—— ORIGINAL ARTICLE ——

A cytological analysis of the oral mucosa adjacent to orthodontic devices

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ABSTRACT

Objectives: The objective of this study, was to evaluate the cytometry and cytomorphology of oral mucosa epithelial cells adjacent to orthodontic accessories using liquid-based exfoliative cytology. **Materials and Methods:** We placed metallic brackets on the upper pre-molars with stainless steel wires and metal or elastic ties on 20 people of both sexes, with an average age of 22.7 years. We evaluated nucleus area (NA), cytoplasm area (CA), and NA/CA ratio before installation (T0, baseline), 30 days after installation (T1), and 30 days after the accessories were removed (T2). **Results:** We verified a reduction in NA and an increase in CA with both types of ties at T1 ($P<0.05$ vs. T0), with a tendency to return to baseline values at T2 ($P<0.05$ vs. T0). At T1 and T2 we observed a predominance of surface cells over subsurface cells. **Conclusions:** The orthodontic appliance causes adaptive cell changes characterized by an increased level of keratinization of the oral mucosa, with a tendency to return to the normal state after removal of the mechanical stimulus of the orthodontic devices.

Key words
Bracket, exfoliative cytology, mucosa

INTRODUCTION

During orthodontic treatment, it is necessary to install accessories such as brackets, arches and ties. These accessories, which come in contact with the oral mucosa, can lead to irritation, hematomas, erosions, and even ulceration of the oral mucosa, which can cause patients to have discomfort and pain.[1-3] Pain arising from friction between the mucosa and these accessories is the reason why some patients abandon orthodontic treatment,[2] although this discomfort tends to disappear after the first four weeks of treatment as the tissues adapt.[4]

Exfoliative cytology is a non-invasive alternative for examining oral lesions with a simple protocol based on the Papinicolau staining technique that is accepted as a reliable method.[5] Relative to exfoliative cytology traditional method, Liquid-based Exfoliative Cytology (LEC) has the advantage of reducing blood contamination, inflammatory cells and mucus, thus allowing a clear base and greater sensitivity, and thereby improving smear quality.[6,7]

Orthodontic treatments should produce as little tissue damage as possible; however, only a small number of studies have examined changes to the oral mucosa caused by friction with orthodontic appliances. Therefore, this study's objectives were to perform cytomorphometric and cytological analyses of the oral mucosa epithelium adjacent to metal brackets, stainless steel wires, and elastic or metal ties.

MATERIALS AND METHODS

The study's experimental protocol was approved by the Research Ethics Committee of (the name was omitted), case no 1389/08.

Sample

We selected 20 participants with a mean age of 22.7 years old (range, 14-36), including 10 women and 10 men. The following exclusion criteria were applied: A history of smoking and alcoholism, debilitating diseases, use of

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mouthwash, treatment with antibiotics or steroids, use of a total or partial removable prosthesis and the presence of any type of lesion in the oral mucosa. Everyone who participated in the research signed an informed consent form.

Installation of the accessories

We placed metal brackets (Generus® Straight Wire "Roth" – GAC Orthomax, Bohemia, NY, USA) on each person's first and second upper right and left pre-molars. To position the brackets on the same horizontal axis, we used a 0.020-inch-thick stainless steel round guide wire (Shiny Bright®, TP Orthodontics, La Porte, IN, USA), which was inserted into the brackets' slots before polymerization of the adhesive (Transbond XT®, 3M Unitek Orthodontic Product, Monrovia, CA, USA). After polymerization, we inserted another segment of the same stainless steel wire into the brackets' slots, folding them on their mesial and distal ends to prevent them from coming unwound. We fixed these wires with 0.012-inch-thick metal ties (Dentalloy®, TP Orthodontics, La Porte, IN, USA) on the left side and elastic ties (Mini Stix Non-Coated®, TP Orthodontics, La Porte, IN, USA) on the right side [Figure 1].

Liquid-based exfoliative cytology

We collected samples at three time points: Before installing the accessories (T0); 30 days after installing the accessories (T1); and 30 days after their removal (T2). Before starting each collection, we asked patients to rinse their mouth with water for 20 seconds. Next, we used a Universal Collection Medium (UCM) kit from the DNA-CITOLIQ system (Digene Brasil LTDA., São Paulo, Brazil) to collect mucosal cells by means of friction with a brush on the mucosa in the region adjacent to the brackets, moving the brush softly in five circular movements, clockwise. After applying this friction, we placed the brushes in the UCM solution, in accordance with the manufacturer's instructions.

