

REVIEW

## Cytomorphometric analysis for Metal Bracket Effects on Human Buccal Mucosa

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### Abstract :

**Objective:** To find out effect of metal brackets on the epithelial cells of the buccal mucosa as compared to normal mucosa without any brackets.

**Patients and Methods:** Oral mucosal smears were obtained for patients visited for orthodontic treatment using a cytobrush. The study group consisted of P1: patients with no brackets, P2: 60 days after placement of metal brackets, P3: 30 days after removal of brackets. 30 individuals of both sexes who are undergoing fixed appliance therapy with bonded brackets were selected. With the use of exfoliative cytology, morphometric and morphologic changes in buccal mucosa cells adjacent to these brackets were determined and were compared at three points.

**Results:** A decrease in nuclear area and an increase in cytoplasmic area occurred in the buccal mucosa cells adjacent to the brackets at P2 ( $P < .01$ ). At P3, this altered morphometry persisted only in cells adjacent to the metal brackets, although to a lesser degree than at P2 ( $P < .01$ ). A greater decrease in nuclear area was noted in cells adjacent to the metal brackets than in those for patients P1 ( $P < .01$ ). At all time points, smears of cells appeared normal or normal with some inflammatory changes.

**Conclusion:** Placement of metal brackets in the buccal cavity induces cellular alterations. These alterations do not suggest malignancy.

**Keywords:** Metal brackets, Buccal mucosa, Cytomorphometry.

### INTRODUCTION:

Placement of orthodontic appliances in a healthy oral cavity can induce a continuous accumulation of dental plaque,<sup>1</sup> alter the normal oral microbiota,<sup>2 and 3</sup> cause lesions in the buccal mucosa,<sup>4</sup> exacerbate periodontal disease, and consequently cause infection.<sup>5</sup> It is known that ulceration in the buccal mucosa is one of the most frequent complaints of patients because of the friction between bracket and mucosa, which causes discomfort for the patient.<sup>6 and 7</sup> Thus, when these ulcerations persist during

treatment, the orthodontist refers the patient to another specialist, who performs additional tests such as taking a biopsy and doing exfoliative cytology, which can detect alterations in the buccal mucosa caused by this irritation.

The use of exfoliative cytology in the diagnosis of buccal lesions was more common during the period from 1955 to 1975. Since then, a decline in its clinical application has occurred because of the subjective nature of its interpretation, and because few abnormal cells can be identified in smears.

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However, this technique has stirred renewed interest because of the possibility of its being complemented with other laboratory techniques such as molecular biology, cytomorphology, and immunohistochemistry.<sup>8</sup> In addition, it offers the advantage of being minimally invasive and painless, without the need for local anesthetic, and it is easy to perform.

The aim of this investigation was to study and compare the epithelial cells of the buccal mucosa adjacent to metal brackets at three time points: baseline, 60 days after placement, and 30 days after removal of the brackets. These cells were examined for morphometric alterations in the area of the nucleus and cytoplasm, alterations in the nuclear/cytoplasmic ratio, morphologic alterations in the nucleus and cytoplasm, and alterations in the cytologic criteria for malignancy.

#### **MATERIALS AND METHODS:**

Individuals who came to Department of Orthodontics, Kamineni Institute Of Dental Sciences, Narketpally, for orthodontic treatment were selected for this study. When they agreed to participate, individuals or their legal guardians signed an informed consent form.

Selected individuals had no related history of smoking, alcoholism, diabetes, anemia, or debilitating diseases and were not being treated with antibiotics or steroids during the study period. They did not use alcohol-based mouthwashes, did not wear prostheses or have tooth restorations with sharp edges, and did not have any type of lesion on the buccal mucosa. The sample of this study comprised 30 individuals (mean age, 16 years; range, 14 to 28 years), 12 males and 18 females.

The locations chosen for bracket placement were first premolar, second premolar, and first and second permanent molars, all on the upper arch. The teeth chosen varied according to the stage of dentition for each at the time of bracket placement.

Full mouth fixed appliances were bonded in each individual with Transbond XT adhesive (3M Unitek Orthodontic Product, Monrovia, CA 91016 USA). Bonded brackets were standard edgewise metal brackets (3M Unitek Orthodontic Product).

Epithelial cells were collected at three times by the same operator: baseline (P1), 60 days after placement (P2), and 30 days after removal of the brackets (P3). P1 was used as a control, and cells were collected from areas of clinically healthy buccal mucosa.

