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ORIGINAL ARTICLE

Bio-Monitoring of the Nuclear Abnormalities in Smokers Using Buccal Exfoliated Cytology

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ABSTRACT
The buccal cell nuclear changes continue to gain popularity as a biomarker of genetic damage in cancer progression. This study was aimed to evaluate and compare the abnormal nuclear changes in buccal epithelial cells of smokers with nonsmokers. The study was carried out on 40 samples classified in two groups of smokers and nonsmokers. The buccal mucosa cytology slides were observed under 400X magnifications. From each subject, a minimum of 500 cells were screened for calculating frequency of nuclear anomalies including Karyorrhexis, Karyolysis, Broken egg and Micronucleus. Finally, repair index calculated and the results were analyzed using student "T" test, the significance level was considerate at P < 0.05. The percentages of Karyorrhexis, Karyolysis, and Micronucleus were significantly higher in smokers vs. nonsmokers. (p=0.032, 0.007 & 0.000, respectively) Also, the percentage of broken eggs was higher in smokers but the difference was not significant. (p=0.662) Micronucleus percentage showed the greatest difference in comparison of smokers and nonsmokers group, Furthermore, repair index showed significantly higher amount in nonsmoker group. (p=0.002). Conclusion: Base on this study the evaluation of Repair Index may be a new bio-monitoring tool to approach the nuclear changes in population with risk of oral cancer.
Keywords; *Karyorrhexis, Karyolysis, Broken egg, Micronucleus, Repair index*

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INTRODUCTION

Oral cancer constitutes the fourth most common cancer among males and the sixth in females. It is recognized as one of the most common cancers in many developing countries [1] and accounts for roughly two percent of all cancers diagnosed annually in the United States; so, approximately 36,500 people are diagnosed with oral cancer each year.[2]

Smoking and other tobacco use are strongly associated with about 75 percent of oral cancer cases; caused by irritation of the mucous membranes of the mouth from smoke and heat of cigarettes, cigars, and pipes. [3] In the study of Chiba et al, relationship between smoking and oral cancer has also been reported. [4] Parkin et al have also reported the relationship between smoking and oral cancer. [5] Although, there has been a strong association between a history of smoking and oral carcinoma in many scientific reports, [6] researchers are now focusing to detect minimally invasive methods for early diagnosis of oral cancer in these high risk population.

The buccal cell nuclear changes were first proposed in 1983 [7] and it continues to gain popularity as a biomarker of genetic damage in numerous applications. It is believed that the number of nuclear changes is related to the increasing effects of carcinogens. It is important to emphasize that this event has already occurred before the clinical symptoms of cancer appear; so early detection of them may be useful in more effective treatment and reduction of its complications. The nuclear anomalies present as nuclear buds

(indicative of gene 3 amplification), bi-nucleation (caused by cytokinesis-failure or arrest), broken egg nuclei and micronucleus (referred to chromosomal alterations) and various forms of cell death measured as condensed chromatin, karyorrhexis, karyolysis, pyknosis as well as the frequency of basal and fully differentiated cells. [8]

Microscopic observations of nuclear aberrations are performed in exfoliated cytopathology sample. [9] A review of literature shows that this technique has been in use since the 1960s and 70s as a cancer evaluation and diagnostic technique with acceptable sensitivity and specificity.[10] Exfoliative cytology technique is a non-invasive method for initial and early diagnosis of cancers as an adjunct to clinical examination. Also, it can be used as a diagnostic technique in detecting early changes of diseases even in the absence of clinical manifestations.

Many researchers have reported significant correlation with smoking and increased frequency of micronucleus and other nuclear abnormalities in smokers, off course with degrees of controversial results. [11-15] Complementary assessment and analysing of extracted results referred to all abnormalities may be confusing; so, this study was aimed to calculate "Repair Index" related to coincidence evaluation of such the most recognized abnormal nuclear changes in buccal epithelial cells of smokers and comparison of them with non-smoker people. In the best of our knowledge, it was done for other environmental toxic exposures but the present study seems to be the first in smoking issue.

MATERIAL AND METHODS

The present study was carried out on 40 individuals including 20 smokers and 20 non-smokers selected from the patients attending the Islamic Azad University, dental branch of Tehran. The smoker group, who used to smoke over five years and more than twenty cigarettes a day, considered as the heavy smokers group. [16] Also, all of them were male, resident of Tehran and matched through category of economic, social parameters and age.

The subjects were classified in two groups: Smokers and non-Smokers which have not reported any other habits such as alcohol intake, etc., and drug consumption and also did not suffer from any kind of systemic disease.

After seeking consent and recording the history, the patients were subjected to sample collection. They were asked to rinse the mouth with drinking water and taking all aseptic precautions. A clean plastic spatula was used to scrape the buccal mucosa, three to four times with firm pressure. The slides were coded and the sample was then spread on the slide.

