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ORIGINAL ARTICLE

Effect of orthodontic fixed appliances on epithelial cells of lower lip oral mucosa during orthodontic treatment

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Abstract:

Background and aim: The aim of this study was to find out influence of the orthodontic devices on the oral epithelial cells.

Materials and methods: Cells of lower lip oral mucosa from 32 orthodontic patients were collected by exfoliative cytology in three times: Day 0 (just before appliance placement), and 14 and 60 days after appliance insertion. Nuclear (NA) and cytoplasmic (CA) areas, NA/CA ratio, cell morphology, and cellularity of smears were analyzed by using cytologic and cytomorphometric methods.

Results: The NA of the cells adjacent to orthodontic devices diminished after appliance placement, and reached to its lower level in day 60 ($p = 0.000$). Investigated epithelial cells showed insignificant changes in CA. Type II inflammatory smears, according to Papanicolau, increased insignificantly after the treatment initiation. Superficial epithelial cells, were predominant cell type in day 14 and 60 ($p= 0.002$)..

Conclusion: Orthodontic appliances cause reduction in NA and induce epithelial hyperkeratosis in lower lip mucosa.

Keywords: Epithelial cells/Exfoliative cytology/Cytomorphometry/ Buccal mucosa/ Orthodontic appliance.

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INTRODUCTION

The oral cavity is covered with a mucous membrane [1]. Epithelial cells of oral cavity may undergo some changes due to diseases, infections, injuries, and metabolic or systemic conditions, resulting in mucosal lesions [2]. Occasionally, these lesions can be a sign of dangerous conditions such as a malignant neoplasm. But most of them develop subsequent to local irritation, and usually, trauma is the main factor that results in clinical alterations of mucosa and common oral lesions [3, 4]. Some lesions are result of acute traumatic injuries, but long lasting irritants with low intensity usually precede chronic conditions which are called reactive lesions [5].

Orthodontic appliances (ligatures, braces, wires, etc.), are one of main sources of trauma in oral cavity. Friction between brackets and mucosa is a kind of chronic trauma [5] and also may cause buccal mucosal ulceration which is one of the most common complaints of patients during orthodontic treatment [6, 7]. On the other hand, presence of orthodontic appliances in a healthy mouth increases plaque accumulation, and changes microbial flora of oral cavity which may deteriorate periodontal and mucosal normal biology [8].

Lower lip oral mucosa is a common site of irritation due to orthodontic appliances. Cellular changes that proceed these insults can be studied by exfoliative cytology. Clinical use of this technique was restricted because it was interpreted subjectively, and difficult to find abnormal cells in smears. However, the researchers show renewed interest to this easy, and painless technique because of advantages such as minimal invasiveness, no need for local anesthetic, and its complementarity with other procedures such as cellular-molecular biology, cytomorphology, cytomorphometry, and immunohistochemistry [8, 9].

Few studies have performed histological assessments on this issue in orthodontics patients under a comprehensive treatment process. The aim of this study, therefore, was to evaluate the effect of the orthodontic appliances irritation, before and during treatment, on the epithelial cells of clinically normal lower lip oral mucosa using cytological and cytomorphometrical techniques.

MATERIAL AND METHODS

According to the study performed by Arruda et al. [10], and getting into consideration of a α equal to 0.05 and power of 80%, a thirty two patients sample size was calculated.

Therefore, 32 patients who referred to the department of orthodontics of dentistry school in Tabriz University of medical sciences, and needed fixed orthodontic treatment, were selected. Informed consent was filled by the patients. Exclusion criteria consisted of: history of smoking, using alcoholic drinks, diabetes, anemia, or any other systemically compromising illness. Patients, who consumed antibiotic drugs or estroidal agents during treatment, were also excluded [8, 9].

All brackets were metal preadjusted ones (American Orthodontics, Mastery Mini Master Series TM, USA) and micro-etched metallic bands (3M Unitek Victory series), along with NiTi arch wires (G&H Company, USA) were used during this study. Canine brackets had hooks and plastic O-rings (American Orthodontics, USA) were used to ligate the arch wires to brackets.

NiTi arch wires are usually used for 60 days, at the beginning of orthodontic treatment, so, the study design did not impose any delay in patients' treatment duration.

Before oral cells collection, the patients were instructed to rinse their mouth with water to remove excess of debris. Epithelial cells were collected for smear from lower labial oral mucosa, in three times: just before placement of braces and also after 14 and 60 days that braces were in place.