Before cell collection, individuals were instructed to rinse the mouth with water to remove possible debris. Following informed consent, brush – cytology scrapings were obtained from buccal mucosa. The surface of the mucosa was rolled or scraped uniformly, and collected epithelial cells were smeared on an appropriately labeled glass slide and fixed with commercially available spray fixative (available with the rapid PAP™ Kit for 15 min). Two cytologic smears were obtained from each site. All the cytology smears were stained with Papanicolaou's stain. All the cytology smears were reviewed in an independent and blind fashion by an oral pathologist.

#### **CYTOLOGICAL STAINING AND EVALUATION:**

All the cytological smears were stained by papanicolaou technique using a commercially available staining kit RAPID PAP™ (sigma-Aldrich Diagnostic, Mumbai, Maharashtra). The slides were mounted with cover glass using DPX mountant. All the slides were observed under light microscope using low magnification (10X) for screening and high magnification (100X). CD and ND of the cells were measured using a calibrated micrometer. By superimposition of the calibrated eyepiece graticule on the cytological smears, direct measurements of individual epithelial cells were made, under 100X objective. Values were obtained in both axes of the cells and nuclei. Only clearly defined cells were measured, avoiding clumped or folded cells and unusually distorted nuclei and cells. One hundred cells were measured for CD and ND from each slide. The mean values of CD and ND were obtained for each case.

The smears also were evaluated qualitatively, according to the cytologic criteria of malignancy, and were classified according to Papanicolaou<sup>9</sup> as follows:

1. Class 0: Material insufficient or inadequate for analysis
2. Class I: Smear normal

3. Class II: Smear normal with inflammatory changes
4. Class III: Dysplastic changes—smear suspect
5. Class IV: Strongly indicative but not conclusive for malignancy
6. Class V: Smear malignant

#### STATISTICAL ANALYSIS:

A correlation analysis was performed between the CD and ND for each time point. The study and control groups were compared in terms of mean cellular diameter, mean nuclear diameter, and mean cytoplasm nuclear ratio using one way ANOVA.

#### RESULTS:

After placement of the brackets, a significant decrease in NA and N/C and an increase in CA of buccal mucosa cells adjacent to metal brackets were observed ( $P < .01$ ). Cells adjacent to metal brackets did show a lower NA and N/C compared with those next to no brackets ( $P < .01$ ). (Table 1-4) and Graph 1.

When the brackets were removed, the buccal mucosa cells adjacent to metal brackets still showed a smaller nucleus, a larger cytoplasm, and a lower nuclear/cytoplasmic ratio than at no brackets ( $P < .01$ ), although with fewer alterations than at P2 ( $P < .01$ ).

With respect to the predominance of cells present in the smears, on the basis of their staining, few 6 slides were found with a predominance of cells of the spinosum and basale stratum in P2.

In P1, the slides had predominance of surface and subsurface cells, and for P2 and P3, a greater number of slides showed a predominance of surface cells. (Fig.1-6)

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 and II were observed at all time points, and no significant difference was noted among the groups ( $P > .05$ ).

#### Discussion:

In this study, metal brackets were bonded with the presence of arches, ligatures, or rubber bands

though these materials could bias the findings.<sup>10</sup> However, it is known that these accessories are used routinely in treatment and they can protect the buccal mucosa from direct friction caused by the bracket.

Ulcerations in the buccal mucosa are frequent complaints among orthodontic patients. Studies indicate that approximately 76%<sup>6</sup> to 95%<sup>7</sup> of patients report ulcers in the buccal mucosa during treatment, and only between 16.5%<sup>7</sup> and 21.1%<sup>6</sup> of patients report ulcers only once. Therefore, because the epithelium of the buccal covering is exposed to aggressive agents, as in the case of brackets that are capable of causing alterations at various times during treatment, exfoliative cytology can be an effective tool in diagnosis to detect and evaluate these alterations, assuming that its limitations are well elucidated and applied<sup>11 and 12</sup>. The clinician should be knowledgeable about this technique because the cells are studied individually and cannot be evaluated with regard to tissue conformation, as in a biopsy.<sup>11</sup> In addition, only the most surface cells of the epithelium are collected for exfoliative cytology.<sup>13</sup> Therefore, the use of brushes to collect epithelial cells allows collection of samples that include cells of all layers of stratified squamous epithelium<sup>14, 15 and 16</sup> in addition to providing a thinner and more dispersed, homogeneous distribution of cells on the slides.<sup>17</sup>

In the present study, placement of brackets in the buccal cavity caused diminution of the nucleus, an increase in cytoplasm, and a lower nuclear/cytoplasmic ratio of buccal mucosa cells that were in contact with the brackets. These results corroborate the findings of Shabana et al,<sup>18</sup> who also reported a statistically significant increase in the size of cells of traumatic keratosis lesions when these were compared with normal cells of the buccal mucosa.

