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Cytomorphological Changes in Oral Mucosa as a Result of Cigarette Smoking in Wad Medani City, Gezira State, Sudan (2021)

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Abstract

Background: Smoking is a common practice and damages almost all organs and systems of the body. Oral cavity is rich of flora and contains many microorganisms that cause local and systemic diseases if microbiological flora is altered. Cigarette smoke renders oral mucosa epithelium to be susceptible for colonization of pathogens. These pathogens can cause or contribute formation of systemic diseases.

Objectives: This study aimed to assess the cytomorphological changes in oral mucosa Duo to cigarettes smoking using Pap stain and H&E stain, to find out the better staining results of the two stains, and to assess the effect of age, duration and frequency of smoking on the oral mucosa.

Materials and Methods: This study was cross-sectional study to assess the cytological changes in oral mucosa of cigarettes smokers using Pap stain in Wad Medani City, Gezira State, sudan (2021).The study included 100 cigarette smokers' samples. Samples tacked by plastic stick were spread on a slide and immediately fixed with fixative spray to avoid exposure to dry air. In the pathology laboratory, the samples were stained with Papanicolaou and Hematotoxlin and Eosin. Each specimen had two slides, which was reviewed by a cytopathologist according to criteria of benign and malignant.

Results: The mean age was 24 years. There was insignificant relation between the age of smokers and the cytological diagnosis of samples (P.Value 0.59), but most of normal cases in less than 24 years and most of a typical cases in more than 24years.There was insignificant relation between the duration of smoking and the cytological diagnosis of samples (P.Value 0.534), but most of a typical cases in more than 2years of smoking.

There was insignificant relation between the frequency of smoking/day and the cytological diagnosis of samples (P.Value 0.190).

Conclusion:The mean age was 24 years.Cytomorphological changes in oral mucosa associated with presence of a typical mucosal cell in the age more than 24years old, and in smoking duration more than two years.There was insignificant relation between the frequency of smoking/day and presence of cytological changes in the samples.PAP stain was more sensitive and specific than H&E stain in the diagnosis ofearly malignant oral lesions.

Key words: Cytomorphological Changes in Oral Mucosa; Cigarettes Smoking;PAP; H&E;Sudan

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Introduction:

In recent years cytology has played important role in the diagnosis of various diseases; specially those of neoplastic origin. Cytology is some time use to differentiate between a benign / reactive process and neoplastic or preneoplastic condition. Reagent and automation are now available, but the technical problems no longer present a major concern in this field (1). Oral cancer is a general designation of all malignant tumors appearing in the mouth. In 2018, the number of new oral cancer cases was approximately 354,000, accounting for 2% of the total number of 36 cancer cases. Oral

squamous cell carcinoma (OSCC) cases constitute 90% of all oral carcinoma cases (2). In 2016, a total of 48330 oral cavity and oropharyngeal cancer incidents were reported, and an increase of 22.5% of human papillomavirus-related oropharynx cancer was recorded (3). The oral cavity and oropharynx considered the upper part of the digestive system. These two regions are differentiated from each other by their pathologic processes, prognosis, and histological grades (4). The oral cavity is the first part of the digestive system where the food is broken into small pieces by teeth, moistened and lubricated by saliva. The oral cavity consists of two parts, namely; the vestibule and the oral cavity proper. The oral cavity is lined by moist oral mucous membrane or oral mucosa which is continuous with the dry skin at the mucocutaneous junction of the lip (5). The oral **mucosa** has several functions. Its main purpose is to act as a barrier; It protects the deeper tissues such as fat, muscle, nerve and blood supplies from mechanical insults, such as trauma during chewing, and also prevents the entry of bacteria and some toxic substances into the body (6). The oral mucosa has an extensive innervation of nerves; which allows the mouth to be very receptive of hot and cold, as well as touch. Taste buds are also located in oral mucosa and are important for recognition of taste (7). The buccal mucosal membrane secretes moisturizing and lubricating fluids for the mouth and upper throat. These fluids are necessary to prevent drying effects, since this mucosa is part of the membrane system that lines the entire gastrointestinal tract, and this is open to exterior surfaces at both ends (8). Nicotine in cigar smoke (pH 8.5) is yields in small cigars averaged 1.24 and 3.49 mg/unit on ISO and CI regimens, respectively, compared with 0.73 and 2.35 mg/unit, respectively, for the research cigarettes. Nicotine yields per puff were similar between small cigars and cigarettes. We also found that FC (Filtered cigarettes) did not differ from LC (Little cigarettes) in nicotine yields. FC and LC differ from each other in many physical design features (unit weight, filter weight, and filter length), but are similar in others (unit length, diameter, and filter ventilation) (9).Oral mucosa is the first part that affected by smoking. The early significant response to cigarette smoke came from the basal and para basal layers of the oral epithelium, which appear as three-dimensional arrangement of the oral mucosa mimicking the inhalation/exhalation cycle during the exposure to cigarette smoke (10). Smoking can be associated with the decreasing gingival blood flow and epithelial changes. During the oral exfoliative cytology the presence of two or more features consistent with a typical change in cells which is a sign of malignancy. This study aimed to assess the cytomorphological changes in oral mucosa duo to cigarettes smoking using Pap stain and H&E, to find out the better staining results of the two stains, and toassess the effect of age, duration and frequency of smoking on the oral mucosa.

