

# Tobacco Consumption in Some Traveling Agencies in Bafoussam in West Region Of Cameroon, Associated To Mouth Cytology and Malondialdehyde (MDA) Profile

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## ABSTRACT

Tobacco is mostly regarded as one of the primary etiologic factor in causing oral cancer. Its use affects the pattern of maturation in oral mucosal cells and results in changes in the appearance of the tissues. Lipid peroxidation (LPO) has been implicated in the pathogenesis of several pathologic disorders, including oral premalignant and malignant lesions. The aim of this study was to determine the prevalence of tobacco use and assess its effects on oral epithelial cells and oxidant/antioxidant balance in the oral cavities of tobacco consumers in some travelling agencies in Bafoussam West Region Cameroon.

A cross sectional study with a total of 120 individuals (divided into two groups; group A (n=55) including non-consumers of tobacco above 21 years old and group B (n=65) including tobacco consumers above 21 years old), voluntarily enrolled in the study. After giving their consents and answering a series of questions from questionnaires, saliva samples and buccal smears were collected from their oral cavities. Buccal smears were stained using Papanicolaou staining technique and the presence of hyperkeratosis, tobacco hyperkeratosis, non-specific inflammatory changes and oral microflora were assessed. [MDA] was determined using the Thiobarbituric acid method. The results showed that male tobacco consumers made 48.3% and most of them were aged between 21-31 years old, while female tobacco consumers made 5.7% of the total sample population. Smoking was the method most used by consumers (41.7%) and 66% of them smoked for more than 10 years while 50% smoked more than 10 sticks of cigarette per day. A statistical significant relationship was found between tobacco consumption and oral epithelial changes ( $p<0.001$ ). Only tobacco consumers presented with tobacco hyperkeratotic buccal smears (20%) when compared with the control group. A good number of hyperkeratotic buccal smears (39.2%) was found in both the case (17.5) and control (21.7%) groups. 24.1% of the total sample population had normal buccal smears, where 8.3% were consumers and 15.8% were non-consumers. Microorganisms were found on buccal smears of 5.9% of individuals and 9.2% had non-specific inflammatory changes. Between tobacco consumption and salivary [MDA] in saliva of tobacco consumers was significantly higher compared to non-consumers ( $p<0.001$ ). A strongly positive correlation was found between salivary [MDA] and oral epithelial changes ( $r=0.204$ ,  $p=0.026$  at 0.05CI). In conclusion, tobacco consumption severely increases the risk for developing oral mucosal proliferative lesions, and the level of oxidative stress in the saliva of individuals in the study population.

**Key words:** Cigarette smoking, Oxidative stress, Malondialdehyde, Premalignant lesion, Oral squamous cell carcinomas.

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## I. INTRODUCTION

Tobacco use is harmful and often results in serious adverse health problems. It is a delivery system for the addictive agent nicotine. While there are many different forms of tobacco (cigarettes, cigars, cigarettos, beedis, kreteks, pipe tobaccos and smokeless products), the most common is the manufactured cigarette. In 2015, the prevalence of tobacco

smoking in the world was estimated to be more than 1.1 billion, with males more than females. Although tobacco smoking is globally declining, its prevalence appears to be increasing in the Eastern Mediterranean and the African Regions [1]. It is well established that tobacco use plays a major role in the etiology of several cancers particularly oral cancer [2], [3]. People play an important role in the beginning and the continuation of tobacco consumption. Family, friends

and role models influence smoking behavior. Young people who have smokers around them are particularly at greater risk of starting to smoke. In adolescence, cigarettes can also be used as a way to rebel against one's parents or to belong to a group. Stress related to working conditions is also a factor that increases the risk of starting to smoke. Tobacco advertising also promotes the consumption of cigarettes. Tobacco advertising aimed at children and adolescents is very widespread and has the effect of trivializing tobacco consumption. All these conditions are perfectly met in travel agencies where there are influencers, working conditions and harsh stress as well as promiscuity.

