CYTOMOPHORLOGICAL ANALYSIS OF BUCCAL MUCOSA SMEARS AMONG CIGARETTE SMOKERS IN OWO TOWN, ONDO STATE, NIGERIA.

ABSTRACT

Cigarette smoking is the practice of burning cigarettes and inhaling the smoke that comes from it. Cigarette smoke elicits carcinogenic effects on the tissues of the body that are exposed to it. About 150 subjects were recruited for this study, of which 100 were active cigarette smokers while 50 were passive cigarette smokers. Active cigarette smokers that have not been smoking daily for at least 5 years were not included for this study and passive cigarette smokers who have smoked cigarette or any other type of tobacco products before were not included for this study. The subjects for both active cigarette smokers and passive cigarette smokers were given questionnaire to fill; clean water was given to them to rinse their mouth before samples were collected from their buccal cavities with the use of sterile spatula. Samples collected were immediately smeared on a clean frosted end slide, fixed in 95% alcohol and stained with Haematoxylin and Eosin and Papanicolaou stain. This study revealed that the prevalence of male involved in cigarette smoking is higher than that of females and there is higher prevalence of youths actively involved in cigarette smoking in Owo town, Ondo state. The stained buccal smears of passive cigarette smokers revealed normal squamous epithelial cells with some smears showing scanty inflammatory cells. The stained buccal smears of active cigarette smokers revealed heavy infiltrates of inflammatory cells, increased nucleo-cytoplasmic ratio, hyperchromatic cells, and squamous epithelial cells looking glycogenated with tiny spherical bodies on the cytoplasm suggestive of fungi infection. Cigarette smoking is one of the most important risk factors for the development of oral mucosal lesions.

Keywords: Cigarette, Glycogenated, Hyperchromatic, Papanicolaou, Squamous.

Introduction

Diseases that are related to cigarette smoking have claimed many lives (Hecht, 2003), this also include those that are affected secondarily such as babies born prematurely due to prenatal maternal smoking and victims of second hand exposure to tobacco carcinogens (Hajek et al. 2014). Based on several studies that have been carried out, it has been revealed that there are about six hundred (600) constituents in cigarettes, when cigarettes are burned, they generate above seven thousand (7,000) chemical compounds which include; arsenic, formaldehyde, cyanide, lead, nicotine, carbon monoxide, acrolein, and other poisonous substances (Csordas and Bernhard, 2013). At least sixty nine (69) of these chemicals are known to cause cancer and some are also poisonous. Most of these chemicals are being found in consumer products, these products always have warning labels while the general public is strictly warned about the dangerous effects of the poisons in these products (Dales et al. 1978). Cigarette manufacturers have described cigarettes as a drug administration system for the delivery of nicotine in an acceptable and attractive form (Cummings, 2015). An individual becomes addicted to Cigarettes smoking because of its nicotine content (Spira et al. 2004). Nicotine is the addictive component of tobacco products (Sobkowiak and Lesicki, 2013). It exerts its addictive action by stimulating the release of the neurotransmitter dopamine in the brain, which happens within seconds of inhaling. Several studies have revealed that cigarette smoking can cause chronic obstructive pulmonary disease (including emphysema and chronic bronchitis), heart disease, strokes, cancer of the lungs, oral cavity, pharynx, oesophagus, stomach, liver, pancrease, kidney, bladder and cervix (Arcavi and Benowitz, 2014). Cigarette smoking leads to dental problems such as bad breath, tooth discoloration, inflammation of the salivary gland openings on the roof of the mouth, increased buildup of plaque and tartar on the teeth, increased loss of bone within the jaw, increased risk of leukoplakia, white patches inside the mouth, increased risk of developing oral cancer. It also causes peripheral vascular disease and hypertension. The chemicals that cause cancer in cigarette smoke can become concentrated in in the buccal cavity which eventually has negative effects on the epithelial lining of the buccal cavity, which can also increase the chances of developing oral cancer. Active cigarette smokers have higher chances of developing buccal cavity related diseases than the passive cigarette smokers (Lyon, 2012). The

risk increases based on the number of cigarette sticks an individual can smoke in a day and the number of years an individual has been smoking. On average, each cigarette smoked is estimated to shorten life by 11 minutes (WHO, 2008)**.** About half of cigarette smokers die of tobacco-related disease and lose an average 14 years of life (Doll et al. 2014)**.** The aim of this study was to analyze the cytology of buccal mucosa smears of active and passive cigarette smokers in Owo, Ondo State, Nigeria.

