RESEARCH ARTICLE

Repair Index in Examination of Nuclear Changes in the Buccal Mucosa of Smokers: A Useful Method for Screening of Oral Cancer

Sareh Farhadi, Maryam Mohamadi, Mehdi Mohamadi*

Abstract

Background: Smoking is one of the major risk factors for cancers, especially in the oral cavity. Nuclear changes occur in the early stages of cancer. The aim of this study was therefore to investigate nuclear changes and calculate a "repair index" for the buccal mucosa of smokers. **Material and Methods:** This historical cohort study was conducted by selecting samples including smokers and non-smokers. In addition, the smoker group were divided into 2 subgroups with a smoking history of >10 and ≤10 years. Buccal mucosa smears were obtained and Papanicolaou staining was employed to detect nuclear changes. Micronuclei, karyorrhexis and karyolysis were assessed and eventually a repair index was calculated. Statistical analysis was performed using the t-test. **Results:** In the 60 samples studied, differences were significant in smopkers vs. nonsmokers for micronuclei, (P=0.002) but not karyorrhexis or karyolysis. (P=0.789 and P=0.578, respectively). Also, the repair index demonstrated no statistically significant variation (P=0.107). Comparison of the two subgroups of smokers demonstrated that the frequency of micronuclei in those with a history >10 years was significantly higher and the RI was significantly lower than with ≤10 years (P=0.0001 and 0.04, respectively). While karyorrhexis and karyolysis were also higher in the longer exposure individuals the differences were not significant (P=0.07 and 0.78, respectively). **Conclusion:** Among the nuclear changes investigated, micronuclei proved the more reliable indicator to assess the adverse effects of smoking on the oral mucosa, becoming prominent with increase in smoking history. In addition, while a "repair index" may have benefits for assessment of nuclear damage caused by smoking, further research is necessary in this field.

Keywords: Micronucleus-Karyorrhexis- Karyolysis- repair index

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Introduction

Oral cancer is usually diagnosed in its advanced stages accompanied by severe complications (Abbasi et al., 2013) because there are no primary diagnostic markers. (Rivera et al., 2017) Therefore use of non-invasive diagnostic techniques in the early stages of cancer development appears to be important.

In many studies, smoking has been considered a major risk factor for cancers of the buccal mucosa (Chiba, 2001; Chang et al., 2000; Stewart et al., 2003; Parkin et al., 2005; Taybos, 2003; Silverman, 2003). Cigarettes contain carcinogenic substances that can affect the DNA in the nucleus, especially in the oral tissue. Nuclear changes occur in the early stages of cancer. These nuclear changes in the buccal mucosa cells were first introduced by Stich in this field (Stich, 1983) and are now used as a biomarker in many cases for genetic damage. The advantage of this method is non-invasiveness, rapidity and ease of application (Kamboj, 2007). In addition, it provides investigation of nuclear changes in cells exposed to carcinogens in the pre-clinical symptoms of cancer (Stich tel, 1984; Saeed et al, 2012). To date, many studies have been performed on nuclear changes as micronucleus in smokers (Kashyap et al, 2012). Micronucleus assay was established by Schmid, who stated that the micronucleus in the cell nucleus is similar but in a smaller size. They have round-to-oval shapes with well-defined margins and the same color as the nucleus of the cell, but their nucleus size is one-third of the main nucleus (Kamboj et al, 2007). Other nuclear changes include karyorrhexis which is a form of nuclear change in which nuclei are pyknotic or partially pyknotic and sliced and necrotic cell nuclei completely disappear with time. Karyolysis also shows the degree of cell death in which basophils of the chromatin disappear (Kumar et al, 2010); changes in broken eggs nucleus occur as a result of damage to the nucleus and the nucleus can be seen as worn (Tolbert et al., 1992). There are some reports regarding significant differences between the frequencies of micronucleus in the buccal mucosa cells of smokers compared to the control group (Kamboj et al., 2007; Stich et al., 1982; Majer et al., 2001; Rosin et al.,

*Oral and Maxillofacial Pathology Department, Dental Branch of Tehran, Islamic Azad University, Tehran, Iran. *For Correspondence: ddsmehdimohamadi@gmail.com*

1987). However, studies to evaluate other nuclear changes in this field simultaneously in smokers are very limited. In addition, calculation of "repair index" allows simultaneous survey of these changes, too. To the best of our knowledge, this index has not considered for smoking. Therefore this study was undertaken to investigate nuclear changes in the buccal mucosa of smokers, calculate the related repair index and compare the results with non-smokers.

Materials and Methods

Sixty patients, including 30 smokers in the case group and 30 non-smokers as the controls, were selected in the Dental Branch of Tehran, Islamic Azad University. Also, smokers group were divided to 2 subgroups with history of >10 and ≤10 years. All the samples were male in order to eliminate the gender effect. Also the two groups were matched in their age. Subjects with recent viral diseases, those taking any specific drug, drug addicts, those with occupations in contact with chemicals and those undergoing radiotherapy were excluded. To collect data in the present historical cohort study, after interviewing and taking a signed consent form, samples were obtained from the oral buccal mucosa by scraping with a wet spatula and the smears were examined after Papanicolaou staining under an optical microscope at ×400 magnification. The case group subjects were smokers with a history of smoking 20 cigarettes per day at least for 5 years (Buamert et al, 2010).