These slides were stained according to Papanicolaou staining technique. [17] The smears were observed under 400X magnifications.(figures 1-3) From each subject , a minimum of 500 cells were screened for calculating frequency of nuclear anomalies including Karyorrhexis , Karyolysis , broken egg and micronucleus . For each sample repair index calculated via formula "KR+KL/BE+MN". The results were analysed using student "T" test, the significance level was considerate at $P < 0.05$.

Fig1: Cytological examination buccal mucosa under 400X magnifications using Papanicolaou staining technique, arrow shows Karyorrhexis.

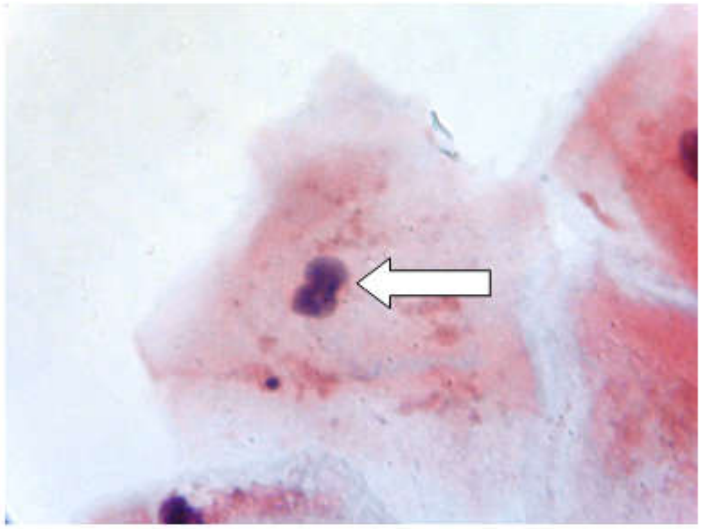

Fig2: cytological examination buccal mucosa under 400X magnifications using Papanicolaou staining technique, arrow shows broken egg nuclei.

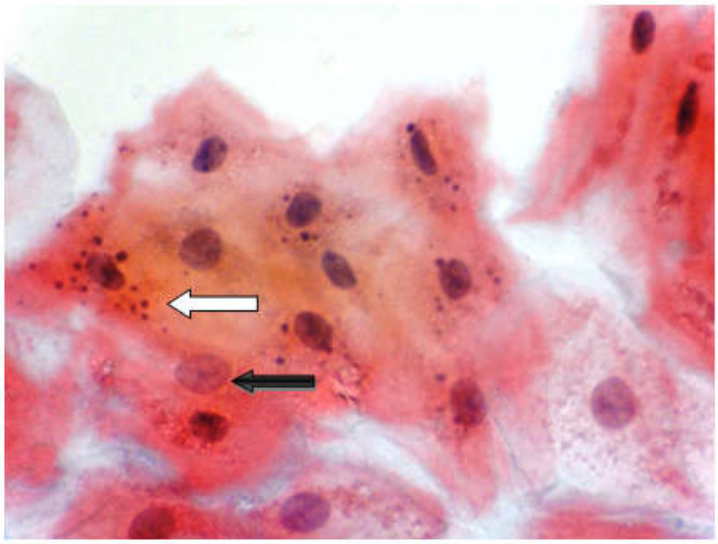

Fig3: cytological examination buccal mucosa under 400X magnifications using Papanicolaou staining technique, white arrow shows Micronucleus & black one point to Karyolysis.

RESULTS

In the present study, exfoliative cytology technique was used to evaluate cellular changes in the buccal mucosa of smokers in comparison with a control group. The age of non-smokers, 34.42 ± 16.38 and the group under study was 36.42 ± 16.17 years old. Table 1&2 show the frequency of nuclear changes in smokers and non-smokers and table 3 explains the statistical analysis through comparison of karyorrhexis, karyolysis, broken Eggs, micronucleus and repair index in the studied groups.

Based on facts shown in table 3, the mean percentage cells containing Karyorrhexis in smokers are 1.25 and in non-smokers are .85, also the mean percentage of Karyolysis cells in smokers is 1.75 and in non-smokers are 1.10, and also the mean percentage of Broken Egg cells in smokers is 1.30 and in non-smokers are 1.20, and also the mean percentage of micronucleus cells in smokers is 5.00and in non-smokers are 1.70.

The percentage of Karyorrhexis was significantly higher in smoker samples. (p=0.032) Also, the similar results were found for Karyolysis and micronucleus which were significantly higher in smokers. (p=0.007

& p<0.001, respectively) Furthermore, the percentage of broken eggs was higher in smokers but it was not significant. (p=0.622)

Among these four variables, micronucleus percentage showed the greatest difference in comparison of smokers and non-smokers group, so it can be reports as the most common nuclear abnormalities in the present study. Also calculating of repair index resulted in significantly higher amount in non-smoker group. (p=0.002)

Table1: Frequency of nuclear changes in smoker groups.