However, in the buccal cells of individuals with malignant lesions<sup>19 and 20</sup> or of smokers,<sup>21 and 22</sup> alterations distinct from those in the present study were found. In individuals with a tobacco-chewing habit and in those with smoking and tobacco-chewing habits combined, an increase in nuclear diameter and a decrease in cell diameter were observed<sup>22</sup> as were seen in samples of individuals

**Table 1: Comparison of Mean Cell Diameter (CD) amongst various time points:**

Time Points	Mean CD $\mu\text{m}^2$	SD	P value
P1	60.2	11.12	P<0.0001
P2	83.6	6.72	P<0.0001
P3	77.9	7.66	P<0.0001

**Table 2: Comparison of Mean Nuclear Diameter (ND) amongst various time points:**

Time Points	Mean CD $\mu\text{m}^2$	SD	P value
P1	18.3	2.96	P<0.0001
P2	16.8	2.13	P<0.0001
P3	17.2	3.60	P<0.0001

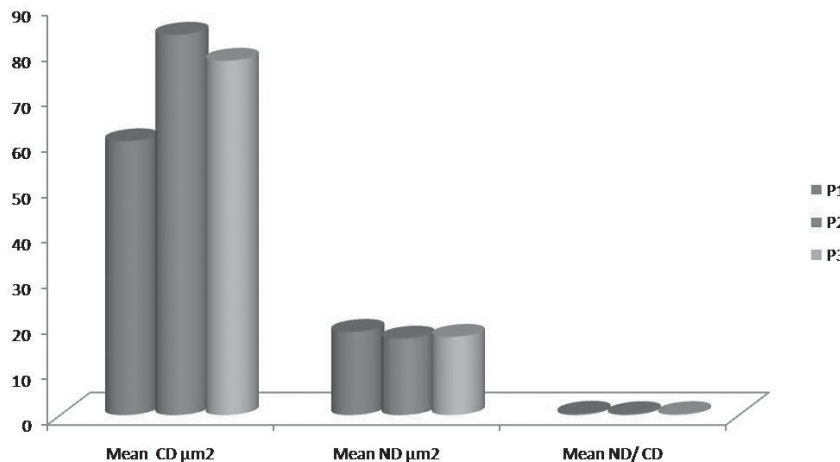
**Table 3: Comparison of Mean (ND) / (CD) amongst various time points**

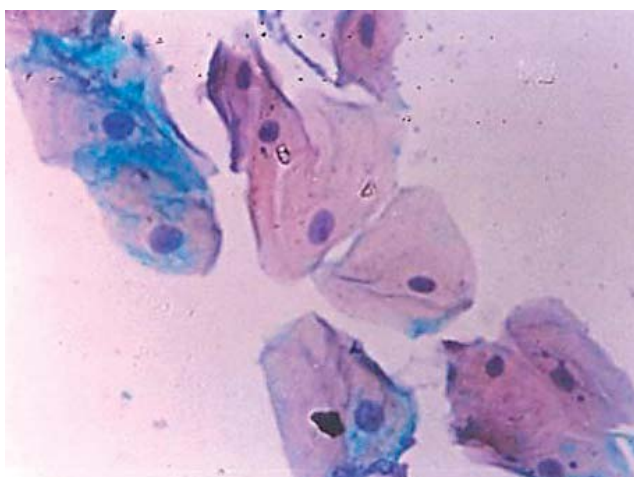
Time Points	Mean ND/ CD	SD	P value
P1	0.30398671	0.83	P<0.0001
P2	0.20095694	0.86	P<0.0001
P3	0.22079589	0.77	P<0.0001

