinsed in 70% and95% alcohol for 10 seconds each before

transferring into Orange G6 for 2 minutes. Smears were rinsed in 2 changes of 95% alcohol for 10 seconds and then transferred into Eosin Azure50 for 2 minutes. The Smears were rinsed in two changes of 95% alcohol for 10 seconds each and then dehydrated in absolute alcohol for 10 seconds, cleared in xylene and mounted with DPX.

Staining Procedures for Giemsa Stain (Avwioro, 2002)**:** Smears were fixedwith 95% Methanol for 30 minutes. The Fixed smears were rinsed in descending grades of alcohol (90%, 80%, 70% and 50%) and water for 10 seconds each. The Smears were stainedin 10% aqueous Giemsa working solution for 30 minutes afterwhich the Smears were rinsed in water, dehydrated in 70% alcohol and absolute alcohol for 10 seconds each then clearedin xylene and mounted with DPX.

Slide Analysis: The Slides were viewed with Olympus CH microscope, connected to Eakins Intra microscopy camera and Compaq Laptop for the Photomicrography using ×10 and ×40 objective lens.

Statistical Analysis: Data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. Analysis of variance (ANOVA) was carried out and data were presented as mean ± SEM. LSD post-hoc test was used. Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representation in the form of bar charts.

RESULTS

Demographic characteristics: As shown in Table 1, a total number of 290 subjects comprising of both males and females were recruited for this study, out of which 220 were active cigarette smokers of which 217(98.6%) were males with 3(1.4%) females. About 20 subjects were of the passive cigarette smokers, comprising of 8(40%) males and 12(60%) females. Around 50 subjects were non-smokers, comprising of 30(60%) males and 20(40%) females.

Table 1:
Sex Distribution of subjects

Parameters	Male n= 258 (%)	Female n= 32 (%)	Total
Active cigarette smokers	217(98.6)	03(1.4)	220
Passive cigarette smokers	8(40)	12(60)	20
Non-smokers	30(60)	20(40)	50

Table 2 shows that among the 290 subjects recruited for this study, subjects within the age group 25-29 years old were 47(21.4%) of active cigarette smokers, 19(38%) passive cigarette smokers, 6(30) non-cigarette smokers; 30-34 years old were 57(25.9%) active cigarette smokers, 13(26%) passive cigarette smokers and 4(20%) non-smokers; 35-39 years old were 41(18.6%) active cigarette smokers, 5(10%) passive cigarette smokers and 3(15%) non-smokers; 40-44 years old were 37(16.8%) active cigarette smokers, 4(8%) passive cigarette smokers and 5(25%) non-smokers; 45-49 years old were 29(13.2%) active cigarette smokers, 5(10%)passive cigarette smokers and 2(10%) non-smokers; 50-54 years old were 6(2.7%) active cigarette smokers, 2(4%) passive cigarette smokers and no subject for non-smokers; ≥ 55 years old were 3(1.4%) active cigarette smokers, 2(4%) passive cigarette smokers and no subject for non-smokers. Age groups did not affect the prevalence of active and passive cigarette smokers when compared to the non-smokers in this study (P=0.349).

Table 2:
Age Distribution of the Subjects

Age (years)	Active Cigarette Smokers n_1=220 (%)	Passive Cigarette smokers n_2=50(%)	Non-Smokers n_3=20(%)	P-Value
25-29	47 (21.4)	19(38)	6(30)	0.349
30-34	57(25.9)	13(26)	4(20)	
35-39	41(18.6)	5(10)	3(15)	
40-44	37(16.8)	4(8)	5(25)	
45-49	29(13.2)	5(10)	2(10)	
50-54	6(2.7)	2(4)	0(0)	
≥ 55	3(1.4)	2(4)	0(0)	

P>0.05

Table 3:
The duration participants have been smoking

Duration of Smoking (Years)	Frequency n_1= 220(%)
5-14	131(59.5)
15-24	58(26.4)
25-34	22(10)
≥35	9(4.1)

Table 3 shows the duration subjects have been smoking cigarettes, with 131(59.5%) being the highest that have been smoking for 5-14 years; followed by 58(26.4%) for 15-24 years; 22(10) for 25-34 years while 9(4.1%) being the least, have been smoking cigarettes for ≥35 years.

