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ORIGINAL ARTICLE

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# Detection of Cytological changes in buccal mucosa among Sudanese alcohol drinkers

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## Abstract

**Aim:** Chronic alcohol consumption led to a number of cytological changes in the mouth including inflammatory cell, fungal infection, bacterial infection, viral infection, dyskaryosis, and malignant changes. The study aimed to detect the cytological changes of buccal mucosa causing by alcohol consumptions.

**Methods:** This laboratory-based study included a total number of 50 patients of alcohol consumption and 25 non-alcohol drinkers which were collected from healthy people. Samples collected were two buccal smears from the buccal mucosa of alcohol consumers, using a tongue depressor, before that washed mouth to avoid contamination of bacteria.

**Results:** The percentage of inflammatory change in age groups 10–20 was 3%, 21–30 was 6%, 31–40 was 20%, 41–50 was 26%, and the last group 51–60 was 45%.

The cytological findings showed in the study population, the normal result there was 28% but the inflammatory changes show is very significant was 60% while the infected changes and dyskaryosis were not significant was 6% for each.

**Conclusions:** This descriptive laboratory study confirmed the effectiveness of alcohol on buccal mucosa which led to abnormality of cytological findings like inflammatory changes, infected changes, dyskaryosis, and malignant changes due to excessive alcohol intake.

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**KEYWORDS**

Dyskaryosis, malignant changes, alcohol intake, Sudan

## INTRODUCTION

Recent studies raised the point that alcohol-containing mouthwashes are an etiologic agent in oral disorders [1–6]. Constant exposure to these alcohol-containing rinses, even in the absence of smoking and drinking, leads to significant increases in the development of oral diseases [7–9]. Previous reports study focused on abusers of alcohol and made no reference to mouthwash. Also, many reports mentioned a clearer link between the cytological findings and their broader implications, such as oral cancer risk or overall oral health in alcohol drinkers. Included global statistics in different regions which is relevant of the findings in Sudan. Any connection between oral cancer and mouthwash is tenuous without further investigation [10–14].

Qiao et al., Sorkina et al., Cheng et al., reports mentioned that, while alcohol is recognized as a risk factor, the mechanisms through which it leads to cytological changes in the oral cavity are explored in more detail [15–17].

The present article provides a strong rationale for studying the effects of chronic alcohol consumption on the buccal mucosa. The references to cytological changes such as inflammatory cells, fungal and bacterial infections, and malignant changes are well-founded.

The study aimed to detect the cytological changes of buccal mucosa due to alcohol consumption.

## METHODS

### *Study design and setting*

This study was carried out at Gharb EL Niel College. Cytological analysis was conducted at the Histopathology & Cytology Department – Gharb

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EL Niel Collage - Sudan. It was a laboratory-based study that included a total number of 50 patients of alcohol consumption and 25 non-alcohol drinkers which were collected from healthy people.

### *Sample collection*

-Samples collected were two buccal smears from the buccal mucosa of alcohol consumers, using a tongue depressor, before that washed mouth to avoid contamination of bacteria.

Patients' inclusion criteria included Sudanese patients whom were alcohol consumers, non-smokers and use mouthwash. While exclusion criteria included non-Sudanese patients, smokers, and non-users of mouthwash.

### *Sample process*

One smear (wet preparation) is put in 95% ethyl alcohol for stain by hematoxylin and eosin (H&E) stain. Another smear (air dry) is put in methanol to be stained by May Grunwald Giemsa (MGG). It's based on the two neutral days and the affinity of the cellular element or acidic and basic days.

### *MGG stain*

Staining process

Rinse the air-dried slide in buffer pH 6.8 and stain in freshly prepared May Grunwald for eight minutes after that drain off the excess stain and stain in freshly prepared Giemsa for ten minutes, differentiate in buffer pH 6.8 and allow smears to dry, if necessary, they can be gently blotted before mounting [18].

### *Mayers Haematoxylin*

Staining process

The haematoxylin component stains the cell nuclei; while eosin stains cells cytoplasm and most connective tissue fibers. Sections were stained with solution of haematoxylin, differentiated in 1% acid alcohol, washed in alkaline water and stained in 1% aqueous eosin, and mounted the sections beneath a cover- slip in Disterene Plastcizer & Xylene (DPX), to preserve permanent staining preparations. Light microscopic examinations were carried out at magnifications of 4 X, 10 X, and 40 X [19].

### *Statistical analysis*

Using the Scientific Package for Social Sciences (SPSS) program for data analysis.

## RESULTS

The results of this study were based on cytological analysis. The frequency of age groups 10–20 was 3(6%), 21–30 was 6(12%), 31–40 was 8(16%), 41–50 was 13(26%), and the last group 51–60 was 20(40%).

The percentage of inflammatory change in age groups 10–20 was 3%, 21–30 was 6%, 31–40 was 20%, 41–50 was 26%, and the last group 51–60 was 45%.