The relationship between oral cancer and tobacco consumption is well documented, but other oral changes are known to occur with tobacco use [4]. Worldwide, it is estimated that an incidence of approximately 274,300 and mortality rate of 127,500 are attributed to cancer of the oral cavity during 2002 [5]. In the developing countries like Cameroon, cancer is one among the ten commonest causes of mortality. This cancer epidemic is due to the combined effect of increased life expectancy and the high or increasing levels of prevalence of cancer risk factors. Tobacco use can irritate the minor salivary glands on the hard palate and directly increase a person's risk for periodontal disease and oral cancer. Oral cancer causes serious morbidity and mortality in older adults [6]. It is predominately a disease of middle-aged men who use tobacco and alcohol. Approximately 95% of carcinomas appear after age 45 years, with an average age of 60 years. Most oral cancers are squamous cell carcinomas, and the vast majority of oral squamous cell carcinomas (OSCC) are preceded by precursor lesions that can present as leukoplakia, erythroplakia, or erythroleukoplakia [6]. Microscopically, these lesions may exhibit oral epithelial dysplasia, a histopathologic diagnosis characterized by cellular changes and maturational disturbances indicative of developing malignancy. Oral cytology is not done in our country that is why patients come with already advanced lesions. Cigarette pyrolysis produces smoke containing combustion byproducts and free radicals which have been defined as carcinogenic. These free radicals can and do react with other additives or other pyrolytic products or living cells and cause DNA damage [7], [4]. ROS and free radicals primarily target peroxidation of polyunsaturated fatty acids (PUFAs) in membrane lipids [7]. They also interact with cellular DNA causing DNA damage and interference with its repair, which is a major cause of pathobiological changes in cancer cells. Peroxidation of membrane lipids generates well characterized, mutagenic lipid peroxidation end products, such as Lipid. Thus, the aim of this study is to assess the effect of tobacco on buccal epithelial cells and oxidant/antioxidant balance in the oral cavities of tobacco smokers in Bafoussam West Region Cameroon.

## II. METHODS

This study was carried out based on the ethical approval from the National Ethical Committee for Research and Human Health in Yaoundé, Cameroon, reference N° 2019/06/37/CE/CNERSH/SP. It was carried out from April 2019 to September 2019 in the dental service of the regional hospital of Bafoussam and in 10 travelling agencies in Bafoussam, West Region of Cameroon. One hundred and twenty (120) subjects of both genders were recruited, 32 from regional hospital and 88 from travelling agencies. The subjects ranged in age from 21 years and above and were categorized into two groups; 55 non consumers of tobacco who constituted the control group and 65 consumers of tobacco who constituted the study (case) group. Were excluded subjects who denied to provide saliva and buccal smears samples after answering questionnaire. Buccal smears were used to assess oral cellular characteristics of premalignant lesions while saliva samples were used to determine the level of MDA in the mouth. Written informed consent was obtained from the patients, after the planned study was explained in detail.

### A. Administration of questionnaire

The questionnaire was structured into demographic data (age, gender, and profession), specific questions on tobacco consumption, method of consumption, and duration of consumption. Participant's medical history and dental history were assessed, where diabetes, hypertension, HIV/AIDS, leukemia, pregnancy, breast feeding, dental caries, gingivitis, periodontal diseases and any other pathology present were checked from hospital books (when applicable) and questions.

The data sheet was structured into three (03) parts including macroscopic analysis, microscopic analysis and MDA determination. Macroscopically, the presence of lesion, location, dimensions, color and aspect of the lesion were determined. Microscopically, the presence of hyperkeratosis, tobacco hyperkeratosis, non-specific inflammatory changes and microflora were assessed. MDA concentration was determined using the thiobarbituric acid method and the optical density was read from a spectrophotometer at 532nm.

### B. Detection of cytological abnormalities

- Buccal smear sample collection and processing

Oral exfoliative cells were collected from the buccal mucosa (covering both cheeks) and any other suspicious areas, using wooden tongue depressors, and the material obtained were directly smeared on clean labelled glass slides and immediately fixed using a cytofixative spray (materials purchased from POLYPHARMA). The fixed slides were taken to the cytopathology laboratory of the university (Cameroon Evangelical University)