Table 4:
Showing the brand of cigarette the Participants normally smoke

Brands of cigarette	Frequency n_1=220(%)
Benson & Hedges	41(18.6)
Philip Morris	53(24.1)
Saint Moritz	58(26.4)
Marlboro	31(14.1)
White London	25(11.4)
Rothmans	11(5.0)
Aspen Export	1(0.5)

Table 4 shows the brand of cigarette the active cigarette smokers normally take. Saint Moritz has the highest number amongst others and Aspen export has the least number.

As shown in Table 5, among the 220 active cigarette smokers recruited for this study, 113(51.4%) being the highest smoked 6-10 sticks of cigarettes daily; followed by 56(25.5%) who smoked 11-15 sticks of cigarettes daily, 36(16.4%) smoked ≤5 sticks of cigarettes daily; 10(4.5%) smoked 16-20 sticks of cigarettes daily while 5(2.3%) being the least, smoked ≥21sticks of cigarette daily.

Table 5:
Number of sticks of cigarette participants smoked per day

Sticks of cigarette per day	Frequency n_1=220(%)
≤5 cigarettes	36(16.4)
6-10 cigarettes	113(51.4)
11-15 cigarettes	56(25.5)
16-20 cigarettes	10(4.5)
≥21	5(2.3)

Table 6:
Distribution of Fungi and Bacilli Infection among the subjects

Infections	Active Cigarette smokers n_1=220(%)	Passive Cigarette Smokers n_2=20(%)	Non-Smokers n_3=50(%)
Fungi Infected	25(11.4)	4(20)	5(10)
Bacilli Infected	15(6.8)	3(15)	3(6)
Both Fungi and Bacilli Infected	20(9.1)	5(25)	4(8)
Non-Infected	160(72.7)	8(40)	38(76)

Table 6 shows the cytomorphological analysis of buccal mucosal smears among the subjects recruited for this study and revealed 25(11.4%) active cigarette smokers, 4(20%) passive cigarette smokers and 5(10%) non-smokers had fungi infection; 15(6.8%) active cigarette smokers, 3(15%) passive cigarette smokers and 3(6%) non-smokers had Bacilli infection; 20(9.1%) active cigarette smokers, 5(25%) passive cigarette smokers and 4(8%) non-smokers had both fungi and bacilli infections; while 160(72.7%) active cigarette smokers, 8(40%) passive cigarette smokers and 38(76%) non-smokers had no fungi or bacilli infection (Figure 1)

Table 7 shows the Comparison of fungal infection, bacilli infection and combined infection among non-smokers, active and passive cigarette smokers. It was observed that there was:
(1) In comparison to non-smokers, there was a substantial increase ($p<0.05$) in fungal infection among active and passive cigarette smokers.
(2) In comparison to non-smokers, there was a significant increase ($p<0.05$) in bacilli infection among active and passive cigarette smokers.
(3) There was a significant increase ($p<0.05$) in fungal and bacilli infection among active and passive cigarette smokers compared to non-smokers.
(4) There was significant increase ($p<0.05$) in non-infection of non-smokers compared to active and passive cigarette smokers.

Table 8 Shows the comparison of micronucleation, hyperchromatism, N/C ratio, mitotic activities and pleomorphism among non-smokers, active and passive cigarette smokers, it was observed that there was
(1) A significant increase ($p<0.05$) in Micronucleation of cells from active cigarette smokers compared to cells from passive cigarette smokers and non-smokers.
(2) A significant increase ($p<0.05$) in Hyperchromatism of cells nuclei in active and passive cigarette smokers compared to nuclei of cells from non-smokers.
(3) A significant ($p<0.05$) increase in Nuclear/cytoplasmic Ratio in cells of active and passive cigarette smokers compared to cells from non-smokers.
(4) A significant ($p<0.05$) increase in mitotic activity of cells of active and passive cigarette smokers compared to cells from non-smokers.
(5) A significant ($p<0.05$) increase in pleomorphism in cells from active and passive cigarette smokers compared to non-smokers.

