



Evaluation of the cytological changes of oral mucosal cells in Smokers by using Exfoliative Pap Stain.

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Abstract

Oral cancer is a major public health issue worldwide; it remains a highly lethal and disfiguring disease. In Iraq little is written regarding the occurrence of oral cancer. The aim of this study is To examine the effect of smoking oral mucosa cytological. Hundred men aged between 40 and 70, comprising 50 smokers and 50 non smokers.

Material for **exfoliative** cytology was collected with a metal spatula from the lateral border of the patient's tongue and from the floor of the mouth and the samples were then submitted for Papanicolaou staining. Then cytologic smears were classified according to Papanicolaou's classification. The results of the current study reveals that there is a significant association between smoking (number of cigarette smoked per day and the duration of smoking (year) and the occurrence of cytological changes in the oral mucosa , $p < 0.001$. and there is a significant association between the age and the papanicolaou's classes , $p < 0.001$.

Key words: oral cancer, exfoliative cytology, papanicolaou's, smoking.

Introduction

Smoking is currently the most preventable cause of diseases and death worldwide and is one of the main risk factors for the development of cancer in different organs. Therefore, smoking patients should be carefully monitored in view of the series of alterations that tobacco can cause ⁽¹⁾.

According to the World Health Organization, cancers of the mouth and oropharynx are the most frequent head and neck tumors, corresponding to 4% of all cancer cases ⁽²⁾. Oral cancer mainly affects the floor of the mouth, lateral border of the tongue and soft palate, although other areas of the mouth may also be involved, and smoking alone or in combination with frequent alcohol consumption represents the main risk factor ⁽³⁾. According to Winn, smoking is the

leading cause of oral cancer in 91% of men and in 59% of women ⁽⁴⁾. Many cases of oral cancer are diagnosed at advanced stage, a fact that results in an unfavorable prognosis and high mortality, in addition to high treatment costs coupled with an increased number of complications. Thus, early diagnosis is of extreme importance in managing this disease.

Considering the possible influence of smoking on the occurrence of oral cancer and of precursor lesions, together with the accessible location of the lesions which permits easy visualization and collection of material for analysis, exfoliative cytology might be a useful tool for the detection and monitoring of initial alterations ⁽⁵⁾ and for the establishment of adequate treatment in smokers .It is a

complementary diagnostic method which presents several advantages such as rapid and easy execution, low cost, diagnostic safety, efficacy and noninvasiveness, and can be repeated several times.^(6,7)

Papanicolaou staining is used as for the analysis of cytological aspects and permits the identification of basic inflammatory, dysplastic or malignant alterations⁽⁸⁾.

In the present study, we examined exfoliative cytology from the lateral border of the tongue and the floor of the mouth, these sites associated with a high incidence of oral cancer, in both smokers and nonsmokers.

This study aimed to detect the cytological changes in oral mucosa among cigarette smokers.

Materials and Methods

This study was designated as Prospective one, it involved hundred men ranging in age from 40 to 70 years who visited dental clinic in Baghdad, because of dental problems, were recruited for this study. Informed consent was obtained from all patients before including them in this study. This study carried on from Jan 2012 to Jan 2013, The following inclusion criteria were applied: not addicted to alcohol, absence of any oral lesion, and with no prior or present history of benign or malignant oral neoplasms⁽¹¹⁾. Two groups were analyzed: fifty smokers and fifty non-smokers. Smokers were defined as those individuals that smoked 10 cigarettes or more per day for at least 20 years. In this research, only smokers of filtered cigarettes were included. Pipe smokers or consumers of tobacco in other ways were not included, because of concentration variation, which may affect the oral mucosal cells with different intensity, and other systemic effects⁽¹⁰⁾ Non-

smokers were defined as people who have never smoked.

Material for exfoliative cytology was collected with a metal spatula from the lateral border of the patient's tongue and from the floor of the mouth and smears were prepared on properly identified glass smears and fixed in 99.3% ethanol. The samples were then submitted for Papanicolaou staining.

After screening cytologic smears were classified according to Papanicolaou's classification⁽¹¹⁾:

Class I (Normal) – Only Normal cells were observed

Class II (atypical) – Presence of minor atypia, but no evidence of malignancy.

Class III (Intermediate) – An in-between cytology. The cells display wide atypia that may be suggestive of malignancy, but they are not clear cut cancer and represent precancerous lesions or in situ carcinoma.

Class IV (Suggestive of cancer) – A few epithelial cells with malignant characteristics or cells with borderline characteristics.