Materials & Methods:

The study design: This study was cross-sectional study to assess the cytological changes in oral

mucosa of cigarettes smokers using Pap stain in Wad Medani City, Gezira State, sudan (2021).

The study area: This study was conducted in Wad Medani City and surround villages, Gezira state, Sudan. Wad Medani the capital of Gezira state which lies in the western bank of the Blue Nile, Central Sudan. The study included 100 cigarette smokers' samples.

The inclusion and exclusion criteria: The study included all patients smoke cigarette, but excluded patients with a history of radiotherapy or chemotherapy for oral or other malignancy, patients with history of alcohol consumption and tobacco users, patients with a history of systemic diseases, and patients with a history benign or malignant oral lesions.

The informed consent: The specimens and information were collected from the individuals under privacy and confidentiality and was not be used for any purposes rather than this study**.** Ethical committee approval has also been obtained from Medical Laboratory Sciences.

Collection and preparation: Participants were asked to rinse their mouths with normal saline before samples were taken to eliminate debris and excess saliva from the oral mucosa. Samples tacked by plastic stick were spread on a slide and immediately fixed with fixation spray to avoid exposure to dry air. In the pathology laboratory, the samples were stained with Papanicolaou and Hematotoxlin and Eosin. Each specimen had two slides, which was reviewed by a cytopathologist according to criteria of benign and malignant. Atypia was assessed cytologically by using the presence of two or more of the following features which were consistent with atypia: nuclear enlargement associated with increased nuclear: cytoplasmic ratio, hyperchromatism, chromatin clumping with moderately prominent nucleoli, irregular nuclear membranes and bi- or multi-nucleation, scant cytoplasm, and variation in size and/or shape of the cells and nuclei (11). The results for each stain compared with positive and negative control samples. Also assessment of other factors such as the most affected age group, duration and frequency of smoking.

Protocol of Papanicolaou stain: Ethyl alcohol fixed smear are hydrated in 95% alcohol for 2 min,through 70% alcohol for 2 min, rinse in water for 1 min, **s**tained in harries Hematoxylin for 5 min, rinsed in water for 2 min, differentiated in 0.5% aqueous hydrochloric acid for 10 seconds, rinsed in water for 2 min, blued in Scott's tap water substitute for 2 min, rinsed in water for 2 min, dehydrated in 70% alcohol for 2 min, dehydrated in 95% alcohol for 2 min, dehydrated in 95% alcohol for 2 min, stained in OG6 for 2 min, rinsed in 2 changed 95% alcohol for 2 min in each, stained in EA50 for 3 min, dehydrated in 95% alcohol for 1 min, through absolute alcohol, cleared in xylene and mounted in DPX (12)**.**

Protocol of Hematoxylin and Eosin: The smear was hydrated in 95% alcohol for 2 minutes and was hydrated in 70% alcohol for 2 minutes, then the smear was rinsed in water in for 1 minute and stained with Harris's Hematoxylin for 5 minutes and was rinsed in 1% acid alcohol for few second and blued by tap water for 5 minutes was stained with eosin for 3 minutes the smear was dehydrated through 70% alcohol for 2 minutes ,95%alcoho for

2 minutes then was dried and cleared by xylene and was mounted by DPX media (13).

Statistical analysis: The data was done by SPSS program. P value less than or equal 0.05 was consider statistically significant(*Chi test* was used for analysis).