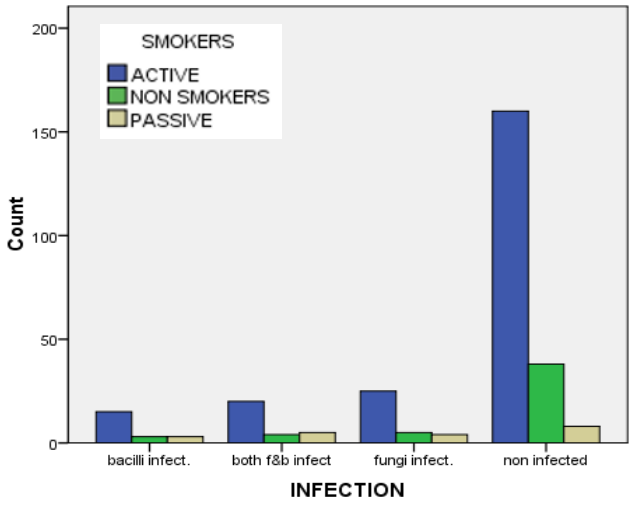

Figure 1:
Bar chat distribution of fungi and bacilli infection among subjects

Table 7:
Shows the comparison of fungal infection, bacilli infection and combined infection among non-smokers, active and passive cigarette smokers. P<0.05

infections	Non-smokers	Active Smokers	Passive smokers	P-value
Fungal infected	110.00±0.00	336.17±0.00*	220.00±0.00*	0.001*
Bacilli infected	66.00±0.00	331.00±0.00*	115.00±0.00*	0.001*
Both fungi and bacilli infected	88.00±0.00	226.60±0.00*	225.00±0.00*	0.001*
Non-infected	776.00±0.00	55.32±0.00*	440.00±0.00*	0.001*

Table 8:
Cytomorphological features among non-smokers, active and passive cigarette smokers.

Cell Features	Non-smokers	Smokers	Passive smokers	P-value
Micronucleation	4.00±0.00	81.82±0.00*	5.00±0.00	0.001*
Hyperchromatism	2.00±0.00	6.82±0.00*	5.00±0.00*	0.001*
Increased nuclear/cytoplasmic ratio	6.00±0.00	61.18±0.00*	10.00±0.00*	0.001*