MATERIALS AND METHOD

Study Area (Site)

This study was conducted on human populations who are active and passive cigarette smokers in Owo town, Ondo State, Nigeria. Owo is the third biggest town in Ondo State, in south western Nigeria.

Inclusion Criteria

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

1. subject for this study included both males and females between the ages of 25years and above, who have been smoking cigarette for at least 5 years.
2. those that consented to participate in the study.
3. apparently healthy age matched males and females who are non-tobacco smokers and users, and have no history of buccal lesions were recruited as passive cigarette smokers.

Exclusion Criteria

Subjects with the following criteria were excluded from this study:-

1. subjects who have not been smoking cigarettes for a period up to five (5) years at the time of sample collection were excluded from this study.
2. subjects with history of any form of buccal disease were excluded from this study.
3. subjects that refused participation and
4. subjects whose ages were below 25 years.

Questionnaire

A well-structured questionnaire bothering on bio-data and socio-demographic characteristics was administered to each participant.

Ethical consideration

Approval for this study was obtained and granted from the Research Ethics Committee of the Federal Medical Centre, Owo, Ondo State, Nigeria.

Study Population

This study was carried out on subjects that only smoke cigarette (active smokers) and subjects who have never been involved in cigarette or any other form of tobacco smoking before (passive smokers). The study population consisted of 100 active cigarette smokers and 50 passive cigarette smokers.

Sample Collection for active and passive cigarette smokers

Questionnaires were given to subjects to fill, to know those eligible for this study. Thereafter, clean water was given to them to rinse their mouth before samples were collected by rolling sterile disposable spatula firmly in their buccal cavity. Samples were transferred to clean sterile frosted slides where smears were made and fixed immediately in 95% alcohol.

Staining Procedures for Papanicolaou Stain

Buccal Smears were fixed in a cytology fixative (95% alcohol) for 30 minutes and briefly rinsed in descending grades of alcohol (80%, 70%, 50%) and water. Hydrated buccal Smears were stained with Harris Haematoxylin for 4 minutes, rinsed in tap water and briefly differentiated in 1% acid alcohol. Smears were rinsed in water and blued in tap water for 10 minutes. Smears were transferred to 70% alcohol, 95% alcohol for a few seconds before they were stained with Orange G6 for 2 minutes. Smears were briefly rinsed in 2 changes of 95% alcohol and then stained with Eosin Azure 50 for 2 minutes. Stained smears were briefly rinsed in 2 changes of

95% alcohol, dehydrated in absolute alcohol for 10 seconds, cleared in xylene and mounted with DPX (Ochei and Kolhatkar, 2000).

Staining Procedures for Haematoxylin and Eosin

Buccal smears were fixed in a cytology fixative (95% alcohol) for 30 minutes and briefly rinsed in descending grades of alcohol (80%, 70%, 50%) and water. Hydrated smears were stained with Harris haematoxylin for 4 minutes, rinsed in water and briefly differentiated in 1% acid alcohol. Smears were rinsed in water briefly and blued in tap water for 10 minutes. Smears were counterstained with 1% eosin for 2 minutes, rinsed in tap water, dehydrated in ascending grades of alcohol for 10 seconds each, cleared with xylene and mounted with DPX (Ochei and Kolhatkar, 2000).

Statistical Analysis

All the information, results and data gotten from this study were analyzed using frequency table distribution and Pearson Chi-Square.

Results

Table 1.0: Prevalence of active cigarette smokers in relation to gender.

Parameters	Number Tested n=150 (%)	Active Smokers n=100 (%)	OR	95% CI	P- value
Male	128(85.3)	83 (83)	1.19	0.6, 2.38	0.618
Female	22(14.7)	17 (17)			

P>0.05

Among the 150 subjects recruited for this study, 128(85.3%) were males, while 22(14.7%) were females. Among the 100 active cigarette smokers, 83 (83%) were males while 17(17%) were

females. In addition, gender did not significantly affect the prevalence of active cigarette smokers in this study (P=0.618) (Table 1.0).