The cytological findings showed in the study population, the normal result there was 28% but the inflammatory changes show is very significant was 60% while the infected changes and dyskaryosis were not significant was 6% for each.

Figure 1 shows the frequency and percentage among different age groups. Figure 2 points out the percentage of inflammatory changes among different age groups. The percentage of different cytological findings among the study population was illustrated in Figure 3.

Table 1 shows the frequency and percentage of different age groups. Table 2 points out the correlation between normal cytological findings and abnormal cytological findings to the study population, while Table 3 illustrates the normal cytological findings and abnormal cytological findings to different age groups by using the cross-tabulation.

## DISCUSSION

The review of existing literature mentions several important factors, including the role of alcohol-containing mouthwashes and their potential link to oral disorders.

The results of this study showed significant cytological changes ($P \leq 0.01$) in the buccal mucosa after consumption of alcohol.

The results are well-presented, and the data tables provide a clear breakdown of the cytological findings across different age groups. The findings that 60% of alcohol consumers exhibited significant inflammatory changes are noteworthy, and the correlation between age and the severity of these changes is well-highlighted.

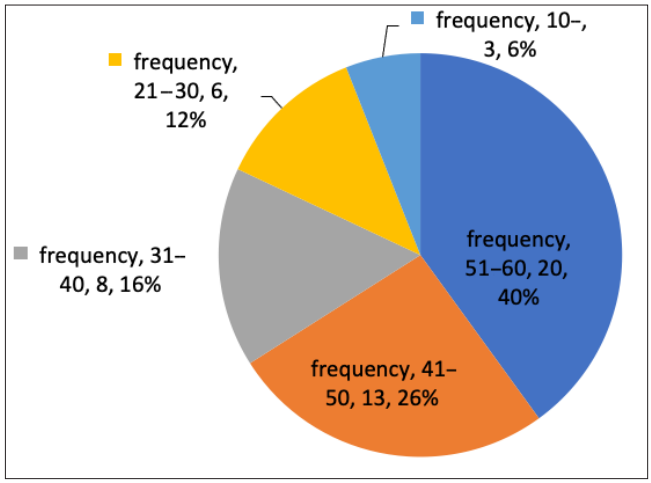


**Figure 1.** The frequency and percentage among different age groups.

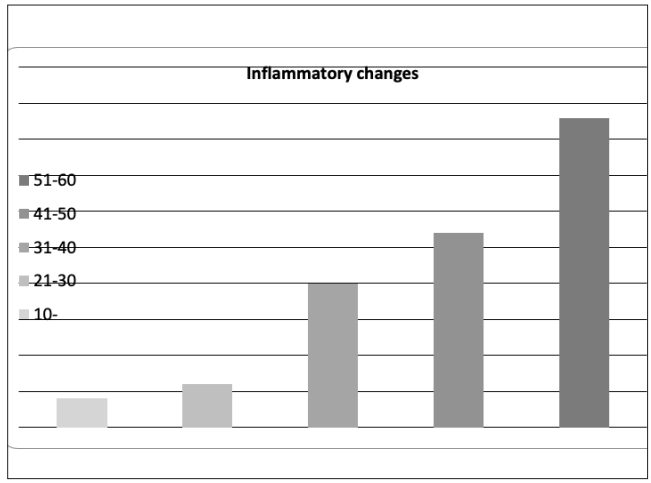


**Figure 2.** The percentage of inflammatory changes among different age groups.

A previous study done to determine the effects of alcohol on the buccal mucosa found that the inflammatory changes were significantly higher ($P < 0.01$) (68%) in the study group [20]. This agreed with our findings which revealed highly significant (60%), due to Alcohol intake.

Regarding the cytological examination, this study revealed normal cells for the control groups. The alcohol manifested inflammatory changes, infected changes, and dyskaryosis.

A previous study reported that all cytological findings abnormality occurred in all study groups (72%) except (28%) of the study population [21–23].

Other previous studies reported that chronic alcohol consumption to study population has led to a number of cytological changes including inflammatory cell, fungal infection, bacterial infection, viral infection, dyskaryosis, and malignant changes. All these changes can lead to oral cancer after drinking alcohol for a long period [24, 25].

Li Y et al., Schwarzinger et al., Hsieh et al., Rao et al., studied the relationship between alcohol consumption and oral cancer. Their reports are in accordance with our findings and support our results [26–29].

Dyskaryosis and malignant changes were present in only 6% of the population. The clinical significance of this finding, in comparison to other studies on alcohol consumption and oral health, showed agreement with our findings [30–35, 7].

The rates of inflammatory changes so high, but the rates of dyskaryosis and malignant changes relatively low, may be affected by other factors, such as diet or oral hygiene practices, which need further studies.