Pleomorphism	2.00±0.00	80.91±0.00*	10.00±0.00*	0.001*

P<0.05

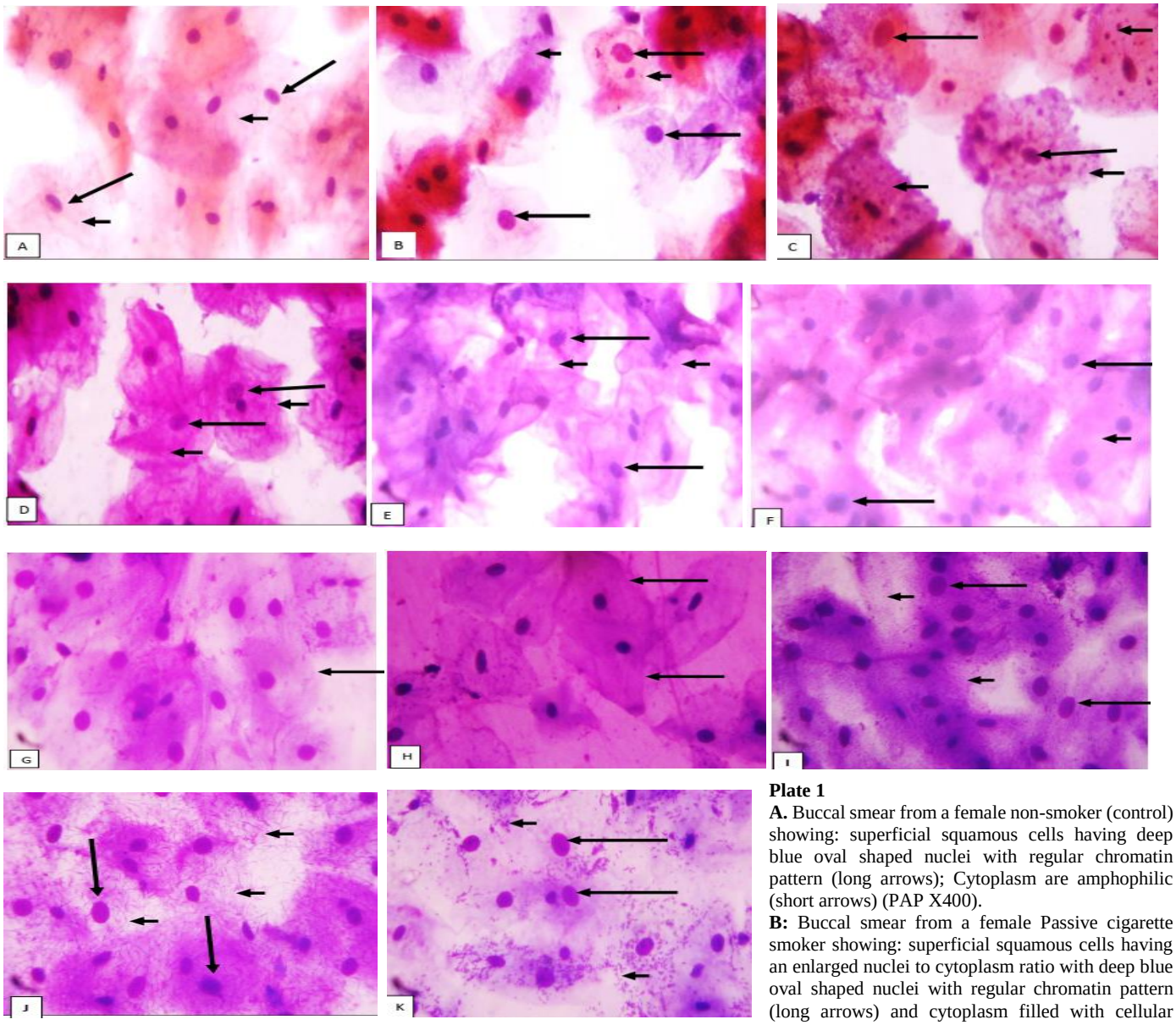

Plate 1

A. Buccal smear from a female non-smoker (control) showing: superficial squamous cells having deep blue oval shaped nuclei with regular chromatin pattern (long arrows); Cytoplasm are amphophilic (short arrows) (PAP X400).

B: Buccal smear from a female Passive cigarette smoker showing: superficial squamous cells having an enlarged nuclei to cytoplasm ratio with deep blue oval shaped nuclei with regular chromatin pattern (long arrows) and cytoplasm filled with cellular granules (short arrows), (PAP X400).

C: Buccal mucosal smear from a male active cigarette smoker (Subject) showing: superficial squamous cells having nuclei with regular chromatin pattern exhibiting micronucleation (long arrows) and Cytoplasm filled with granules (short arrows) (PAP X400).

D: Buccal smear from a female non-smoker (control) showing superficial squamous cells having: oval shaped nuclei with regular chromatin pattern (long arrows), cytoplasm (short arrows) (H&E X400).

E: Buccal smear from a female passive cigarette smokers showing superficial squamous cells having: deep blue oval shaped nuclei with regular chromatin pattern (long arrows), cytoplasm (short arrows) (H&E X400).

F: Buccal smear from a male active cigarette smoker (Subject) showing: superficial squamous cells having: an increased nuclei to cytoplasmic ratio with oval nuclei with regular chromatin pattern (long arrows), cytoplasm (short arrows) (H&E X400).

G: Buccal smear from amale non-smoker showing: superficial squamous cells having oval nuclei and Cytoplasm, no fungal and bacilli seen (long arrow) (Giemsa X400).

H: Buccal smear of male passive cigarette smoker showing: superficial squamous cells having: pyknotic oval nuclei, cytoplasm, no fungal hyphae or bacilli seen on the plate (Giemsa X400).