Table 2.0: Prevalence of passive cigarette smokers in relation to gender.

Parameters	Number Tested n=150 (%)	Passive Smokers n=50 (%)	OR	95% CI	P- value
Male	128(85.3)	45 (90)	0.65	0.23, 1.81	0.403
Female	22(14.7)	05 (10)			

P>0.05

Among the 50 passive cigarette smokers, 45(90%) were males, while 5(10%) were females. However, gender did not significantly affect the prevalence of passive cigarette smokers in this study (P=0.403) (Table 2.0).

Table 3.0: Age group of active and passive cigarette smokers.

Parameters (Years old)	Active Cigarette Smokers n=100 (%)	Passive Cigarette Smokers n=50 (%)	P value
25-34	47 (47)	21(42)	0.752
35-44	13(13)	10(20)	
45-54	19(19)	10(20)	
55-64	11(11)	6(12)	
65 years and above	10(10)	3(6)	

P>0.05

Among the active cigarette smokers, subjects within the age group 25-34 years recorded the highest prevalence (47%) involved in cigarette smoking followed by 45-54 years (19%); 35-44 years (13%); 55-64 years (11%) while subjects that are 65 years and above had the lowest prevalence (10%). According to this study, subjects within the age group 25-34 years recorded the highest prevalence (42%) of passive cigarette smokers, followed by 35-44 years and 45-55years (20%); 55 – 64 years (12%) while subjects that are 65 years and above had the lowest prevalence rate of 6%. Age group did not significantly affect the prevalence of active and passive cigarette smokers in this study (P=0.752) (Table 3.0).

Table 4.0: Frequency distribution of Numbers of Cigarette sticks smoked per day among the active cigarette smokers

Cigarette Range (Cigarettes)	Frequency
≤5	23 (23%)
6-10	36 (36%)
11-15	22 (22%)
16-20	10 (10%)
21-25	03 (3%)
26-30	04 (4%)
≥31	02 (2%)
Total	100 (100%)

About 36% of active cigarette smokers smoked 6-10 cigarette sticks per day which was the highest followed by 23% smoked ≤5 cigarette sticks per day; 22% smoked 11 – 15 cigarette sticks per day, 10% smoked 16- 20 cigarette sticks per day; 4% smoked 26-30 cigarette sticks per day, 3% smoked 21-25 cigarette sticks per day while 2% smoked ≥31 sticks per day (Table 4.0).

Table 5.0: Different cell types seen in the urinary cells of active and passive cigarette smokers

CELL TYPES	Active Cigarette Smokers n=100(%)		Passive Cigarette Smokers n=50		**P-value**
	YES	NO	YES	NO	
Normal Squamous Epithelial cells	37(37)	63(63)	47(94)	03(6)	0.0001*
Inflammatory cells	58(58)	42(42)	07(14)	43(86)	0.0001*
Hyperchromatic cells	35(35)	65(65)	01(2)	49(98)	0.0001*
Degenerating cells	22(22)	78(78)	00(0)	50(100)	0.0001*
Increase in Nucleo-cytoplasmic ratio	21(21)	79(79)	00(0)	50(100)	0.002*

*Significant at $P \leq 0.05$

The buccal smears of active cigarette smokers revealed various degrees of morphological cell types, with inflammatory cells being the most dominant seen in about 58% of subjects, followed by normal squamous epithelial cells (37%), hyperchromatic cells (35%), degenerating cells (22%) and increase in nucleo-cytoplasmic ratio (21%). The buccal smears of passive cigarette smokers revealed normal squamous cells among 94% of the subjects being the highest, followed by scanty inflammatory cells (14%); hyperchromatic cell (2%). None of the buccal smears of passive cigarette smokers revealed degenerating cells and increase in Nucleo-cytoplasmic ratio (Table 5.0).