At the cytopathology laboratory of the Evangelical University of Cameroon, the slides were stained using the Papanicolaou staining technique [22]. The slides were then observed under the light microscope to look for cellular changes like dysplasia, dyskeratosis, parakeratosis,

hyperkeratosis, hypergranulosis (cytoplasmic granulosis), basal cell hyperplasia, binucleation, perinuclear hallos, nuclear enlargements, as well as the presence of oral microflora and inflammation. A normal buccal smear was defined as on which superficial and intermediate cells are well represented, with a clear background (i.e. absence of microorganisms, inflammatory cells, debris...). Parakeratotic cells where keratinized small superficial squamous cells with persistent picknotic nuclei. Shapes vary from round or oval to polygonal or spindle shaped. Cytoplasmic staining usually is dark or light eosinophilic, rarely cyanophilic. They can be isolated or in plaques or sheets. It shows anucleated single cells with cells with non-refractive pink yellow, or orange cytoplasm and plaques of anucleate cells. On this image is also found epithelial cells with granules in their cytoplasm, similar to cells of the granulated layer of an epidermis.

- Detection of oxidative stress levels

To minimize diurnal variations, saliva samples were collected in the morning (from 08am to 10am). Unstimulated saliva pooled in the floor of the mouths was collected by asking the patient to spit in a sterile labelled glass tube. These samples were stored at low temperature in glass flask and immediately transported to the laboratory for analysis. The concentration of MDA was determined using the Thiobarbituric acid method [21]. In glass tubes, 0.3ml of collected saliva was mixed with 3ml of 0.025 M Tris-HCL and 0.175 M KCL buffer at a pH of 7.4. The mixtures were centrifuged at 4000 g for 10 minutes and both the precipitates and supernatants were stored in the school laboratory freezer.

- Saliva sample processing

The determination of MDA was done as follows; in dry glass tubes, five hundred microliters (500 µl) of 1% orthophosphoric acid and 500 µl of precipitation mixture (1% thiobarbituric acid in 1% acetic acid) were added to 100 µl of homogenate (sample + Tris HCL-KCL buffer). The mixture of each tube was homogenized by vortexing and passed on a boiling water bath at 95°C - 100°C for 15 minutes. The tubes were then cooled in an ice bath and the mixture was centrifuged at 3500 g for 10 minutes. The absorbance of the supernatants was read at 532 nm against the control. Lipid peroxidation was calculated on the basis of the MDA molar extinction coefficient and expressed in micromoles of MDA per milliliter of sample using the Lambert-Beer formula where  $DO = \epsilon LC$ .

### C. Statistical analysis

Data was statistically analysed using SPSS. MDA raw data were subjected to the one-way ANOVA analysis of variance and to using the Waller-Duncan test at 0.05 Confidence Interval (CI). Demographic data, prevalence, duration and quantity of tobacco consumption and oral epithelial changes were described in terms of percentages and were subjected to the Chi Square statistical test at 0.05 CI. The Pearson correlation was used to research the degree of links between the various quantitative parameters at the threshold of 0.05 or 0.01.

## III. RESULTS AND DISCUSSION

### A. General characteristics of the study population

The study population consisted of 120 subjects including 91 (54.2%) of men and 55 (45.8%) of women (Table 1). The most represented age group was that of [21-31] years consisting of 36 (30 %) people including 29 (24.1%) men for 7 (5.8%) women. The least represented age groups were [61-71] and > 71 years old, with 5 (4.2%) and 1 (0.8%) respectively. In terms of medical history, the majority of participants (84 (70%)) declared that they had no known health problems, with up to 66 (55%) men for only 18 (15%) of women. However, 18 (15%) of participants had gastritis including. Only 10 (8.3%) participants were hypertensive, equally distributed between men and women. Regarding dental history, 59 (49.2%) of the participants had no known problems while gingivitis and periodontitis (20; 16.7%), plaque and calculus (18; 15%) and dental caries (14; 11.7 %) were the most common diseases in the study population.