I: Buccal smear from a male active cigarette smoker (Subject) showing: superficial squamous cells having: an increased nuclei-cytoplasmic ratio (long arrow),presence of fungal hyphae at the background (short arrow) (Giemsa X400).

Plate J: Buccal smear from a male active cigarette smoker (Subject) showing: superficial squamous cells having: an increased nuclei-cytoplasmic ratio (long arrows) and high pseudohyphae of candida at the background (short arrows) (Giemsa X400).

Plate K: Buccal smear of male cigarette smoker (Subject) showing: an enlarged nuclei (long arrows) and heavy presence of bacilli infection at the background (short arrows) (Giemsa X400).

DISCUSSION

Cigarette smoking is the leading cause of lung cancer which is one of the deadliest cancer types. Exposure to the carcinogens in cigarette smoke damages lung and airway epithelial cells, and over time, chronic exposure can lead to cancer (Schwartz et al., 2003). The effects of cigarette smoke as a risk factor for oral cancer depends on the number of cigarette sticks an individual smokes daily and the number of years an individual has been smoking. Individuals who have been smoking for 10 years or more, and/or over 2 cigarette packs a day, are defined as heavy (active) smokers (Ayanian and Cleary,1999; Sayette et al., 2001). Shiffman et al., 2002 considered individuals to be heavy (active) smokers if they smoke over a pack of cigarette a day. In this study, individuals comprising the study group smoked at least 5 cigarette sticks a day and has been smoking for at least 5 years.

The present result reported the causes of oral cell changes among cigarette smoking subjects, which are strongly related to cancer risks, Data suggested healthy smokers were at increased risk for pre-malignant transformation of oral keratinocytes because of the changes (Schwartz et al., 2003).
A total number of 290 subjects comprising of both males and females were recruited for this study, out of which 220 were active cigarette smokers, which consisted of 217(98.6%) males and 3(1.4%) females. This finding is in tandem with a similar study carried out by WHO, 2008 revealing a higher prevalence of male subjects involving in cigarette smoking than their female counterparts. About 20 subjects were passive cigarette smokers, comprising of 8(40%) males and 12(60%) females, while, 50 subjects were non-smokers, comprising of 30(60%) males and 20(40%) females (Table 1.0).

Among the 220 active cigarette smokers recruited for this study, subjects between the age group of 30-34 years old had the highest prevalence of 25.9%, followed by 25-29years (21.4%); 35-39 years old (18.6%); 40-44 years old (16.8%), 45-49years old (13.2%); 50-54 years old (2.7%), while active cigarette smokers that are 55 years old had the lowest prevalence of 1.4%. This shows that a higher percentage of youths are actively involved in cigarette smoking. This is in line with the report from other works by Takure et al., 2015 and Ajileye et al., 2021

The duration at which subjects (active cigarette smokers) have been smoking cigarettes varies, with 131(59.5%) being the highest number of them that have been smoking for 5-14 years; followed by 58(26.4%) for 15-24 years; 22(10) for 25-34 years while 9(4.1%) being the least, have been smoking cigarettes for ≥35 years. This finding is in agreement with Inyang et al., 2018.

Among the 220 active cigarette smokers recruited for this study, 113(51.4%) being the highest, smoked 6-10 sticks of cigarettes daily; followed by 56(25.5%) who smoked 11-15 sticks of cigarettes daily; 36(16.4%) smoked ≤ 5; 10(4.5%) smoked 16-20; while 5(2.3%) being the least smoked ≥21 sticks of cigarette per day.

The cytomorphological features observed among the buccal mucosal smears of active cigarette smokers include: Nuclear enlargement associated with increased nuclei-cytoplasmic ratio, Pleomorphic cells, Micronucleation, cellular Debris, Fungal infection/Oral candidiasis, and Bacteria infection. This is similar to the report by Inyang et al., 2018 and Ajileye et al., 2021.