Results:

The mean age was 24 years (table 1). The duration of smoking of most of the study population was more than 2 years in 52 sample (56.5%) and in duration less than 2 years was 40 samples (43.5%) (table 2). Most of study population frequency of smoking was only one time per day in 52 samples (57.1%), followed by two time per day 21 samples (23.1%), and finally three time per day in 17 samples (18.7%)%) (table 3). Most of samples were normal with H&E stain (91samples) and only one sample was Atypical, in PAP stain the normal samples were 78 , 3 inflammatory samples and 11 Atypical (P.Value 0.7)(table 4). The sensitivity of H&E samples was 100 % and specificity 1.4%. The sensitivity of PAP stain 100% and specificity 100% (Figure1). There was insignificant relation between the age of smokers and the cytological diagnosis of samples (P.Value 0.59), but most of normal cases in less than 24 years and most of a typical cases in more than 24years) (table 5).There was insignificant relation between the duration of smoking and the cytological diagnosis of samples (P.Value 0.53), but most of a typical cases in more than 2years of smoking(table 6). There was insignificant relation between the frequency of smoking/day and the cytological diagnosis of samples (P.Value 0.190) (table7). *Chi test* was used for analysis.

Table 1: age of study population

Age	Frequency	Percent
<24 years	50	54.3
>24 years	42	45.7
Total	92	100.0

Table 2: Duration of smoking of study population

Duration	Frequency	Percent
1-2	40	43.5
>2	52	56.5
Total	92	100.0

Table 3: The frequency of smoking of study population

No	Frequency	Percent
1	53	57.6
2	21	22.8
3	18	19.6
Total	92	100.0

Table 4: The cytological diagnosis of H&E stain and Pap stain

Stain	Normal	Inflammation	Cytological a typia	P.Value
H & E	91	-	1	0.7
Pap	78	3	11	

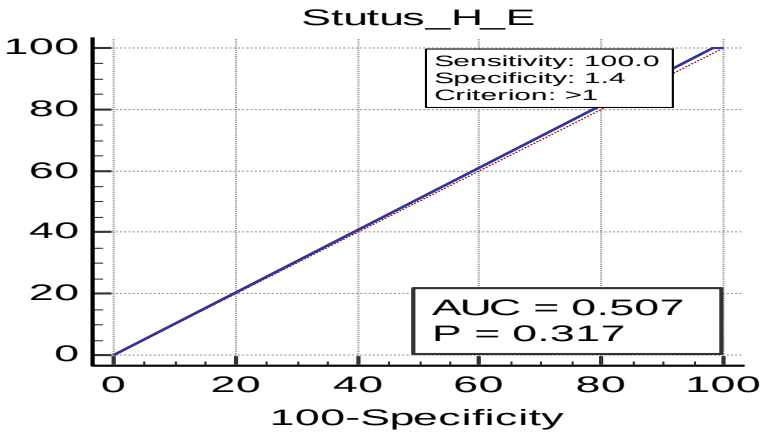

Figure 1: The sensitivity and specify of PAP stain and H&E stain

Table 5: comparison between the age of the smokers and cytological diagnosis of the study population

Age	<24 years	>24 years	Total	P.Value
normal	44	34	78	0.59
Inflammation	2	1	3	
Cytological a typia	4	7	11	
Total	50	42	92	

Table 6: comparison between duration of smoking and cytological diagnosis of the study population

Cytology diagnosis	1-2	>2	Total	P.Value
normal	34	44	78	0.249
Inflammation	2	1	3	
Cytological a typia	3	8	11	

Table 7: comparison between frequency of smoking/day and cytological diagnosis of the study population

Cytology diagnosis	1/day	2/day	3/day	Total	P.Value
normal	45	19	14	78	0.190
Inflammation	1	0	2	3	
Cytological a typia	7	2	2	11	
Total	53	21	18	92	

Discussion:

This study was cross-sectional study to assess the Cytological Changes in Oral Mucosa of Cigarettes Smokers using Pap stain in Wad Medani City, Gezira State, sudan (2021).