**Table 4: Comparison of (CD), (ND), and (ND) / (CD) amongst various time points.**

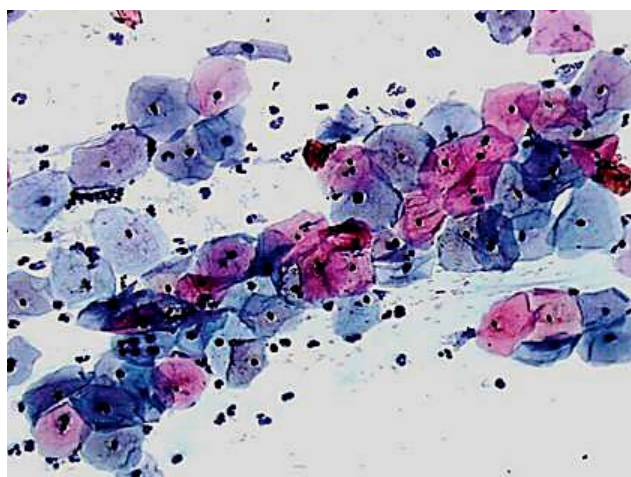
Time Points	Mean CD $\mu\text{m}^2$	Mean ND $\mu\text{m}^2$	Mean ND/ CD
P1	60.2	18.3	0.30398671
P2	83.6	16.8	0.20095694
P3	77.9	17.2	0.22079589

**Graph - 1**

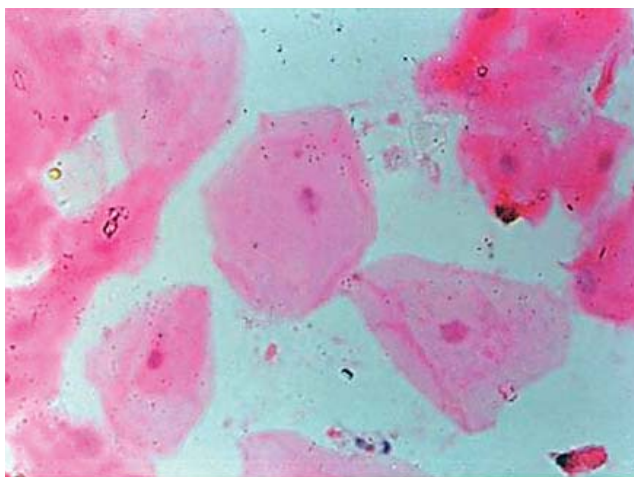




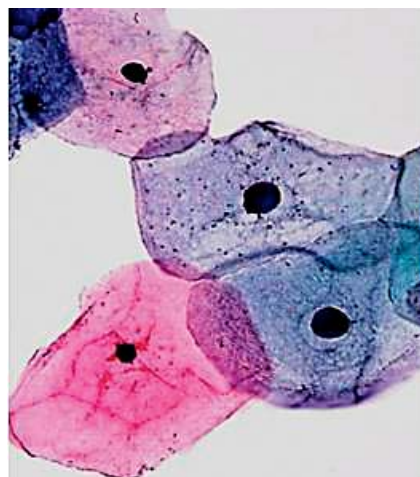
**Fig 1:** Epithelial cells of clinically healthy buccal mucosa at P1.



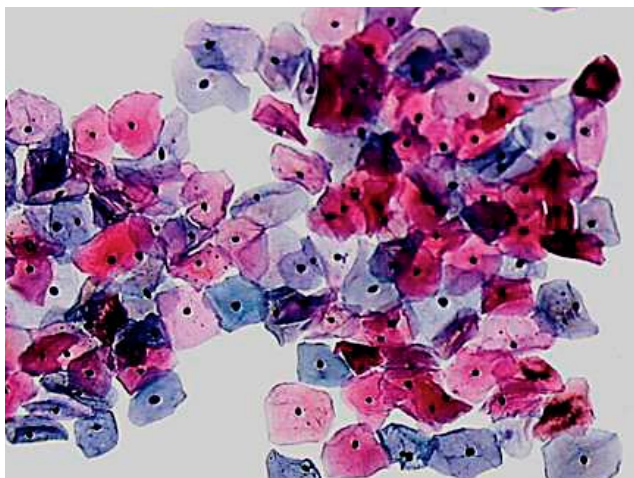
**Fig 4:** Epithelial cells of buccal mucosa adjacent to metal brackets P2 along with leukocytes.