BUCCAL SMEARS STAINED WITH H & E

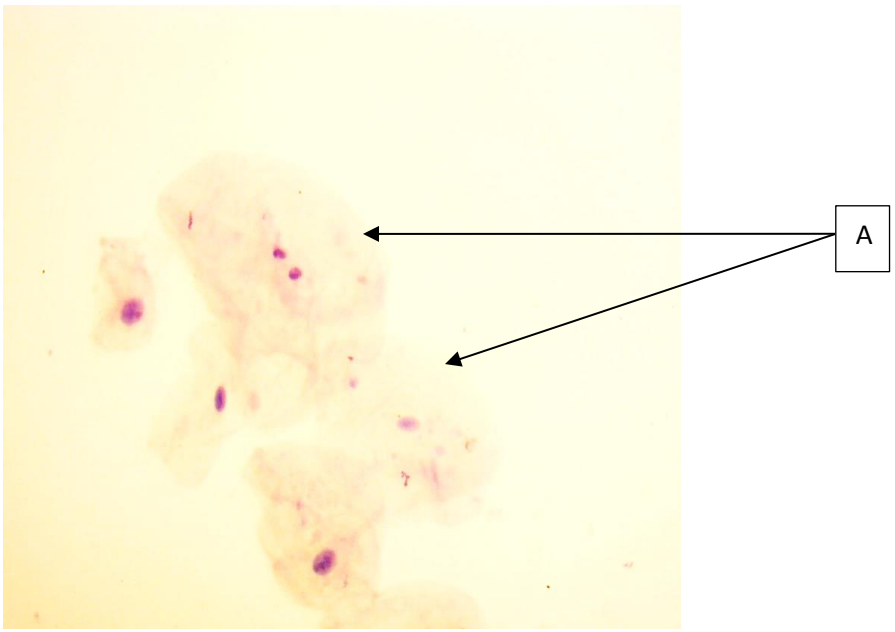

Plate 1. Buccal smear from a Passive cigarette smoker, showing **[A]** Normal Squamous Epithelia Cells (H&E X400)

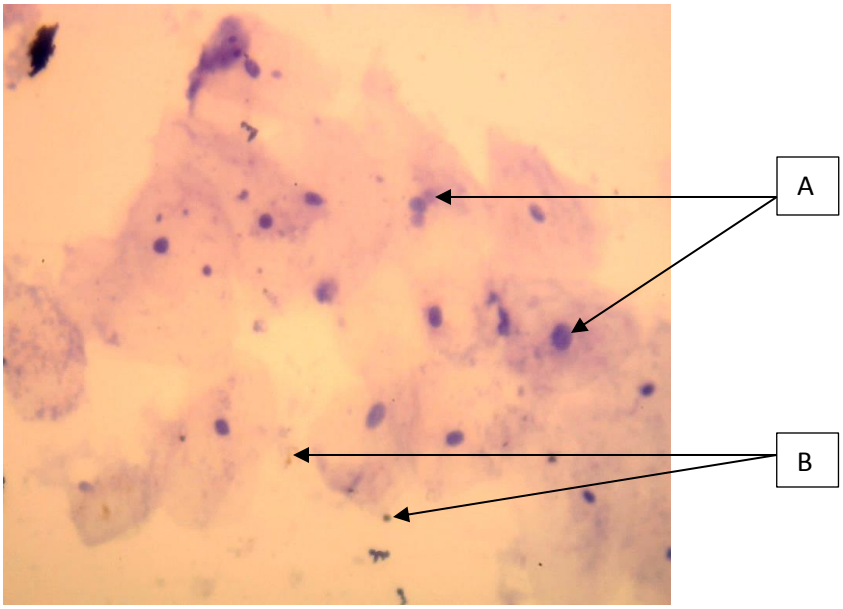

Plate 2. Buccal smear from an active cigarette smoker, who smokes 16 to 20 cigarette sticks daily, showing **[A]** squamous epithelia cells with slight increase nucleo-cytoplasmic ratio **[B]** inflammatory cells (H&E X400).