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY POPULATION

Characteristics	Male n(%)	Female n(%)	Numbers n(%)
<b>Age ranges (years)</b>			
[21-31]	29(24,1)	7(5,8)	36(30)
[31-41]	18(15,0)	5(4,2)	23(19,2)
[41-51]	19(15,8)	6(5,0)	25(20,8)
[51-61]	21(17,5)	9(7,5)	30(25)
[61-71]	4(3,3)	1(0,8)	5(4,2)
≥71	0(0)	1(0,8)	1(0,8)
<b>Total</b>	91(75,8)	29(24,2)	120(100)
<b>Medical history</b>			
Healthy (No known health problems)	66(55.0)	18(15.0)	84(70)
Hypertension	5(4.2)	5(4.2)	10(8.3)
HIV/AIDS	0(0.0)	1(0.8)	01(0.8)
On medications	1(0.8)	2(1.6)	03(2.5)
Pregnancy	-	1(0.8)	01(0.8)
Gastritis	17(14.2)	1(0.8)	18(15.0)
Diabetes and hypertension	2(1.6)	1(0.8)	03(2.5)
<b>Dental history</b>			
Healthy oral cavity (No known oral health problems)	45(37.5)	14(11.7)	59(49.2)
Dental caries	12(10.0)	2(1.7)	14(11.7)
Gingivitis and periodontitis	14(11.7)	6(5.0)	20(16.7)
Plaque and calculus	14(11.7)	4(3.3)	18(15.0)
Dental caries, gingivitis and periodontal diseases	5(4.2)	3(3.3)	08(6.7a)
Apical abscess	1(0.8)	0(0.0)	01(0.8)

### B. Distribution of tobacco consumption according to population characteristics

Tobacco use was higher among men (48.3%) than women (5.8%). This consumption was unevenly distributed across the different age ranges. Thus, in men, tobacco consumers were more recruited in the age groups of [21]-[31], [41]-[51] and [51]-[61] years, while in women they were mostly in the age groups [41]-[51] and [51]-[61]. The difference of tobacco consumption according to age range in each sex was not

significant while the tobacco use was significantly higher in men compared to women ( $p=0.0001$ ).

Among tobacco users, four methods of consumption were identified: smoking, inhaling, chewing and application on the teeth (Table 2). The last two methods were combined to smoking only in men: smoke + inhale (9.2%) and smoke + chew (6.1%). Tobacco smokers (76.9%) were most represented in the study population, with 73% of men and only 3.1% of women. Smokers were significantly more represented ( $p=0.001$  in male population. These smokers are mainly recruited in the age group of [21]-[31] and [61]. Very

few participants admitted to applying tobacco on their teeth (7.7% of women against 3.1% of men). The vast majority of smokers (66%) have been smokers for more than 10 years and are recruited in the age groups of [41-51] and [51-61] which represent respectively 24% and 26% of smokers (Table 3). The number of cigarette sticks consumed per day varies with the age of smokers. The biggest consumers are recruited in the age groups [31]-[41], [41]-[51] and [51]-[61] and the people who smoke more than 10 sticks per day constitute 50% of the smoker population (Table 4).

TABLE 2. DISTRIBUTION OF TOBACCO CONSUMERS ACCORDING TO THE METHOD OF CONSUMPTION WITH RESPECT TO AGE AND GENDER

Sex	Method	Age range						P-value
		[21-31]	[31-41]	[41-51]	[51-61]	[61-71]	$\geq 71$	
male	smoke	13(20.0)	7(10.8)	13(20.0)	12(18.5)	3(4.6)	/	0.0001
	applied on tooth	2(3.1)	/	/	/	/	/	
	Smoke + crew	1(1.5)	1(1.5)	/	/	/	/	
	Smoke + inhale	2(3.1)	/	/	4(6.2)	/	/	
	Total	18(27.7)	8(12.3)	13(20.0)	16(24.6)	3(4.6)	/	
female	smoke	/	/	0	1(1.5)	/	1(1.5)	0.052
	applied on tooth	/	/	2(3.1)	3(4.6)	/	/	
	Total	/	/	2(3.1)	4(6.2)	/	1(1.5)	
Total	smoke	13(20.0)	7(10.8)	13	13(20.0)	3(4.6)	1(1.5)	0.0001
	applied on tooth	2(3.1)	/	2(3.1)	3(4.6)	/	/	
	Smoke + crew	1(1.5)	1(1.5)	/	/	/	/	
	Smoke + inhale	2(3.1)	/	/	4(6.2)	/	/	
	Total	18(27.7)	8(12.3)	15(23.1)	20(30.8)	3(4.6)	1(1.5)	

TABLE 3. DISTRIBUTION OF TOBACCO SMOKERS ACCORDING TO THE DURATION OF SMOKING WITH RESPECT TO AGE.

