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Morphological Changes and Cellular Proliferation in Epithelial Oral Mucosa among Children Streets Chloroprene Users, Sudan

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Abstract : *Chloroprene is polymerized to form polychloroprene (neoprene), a synthetic rubber used for wire and cable covers, gaskets, automotive parts, adhesives, caulks, flame-resistant or high gum strength cushioning and other applications requiring chemical, oil and weather rubber[(1)].This case control study was conducted in Gezira State Wad Madani from December 2016 - October 2017, to assess the cytological atypia and agyrophilic nucleolar organizer regions Count in Epithelial Oral mucosa exposed to chloroprene among Sudanese users. Fifty individuals were chloroprene addicted as cases, and fifty individual as control, their age ranged between (11-43) years with a mean age (17.4) years. The samples were prepared and stained by Papanicolaou stain and silver stain. The result revealed that epithelial atypia in 46% by Papanicolaou stain. The mean agyrophilic nucleolar organizer regions increased in* **%62** *of studied population. There was strong association between duration of chloroprene ues and severity of cytological atypia, and increase mean agyrophilic nucleolar organizer regions with p.value (0.000) for both. The amount of chloroprene use was positively correlated with increase mean agyrophilic nucleolar organizer regions with p.value (0.000). The study conclude that chloroprene is one of the risk factors for change normal epithelial to abnormal. Chloroprene users should undergo continuous screening program. Oral exfoliative cytology is a simple, non-invasive technique, cytological analysis of exfoliated cells should be done for screening and regular follow up of premalignantlesions.*

Keywords; morphological, proliferation, oral, mucosa, Children Streets, Chloroprene, Sudan

Introduction: Oral cancer is one of the six most common cancers in the world**,[(2)]**. It is one of ten major causes of death across the globe[(3)] It is a disfiguring disease prevalent among middle-aged adults and is associated with low survival rate[(4)]. Two thirds (2/3) of oral squamous cell carcinoma and 75% of head and neck cancer can be attributed to tobacco use and alcohol consumption[(5 ,6)] the risk increases with the frequency of exposure. A recent study showed that 48% of head and neck cancer patients were alcoholics[(7)]. While, tobacco smoking has been observed to beassociated with increasing risk of oropharangeal cancer and oral leukoplakia[(8]. Most oral cancers are preceded by precursor lesion, which could be of great help in early diagnosis. Oral cytology, which is largely based on the presence of nuclear or cytoplasmic alterations, can easily be performed to detect cancer at an early stage and to establish quantitative techniques[(9 ,10,11)].The oral mucosa is subjected to numerous physical insults. It is exposed to vast numbers of microorganisms and to food and other material introduced into the mouth. Oral epithelium has a high rate of cell turnover. In almost all lesion of oral mucosa, physical trauma and infection have a role and this may be superimposed on previously normal or abnormal mucosa[(12)].Squamous cell carcinoma accounts for over 95% of malignant tumors of oral cavity. Men are more affected than women; the major risk factors are tobacco chewing alcohol, smoking and betel chewing[(13)].Statistical studies confirmed that histopathologic differentiation of oral squamous cell carcinoma lesions is insignificant as far as prognosis is concerned. On the other hand, staging is a critical factor in prognosis of oral cancer. Staging is a procedure utilized in determining the magnitude of progress of the tumor by measuring the size of the tumor, invasion of surrounding structures, metastasis to related lymph nodes and distant metastasis to other organs. Epidemiologic studies established associations between a number of environmental factors and oral cancer. The most important among these factors are tobacco and alcohol. Separately these two factors increase the risk of oral cancer. Onusing both, the risks of developing oral cancer are multiplied. It is a very important that a methodical approach to examination of oral mucosa should be adopted by all dentists. Examination of the oral mucosa of lips, tongue, alveolar mucosa, floor of the mouth, palate, cheek and oropharynx should be routinely carried out for every patient presenting at a dental office.Since oral cancer in early stages is painless, it is not likely that the patient will seek dental consultation for an early cancerous lesion. The more likely scenario is a patient seeking consultation for a painful carious lesion, periodontal condition or routine checkup and the cancerous or precancerous lesion is discovered as incidental finding.In most patients the lesion ultimately becomes painful due to secondary infection. Eventually, the patient might complain of difficulty in chewing or swallowing, difficulty in moving the tongue or mandible or numbness of the tongue or other areas of the mouth. These changes are reflections of infiltration of related muscles or nerves. If left untreated eventually firm swelling develops in related lymph nodes. A variety of diagnostic aids and adjunctive techniques are available to assist in screening healthy individuals for evidence of oral cancer. These aids include toulidine blue, brush cytology, tissue reflectance and autofluorescence[(3)]. Histological examination of tissue remains the gold standard for diagnosis and identification of malignant oral lesions. Biopsy is an invasive technique with surgical implications, technique limitations for professionals and psychological implications for most patients. Recently we have dramatic switch from histopathological to cytopathological as a rapid, simple and unexpensive methods for diagnosis.Exfoliative cytology (cell scrapings) serves as an adjunct to clinical diagnosis, as it enables more extensive screening and provides