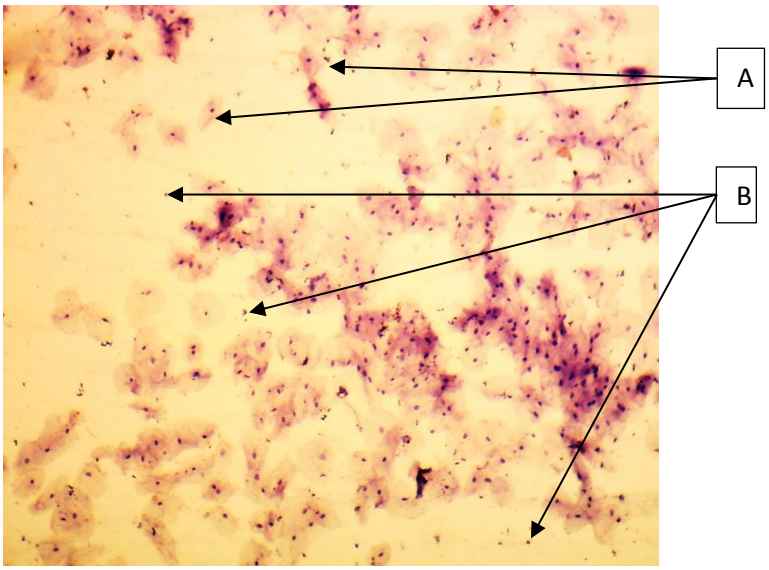

Plate 3. Buccal smear from an active cigarette smoker, who smokes 21 to 25 cigarette sticks per day showing **[A]** hyperchromatic squamous epithelia cells **[B]** infiltrate of inflammatory cells (H&E X100).

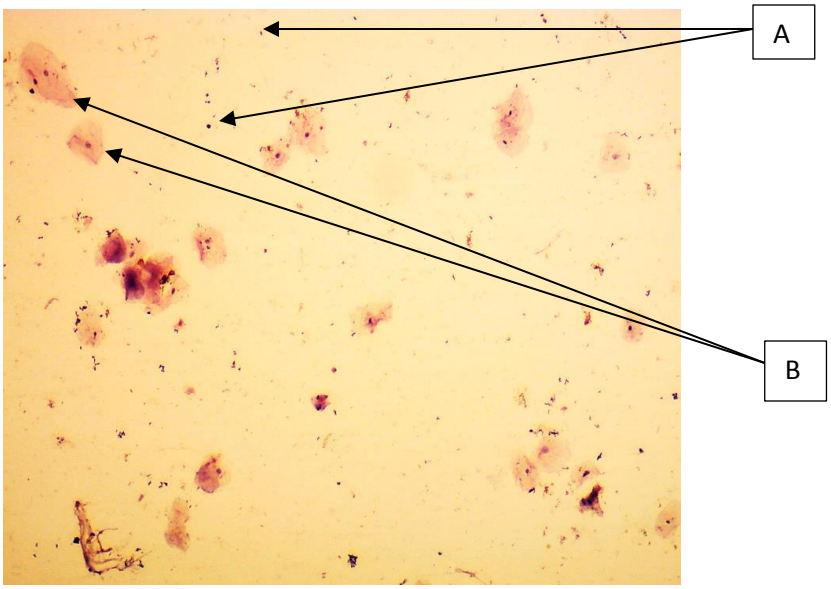

Plate 4. Buccal smear from an active cigarette smoker, who smokes 26 to 30 cigarette sticks per day showing **[A]** Heavy infiltrate of inflammatory cells **[B]** normal Squamous epithelial cell (H&E X100).