This study found a significant decrease in cytoplasmic area and an increase in nuclei area and nuclei cytoplasmic ratio in oral squamous cells of active and passive cigarette smokers as compared to non-smokers, which is consistent with Sharma et al., 2015, who found a significant decrease in cytoplasmic area and an increase in nuclei area and nuclei cytoplasmic ratio. The increased nuclei size is related to an increase in the nuclei contents needed for reproduction, while the mean cellular size decreases (Cowpe et al., 1993). Dehydration, a type of cell adaptation in reaction to a decrease in fluids, particularly saliva around the cell, can be blamed for the reduction in cytoplasmic diameter in smokers (Seifi et al., 2014). Exfoliative cytology may be effective for monitoring clinically suspected lesions and early identification of malignancy, according to several researches. Increased nuclei diameter and reduced cellular diameter are useful early indications of malignant transformation, according to various studies (Singh et al., 2014). In the study by Ahmed et al., 2003, who reported an increase in nuclei size, nuclei-cytoplasmic (N/C) ratio and multi-lobed nuclei, while a decrease in size of cytoplasm in active and passive smokers as compared to non-smokers (Ahmed et al., 2003). The study of Seifi et al., 2014, had also revealed an increase in cytoplasmic size and N/C ratio, while a decrease in size of cytoplasm in Tobacco users as compared to the non-smokers (control group). They have reported more atypical changes in smokers in comparison to non-smokers.

It was also discovered in this study that the size and shapes of cells and nuclei differs significantly among cigarette smokers when compared to non-smokers. As it was discussed by Joshi et al., 2011, that the size and shape of cells and nuclei in dysplasia usually differs (pleomorphism) from normal cells of the same origin, and cellular pleomorphism was seen more among the active cigarette smokers. This may be due to the fact that these individuals are already addicted to cigarette smoking, thereby inhaling a higher percentage of the carcinogenic substances present in the cigarette smoke.
According to this study, the occurrence of micronuclei in buccal mucosa smears of active cigarette smokers was higher when compared to the passive cigarette smokers while that of the non-smokers was low. The result was similar with the previous studies by Naderi et al., 2012 and Joshi et al., 2011, where they revealed in their studies an increase in number of micronuclei in the buccal mucosa smears of cigarette smokers when compared to the non-smokers. De-Geus et al., 2018 conducted a comprehensive analysis of clinical studies to compare the frequency of micronuclei exfoliated cells in the oral mucosa of smokers and non-smokers in adult patients and

found that smokers had a greater frequency of micronuclei exfoliated cells than non-smokers (De-Geus et al., 2018).

It was also seen in this study that there was a significant increase in candida infection leading to oral candidasis. Semlali et al., 2014 study showed that smokers are more prone to fungal infection compared to non-smokers. The opportunistic bacterial infection with fungal infection found in this study agrees with Soysa and Ellepola, 2005 findings. The appearance of bacteria in smears of the studied groups coupled with the presence of candida indicated that oral infections may be established in association with cigarette smoking (Abdelaziz and Osman, 2011). The cytological features (micronucleation, hyperchromatism, pleopmorphism and increased nuclei-cytoplasmic ratio) observed in the buccal mucosa smears of active cigarette smokers are significantly raised than the passive cigarette smokers and the non-smokers. This is in line with a similar finding by Twinky et al., 2017, where it was revealed that cytology of buccal smears of smokers showed more clumping of cells, pleomorphism, irregularity of nuclei membranes, increased keratinization, multi-nucleation and micronuclei compared to non-smokers, in which their smears revealed more of normal cells. It is usually known that the transformation of a normal cell into a malignant cell necessitates the presence of a precursor non-malignant cell with enhanced DNA alterations, cell proliferation, and death (Ahmed and Babiker, 2009).

Cigarette smoking has caused various alterations in the oral mucosa of many people, and it has been linked to a variety of pathologies ranging from benign and reversible lesions to mouth cancer in the mucous membranes (Sham et al., 2003). The results obtained in this study revealed that exfoliative cytology aided with image analysis can be a useful diagnostic tool in identifying risk markers like increase in nuclei diameter and decrease in cytoplasmic diameter thereby detecting the pre- malignant changes before the occurrence of any visible change in oral mucosa.