microscopic material if there is a delay in or contraindication to biopsy. The most effective way to control oral cancer is to combine early diagnosis with determination of an appropriate treatment. Oral exfoliated cytology serves as an important to early detection of premalignant lesions; Particularly if employed for screening at risk population[14]. Chloroprene is polymerized to form polychloroprene (neoprene), a synthetic rubber used for wire and cable covers, gaskets, automotive parts, adhesives, caulks, flame-resistant or high gum strength cushioning and other applications requiring chemical, oil and weather rubber. The International Agency for Research on Cancer (IARC) has classified chloroprene as a Group 2B carcinogenic to humans[1]. Chronic exposure to these risk factors causes changes in the oral mucosa and these changes are visible as pre-cancer lesions. Overtime, malignancy may develop in these lesions.

Materials and Methods:

Study design: This is a case control study conducted in Gezira State (Wad Madani) from December 2016 to September 2017 to evaluate the effect of chloroprene consumption on buccal mucosa by using conventional cytology Papanicolaou stain (PAP), and silver stain.

Collection and preparation: Oral examinations were performed using a mouth mirror and artificial light. Participants were asked to rinse their mouths with normal saline before samples were taken to eliminate debris and excess saliva from the oral mucosa. Exfoliated epithelial cells were obtained from the right buccal mucosa with the help of a tongue depressor. Samples were spread on a slide and immediately fixed with fixation spray (Merckofi x, Merck, Darmstadt, Germany) to avoid exposure to dry air (otherwise the cells will degenerate). In the pathology laboratory, the samples were stained with Papanicolaou and silver stain.

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 [15

Protocol of silver nitrate for AgNORs protein sites: Ethyl alcohol fixed smear are hydrated in 95% alcohol for 2 min, through 70% alcohol for 2 min, rinse in water for 1 min, then incubated in freshly working prepared solution for 45 minutes at room temperature, washed by deionized water, dehydrated in two change of absolute alcohol, cleared in xylene and mounted in DPX [15]

Result interpretation: Atypia was assessed cytologically by using the criteria described elsewhere. The presence of two or more of the following features 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[16]. AgNORs visible as black dots located within the nuclei of the cells, the mean numbers of AgNORs were counted in hundred squamous epithelial cell nuclei per smear, the normal range of AgNORs are (2-4 black) dots per nucleus

Results: This is case control study aimed to assessed atypical change, mean AgNORs count among 50 individuals, their ages ranges ranging from 11 to 43 years, with a mean age (17.4) years. The majority of the study population were at age group (11-21) years which constitutes 30 (60%), followed by the age group (22-32), (33-43), wears which constitute 16 (32%), 4 (8%) respectively**.**

Table (1):Distribution of age among study group**.**

Age	Frequency	Percent%
11-21 years	30	60
22-32 years	16	32
33-43 years	4	8
Total	**50**	**100**

Table (2):Distribution Of Cytological Atypia By Duration Of Chloroprene Addicted Per Year.