Age range	<1 Year	1-5 Years	5-10 years	>10 years	Total
[21-31]	2(4)	9(18%)	2(4%)	1(2%)	14(28%)
[31-41]	2(4%)	1(2%)	5(10%)	8(16%)	
[41-51]		1(2%)	11(22%)	12(24%)	
[51-61]			13(26%)	13(26%)	
[61-71]			2(4%)	2(4%)	
$\geq 76$			1(2%)	1(2%)	
Total	2(4%)	12(24%)	3(6%)	33(66%)	50(100%)

TABLE 4. DISTRIBUTION OF TOBACCO SMOKERS ACCORDING TO THE NUMBER OF STICKS THEY SMOKE PER DAY WITH RESPECT TO AGE

Age range	2-5 sticks	5-10 sticks	More than 10 sticks	Not determine	Total
[21-31]	1(2%)	6(12%)	1(2%)	12(24%)	9(18%)
[31-41]	2(4%)	6(12%)	6(12%)	8(16%)	
[41-51]	1(2%)	3(6%)	6(12%)	2(4%)	12(24%)
[51-61]	4(8%)	1(2%)	9(18%)	3(6%)	17(34%)
[61-71]			3(6%)		3(6%)
$\geq 76$				1(2%)	1(2%)
Total	8(16%)	10(20%)	25(50%)	7(14%)	50(100%)

### B. Mouth cytology of tobacco consumers and non-consumers

Characteristics of cellular abnormalities found on a hyperkeratotic buccal smear of individuals in the study population are represented on the figure 1. The anomalies observed on the buccal smears include parakeratotic lesions with pycnotic nuclei (Fig 1.b), abnormal keratinization of epithelial cells with or without sparingly nuclei (Fig 1.c), cells with multiple granulation (Fig 1.d), intermediate cells with

active binuclearisation but a normal-sized nucleus (Fig. 1.e), epithelial cells presenting a small clear halo around the nucleus (fig.1.f), epithelial cell whose morphology suggests a tadpole appearance, in particular with a fairly apparent cytoplasmic extension, the presence' a population of filamentous elements reflecting the presence of candida species (Fig. 1.h).

The distribution of oral epithelial atypia found on tobacco hyperkeratotic buccal smears of tobacco consumers in the study population is represented in the Figure 3. From this figure, hyperorthokeratosis + hyperparakeratosis were the changes mostly seen on tobacco hyperkeratotic buccal smears (59%), followed by hyperorthokeratosis + hyperparakeratosis + cytoplasmic granulositis (27%) and hyperorthokeratosis + cytoplasmic granulositis were the least seen (14%). The tobacco consumption was significantly correlated to the presence of oral epithelial cellular changes ( $p<0.001$ ).