Further study included figures useful in illustrating the distribution of cytological changes, by including more detailed explanations or captions to make the graphs easier to interpret.

### *Study limitation*

Sample size (50 alcohol consumers and 25 non-consumers), that the sample size was small in both groups. The study mentions the potential for alcohol to lead to oral cancer, but the mechanisms in more depth not mentioned. Chronic alcohol consumption contributes to the development of dyskaryosis and malignancy, and its preventive measures based on these findings are not discussed. The cross-sectional design limits the ability to draw causal conclusions about the effects of alcohol on buccal mucosa. A longitudinal study would provide stronger evidence of the progression from inflammation to malignancy. Additionally, the reliance on self-reported alcohol consumption could introduce bias, as participants may underreport their alcohol intake.

## CONCLUSIONS

This laboratory study confirmed the effectiveness of alcohol on buccal mucosa which led to abnormality of cytological findings like inflammatory changes, infected changes, dyskaryosis, and malignant changes due to excessive alcohol intake. The achieved results lead to a conclusion that chronic alcohol consumption to study population has led to a

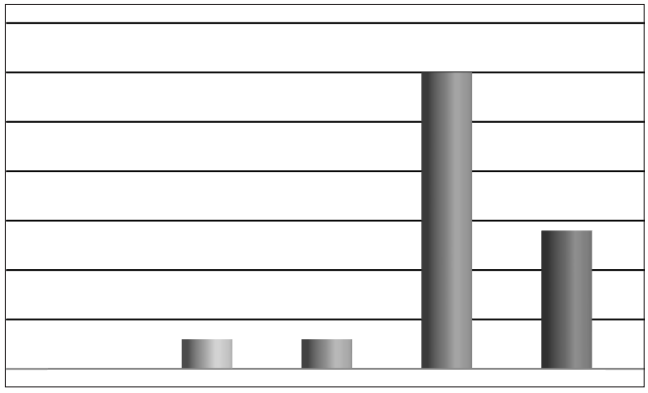

**Figure 3.** The percentage of different cytological findings among the study population.

number of cytological changes including inflammatory cell, fungal infection, bacterial infection, viral infection, dyskaryosis, and malignant changes. All these changes can lead to oral cancer after drinking alcohol for long period.

The study recommends widely spread public health awareness to reduce alcohol-related oral health issues in Sudan, and enhance the role of dental health professionals in early detection of mucosal changes by increase number of clinics in many regions and states in Sudan.

### *List of abbreviations*

**DPX**: Disterene Plastcizer & Xylene
**H&E**: Hematoxylin and eosin
**MGG:** May Grunwald Giemsa
**SPSS**: Scientific Package for Social Science

## DECLARATIONS

### *Ethics approval and consent to participate*

Ethical approval was obtained from the Ministry of Health Ethical Research Committee in accordance with the Declaration of Helsinki Principles, and the agreement was taken from the Dentistry hospital administration before sample and data collection. Informed consent (Consent to Participate and Consent to Publish) was obtained from all participants or, if participants are under 18, from a parent and/or legal guardian. The patient's information was highly secured and not used for other purposes than scientific inquiry.

**Table 1.** The frequency and percentage of different age groups.

| Age group | Frequency | Percentage (%) |
|---|---|---|
| 10–20 | 3 | 6 |
| 21–30 | 6 | 12 |
| 31–40 | 8 | 16 |
| 41–50 | 13 | 26 |
| 51–60 | 20 | 40 |

**Table 2.** The correlation between normal cytological findings and abnormal cytological findings to the study population

| Study group | Frequency | Percentage (%) |
|---|---|---|
| Normal group | 14 | 28 |
| Abnormal group | 36 | 72 |

= ($P < 0.001$).

## ETHICAL CLEARANCE CODE NUMBER

MH-RES/21-022-11, Date**:** 5/11/2022

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Table 3.** Normal cytological findings and abnormal cytological findings to different age groups by using cross-tabulation.

| Study group | Normal | Inflammatory changes | Infected changes | Dyskaryosis | Malignant change |
|---|---|---|---|---|---|
| **10–20** | 2(4%) | 1(2%) | 0(0%) | 0(0%) | |
| **21–30** | 4(8%) | 2(4%) | 0(0%) | 0(0%) | |
| **31–40** | 6(12%) | 2(4%) | 0(0%) | 0(0%) | |
| **41–50** | 1(2%) | 9(18%) | 2(4%) | 1(2%) | |
| **51–60** | 1(2%) | 16(32%) | 1(2%) | 2(4%) | |
| **Total** | 14(28%) | 30(60) | 3(6%) | 3(6%) | |

= ($P < 0.001$).

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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## AUTHORS' CONTRIBUTIONS

MAM and AA1 conceived the design and carried out the experiments. HMY obtained, analyzed, and interpreted the data. MAH and MAE wrote and revised the manuscript. AAI provides financial support for all experiments. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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