In conclusion, it was observed from this study, that active and passive cigarette smokers are predisposed to developing atypical cellular changes, premalignant lesions, oral candidiasis and oral bacterial infections. This study contributed to the fact that exfoliative cytology is a simple, good, noninvasive diagnostic technique for identifying cancerous changes in oral mucosa at an early stage for early intervention..

Ethical Approval

Approval for this study was sought and approved by the Research and Ethics Committee of Federal Medical Centre, Abeokuta, Ogun state, Nigeria. With reference number FMCA/470/HREC/01/2019/11

Consent

Written informed consent was sort from the participants as well as giving assurance the health history of the patients obtained will not in any way be linked with the true identity of the patient when recording the outcome of the findings.

Authors' Contributions

ADO, EGI and ABA conceived and designed the work, conducted research, provided research materials, ADO, EGI and ABA collected and organized data. ADO, EGI and ABA analyzed and interpreted data. ADO, EGI and ABA wrote initial and final draft of article, and provided logistic support. ADO, EGI and ABA critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

REFERENCES

Abdelaziz, M. S. and Osman, T. E. (2011). Detection of Cytomorphological Changes in Oral Mucosa among Alcoholics and Cigarette Smokers. *Oman Medical Journal.* 26(5):349–352.

Ahmed, H. G. and Babiker, A. E. (2009). Assessment of cytological atypia, AgNOR and nuclear area in epithelial cells of normal oral mucosa exposed to toombak and smoking. *Rare tumors.* 1(1): e18.- e21

Ahmed, H. G., Idris, A. M. and Ibrahim, S. O. (2003). Study of oral epithelial atypia among Sudanese tobacco users by exfoliative cytology. *Anticancer Research.* 23(2):1943–1949.

Ajileye, A. B., Akintoke, T. and Fasogbon, S. A. (2021). Cytomorphorlogical Analysis of Buccal Mucosa Smears Among Cigarette Smokers in Owo Town, Ondo State, Nigeria. *Journal of Experimental Research.* 9(1):7-14.

Alberg, A. J., Brock, M. V., Ford, J. G., Samet, J. M. and Spivack, S. D. (2013). Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd edition. American College of Chest Physicians evidence-based clinical practice guidelines. *Chest.* 143(5): 01–29.

Arcavi, L. and Benowitz, N. L. (2004). Cigarette smoking and infection. *Archives of Internal Medicine.* 164(20): 2206–2216.

Avwioro, O. G. (2002). Histochemistry and tissue pathology. 1st edition, Claverianum Centre, Ibadan. Pp. 283-292.

Ayanian, J. Z. and Cleary, P. D. (1999). Perceived risks of heart disease and cancer among cigarette smokers. *JAMA.* 281: 1019–1025.

Bancroft, J. and Marilyn, G. (2007). Theory and practice of histological techniques. *Churchill Livingstone.* Pp. 45-47.

Bardi, J. (2012). Cigarette Pack Health Warning Labels in U.S Lag Behind World. *Tobacco Control.* 23(1): 2 – 5.

Breland, A. B., Spindle, T., Weaver, M. and Eissenberg, T. (2014). Science and Electronic Cigarettes. *Journal of Addiction Medicine.* 8(4): 223–233.

Cowpe, J. G., Ogden, G. R. and Green, M. W. (1993). Comparison of planimetry and image analysis for the discrimination between normal and abnormal cells in cytological smears of suspicious lesions of the oral cavity. Cytopathology: *Official Journal of the British Society for Clinical Cytology*. 4(1), 27–35.

De-Geus, J. L., Wambier, L. M., Bortoluzzi, M. C., Loguercio, A. D., Kossatz, S. and Reis, A. (2018). Does smoking habit increase the micronuclei frequency in the oral mucosa of adults compared to non-smokers? A systematic review and meta-analysis. *Clinical Oral Investigation.* 22: 81-91.

Doll, R., Peto, R., Boreham, J. and Sutherland, I. (2004). Mortality in relation to smoking: 50 years' observations on male British doctor. *BMJ* (Clinical research edition). 328 (7455): 1519.