Class V – Positive cancer cells that are obviously⁽¹¹⁾

The results were reported as mean \pm standard deviation. The results were submitted to statistical analysis, ANOVA (analysis of variances), chi square test, and t test were used to detect the significances between variables of our study.

The comparison of significant (P-value) in any test were:

S= Significant difference (P<0.05).

NS= Non Significant difference (P>0.05).

All the statistical analysis was done by SPSS (statistical package for social sciences) version 17, and **Excel 2007** programs.

Results

A total number of 100 cases were included in the present study. Cases were submitted for Papanicolaou staining including 50 smokers and 50 non smokers.

The number of smears adequate for analysis of Papanicolaou staining was 40 for smokers and non smokers each. This smaller number was due to technical errors.

Age distribution:

The mean age of the smokers in this study was 50.92 ± 8.70 years while the mean age for non smokers was 54.42 ± 8.85 . As shown in table 1 and figure 1. The patients age in this study ranged between 40 to 70 years.

The association between cytological changes (papanicolaou classes) and period of smoking (year):

In the present study cases with Papanicolaou class I :have mean period of smoking of 20.41 ± 1.44 years, cases with class II with mean period of smoking of 27.70 ± 5.31 years and finally class III cases the mean period of smoking 26.62 ± 7.01 . In the current study there is a significant relationship between the cytological changes and the period of smoking , $p < 0.001$. See table 2.

The association between cytological changes (papanicolaou classes) and number of cigarette per day:

The relation between cytological changes (Papanicolaou classes) and number of cigarette per day. 12 cases of Papanicolaou class I with mean number of cigarette smoking per day was 16.25 ± 3.76 , 24 cases of class II with mean number of cigarette per day was 25.62 ± 7.84 and 4 cases of class III with mean number of cigarette per day was 36.25 ± 7.50 . There is significant relationship between Papanicolaou class and number of cigarette per day, p value < 0.001 . As shown in table 3.

Association between cytological changes (Papanicolaou classes) and age:

There is significant relation between Papanicolaou classes and the age ,i.e. the older patient have more risk to develop cytological changes in the oral mucosa , p value < 0.001 . As shown in table 4.

The association between cytological changes (papanicolaou classes) and pack year:

Number of pack years = (number of cigarettes smoked per day \times number of years smoked)/20 (1 pack has 20 cigarettes). In the present study there is a significant relationship between the Papanicolaou class and pack year , p value < 0.001 . As shown in table 5.

Association between cytological changes (Papanicolaou classes) and smoking :

In the present study the Papanicolaou class I : 24 cases were non smoker and 12 cases were smokers ,class II: 16 cases were non smoker and 24 cases were smokers, finally class III all are smokers. As shown in table 6 and figure 2.

Discussion

The effect of smoking, as a risk factor for oral cancer, depends on the number of cigarettes smoked daily and the duration of smoking. Smoking has led to different changes in the oral mucosa of many individuals. Smoking has been shown to be related to many pathologies, which range from harmless and reversible lesions, to oral cancer in oral mucous membranes.⁽⁷⁾

Age distributon:

In the present study The mean age of the smokers was 50.92 ± 8.70 years while the mean age for non smokers was 54.42 ± 8.85 ,this is similar to other studies^{(6),(12)}.

Relationship between cytological changes and period of smoking per year:

In the current study : papanicolaou class I with the mean period of smoking was 20.41 ± 1.44 years, class II with mean period of smoking 27.70 ± 5.31 years and finally class III the mean period of smoking 26.62 ± 7.01 . There is a significant relation between the Papanicolaou classes and the period of smoking , the more the period of smoking , the more cytological changes in the oral mucosa , $p < 0.001$. This is comparable to other studies.^(7,12,14)

Relationship between cytological changes and number of cigarette per day:

There was significant relation between Papanicolaou classes and number of cigarette per day, p value < 0.001 . this is similar to other studies.^(7,13,14)

Relationship between cytological changes and the age :

There is significant positive relationship between cytological changes and the patients age ,i.e. These changes increased with older patients , $p < 0.001$. this is comparable to other studies.^(4,14,15)

The association between the cytological changes and the pack year:

In the present study there was significant relationship between cytological changes and the pack year, this is similar to the results which are reported by Zheng T .⁽¹³⁾

Association between Papanicolaou classes and smoking:

In the present study, cigarette smokers are more common with oral atypical cellular changes than non smokers ,this is similar to the result which is reported by Mohammed S. Abdelaziz etal⁽¹⁴⁾.

