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Table of Content

No.	Title	Pages
01	Assessment and measurement of Wild Animal welfare in captivity, case study: KuKu zoo , Khartoum state. Sudan	01-13
02	Adomian Decomposition Method for Application of Viscose Flows in Cylindrical Polar Coordinates	14-24
03	Homotopy Perturbation Method for Application Viscous Flows in Cylindrical Polar Coordinates	25-43
04	Antimicrobial, Antioxidant Activities and total phenolics contents of Cassia grandis., Leaves	44-50
05	Evaluating and Development of hospital performance using the goal programming (Case study of MakNimir University Hospital-Department of surgery) (2024)	51-65
06	The Spectro-Chemical Analysis of Some Sudan Soil	66-79
07	Use of Gel Permeation Chromatography in Studies of *Prosopis Chilensis* Gum	80-85
08	Comparison of Genotoxic effect and cellular changes in oral mucosal cells Between Water Pipe and Cigarette Smoker in Shendi town, Sudan	86-98

Comparison of Genotoxic effect and cellular changes in oral mucosal cells Between Water Pipe and Cigarette Smoker in Shendi town, Sudan

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Abstract

Background: Cigarette and water pipe smoking is one of the most important etiologic factors of oral cancer. Micronucleus (MN) assay is simple biomonitoring test for the biologic effects of smoking on tissues.

Aim: To compare the genotoxic effect and cellular changes in human buccal mucosal cells between waterpipe and cigarette smoker.

Methods: This is a case–control study conducted in shendi town among Seventy five individual (60 smokers and 15 nonsmoker). Cytology smears have been collected by brush from buccal mucosa and then stained by PAP stain and Feulgen technique. Smears were cytologicaly evaluated and micro nuclei formation was assessed.

Results: Abnormal cytomorphological changes atypia were observed among53/60(88%) of smoker versus0/15(0%) of nonsmoker buccal cells. Dysplastic cells observed only among water pipe smoker 4/30 (13%)mean while micronuclei formation was similar 21/30(70%) in cigarette smoker versus 18/30 (60%) among water pipe smoker, Inflammatory cells (66%) versus 70% , bacteria 76% versus 90% respectively. All smokers with smoking habitats more than 5smoke/day shows abnormal oral cytology. in this study increasing of smoker age was associated with abnormal Pap result (P.value 0.00).

Conclusions: Water pipe smoking is harmful as cigarette smoking for oral mucosal cells and increases the risk for oral cancer. Study recommends Cytology oral screening program and gene monitoring for smokers.

***Keywords:** smoker, cigarette, water pipe, cytomorphological changes , cytotgenetic, micronuclei, microbes*

Introduction:

Oral squamous cell carcinoma is the most common type of cancer of oral cavity worldwide.(1) Its affects 274,000 people worldwide annually (2) Oral cavity is one of the easily accessible areas for cytological analysis in the body. Oral cancer may appear as innocuous and asymptomatic lesions in the beginning stages. Symptomatic changes are seen in the advanced stages of tumor and the patients report to the clinician in this stage.(3)

Buccal mucosal cells are stratified squamous epithelium ,the mature cells called superficial cells, they have small, pyknotic nucleus that is 5 to 6 micrometer in diameter, intermediate cells have larger nucleus 8 micrometer in diameter, and not pyknotic nucleus has finely granular texture. Intermediate cells are occasionally bi-nucleated and even multinucleated. Both intermediate and superficial cells are large polygonal cells with rent pink or green cytoplasm. They are the main predominant .The immature squamous cells are called parapasal and basal cells, not usually sampled, they are round or oval rather than polygonal and have variably sized nucleus (4).