Next, we transferred 200 μl of the loaded UCM solution to a polycarbonate membrane (Filtrogene, Digene Brasil LTDA., São Paulo, Brazil) using a pipette and a metal press (Prepgene, Digene Brasil LTDA., São Paulo, Brazil). We maintained the press closed for 10 seconds to generate an imprint of the material on the histology

Figure 1: Photographs of brackets and write fixed with (a) metal and (b) elastic ties

slide (Lamigene, Digene Brasil LTDA., São Paulo, Brazil). We immersed the slides in an absolute ethyl alcohol solution for 20 minutes to fix them and then stained them using the Papanicolau technique.[8]

Cytomorphometry

We analyzed the smears using a binocular optical microscope (Olympus BX50, Center Valley, PA, USA) with a WH 10×-H/22 lens and PLAN 40×/0.65 objectives. A calibrated examiner examined 50 cells on each slide.[9] Areas in which the cells appeared doubled, agglomerated, or superimposed were avoided. Image capture of the cytological fields was performed with 400 times amplification using a CCD camera (model DXC-107A, Sony, New York, NY, USA). We evaluated the images using the Image Pro Plus program (version 4.5.029, Media Cybernetics, MD, Silver Spring, USA), in which, we defined the nucleus and cytoplasm of each cell. We then measured nucleus area (NA) and the cytoplasm area (CA) in μm^2. Finally, we calculated the ratio between the nucleus area/cytoplasm area (NA/CA) for each cell.[10]

Cytomorphology

For this analysis, we used the same binocular microscope used for cytomorphometry with a WH 10×-H/22 lens and PLAN 10×/0.25, 20×/0.40 and 40×/0.65 objectives. The slide was scanned completely and the smears were classified on the basis of the predominance of cells present, according to Sugerman and Savage.[11]

We performed a qualitative analysis of the smears to identify Papanicolaou's cytological criteria of malignancy.[8] Briefly, class 0 indicates insufficient or inadequate material for analysis, class I indicates a normal smear, class II indicates a normal smear with inflammatory changes, class III indicates dysplastic changes (suspicious smear), class IV indicates that the smear is strongly indicative of malignancy but not conclusive and class V indicates that the smear is malignant.

Statistical analysis

We tabulated all the data and performed statistical tests using the Statistical Package for the Social Science 13.0 for Windows program. First, we performed the Kolmogorov-Smirnov test to verify whether the samples for the different groups (metal and elastic ties) and time points (T0, T1 and T2) were normal and Levene's homogeneity test to evaluate whether the data had variance homogeneity. Next, we applied the two-way analysis of variance (ANOVA) to compare the NA, CA and NA/CA variables according to type of tie (metal and elastic) and time point (T0, T1 and T2). When the ANOVA indicated that there was significant variance between the groups, we used Tukey's HSD multiple comparisons test to detect which of the groups differed from each other. To analyze the predominance of cells present, we determined the difference of two proportions test.

RESULTS

The NA, CA, NA/CA means and standard deviations for the two types of ties at each of three experimental time points are reported in Table 1. Tukey's HSD multiple comparisons test results for NA, CA, NA/CA are reported in Table 2.

We observed a reduction in NA values ($P<0.05$) and an increase in CA values ($P<0.05$) between T0 and T1 for both metal and elastic ties [Table 2]. These data verify that after 30 days of contact and friction with orthodontic devices, oral mucosa cells examined by exfoliative cytology responded to this irritation by increasing cytoplasmic volume and reducing nuclear volume. In addition, there was an increase in NA values ($P<0.05$) and a reduction in CA values ($P<0.05$) between T1 and T2, 30 days after removing the orthodontic devices [Table 2]. These observations demonstrate that mucosa cells tend to return to their normal morphology after the mechanical irritant has been removed. For the group with elastic ties, NA and CA values did not change significantly ($P>0.05$) between T0 and T2, which indicates that oral mucosa cells can recover completely to their normal morphology within 30 days after the removal of orthodontic devices combined with elastic ties [Table 2].

None of the variables (NA, CA, and NA/CA) differed significantly between men and women at any of the time points. Moreover, the NA/CA ratio did not show any statistically significant group differences ($P>0.05$) at any of the experimental periods.

With respect to the predominance of cells present in the smears, no slide was found with a predominance of cells of the spinosum and basale stratum. In T0, the number of slides with a predominance of surface and subsurface cells was similar ($P>0.05$) in both metal and elastic ties groups. Between T1 and T2, a significantly higher number ($P>0.05$) of slides showed a predominance of surface cells only in the group with metal ties. Between T1 and T2, was not observed a statistically significant change in the predominance of cells present in the smears for both groups [Table 3].

Smears that were examined exhibited no instances of Papanicolaou Classes 0, III, IV, and V when the cytologic criteria for malignancy were determined. Classes I (90.83%) and II (9.16%) were observed at all time points, and no significant difference was noted among the groups ($P>0.05$).

DISCUSSION

The present study, demonstrated that orthodontic brackets and the wires associated with metal and elastic ties have the potential to induce an adaptive response in the oral mucosa. We observed the development of hyperplasia and light epithelial hyperkeratosis in response to the mechanical stimulus of orthodontic accessories.