The mean age was 24 years (table 1). This result agree with Wen-Jiun Lin, *et al*,(2011), they investigated the association between smoking, alcoholic consumption, and betel quid chewing with oral cancer in a prospective manner. All male patients' age ≥18 years were included. They found that habitual cigarette smokers, alcohol consumers, and betel quid chewers have a higher risk of contracting oral cancer and should receive oral screening regularly to be detected as early as possible (14). The report from the Institute of Medicine (2007) published that tobacco kills more Americans yearly than other hazards**,** many studies detect the relation between the use of tobacco and the development of oral cancer, like a study done at the University of California and San Francisco, found that more than eight out of ten oral cancer patients were smokers (15). When we compare the age of smokers with the cytological diagnosis of samples, we found that there was insignificant relation between the age of smokers and the cytological diagnosis of samples (P.Value 0.59), but most of normal cases in less than 24 years and most of a typical cases in more than 24years) (table 5). Our results agree with Suhail,*et al*, (2014), they diagnosed the association between water-pipe smoking and the age of patients when diagnosed with oral cancer, they concluded that water-pipe smoking is a risk factor associated with the appearance of oral cancer at a younger age (16). Beth Israel Deaconess Center (2019), published According to the Mouth Cancer Foundation, approximately 90% of people with oral cancer are tobacco users, and smokers are six times more likely than non-smokers to develop oral cancer. Mouth cancer affects more men than women. A typical person at high risk for mouth cancer is male, more than the age 40, who uses alcohol and tobacco (17).

The duration of smoking of most of the study population was more than 2 years in 52 samples (56.5%) and in duration less than 2 years was 40 samples (43.5%) (table2). When we compared the duration of smoking with the cytological diagnosis of samplesThere was insignificant relation between the duration of smoking and the cytological diagnosis of samples (P.Value 0.534), but most of a typical cases in more than 2years of smoking (table 6). These findings agree with Ahmed HG and Babiker AA (2009), they established that, chemical carcinogenesis is a prolonged process and progressed with increasing of exposure (18).

Most of study population frequency of smoking was only one time per day in 52 samples (57.1%), followed by two time per day21 samples (23.1%), and finally three time per day in17 samples (18.7%) (table3). When we compare the frequency of smoking/day with the cytological diagnosis of samples, we found there was insignificant relation between the frequency of smoking/day and the cytological diagnosis of samples (P.Value 0.190) (table7). Our results agree with Brian L (2004), They demonstrated that approximately one third of patients with oral squamous cell carcinoma will

report that they have never smoked. There was a strong association between a history of smoking and carcinoma involving the posterolateral tongue and floor of mouth (19). Our results agree with Julien Berthiller (2016), they suggest that low frequency of cigarette use leads to the development of head and neck cancer, also smoking duration play a major role in the development of head and neck cancer (20).

Most of samples were normal with H&E stain (91samples) and only one sample was Atypical, in PAP stain the normal samples were 78 , 3 inflammatory samples and 11 Atypical (P.Value 0.7)(table 4). The sensitivity of H&E samples was 100 % and specificity 1.4%. The The sensitivity of PAP stain 100% and specificity 100% (Figure1). When H&E and PAP Stain compare together we found there was no significant difference between the two stains but in spite of that PAP stain was accurate(Atypical cases 20) in the diagnosis than H&E stain (Atypical cases only one). PAP stain was more sensitive and specific than H&E stain. PAP Stain was better because it contain three stains (harries Hematoxylin, OG6 and eosin) .and it give us more details about the maturity of cell's nucleus and cytoplasm.This study agree with(Shukla, *et al*, 2015), they found increasing in the severity of the lesion (21), Beside that Papanicolaou (PAP) stain was found to be the most suitable stain . The study also agree with Rajput, *et al* (2010) they found that Sensitivity and specificity of PAP analysis in the oral smears for detection of oral cancer and normal cells was 91.176% and 100% (22). Gupta *et al* (2019) they concluded that PAP stain was the most suitable stain for screening of oral cancer and can be used as a prognostic indicator (23). Study not agree with Zafar, *etal*, (2020) they found that H&E stain showed sensitivity 44%, Pap 35%,. NPV–H&E 70%, Pap 66% (24).

Cigarettes, the most common form of tobacco used, causes about 90% of all lung cancers, according to the American Lung Association. Smokers are also at a 10 times higher risk for oral cancer compared to non-smokers. Smoking is linked to increased risk for more than 12 other types of cancer, too. In addition, cigarette smoking is linked to nearly 1 in 5 deaths in the U.S. Cigarettes contain more than 60 known cancer-causing agents (25)

Oral cancer ranks eighth among the most common causes of cancer-related deaths worldwide, and tobacco is one the most important carcinogenic factor (26).

Conclusions

The mean age was 24 years. Cytomorphological changes in oral mucosa associated with presence of a typical mucosal cell in the age more than 24years old, and in smoking duration more than two years. There was insignificant relation between the frequency of smoking/day and presence of cytological changes in the samples.PAP stain was more sensitive and specific than H&E stain in the diagnosis of early malignant oral lesions.

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