Duration/ Year	Absent	Mild	Moderate	Number	Percent
3-8	21	10	0	31	62%
9-14	3	6	1	10	20%
15-20	3	1	2	7	14%
21-26	0	0	3	2	4%
Total	**27**	**17**	**6**	**50**	**100%**

Table (3):Distribution of Cytological atypia by amount of chloroprene intake per day.

Chloroprene intake / day	Absent	Mild	Moderate	Number	Percent
1-3	16	10	2	28	56%
4-6	9	3	2	14	28%
7-9	2	4	2	8	16%
Total	**27**	**17**	**6**	**50**	**100%**

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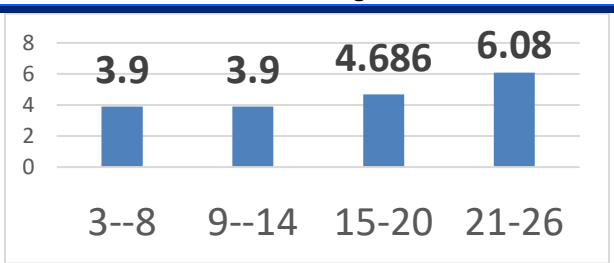


Figure (1): Distribution of AgNOR mean according to duration per years.

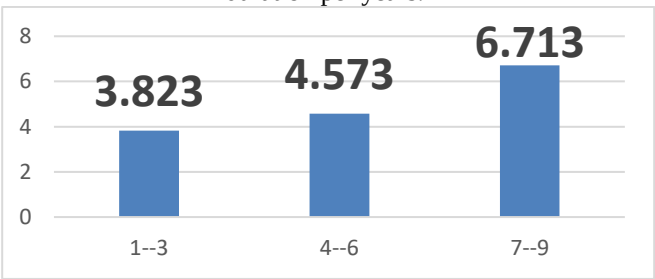
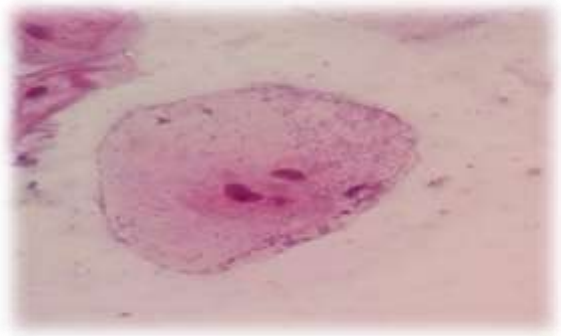
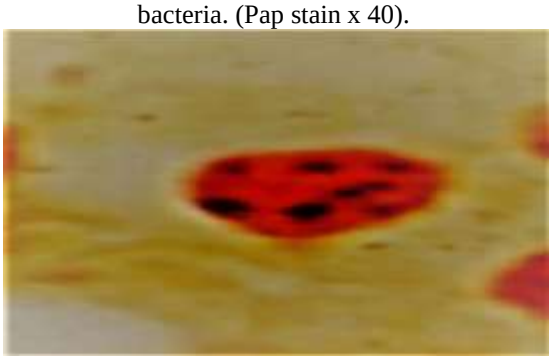


Figure (2): Distribution of AgNOR mean according to amount of intake / day.



Microphotograph (1): Buccal smear from 15 years old male, showing superficial cells with binucleation and intracellular bacteria. (Pap stain x 40).