Exfoliative cytology of the oral cavity is an easy diagnostic method [5, 6] can be used for early detection of oral potentially malignant and malignant disorders.[6] It's a valuable site for biomarker assay to identify chromosomal aberrations. The most important biomarker used as an indicator of structural and numerical chromosomal aberrations is micronuclei.(5)

Micronuclei are small chromatin bodies in the cytoplasm formed by condensation of acrocentric chromosomal fragments or by whole chromosomes, lagging behind the

cell division and are sensitive indicators of genetic damage. It is the only biomarker that allows simultaneous evaluation of mutagenic and an eugenic effects in multiple cells, easily detected in the interphase.(7) Micronucleus test serve for genomic damage risk assessment and is an important strategy for monitoring pre-neoplastic oral lesions, thereby guiding the therapeutic approach to be adopted.(8)

Tobacco has been considered the greatest disease producing chemical to humans. Usage of tobacco products can be in two forms,
namely, smoking form as well as the smokeless tobacco chewing form.(9) Smoking forms of tobacco include various kinds of cigarettes (manufactured, hand-rolled, filtered, unfiltered, and flavored), cigars, and pipes.(10)

For many centuries, waterpipe is used for tobacco smoking in Asia and Africa. Traditionally, most waterpipe users are concentrated in North Africa and South-east Asia (11). Nowadays, waterpipe smoking is a global problem. Based on published data numbers of waterpipe users are increasing among women and teenagers. Waterpipe smoking delivers high levels of nicotine to mucosal cells of oral cavity and respiratory tract. The inhaled nicotine contains toxic materials comprising carbon monoxide and carcinogenic materials (12). The risk of carcinomatous changes intensifies by waterpipe smoke (13, 14).

This study was conducted among smokers in shendi town to study the cytotoxic effect of smoking on oral mucosal cells using micronuclei estimation cytochemical assay as indicator for DNA destruction.

Methodology:

This study is comparative crossectional analytical study, conducted during period from January to march 2020. Sample were collected from different populations in Shendi town. Sixty male volunteers who smoke cigarette or water pipe were included as case group verus15 nonsmoker of cigarette or water pipe as control group. Smokers used snuff, uptake alcohols and drugs were excluded in this study. Two Buccal cavity smears were collected using tooth brush and then smeared in clean, dry, glass slides and immediately fixed (while the preparation was wet) in 95% ethyl alcohol for15minutes. Then one smear was stained by Harris haematoxlin 5min, while the counter stain will be EA50 2 min and O.G6 2 min. the second smear stained using . Feulgen method as follows: dipping the slides in 1M HCl at 60°C for 8min, rinsing in1M HCL for 3 min, immersing in Schiff's reagent for30 min, treat with tap water 10 min , staining with 1% light green for 3min After staining were applied smears dehydrated cleared and finally mounted. All participants were informed about the objective and the purpose of the research before collection of samples, also the procedure was explained to them, then written agreement was signed of participants. Data collected using Questionnaire and laboratory test results. Data analysis all data have been analyzed by SPSS 16 and result presented in form of tables and figures and picture.

RESULTS:

The study included 75 participants 60 were smoker (30 cigarette ,30 water pipe)and 15 were control group (fig1). Majority of smoker were in ages 15-30 years old 45/60(75%) and Due to the fact that smoking is considered as

social stigma, all of the study subjects in the present study were males.

No cytomorphological changes observed among nonsmoker in buccle mucosal cells while smokers shows abnormal cytomorphology in 53/60 (88%) of cases classification was based on Pap staining result. NO micronuclei formation assessed among non-smoker while micronuclei formation was detected among 65% of smokers. Dysplastic cells were detected among water pipe smoker only 4/30 (13%) , inflammatory cells (68%), bacterial infection (76%) (table2). Cytomorphology of viral infection (hyperkeratosis, bi-multinucleation, cytoplasmic vaculation, koilcytosis) were detected in more than half 56% of cigarette smoker. All smokers with ages above 30 years old shows abnormal cytomorphology while 84% of 15- 30 years old shows abnormal morphology. Only age factor was statistically significant when associated with abnormal findings. None of number of smokers per day or period of smoking was having a correlation to abnormal morphology.