The epithelial cells were collected using cytobrush by scraping and slight rolling movement in the lower lip oral mucosa region. The cells collection was done by the post- graduate student of orthodontics and the smear was prepared by pulling the cytology brush over a glass slide [11].

The collected material was immersed in 96% alcohol for 20 minutes to be fixated. The samples were hold in 4 degrees of centigrade. Smears were then stained with Papanicolau staining [11].

A pathologist studied the smears. Each slide was assessed using the light microscopy by binocular Olympus microscopy TM (Olympus Cl 4- BI45- T- 2, Taiwan) linked to a computer that had the Motic 2 Image plus version 2- OML camera software at x 400 magnification.

The assessment consisted of qualitative analysis including cell morphology (using Papanicolau technique) and cellularity (the type of predominant cell) and quantitative analysis (the nuclear (NA) and cytoplasmic (CA) areas and NA/CA ratio by image analysis system). The nuclear (NA) and cytoplasmic (CA) areas were obtained by drawing around areas the nuclear and cell boundaries using the digitizer cursor.

Statistical analysis

Mean and standard deviation were assessed for the values of descriptive statistical analysis, and evaluated by the Mauchly's test of Sphericity to check normal distribution of the data. Because the obtained data did not have a normal distribution, non-parametric tests were used. The Friedman test was used to compare the cell morphology in different times, and if the differences were significant, Wilcoxon signed ranks test was done. Comparing NA and CA in different times was done by the repeated measures ANOVA, and if the difference was significant, subsequent T-test analysis was done. Level of significance was considered a *p* value equal or below 0.05.

RESULTS

This study assessed 4800 cells obtained from thirty two patients. Mean values in the nuclear area showed statistically significant difference. The NA was diminished in cells under the friction and irritation of orthodontic appliances in each time after the first time, and reached its lower level in day 60 after appliance placement (F=17.953, df= 2, *p* value= 0.000). The T-test showed that difference in NA between day 0 and day 14 (*p* = 0.000) and between day 0 and day 60 (*p*= 0.000) were statistically significant (Table 1).

Mean values in the cytoplasmic area increased 14 days after appliance placement, and then had a reduction to under the zero day in day 60, but the changes in size were not significant (F=0.780, df= 1.072, *p*= 0.399). Mean NA/CA decreased in day 14 and got to its least amount in day 60 and the differences were significant (F= 3.883, df = 2, *p* = 0.026) (Table 1), also in pairwise comparisons only the difference between day 0 and day 60 was statistically significant (*p* = 0.009).

Table 1: Cytological measurements in epithelial cells in different times.

Groups variables	Day 0 Mean± SD	Day 14 Mean± SD	Day 60 Mean± SD	P value
NA *	2077 ± 314.68	1762 ± 225.34	1703 ± 263.21	0.000
CA **	74395 ± 13746.12	88561 ± 18892.53	69666 ± 14652.96	0.399
NA/CA ***	0.028 ± 0.005	0.025 ±0.006	0.025 ± 0.005	0.026

*NA: nuclear area
**CA:cytoplasmic area
***NA/CA: nuclear/cytoplasmic area ratio

The smears also were classified according to Papanicolau [8]:
Class 0: material insufficient or inadequate for analysis
Class I: smear normal,
Class II: smear normal with inflammatory changes,
Class III: dysplastic changes-smear suspect,
Class IV: strongly indicative but not conclusive for malignancy,
Class V: smear malignant.
In our study, class I smears were predominant one in all times, but the number of class II smears increased in times 14 days and 60 days after appliance placement, and the most number was related to the day 14, but the differences were not statistically significant (df = 2, p= 0.338) (Table 2).

Table 2. Characterization of smears by Papanicolaou system classification.

Papanicolaou classification	Day 0 n	Day 0 %	Day 14 n	Day 14 %	Day 60 n	Day 60 %
Class I	30	93.8	26	81.2	27	84.4
Class II	2	6.2	6	18.8	5	15.6
Class III	0	0	0	0	0	0
Class IV	0	0	0	0	0	0
Class V	0	0	0	0	0	0
Total	32	100	32	100	32	100

Epithelial layer of oral mucosa consists of four distinct cellular types: basal cells, para-basal cells, intermediate cells (subsurface cells), and superficial cells. In days14 and 60, superficial epithelial cells were dominant quantitatively with a significant difference (df = 2, *p*=

0.002). The difference was significant when comparing day 0 to day 60 (p=0.001) and between day 0 and day 14 (p=0.008), but it was not statistically significant between days 14 and 60 (p= 0.467). The most number of superficial cells was observed in day 14.