We chose to expose the participants' oral mucosa to orthodontic devices for a period of 30 days because the period needed for total renewal of the epithelium of the cheek mucosa is 25 days. This epithelial renewal occurs under physiological conditions or when influenced by external stimuli. This period is the one needed for young basal epithelial cells to differentiate, reach the surface-most beds, and exfoliate.[12]

In analyzing the effects of the mechanical irritation promoted by orthodontic accessories on the NA and CA of mucosal epithelial cells, we found that NA decreased and CA increased significantly over a 30-day period after installation of brackets. These results are in line with those of a study in which Pereira *et al.*[13] observed similar cellular changes caused by friction between the oral mucosa and metal and ceramic brackets. These changes to epithelial cells are part of an adaptive process that occurs together with an increase in the number of surface and subsurface cells[14] of the

Table 1: Descriptive statistics for NA, CA, and NA/CA at each time point

Variables	Time	Metallic (*n*=20)		Elastic (*n*=20)	
		Mean	S.D.	Mean	S.D.
NA	T0	66,853	7,697	64,073	5,805
	T1	52,208	7,333	53,321	4,544
	T2	61,594	6,765	59,637	4,173
CA	T0	2,286,349	227,107	2,338,914	188,439
	T1	2,654,724	205,881	2,608,837	193,384
	T2	2,410,223	196,529	2,439,367	181,960
NA/CA	T0	0,030	0,003	0,054	0,081
	T1	0,028	0,036	0,021	0,002
	T2	0,026	0,002	0,026	0,003

NA – Nucleus area; CA – Cytoplasm area; T – Time; S.D. – Standard deviation

Table 2: Intra-group analysis of NA, CA, and NA/CA using Tukey's HSD test

Variables	Metallic			Elastic		
	T0×T1	T0×T2	T1×T2	T0×T1	T0×T2	T1×T2
NA	0.000122*	0.000441*	0.000122*	0.000122*	0.004011*	0.000129*
CA	0.000122*	0.034358*	0.000122*	0.000122*	0.142981	0.001028*
NA/CA	0.999991	0.999498	0.999971	0.050000	0.128840	0.998774

NA – Nucleus area; CA – Cytoplasm area; T – Time; *Statistically significant ($P<0.050$)

Table 3: Quantity of smears according to cellular predominance for each tie-type group and each time point

Time\Tie	Superficial	Sub-superficial	Spinosum	Basale
To metallic	11	9	0	0
To elastic	12	8	0	0
T1 metallic	17	3	0	0
T1 elastic	16	4	0	0
T2 metallic	14	6	0	0
T2 elastic	14	6	0	0
Total	84	36	0	0

oral mucosal epithelium (hyperplasia together with reactive hyperparakeratosis), following interaction with an irritant.

The CA of epithelial cells increases to allow an augmentation in metabolism and the synthesis of proteins important to tissue adaptation.[12] Shabana *et al.*[15] also observed this process, verifying an increase in the size of cells subjected to traumatic lesions in comparison to normal cells.

After removing the mechanical stimulus, the epithelial tissue should return to its normal structure.[12] Our study, revealed a tendency to return to conditions of morphological cell normality by 30 days after removal of orthodontic devices, as demonstrated by significantly increased NA and significantly reduced CA at T2 relative to T1 [Table 2]. Pereira *et al.*[13] also observed this tendency of epithelial cells to return to normal conditions after withdrawal of orthodontic accessories. The lack of a gender effect in our study agrees with the findings of Cowpe *et al.*[9]

According to Berstein and Miller,[16] normal cell morphology is characterized by abundant cytoplasm and a small, centralized singular nucleus. On the other hand, in oral mucosa cells that have some degree of dysplasia, there are morphological changes. Using cytomorphometric analysis, Ramaesh *et al.*[14] demonstrated that dysplastic lesions and spinocellular carcinomas are associated with a gradual reduction in the diameter of the cytoplasm, relative to normal cells, in conjunction with a gradual shrinkage of nuclei. In our study, we did not observe changes in the morphological characteristics of smear cells obtained from mucosa that was in contact with orthodontic accessories. In fact, 90.83% of the smears were classified as Papanicolaou class I (normal) and 9.16% as class II (inflammatory). These findings are in line with those of Pereira *et al.*,[13] who verified that cellular changes in the oral mucosa arising from friction with orthodontic accessories are not suggestive of malignant transformation.

It is also important to consider other factors beyond

mechanical stimulation that might contribute to the cellular changes observed in this study. One of these factors is cytotoxicity due to ion liberation from metallic orthodontic accessories. Prior studies[17-20] have demonstrated that ions liberated by stainless steel orthodontic accessories (18% chromium and 8% nickel)[21] have a genotoxic effect on oral mucosa cells. However, this effect is reversible owing to cellular DNA repair mechanisms.[17,18]

CONCLUSIONS

Metal brackets, stainless steel wires and metal and elastic ties can induce cytomorphometric and cytomorphological changes to adjacent oral mucosa cells. This fact suggests an adaptive response to the physical stimulus, which is characterized by reactive hyperparakeratosis of the epithelium but which regresses when the irritant stimulus is withdrawn. Considering all the esthetic, functional and oral health benefits that orthodontic treatment brings and the fact that the mucosa's adaptive response to the injury caused by these accessories is reversible, we believe that the benefits of this treatment outweigh the disadvantages of discomfort and possible lesion of the oral mucosa.

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