Figure 1 shows study group distribution:

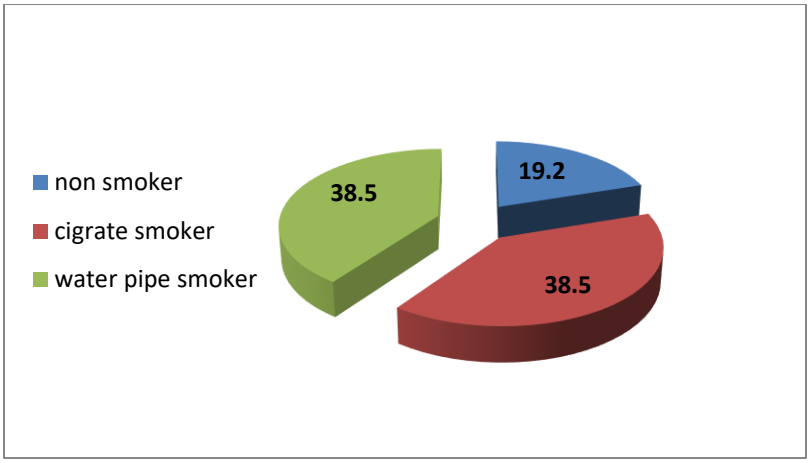


Table 1 : Shaw the correlation between smoking and buccal smear cellular morphology:

	Cytomorphological changes pap stain		
	Abnormal	normal	
non smoker	0	15	15
Cigarette smoker	25	5	30
water pipe smoker	28	2	30
Total	53	22	75

P value: 0.00

Figure 2 Shaw the correlation between smoking and buccal smear cellular morphology:

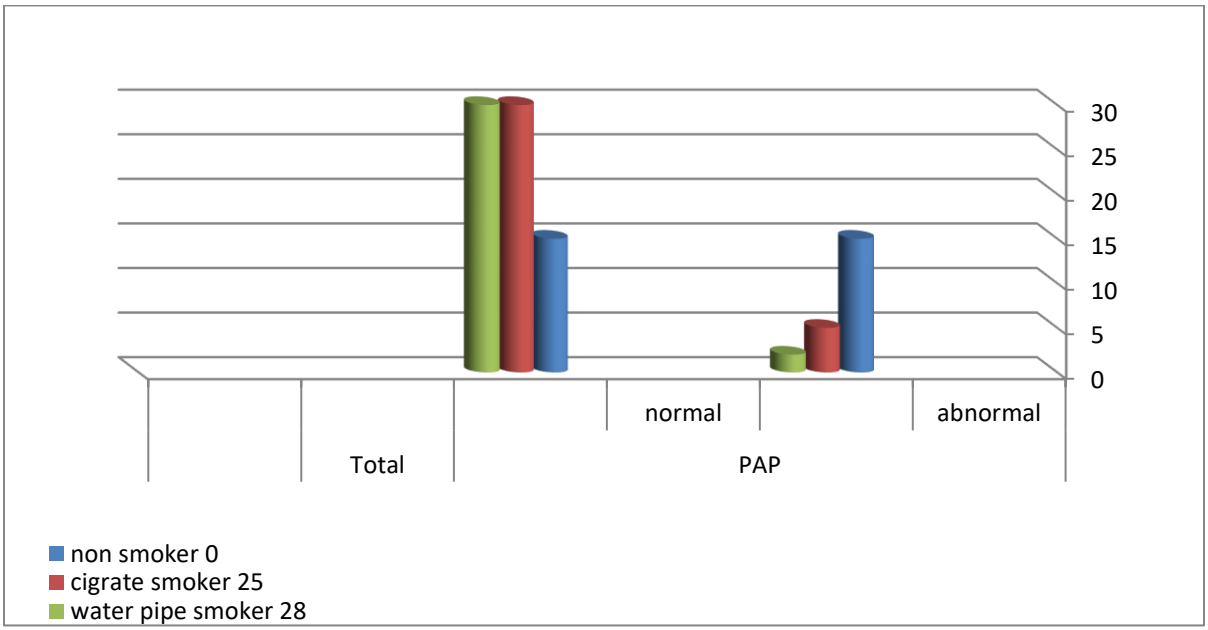


Table 2:cytomorphological finding in buccal smear of smokers:

Cytological criteria	Cigarette smoker	Water pipe smoker	All smoker
Nuclear atypia	83%	93%	88%
Dysplastic cell	0%	13%	0.07%
Micronuclei formation	70%	60%	65%
Inflammatory cells	66%	70%	68%
Bacteria	63%	90%	76%

Table 3 : Show factors increasing risk of smoking according to cytology classification:

Factor	P.value
Increase of age	0.00
smoking for more than 5 years	0.07
Smoking habitat	0.12

Discussion:

The present study tested the destructive effect of smoking on oral cells which might consequently progress into oral precancerous and cancerous lesion among smokers.