Henley, S. J., Thun, M. J., Chao, A. and Calle, E. E. (2004). Association between exclusive pipe smoking and mortality from cancer and other diseases. *Journal of the National Cancer Institute.* 96(11): 853–861.

Inyang, I. J., Bassey, I. E., Udonkang, M., Ugori, C. and Udoka, C. (2018). Urine cytology screening for bladder cancer among tobacco cigarette smokers in Calabar metropolis. *International Journal of Medical Laboratory Research.* 3(2): 52-58.

Joshi, M. S., Verma, Y., Gautam, A. K., Parmar, G., Lakkad, B. C. and Kumar, S. (2011). Cytogenetic alterations in buccal

mucosa cells of chewers of areca nut and tobacco. *Archives of oral biology*. 56(1): 63–67.

Naderi, N. J., Farhadi, S. and Sarshar, S. (2012). Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. *Indian Journal of Pathology and Microbiology*. 55(4): 433-438.

Naing, L., Winn, T. and Rusli, B. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences.* 2(1): 9-14.

Parmar, N. D., Master, N. and Gupta, D. S. (2020). Study of effect of smoking on cytomorphometry of buccal mucosal cells among smokers in South Gujarat Region. *National Journal of Clinical Anatomy.* 9: 90-96.

Sayette, M. A., Martin, C. S., Wertz, J. M., Shiffman, S. and Perrott, M. A. (2001). A multi-dimensional analysis of cue-elicited craving in heavy smokers and tobacco chippers. *Addiction.* 96: 1419-1432.

Schwartz, J., Muscat, J., Baker, V., Larios, E., Stephenson, G. D., Guo, W., Xie, T., Gu, X. and Chung, F. L. (2003). Oral cytology assessment by flow cytometry of DNA adducts, aneuploidy, proliferation and apoptosis shows differences between smokers and non-smokers. *Oral Oncology*. 39(8): 842–854.

Seifi, S., Feizi, F., Mehdizadeh, M., Khafri, S. and Ahmadi, B. (2014). Evaluation of cytological alterations of oral mucosa in smokers and water pipe users. *Cell Journal.* 15: 302–309.

Semlali, A., Killer, K. and Alanazi, H. (2014). Cigarette smoke condensate increases C. albicans adhesion, growth, biofilm formation, and EAP1, HWP1 and SAP2 gene expression. *BMC Microbiology*. 14:61-68.

Sham, A. K., Cheung, L. K., Jin, L. J. and Corbet, E. F. (2003). The effects of tobacco use on oral health. *Hong Kong Medical Journal.* 9: 271-277.

Sharma, D., Sandhu, S. V., Bansa, l. H. and Gupta, S. (2015). Distinct perturbations of oral squamous cell carcinoma patients: A quantitative cytomorphometric analysis. *International Journal of Health Sciences*. 9(3): 283–291.

Shiffman, S., Rolf, C. N., Hellebusch, S. J., Gorsline, J., Gorodetzky, C. W. and Chiang, Y. (2002). Real-world efficacy of prescription and over-the-counter nicotine replacement therapy. *Addiction.* 97: 505-516.

Singh, M., Sircar, K., Tandon, A., Chowdhry, A. and Popli, D. B. (2014). The role of tobacco as an etiological agent for oral cancer: Cytomorphometrical analysis of the buccal mucosa in tobacco users. *Dental Research Journal.* 11(6): 649–655.

Soysa, N. S. and Ellepola, A. N. (2005). The impact of cigarette/tobacco smoking on oral candidosis: an overview. *Oral diseases.* 11(5): 268–273.

Takure, A. O., Olubayo, M. O., Adebayo, S. A. et al. (2015). Histopathologic pattern of bladder cancers in Ibadan, South West Nigeria: An update. *J. West Afri. Coll. Surg.* 5(2):17-42.

Twinky, M., Maya, R. and Sekar, B. (2017). Study of exfoliated cells in the buccal mucosa of smokers and non-smokers – A cytological comparative study. *International Journal of Current Advanced Research.* 6(8): 5010-5014.

World Health organization. (2008). The global burden of Disease Geneva: World Health Organization.