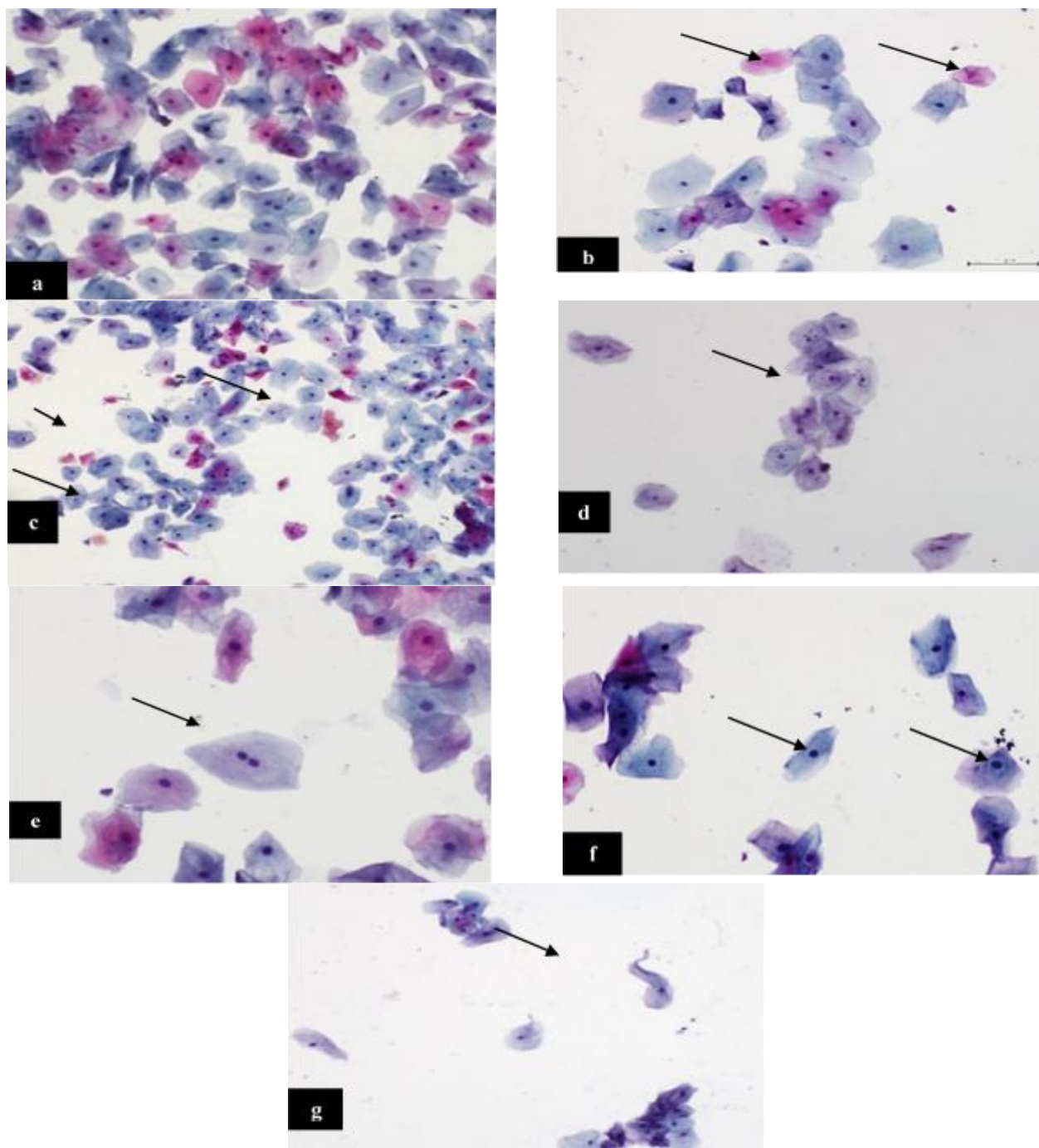


Figure 1: Cellular characteristics seen on buccal smears in the study population.

**a** shows cellular aspects related with a normal buccal smear. We can see a desquamation of superficial and intermediate cells strata (Pap X10). In **b**, the pycnotic aspect of the nucleus (arrow) in a cell with an orangeophilic cytoplasm coincides with parakeratotic lesions (Pap X40). In **c**, epithelial cells sometimes isolated, sometimes tattered, with cytoplasm expressing an abnormal keratinization, but with erased nuclei, are sparingly noted. These aspects coincide with benign hyperkeratosis (Pap X10). Image **d** shows a closet of oral cells with multiple cytoplasmic granulations. In **e** we can easily see an intermediate cell with reactive binucleation. However, nucleus retain a normal shape and size (Pap X40). Image **f** shows epithelial cells marked with a small clear halo around the nucleus (Pap X10). Image **g** shows an epithelial cell whose morphology suggests a tadpole appearance, in particular with a fairly apparent cytoplasmic extension (Pap X40). Finally, in **h**, the inflammatory context marked by the presence of polynuclear cells is justified by the presence of a filamentous population of elements of the oral microflora.

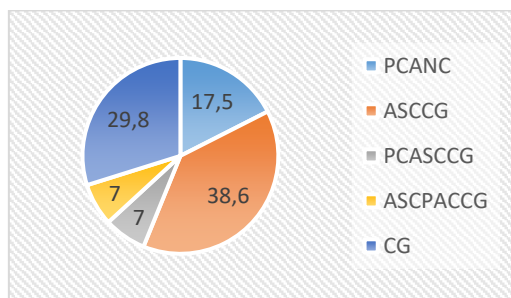


Fig. 2: Distribution of oral epithelial atypia found on hyperkeratotic buccal smears of individuals in the study population.