Some of the benign changes in the squamous cells of the buccal mucosa are hyperkeratosis (a nucleated cells) and parakeratosis (orangeophilic cytoplasm and nuclei). When this characters shows nuclear atypia in the form of enlargement and membrane irregularity with hyperchromasia, they are called “dyskeratocytes” or” atypical parakeratosis” and should be categorized as an epithelial cell abnormality. Other benign condition, small nonspecific halos, they are seen in association with infections. Classic koilocyte (humanpapilloma virus infection) have large, sharply defined prenuclear cytoplasmic cavities surrounded by dense rim cytoplasm. (4).

According to screening result of cytology done by expert cytotechnologist and using sum of the cytology criteria in [table 2] smears classified into normal and abnormal [table 1] In this study all abnormal cytomorphological changes were appeared with higher frequencies among water pipe smokers, None of those cytomorphological changes appear among nonsmoker. This difference was of high statistically significant

value.

Dysplasia shown only among water pipe smoker as shawn in [table 2]. This results was agree with **Mohammed S. Abdelaziz et.al** whose found the similar percent 11.4 % (4/35) of atypical cells among water gather shisha smoker.[15] all results above approve that cigarettes and water pipes smoking are risk factors for oral atypical cellular changes what agree with the review article by **de Geus et al.** who conducted a systematic review of clinical studies to evaluate the frequency of micronuclei in the oral mucosa of smokers and non-smokers in adult patients and concluded that there was a higher frequency of micronuclei exfoliated cells in smokers compared to non-smokers.[16]

The finding of this study water pipe is more harmful dysplastic for buccle mucosal cells is in agreement with **Jalayer Naderi N .et.al** in waterpipe smoking is not safer than cigarette smoking thus waterpipe smoking session is longer than cigarette almost 1/2 h or more. One session of waterpipe smoking is equivalent to smoking of 10 cigarettes.[17]

In this study inflammatory changes, bacterial infection were exist among smokers [table 2] what agree with **Mohammed S. Abdelaziz et.al** but in higher frequencies.[15]

Cells with a high frequency of MN in healthy individuals are very rare but can be increased in the cells of individuals exposed to genotoxic agents, since they are indicators of chromosome ruptures or abnormal cell divisions.[18] The advantage of MN assay lies on its simplicity, as the scoring of micronuclei is rapid and does not require much expertise. Thus, MN in a cell represents an “internal dosimeter” to estimate exposure to genotoxic and carcinogenic

agents.[19] Feulgen reaction a specific test for DNA was chosen to detect the abnormal number of (MN) among smokers oral smears cells [table 2].

In this study increased MN count appear among 65% smokers. This result going with **Chadha and Yadavwhose** whose showed a significant positive correlation between the age of individuals and mean percentage of micronucleated cell count.[20] A correlation found between smoking and increase of age (above 30) this result agree Venkatesh Vishwanath Kamath.et al[21] that no correlation found with habits in smoking or duration of smoking.

Based on study results cigarettes and water pipes are genotoxic , risk factors for oral atypical cellular changes, Nuclear fragmentation and possibly of oral infection susceptible for invasion by bacteria and oncogenic viruses. Water pipe smoking was more harmful for oral mucosa than cigarette smoking. Cytology evaluation and micronuclei assay of significant value for oral health among smokers.

Conclusions:

waterpipe smoking have genotoxic effect on oral buccal mucosal cells as cigarette smoking and induce dysplasia . Micronuclei cytochemical test feasible prognostic test for oral cancer. This study recommended an oral cytology screening program for smokers for early intervention with health education on bad effects of smoking generaly and water pipe especially.

Aknowledgment: for staff of histopathology department in MLS faculty in shendi university

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