DISCUSSION

In our study, we used cytological and cytomorphometrical methods to find out effect of orthodontic appliance irritation on epithelial cells of oral cavity. The results showed that although the NA/CA ratio did not change significantly, the NA of the cells in vicinity of orthodontic appliances was reduced significantly after the beginning of the treatment. This NA reduction was similar to findings of Arruda et al. 10, who showed that NA of the cells in contact with brackets bonded to the incisors and the band tube reduced.

Orthodontic braces can give rise to cellular alterations. Biocompatibility is essential to prevent irreversible tissue changes, and exfoliative cytology can be used effectively to trace histological changes. In 2009, Pereira et al. 8 observed that the stimulus of orthodontic braces lead to reduction of the nuclear area, increase in the cytoplasmic area, and a lower nuclear/cytoplasmic ratio of epithelial cells that were in contact with the orthodontic accessories. Similarly, the results of our study showed that orthodontic appliances could affect the oral mucosal cells and revealed it substantially as nuclear changes.

NA and CA were both reduced during treatment in day 60 after bracket placement. Although the changes of CA were insignificant, this type of cellular change is referred as atrophy. Cellular atrophy is a kind of response to environmental pressures to reduce cell need for energy by discontinuing most cellular activities, and therefore, let the cell to survive in such situations. In day 60, besides the NA decrease, the reduction in the CA occurred, and this shows that braces impose environmental irritation to mucosal cells of oral cavity. Thus, these results suggest that mechanical friction might also be a potential factor, besides other ones, such as reduced blood supply, nutrition deficiencies, hypoxia, and compression, which changes cellular environment and causes NA and CA changes. Arruda et al. 10 also observed a similar condition in mucosal cells adjacent to orthodontic braces.

Morphological analysis revealed that there was an insignificant increase of class II (inflammatory) smears in the region, which may be due to a nonspecific inflammatory response promoted by common irritants in oral cavity besides installed appliances.

The qualitative evaluation in our study showed a predominance of superficial layer cells in day 14 and 60 after treatment beginning. Basal layer cells usually are scarce in the smears obtained by exfoliative cytology because they lay deeply in an intact oral epithelium and usually are not removed by the cytobrush. Mei et al. 12 also observed light heperkeratosis of epithelial cells in contact with orthodontic braces. Silverman et al. 13 reported that in the oral mucosa, intermediate layer cells are predominant. In the study of Arruda et al. 10 in 2011, in the area in contact with the mandibular incisor brackets, and in the bottom area of the vestibule (control region), there was a balance between the superficial and intermediate layer cells, and there was a predominance of superficial cells in the area near the band tube. High numbers of superficial cells in any mucosal region is an indicative sign of keratinization process occurring in corneum stratum to make it thicker, which is an adoptive reaction to environmental irritants. Kwon et al. also found that chronic irritation caused by tobacco precedes greater number of superficial layer cells 14. Pereira et al. observed an increase in the number of superficial cells in the mucosa adjacent to orthodontic braces 8.

Few studies have evaluated effect of orthodontic appliances irritation in cellular levels. We tried to evaluate adoptive response to orthodontic braces that occurs during a real orthodontic treatment process and considering most common accessories that are used for this type of treatment. Biopsy is the gold-standard method for assessment of mucosal pathologies, but non-invasive method of exfoliative cytology is sensitive enough for detection of cellular changes in an apparently healthy mucosa. Exfoliative cytology assessments could be used as a para-clinical tool to evaluate cell damage before clinical signs appear. Appliances are physical agents that irritate oral mucosa, and higher amount of keratinized cells, and inflammatory smears, found in the mucosa may be explained by the fact that the area in vicinity of braces is under considerable friction.

CONCLUSIONS

Placement of orthodontic braces induces cellular changes in lower lip oral mucosa. Diminution of the nuclear area and keratinization are the main changes that occur subsequent to orthodontic appliance irritation. Exfoliative cytology is a useful method to investigate cellular alterations and can be integrated to routine clinical procedures to help evaluation of treatment adverse effects in cellular level.

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