BUCCAL SMEARS STAINED WITH PAP

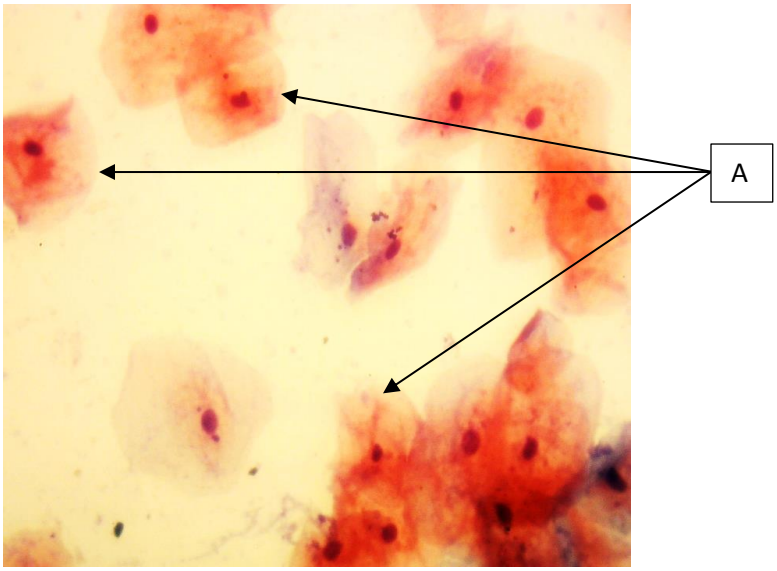

Plate 5. Buccal smear from a Passive cigarette smoker, showing **[A]** Normal Squamous Epithelia Cells (PAP X400)

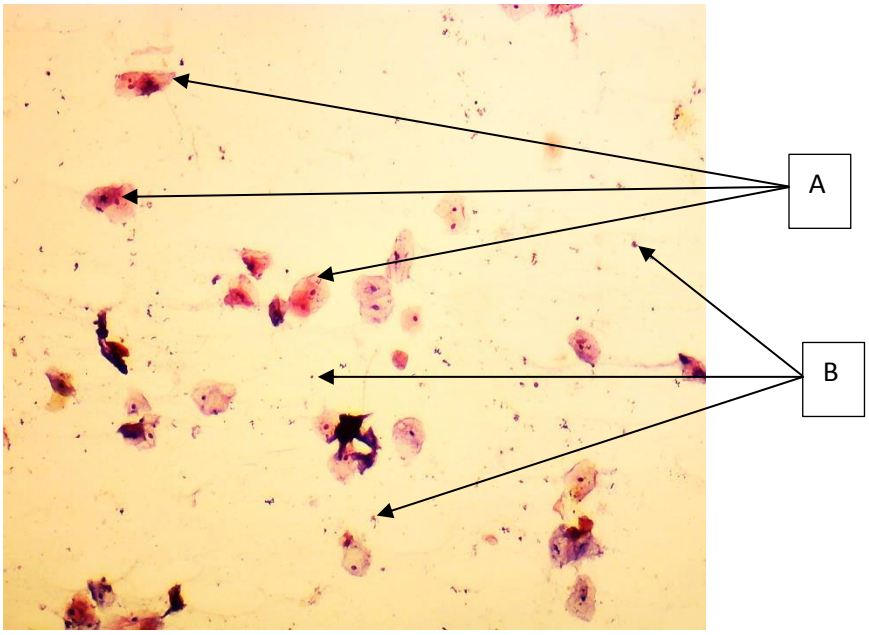

Plate 6. Buccal smear from an Active cigarette smoker, who smokes 6 - 10 cigarette sticks per day showing **[A]** Normal Squamous Epithelia Cells **[B]** Infiltrates of inflammatory cells (PAP X100).