Conclusions

Our results revealed that the cytological changes in the oral mucosa is higher in smokers than non smokers .and the effect of smoking, as a risk factor for oral cancer, depends on the number of cigarettes smoked daily and the duration of smoking(years).

Recommendations:

- 1-Further prospective studies with a large number of cases are needed to validate the results of this study.
- 2-Study the relationship between oral mucosal changes and several risk factors together (like smoking and alcohol consumption).
- 3- Perform the same study by using advanced imaging techniques, computerized systems, and the use of quantitative techniques to verify the reliability of cytomorphometric analysis.

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Table 1: Mean differences of smoking by age.

| Age | groups | Mean age \pm SD | P value |
|------------|------------------|-------------------|---------|
| | Smoker | 50.92 \pm 8.70 | 0.079 |
| Non smoker | 54.42 \pm 8.85 | | |

Table 2: association of cytological changes and period of smoking (year)

| Papanicolaou class | N | Mean period of smoking (year) \pm SD |
|--------------------|----|--|
| I | 12 | 20.41 \pm 1.44 |
| II | 24 | 27.70 \pm 5.31 |
| III | 4 | 38.75 \pm 7.50 |
| Total | 40 | 26.62 \pm 7.01 |

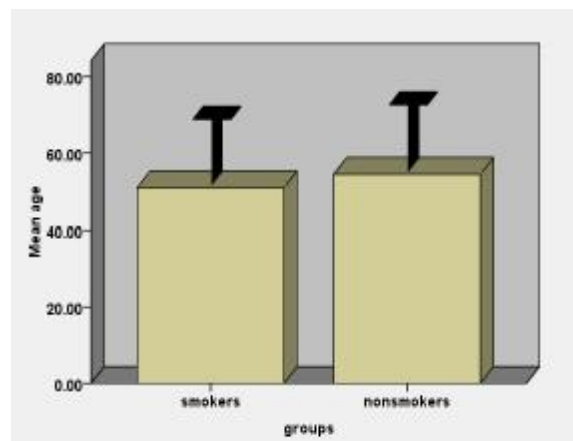


Figure 1: Mean differences of smoking by age

Table 3: association between Papanicolaou classes and number of cigarette per day

| Papanicolaou class | N | Mean age(year) \pm SD |
|--------------------|----|-------------------------|
| I | 12 | 43.08 \pm 2.02 |
| II | 24 | 52.75 \pm 7.63 |
| III | 4 | 63.50 \pm 6.35 |
| Total | 40 | 50.92 \pm 8.70 |

Table 4: Association between Papanicolaou classes and age

| Papanicolaou class | N | Mean number of cigarette per day \pm SD |
|--------------------|----|---|
| I | 12 | 16.25 \pm 3.76 |
| II | 24 | 25.62 \pm 7.84 |
| III | 4 | 36.25 \pm 7.50 |
| Total | 40 | 23.87 \pm 8.95 |

Table 5 : Mean differences of Papanicolaou class by pack year :

| Papanicolaou class | N | Mean pack year \pm SD |
|--------------------|----|-------------------------|
| I | 12 | 16.66 \pm 4.43 |
| II | 24 | 36.40 \pm 16.22 |
| III | 4 | 70.93 \pm 22.98 |
| Total | 40 | 33.93 \pm 20.91 |

Table 6: Association between Papanicolaou class and smoking

| Papanicolaou class | Groups | | Total |
|--------------------|---------|-------------|----------|
| | Smokers | Non smokers | |
| I | 12 | 24 | 36 |
| II | 24 | 16 | 40 |
| III | 4 | 0 | 4 |
| total | 40 | 40 | 80(100%) |

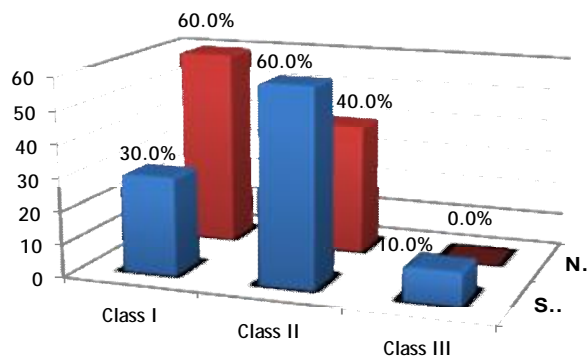


Figure 2: Association between Papanicolaou class and smoking (blue for smokers and red for non smokers)