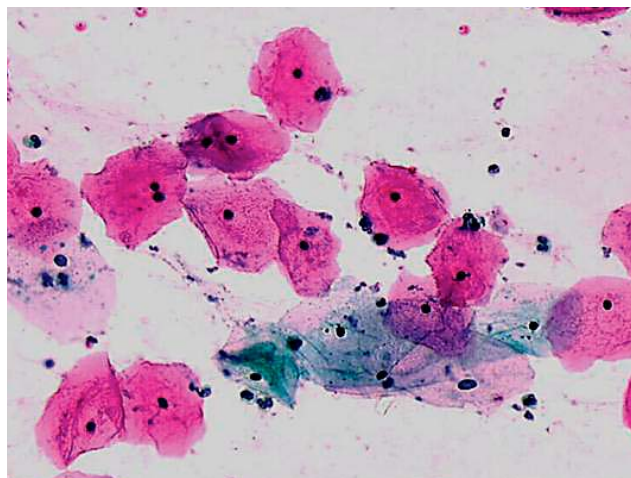
**Fig 2:** Epithelial cells of buccal mucosa adjacent to metal brackets P2.



**Fig 5:** Epithelial cells of buccal mucosa adjacent to metal brackets P2 showing reduction in nuclear size.



**Fig.3:** Epithelial cells of buccal mucosa at P3 after removal of brackets.



**Fig 6:** Epithelial cells of buccal mucosa adjacent to metal brackets P2 with superficial cells.

with tumors in the mouth floor.<sup>20</sup> Cowpe et al<sup>19</sup> did not find size changes in the nucleus in samples of suspicious lesions of the buccal mucosa and the mouth floor but did observe a decrease in cytoplasmic area in lesions of the buccal mucosa. Ogden et al<sup>21</sup> observed an increased nuclear area only in the buccal mucosa cells of smokers and did not note an alteration in the cytoplasmic area. Normal cells of the buccal mucosa have abundant cytoplasm and a single, small centralized nucleus; malignant cells have a broad, enlarged nucleus that occupies a large area of the cytoplasm, with well-stained chromatin and an irregular nuclear membrane.<sup>11</sup> Therefore, the cellular changes that occurred in the buccal mucosa adjacent to the metal brackets in the present study do not suggest malignancy. When this diagnosis was confirmed by the evaluation of cytologic criteria for malignancy, smears of only Classes I and II of Papanicolaou were noted.

Alterations in sizes of the nucleus and cytoplasm as demonstrated here suggest hyperkeratinosis of the stratified squamous epithelium of the buccal mucosa adjacent to the brackets. This would cause an increase in the number of cells in the corneum stratum of the epithelium that show smaller nuclei than cells from deeper layers. This hyperkeratinosis can be confirmed by an increase in the number of slides with a predominance of surface cells at P2 and P3.

Greater cell alterations on the side with the<sup>4</sup> and<sup>23</sup> stainless steel bracket may have been caused by trauma to the buccal mucosa caused by the physical characteristics of brackets, in other words, because of the fact that the wings were less rounded, or because of the cytotoxicity of stainless steel, which has been observed in other studies.<sup>24</sup> and<sup>25</sup>

In this study, buccal mucosa cells were evaluated only 30 days after removal of the brackets, because Jones et al<sup>12</sup> recommend that if a lesion persists for longer than 14 days after removal of the causative factors, a biopsy should be performed immediately. Therefore, within 30 days, cells should have returned to their initial size. In future studies, the buccal mucosa cells should be analyzed after longer periods to determine whether these alterations persist in the buccal mucosa.

This study was undertaken to describe cellular changes in the buccal mucosa adjacent to metal brackets. Because brackets are essential components of fixed orthodontic appliances, biocompatibility is needed to prevent irreversible deleterious damage to tissues. And our findings are consistent with studies of Betina et al<sup>26</sup>. Although results of this investigation suggest that brackets do not cause any malignant changes in the buccal mucosa, the origin of the observed changes remains uncertain. Future studies in this regard will explore ways to prevent these alterations.

### CONCLUSION:

Placement of metal brackets in the buccal cavity induces cellular alterations. These alterations do not suggest malignancy. But they may occur as mild inflammation. Buccal mucosa cells adjacent to the metal brackets tend to return to the initial morphology after removal of the brackets but not to a full extent as was before placement, may be further follow up will give the exact clue about the morphological changes seen in the cells of buccal mucosa.

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