PCANC : parakeratotic cells and anucleate single cells, ASCCG : anucleate single cells and cytoplasmic granulositis, PCASCCG : parakeratotic cells, anucleate single cells and cytoplasmic granulositis, ASCPACCG : anucleate single cells, Plaques of anucleate cells and cytoplasmic granulositis, CG : cytoplasmic granulositis

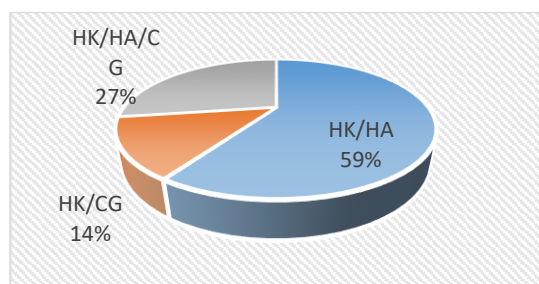


Fig. 3: Distribution of oral epithelial atypia found on tobacco hyperkeratotic buccal smears of tobacco consumers in the study population.

#### C. MDA concentrations in tobacco consumers and non-consumers

The average MDA ( $0.28 \pm 0.163$ ) in the mouth of tobacco users with Tobacco hyperkeratosis was significantly higher ( $p < 0.001$ ) than that of those who did not have this abnormality. Overall, levels of MDA in the mouth of non-tobacco users was significantly lower than among consumers. The majority of non-tobacco users had low MDA levels (between 0.010 and 0.25  $\mu\text{M/ml}$ ). On the other hand, even if the majority of smokers also presented values of MDA contained in this range, it is in this group that high rates of MDA ( $> 0.25$ ) were observed, with a cumulative percentage of 16.7%.

The average MDA in the mouth of tobacco consumers with hyperkeratosis was significantly higher than in those who did not have thus abnormality. Overall, the level of MDA in the mouths of our tobacco consumers was significantly lower than in consumers.

TABLE 5. INFLUENCE OF TOBACCO CONSUMPTION ON SALIVA MDA LEVEL IN PARTICIPANTS TO THE STUDY

	Consumers		Non-consumers	
	N	[MDA] $\pm$ SD ( $\mu\text{M/ml}$ )	N	[MDA] $\pm$ SD ( $\mu\text{M/ml}$ )
Oral epithelial cellular changes				
Normal	10	$0.13 \pm 0.110^a$	19	$0.10 \pm 0.066^*$
Hyperkeratosis	21	$0.16 \pm 0.101^a$	26	$0.09 \pm 0.069^*$
Tobacco hyperkeratosis	25	<b><math>0.28 \pm 0.163^b</math></b>	0	/
Non-specific inflammatory changes	6	$0.19 \pm 0.153$	5	$0.10 \pm 0.086^*$
Oral microflora	3	$0.16 \pm 0.103^a$	5	$0.08 \pm 0.047^*$

<sup>a,b</sup>: in columns, means carrying the same subscript letter are not significantly different ( $p > 0.05$ , Waller-Duncan test).

\*: significantly lower than tobacco consumers.

#### IV. DISCUSSION

Tobacco, because of the nicotine it contains, is a drug. Nicotine addiction is as powerful as that caused by cocaine and heroin due to the combination of physical and psychological dependence. Whether the situation is pleasant or unpleasant, smoking makes the user feel good. This phenomenon pushes consumer to continue using tobacco to feel the same well-being. As smoking declines in developed countries, its prevalence increases in developing countries. This is the case in Cameroon, where the smoking prevalence rate is around 17.5% including 28.8% men and 8.1% women [23]. In this study, the prevalence of tobacco use was very high (54.1%) and this prevalence was higher in men 48.3% than in women (5.8%), contrasting with others published results in Cameroon. For instance, Pefura et al. [24], got a prevalence of 8.4% in adult peoples in Yaoundé. Working conditions in the travelling agencies in Bafoussam is very difficult and may explain this high rate of tobacco consumption by the employers. According to a Dares analysis, the work environment sometimes encourages employees to light a cigarette more easily. Physical constraints, heavy loads, exposure to noise, cold, heat, humidity, dirt, fear of losing one's job are aggravating factors of smoking [24]. All these factors are encountered in the visited travelling agencies in Bafoussam.