Before scraping of the buccal mucosa cells, the subjects were asked to wash their mouth thoroughly with water. Buccal mucosa cells were scraped by a wet spatula on small clean glass slides. The smears on slides prepared were fixed using Pathofix spray. The slides were allowed to dry at room temperature. Then Papanicolaou staining was used for micronucleus assay. Evaluation of nuclear changes was conducted using the criteria of Tolbert et al ., (1992). In randomly selected fields, 500 cells were counted under a magnification of ×400 (JalayerNaderi et al, 2012). In both groups, mean percentages of nuclear changes, including micronucleus, karyorrhexis, karyolysis and broken eggs, was reported and eventually repair index was reported using the formula RI = KR + KL/BE + MN. T-test was used to compare the results between the two groups.

Figures 1 to 4 present the nuclear changes under ×400 magnification:

Table 1. The Mean Percentages of Nuclear Anomalies and Repair Index in The Buccal Mucosa Cells in Smokers or Non-Smokers

	Smokers	Non-smokers	P-value
mean percentages of MN	3.70±1.22	2.73±1.09	0.002
mean percentages of KR	1.57±0.57	1.51±0.62	0.789
mean percentages of KL	1.37±0.53	1.29±0.59	0.587
mean percentages of BE	0.98±0.56	0.90±0.38	0.748
mean percentages of RI	0.68±0.34	0.82±0.34	0.107

Results

60 samples were evaluated in this study with mean age of 38.4±2.9. Table 1, demonstrates mean percentages of nuclear anomalies and repair index in the buccal mucosa cells in smokers or non-smokers. Statistical analysis clear that, mean percentage of micronucleus in the buccal mucosa cells of smokers is significantly higher than nonsmokers. (P=0.002) Although, other nuclear anomalies as Karyolysis, Karyorrhexis and Broken eggs even have higher rate of presence but do not show significant differences vs. nonsmokers. (P=789, P=0.578 and P=0.748, respectively) Also, Repair indexes has lower level in smokers but the difference is not statistically significant difference vs. nonsmokers (P=0.107).

In order to better interpretation of the results, the given data are shown as scatter plots, too (Diagram 1-4).

The frequency of nuclear abnormalities assessed in smoker group including two subgroups of smoker with history of >10 and ≤10 years is shown in Table 2. The given results related to statistical analysis exhibit that MN and BE of smokers with history of >10 years were significantly higher and RI of this subgroup was significantly lower than smokers with history of ≤10 years. (P=0.0001, 0.01 and 0.04, respectively) also, KR and KL of smokers with history of >10 years were higher than

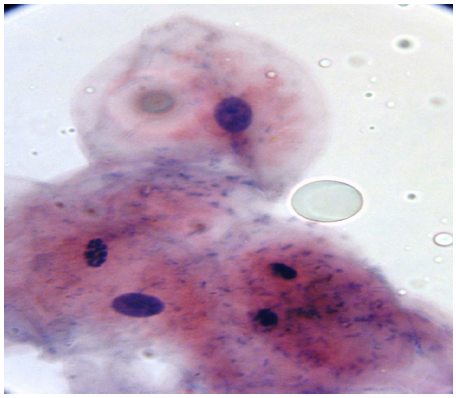


Figure 1. Karyorrhexis is Marked by an Arrow

Table 2. The Mean Percentages of Nuclear Anomalies in The Buccal Mucosa Cells of Smokers in Subgroups with History of >10 Years and ≤10 Years

	Smokers with history of >10 years	Smokers with history of ≤10 years	P-value
mean percentages of MN	4.74±0.83	2.90±0.80	0.0001
mean percentages of KR	1.78±0.53	1.40±0.56	0.07
mean percentages of KL	1.33±0.57	1.39±0.52	0.78
mean percentages of BE	1.26±0.67	0.76±0.33	0.01
mean percentages of RI	0.53±0.18	0.79±0.40	0.04