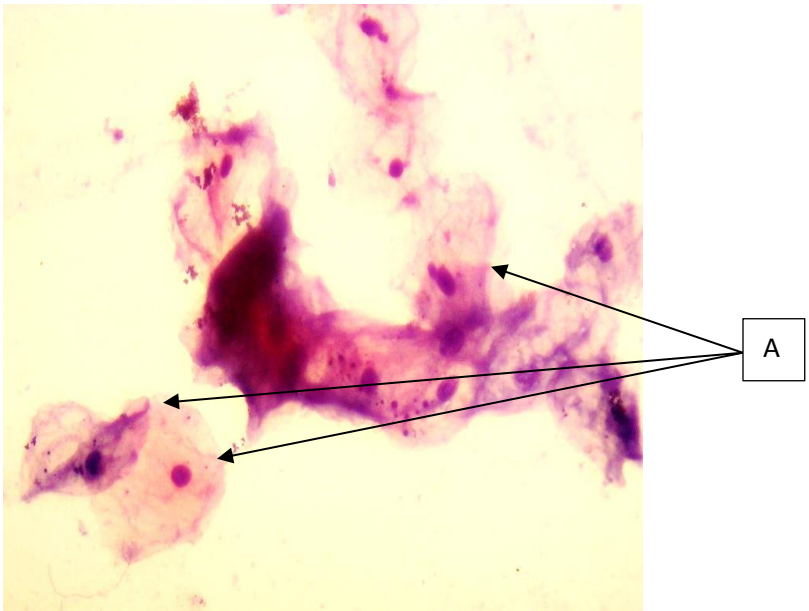

Plate 7. Buccal smear from an active cigarette smoker, who smokes 11 to 15 cigarette sticks per day, showing **[A]** Squamous Epithelia Cells with tiny spherical bodies on the cytoplasm also revealing slight increased nucleo-cytoplasmic ratio (PAP X400).

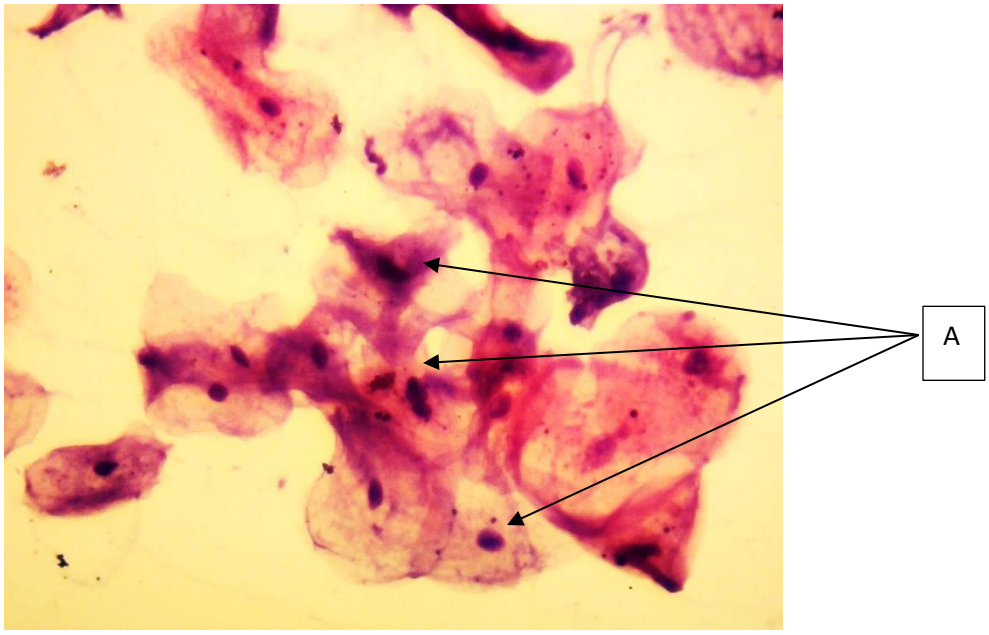

Plate 8. Buccal smear from an active cigarette smoker, who smokes ≥31 cigarette sticks per day, showing **[A]** Hyperchromatic Squamous Epithelia Cells looking glycogenated with degenerating features (PAP X400)

DISCUSSION

The effects of cigarette smoke on the buccal cavity of an active cigarette smoker depends on the number of years an individual has been smoking and the number of cigarette sticks an individual can smoke per day (Arcavi and Benowitz, 2014). This study revealed that the prevalence of active cigarette smokers was 83% males while 17% were females. This finding is consistent with the reported prevalence value given by WHO, (2008) indicating a higher prevalence of male cigarette smokers when compared to that of female cigarette smokers in south-western geo-political region in Nigeria and a previous study carried out by Ajileye et al. (2016) indicating 87% of males and 13% of females to be active cigarette smokers in Owo Town, Nigeria. The number of passive cigarette smokers recruited for this study was 50, of which 45 were males while 5 were females. The age range of subjects recruited for this study ranged from 25 years to 65 years and above. Among the 100 active cigarette smokers recruited for this study, subjects between the ages of 25 to 34 years recorded the highest prevalence of 47%, followed by 35 to 44 years (13%), 45-54 years (19%), 55-64 years (11%), the least prevalence recorded for the active cigarette smokers were subjects that are 65 years and above (10%).