The trend of tobacco consumption in males based on their ages was; the highest (31.0%) level of consumption was among younger adults (aged between 21-31 years), higher (27.6%) among males aged between 54-64 years, high (22.4%) among male aged 43-53 years and lowest among older adults (aged between 65-75 and 76-86 years). It shows that tobacco consumption is fast increasing among young adults. This is in contrast with Fred Pampel's study [25] on tobacco use in sub-Sahara Africa among men aged 15-54 years and women aged 15-49 years in 14 nations, where he found that, male cigarette smokers are more likely to be older. Inhaling and chewing tobacco were methods least used by the study population. These results coincide with previous studies [26] where they found that the predominant use of tobacco is by smoke inhalation of cigarettes, pipes, and cigars.

The present study showed a statistical significance between tobacco consumption and oral epithelial abnormalities ( $p < 0.001$ ). In line with the study carried out by Seifi et al. [11], Shetty et al. [12] and Divyasri et al. [13], increase in keratinized cells (tobacco hyperkeratosis) was present only on buccal smears of tobacco consumers (cases). Hussain et al. [14] had similar findings, they found that smokers with clinically normal mucosa show a greater percentage of keratinized cells and a greater nucleolar activity, suggesting that cigarette smoking influences the cellular activity of the mucosa. The increase in the size of the nucleus as observed in some participant to his study may be related to the increased amount of DNA required for cell replication. The reduction in cytoplasmic in smokers can be attributed to dehydration which is a kind of cell adaptation in

response to the decrease in fluids, especially saliva around the cell [11].

It was noticed an increased number of hyperkeratosis (characterized by parakeratosis, cytoplasmic granulosis, anucleated single cells) in the control group (non-consumers). This important increase may be due to systemic diseases, age related cellular modifications [15] or the effect of other agents or substances on buccal squamous cells like alcohol, hot meals, peppers [4] which could lead to keratinization of cells. The presence of keratinization supports the fact that exposure to alcohol and other agents affects the pattern of maturation in oral mucosal cells [16].

Non-specific inflammatory changes with increased inflammatory cells, which infiltrated were observed in both groups in this study; this increase might be a result of epithelial irritation caused by smoking and other irritating substances. Such a finding was previously reported [17]. Cigarette smoke has effects on saliva, oral commensal bacteria, and fungi, mainly *Candida*, which causes oral candidiasis, the most common opportunistic fungal infection in man [18] and how cigarette smoke affects oral *Candida* is still controversial.

Saliva being the first biological fluid met by external substances ingested as food, drinks, inhaled volatile cigarette smoke, microorganisms, it represents the first line of defense against oxidative stress [27]. Oxidative stress represents the imbalance between the production of highly reactive molecular species (ROS, reactive nitrogen species) and antioxidant defense systems [28]. In the present work, the concentration of MDA in saliva was significantly higher in smokers compared to non-smokers, like the results reported by [19] who showed that oxidative stress is increasing in the smoker. This elevation can be explained by the increase in the number of inflammatory cells in the oral cavity that releases large quantities of anion superoxide and hydrogen peroxide by various mechanisms. The present study shows a significantly higher mean MDA concentration in tobacco consumers compared to non-consumer, and we notice an increased mean MDA concentration in individuals with tobacco hyperkeratotic changes on their buccal smears. These results are similar to those of Rashmi and Saumya [29]. Increases in MDA levels (in saliva and serum) are not only due to tobacco consumption, but also as a consequence of the magnitude of oxidative stress, supporting the hypothesis that cancer cells have markedly altered ROS metabolism, leading to production of large amounts of ROS as compared to non-neoplastic cells, and the suppression of the antioxidant system that mediate body's defense mechanisms [30]. Tobacco use is one of the important causes of death and a leading public health problem worldwide [8]. Tobacco use is considered one of the risk factors for oral cancer.

## V. CONCLUSION

Based on the results of the present study which had as general objective to determine the effect of tobacco on oral epithelial cells and on the oxidant/antioxidant balance in the oral cavities of participants in the study population, we can conclude that tobacco consumption severely increases the risk for developing oral mucosal proliferative lesions, and the level of oxidative stress in the saliva of individuals in the study population.

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