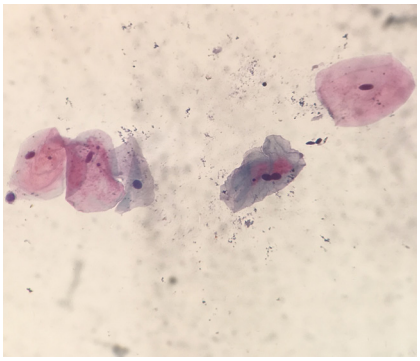


Figure 2. Broken Eggs are Marked by an Arrow

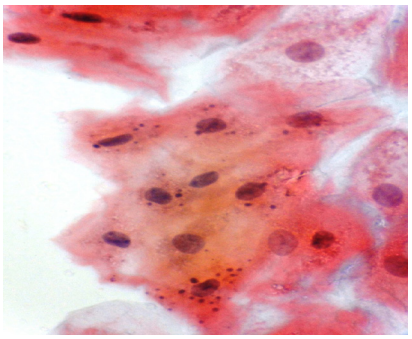


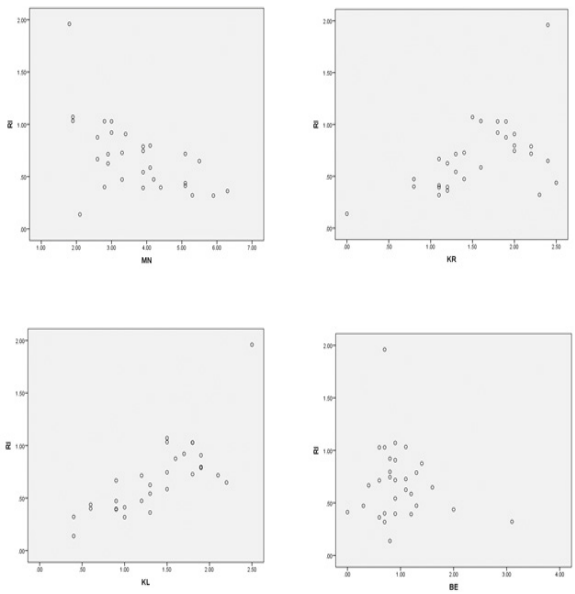
Figure 3. Micronucleus and Karyorrhexis are Marked by an Arrow

other subgroup although, the difference was not significant (P=0.07 and 0.78, respectively).

Discussion

This study showed an increase in nuclear changes, including micronucleus, karyolysis, karyorrhexis and broken eggs in smokers compared with non-smokers. Furthermore, a higher level of repair index was shown in non-smokers compared to smokers.

Sharma et al., (2013) reported karyolysis as the most



Diagrams 1-4. Scatter Plot Diagrams Related to Mean Percentages of MN, KR, KL and BE

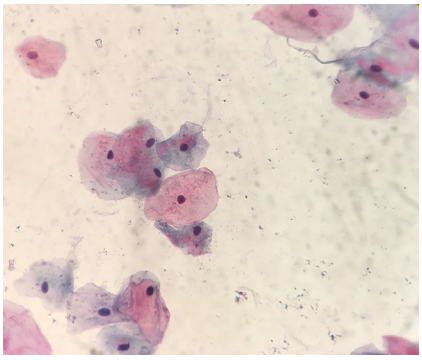


Figure 4. Cytological Appearance of Normal Mucosa Related to Control Group.

common nuclear change and condensing chromatin as the rarest one. On the other hand, Oliveria et al., (2012) demonstrated a significant difference in karyolysis and binucleus in smokers compared with the control group, but this difference was not significant for karyorrhexis. In addition, in a study by Susha et al., (2004) there was a significant correlation between smoking and oral mucosa nuclear changes.

A study by Jyoti et al., (2015) showed a direct relationship between buccal mucosa nuclear changes and exposure to cigarette smoke and alcohol. Furthermore, Sarto et al., (1987) reported an increase in nuclear changes almost twice that in smokers vs. non-smokers. In this regard, Farhadi et al., (2016) reported significant differences between smokers and non-smokers in nuclear changes, too.

These previous studies, in line with the present study, show that increased nuclear changes may have a positive correlation with smoking. It seems sample size, smoking rate, method of sample selection and oral habits can explain the differences between these results. Evaluation of nuclear changes in the buccal mucosa cells using this non-invasive method can provide the possibility of cytological studies in cells exposed to carcinogens through this before the onset of clinical manifestations of cancer (Stich, 1984). Currently, micronucleus is considered as a biomarker in genetic pathologies of carcinogens (Palaskar et al, 2010), but it is believed that evaluation of other nuclear changes provides a better examination in this field. Recently, repair index has been introduced for examination of four nuclear changes (MN, KR, KL, BE), simultaneously. Celik et al., (2010, 2013) in two separate studies on workers in road construction and painters, assessed the repair index and showed that this index was lower in the case group than in the control group. KL and KR represent the degree of cell damage, leading to dismantling of nuclear and ultimately cell death, which occur during the process of apoptosis and cell death by an injury, but MN and BE in most cases are indicative of cell damage. Therefore in cell damage, comparing KR and KL to MN and BE showed smaller amounts, consistent with two studies by Celik et al. To the best of our knowledge, this index has not been evaluated in relation to smoking and present study is the first study on the subject. According to the results of the present study that are consistent with those of studies by Celik, (2010)

it can be useful in evaluation of cell pathology in addition to nuclear changes. Furthermore, in the present study the number of MNs was consistent with the study by Jalayer Naderi et al., (2012); in both studies the frequency of MN was significantly higher in smokers than controls.

In conclusion, the present study showed that, among the nuclear changes investigated, micronucleus was a more reliable indicator to assess the adverse effects of smoking on oral mucosa and this reliability was more prominent with increasing of smoking history. In addition, "repair index" can probably be used for detection of nuclear damage caused by smoking. However, further research is required in this field.

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