The number of cigarette sticks smoked per day among the active cigarette smokers varied with 36% smoking 6 to 10 cigarettes stick per day which was the highest, followed by 23% smoking ≤5 cigarette sticks per day; 22% smoking 11 to 15 cigarette sticks per day; 10% smoking 16 to 20 cigarette sticks per day; 4% smoking 26 to 30 cigarette sticks per day; 3% smoking 21 to 25 cigarette sticks per day while 2% smoked ≥31 cigarette sticks per day. This finding is in agreement with Inyang et al. (2018) who revealed similar prevalence of number of cigarette sticks smoked per day among cigarette smokers in Calabar city, Nigeria. According to Onur et al. (2017) the effects of cigarette smoke on the teeth and oral tissues are based on the amount of cigarette sticks smoked per day and duration of usage.

In this present study, the buccal smears of passive cigarette smokers revealed normal squamous epithelial cells, with few smears showing mild inflammatory cells. Similar study was also noticed by Hamam and El-waseef, (2018). It was reported that the effects of secondhand

cigarette smoke on non-smokers, depend on the level of exposure and the amount one has been able to inhale over a period of time.

In this study, heavy inflammatory cells were noticed in the buccal smears of active cigarette smokers. This is in agreement with Hamam and El-waseef, 2018**.** It was reported that tobacco components produce pro-inflammatory cytokines that cause chronic inflammation. Cytokines are inflammatory factors that are normally secreted due to tissue injury to promote repair process. The cytokines that have been found to increase after exposure to cigarette smoke are the interleukins IL-1, IL-6, IL-8, IL-10, tumor necrosis factor-α, as well as transforming growth factor-β, granulocyte-macrophages, colony stimulating factor and monocytes chemoattractant protein. The role of the inflammatory cytokines is to recruit the immune cells during the infection.

This study also revealed that the buccal smears of active cigarette smokers showed a slight increase in nucleo-cytoplasmic ratio. This is in agreement with a similar study carried out by Hande and Chaudhary, (2010**)** where they conducted a cytomorphometric analysis of buccal mucosa of tobacco (cigarette) smokers and reported an increase in the nucleo-cytoplasmic ratio.

Tiny spherical bodies on the cytoplasm suggestive of fungi infection was seen in the buccal smears of active cigarette smokers who always smoke 11 to 15 and 21-25 cigarette sticks per day. This finding is in agreement with a reported study by Giannopoulou et al. (2001), where they stated that smoking makes the binding of some pathogenic organisms easier to the epithelia lining of the buccal cavity, as smoking affects directly periodonto pathogen colonies, sub-gingival ecology and increases colonization of the mouth by potential pathogen organisms.

This study revealed that the buccal smears of active cigarette smokers who always smoke 26 to 30 showed hyperchromatic squamous cells. Also, squamous epithelial cells looking glycogenated with degenerating features were detected among active cigarette smokers who always smoke between 21 to 25 and ≥ 31 cigarette sticks per day. These findings are caused as a result of cigarette smoke which contains many constituents of hazardous chemicals and free radicals such as nicotine, ammonia, acrolein, phenols, acetaldehyde, benzopyrenen nitric

oxides, carbon monoxide, polonium, radium and thorium which penetrate the cells lining the buccal mucosa to induce cellular damage and DNA damage (Pasupathi et al. 2009). These cytology features noticed in this study were statistically higher among the buccal smears of active cigarette smokers when compared to that of passive cigarette smokers ($P < 0.05$). This shows that cigarette smoke has significant effects on the buccal cavities of cigarette smokers but this depends on the number of years an individual have been smoking and the number of cigarette sticks one can smoker per day.

CONCLUSION

Based on this study and many other relevant literatures, it can be concluded that cigarette smoke renders buccal mucosa epithelium to be susceptible to colonization of pathogens and it is one of the most important risk factors for the development of buccal mucosa lesions.

Conflicts of interest: the authors declared that this manuscript was approved by all the authors in its form and that no competing interest exists.

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