No	karyorrhexis	Karyolysis	broken eggs	micronucleus	Repair index
1	2	2	1	6	0.57
2	1	3	2	8	0.40
3	1	1	3	2	0.40
4	2	2	1	7	0.50
5	2	1	2	9	0.27
6	0	1	1	4	0.20
7	1	1	1	3	0.50
8	1	1	1	7	0.25
9	1	2	2	1	0.38
10	1	1	1	1	1.00
11	2	2	0	4	1.00
12	1	2	1	6	0.43
13	1	3	1	8	0.44
14	1	4	1	7	0.63
15	1	1	2	3	0.40
16	2	1	2	6	0.38
17	2	1	1	3	0.75
18	1	2	1	5	0.50
19	1	2	1	3	0.75
20	1	2	1	7	0.38

Table2: Frequency of nuclear changes in nonsmoker groups.

No	karyorrhexis	karyolysis	broken eggs	micronucleus	Repair index
1	1	1	2	3	0.40
2	0	1	2	1	0.33
3	0	2	1	1	1.00
4	1	1	2	2	0.50
5	0	1	1	1	0.50
6	1	1	1	1	1.00
7	1	2	1	1	1.50
8	0	1	0	2	0.33
9	2	1	1	2	1.00
10	1	1	0	1	2.00
11	1	2	2	1	1.00
12	1	1	1	2	0.67
13	1	1	1	3	0.50
14	1	1	1	1	1.00
15	0	1	1	1	0.50
16	1	0	2	1	0.33
17	1	0	1	1	0.50
18	1	2	1	3	0.75
19	2	1	2	4	0.50
20	1	1	1	1	1.00

Table3: Statistical analysis of Karyorrhexis, Karyolysis, Broken Egg, Micronucleus and Repair index in the studied groups.

Nuclear characteristic	smoking status	N	mean	SD	P value
Karyorrhexis	Smoker	20	1.25	0.550	0.032
	Nonsmoker	20	0.85	0.587	
Karyolysis	Smoker	20	1.75	0.851	0.007
	nonsmoker	20	1.10	0.553	
Broken Eggs	Smoker	20	1.30	0.657	0.622
	nonsmoker	20	1.20	0.616	
Micronucleus	Smoker	20	5.00	2.428	0.000
	nonsmoker	20	1.70	0.923	
Repair Index	Smoker	20	0.5065	0.22160	0.002
	nonsmoker	20	0.7655	0.43093	

DISCUSSION

The present study reported higher percentage of nuclear abnormalities including Karyorrhexis, Karyolysis, Broken egg and micronucleus in smoker group compare to non-smokers and higher amount of repair index in non-smokers group. Also micronucleus was present as the most common nuclear abnormalities in the present study.

Sharma VL et al. reported that, the most common nuclear pathology seen was Karyolysis in all groups and the least common observed was the condensed chromatin. [9] On the other hand, Oliveria et al. showed significant alterations in the frequency of binucleation, and karyolysis in chronic smokers, but not in the frequency of Karyorrhexis. [18]

Also, in a study conducted by Suha et al a significant correlation between the habit of smoking and the frequency of the oral mucosal nuclear changes was observed. [19] Research done by Chan Yu Jung indicates that the frequency of the buccal mucosal nuclear changes has a positive relation with the exposure to consumption of cigarette and alcohol. [20] Also, Sarto et al. reported an increased frequency of nuclear pathology resulting from chromosome breaks and spindle disturbances in healthy in healthy subjects. The result relating to the frequency of nuclear aberrations was approximately twice as high in smokers compared with non-smokers ($p<0.01$) [21]

These previous studies coordinated with current studies shows the variety of nuclear changes is directly related to the smoking habit and depend on the quantity of samples, method of data gathering and other oral habits, reports were varied; but seems like a new subject called Repair Index which is analysing the four obvious nuclear changes at the same time and combine with a minimal invasive method, sure it will have a more accurate result from above changes. In 2010 & 2013 two different studies were done. On road construction workers and painters by Celik et al. In all cases the level of repair index was higher in controlled group than the target group. [22, 23] Based on the best of our knowledge, there was no research toward evaluation and comparison of Repair Index in smokers and non-smokers. So, the result from the present work about this new method awaits further studies in the future.

CONCLUSION

Current study shows, nuclear changes includes Karyorrhexis, Karyolysis, Broken egg and Micronucleus in the smokers group were higher than non-smokers, while the level of Repair Index in smokers were lower than non-smokers.

Base on this study the evaluation of Repair Index may be a new bio-monitoring tool to approach the nuclear changes in population with risk of oral cancer.

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