Microphotograph (2): AgNOR in the epithelial cell of chloroprene user, increase number of AgNOR for chloroprene user, normal range (2-4 black) dots(silver stain X40)

Discussion: Exfoliative cytology is based on epithelial physiology. A normal epithelium is exposed to regular exfoliation, namely the loss of cell surface, and the thickness of the epithelium is constant[17]. Under normal conditions, epithelial cells are strongly held in place. However, the presence of benign diseases or the occurrence of malignant epithelial formations causes the cells to lose their cohesive force, and results in exfoliation. Loss of cohesion between the cells enables the collection of the exfoliated cells for microscopic examination[18]. Cytomorphology is the most widely used method of oral exfoliative cytology, and assesses parameters such as cellular diameter (CD), nuclear diameter (ND), nuclear area (NA), cytoplasmic area (CA), NA/CA ratio, nuclear shape, nuclear membrane continuity, optical density, and nuclear texture [18]. These parameters, especially NA and NA/CA ratio, have been shown to provide meaningful results in the diagnosis of oral lesions [18,19] .

Many cytomorphological studies have been conducted on premalignant and malignant lesions in the oral cavity [20,21,19,22,23]. Quantitative cytomorphometric evaluation of exfoliated buccal mucosa cells obtained from premalignant and malignant lesions has revealed significant differences at the cellular level[22,23,24]. This case-control study assessed the oral cytological changes in buccal mucosa, among Sudanese chloroprene users, these habits are socially not for female, therefore the all of the study populations were males. Chloroprene in Sudan is widely distributed among homeless people as an addictive substance which is used in worldwide as adhesive agents in the car wheels and others rubbers; there is a limited knowledge about its carcinogenic potential socially not for female, therefore the majority of the study populations were males. Of the 50 studied subjects, cytological atypical change was detected among 23 (46%) individuals all of them are exposed to chloroprene , found that chloroprene is a reasonably human carcinogen based on evidence of benign and malignant tumor formation at multiple sites including oral cavity mucosa. In this study, among the 23 atypical subjects, chloroprene users group showed 17 (74%) mild, 6 (26%) moderate and no case show severe atypia.

According to the duration of chloroprene intake / year, the study showed that high frequency cytological atypia was found in the duration of (3-8years) which constituted 10 atypia (mild 10, moderate 0) (43.4%) followed by (9-14 years), (15-20 years) and (21-26 years) that represented 7 atypia (mild 6, moderate 1) (30.4%), (3mild 1, moderate 2) (13%) and 3(mild 0, moderate 3) (13%) respectively. The study approved that there was significant association between duration of chloroprene intake / year and severity of cytological atypia.

Therefore, there is no significant association between cytological atypia and the amount of chloroprene intake/ day, and these changes may be due to continuous contact of chloroprene use during the day, the severity was not related to the daily amount of the habit.

NORs are intimately related to cell cycle and thus may be related to proliferation. In rapidly, proliferating cell nuclear disaggregation may take place resulting in dispersion of individual AgNORs, which appear as black brown dots of varying size in the nucleus. Because of its simple technique and high reliability for cellular proliferation AgNOR staining was used. However, there are certain limitations to this such as risk of obscuring some AgNORs by superimposition and fusion of small AgNOR dots due to continuous deposition of

silver for a long time [(25)]. AgNOR counts have been great value for assessment of cellular proliferative activity that is frequent encountered in pre-malignant and malignant changes [(25)] . A number of studies have pointed out that the AgNOR count is rapid and an easily reproducible method which permits a clear distinction between malignant and benign cells [(26)] .

There was statistical significant difference in correlation between chloroprene users and increase mean AgNOR count according to duration and amount of intake which constituted p.value (0.000) for both. To the best our knowledge this is first study used cytological atypia and mean AgNOR count to assess the oral mucosal cells exposed to chloroprene.

Conclusion:

- The study concludes that exposure to chloroprene may be associated with carcinogenic cytologic changes in the oral mucosa, cellular atypia detected by using PAP stain in 46%, increase in AgNORs score to 62% in chloroprene users.

- The importance of early detection of oral cancer is emphasized by the fact that the most significant factor in prognosis of a cancerous lesion is the stage in which the lesion is detected. This fact underlines the importance of cytological approach in examination of oral mucosa so screening program should be established, particularly for high-risk/high-prevalence communities, and users of chloroprene.

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