Changes in epithelial cells adjacent to orthodontic devices

Alterações nas células epiteliais adjacentes a dispositivos ortodônticos

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ABSTRACT

Objective: The majority of oral lesions in the epithelial tissue are caused by local irritation so the purpose of this study is to compare between the patients have fixed orthodontic device and patients without orthodontic device and to perform cytomorphometric and cytological examinations of the oral mucosa epithelium close to metal brackets and band areas. **Material and Methods:** The study comprises 40 participants, divided into two distinct groups: a control group of 20 patient without fixed orthodontic appliance and the second group is 20 patients with fixed orthodontic appliance. Smears were collected from the oral mucosa adjacent to the orthodontic bracket and adjacent to the band area in the orthodontic patients while cell collection from non-orthodontic patients taken from buccal mucosa then the smears stained and analyzed using an alight microscope. **Results:** This study shows a significant difference in Nuclear area,cytoplasmic area and nuclear cytoplasmic area ratio between control, bracket and molar area, showing alteration change in the cell of the epithelial tissue adjacent to molar band then bracket area and appearance of type II inflammatory smears, according to Papanicolau classification especially in band area. **Conclusion:** Metal brackets and molar band cause cytomorphometric and cytomorphological changes adjacent oral mucosa cells.

KEYWORDS

Epithelial cell; Fixed appliances; Oral mucosa; Orthodontic appliance; Papanicolau Classification.

RESUMO

Objetivo: A maioria das lesões orais nos tecidos epiteliais são causadas por uma irritação local, deste modo, o objetivo deste estudo é comparar pacientes que usam aparelhos ortodônticos fixos e pacientes sem aparelho ortodôntico fixos e realizar exames citomorfimétricos e citológicos do epitélio da mucosa próximo às áreas das bandas e brackets metálicos. **Material e Métodos:** O estudo foi composto por 40 participantes, divididos em dois grupos distintos, um grupo controle com 20 pacientes sem aparelho ortodôntico fixo e o segundo grupo com 20 pacientes com aparelho ortodôntico fixo. Foram coletados esfregaços da mucosa oral adjacente aos brackets e bandas ortodônticos dos pacientes com aparelho, enquanto a coleta das células dos pacientes que não usavam aparelho foi colhida da mucosa bucal. Em seguida, os esfregaços foram corados e analisados usando um microscópio ótico. **Resultados:** Este estudo mostrou uma diferença significativa na área nuclear, na área citoplasmática e na área citoplasmático nuclear proporcionais entre o controle, área do bracket e área de banda molar, apresentando alterações nas células do tecido epitelial adjacente à banda molar e à área do bracket e aparecimento de esfregaços inflamatórios do tipo II, de acordo com a classificação de Papanicolau, especialmente na área de banda. **Conclusão:** Brackets metálicos e bandas nos molares causam mudanças citomorfométrica e citomorfológica nas células da mucosa oral adjacente.

PALAVRAS-CHAVE:

Celula epitelial; Aparelhos fixos; Mucosa bucal; Aparelho ortodôntico; Classificação Papanicolau.

INTRODUCTION

The epithelial layer is the layer that covers the oral mucosa in the oral cavity [1]. Therefore, maintaining the integrity of the mucous membrane is crucial for oral health. However, Epithelial cells can undergo alterations caused by oral cavity disease, infections, traumatic agents, or metabolic circumstances, leading to various clinical changes [2,3]. When undergoing treatment with a fixed orthodontic appliance, attaching additional components such as brackets, arches, and molar bands is essential. These accessories, which attach to the oral mucosa, may cause irritation, ulceration, hematomas, and erosions, which can cause discomfort and pain to the orthodontic patient [3-6].

Most oral sores arise due to localized irritation and do not warrant worry [3,7]. Infrequently, they may serve as an initial indication of more severe oral problems. Typically, oral lesions are mainly caused by trauma like a trauma from orthodontic appliance that does not fit properly, is not smooth or cracked denature or may be a sharp tooth surface which may affect the nearby soft tissues. Oral tissues can undergo significant alterations at a deeper level due to acute stress [3].

Additionally, the orthodontic device promotes plaque buildup and alters the microbial population in the mouth, which has a detrimental impact on the oral epithelial tissue surrounding the fixed orthodontic appliance [8,9]. Orthodontic treatments should aim to correct dental or /and skeletal discrepancies without tissue damage. Nevertheless, there are a restricted number of researches that have investigated the alterations to epithelial cells caused by the friction produced by fixed orthodontic appliances [3,9,10].

Therefore, the purpose of this study was to examine the differences between patients who were receiving orthodontic treatment and those who were not. In addition, the purpose of the study was to conduct cytomorphometric and cytological exams in order to carry out extensive investigations of the oral mucosa epithelium in the vicinity of metal brackets that were equipped with stainless steel wires and the area surrounding the molar band.

MATERIAL AND METHODS

The experimental protocol for the current study received approval from the Human Research Ethics Committee at Anbar University number 116 in 11/8/2023. The present study was conducted with 40 subjects aged 18 to 35 years with good oral hygiene. The participants were selected from a private dental clinic and separated into two groups. In the control group, 20 patients without fixed orthodontic appliances came to the clinic for different reasons. The second group is 20 patients with fixed orthodontic appliances exclusion criteria included patient with history of any lesion in the oral cavity ,smoking, any systemic disease and bad oral hygiene patients. The fixed appliances include a metallic bracket (Roth bracket slot 0.22 and stainless wire) and an orthodontic band on the first molar.

Oral exfoliated cells were obtained from buccal mucosa by liquid exfoliation cytology. Initially, the mouth of the patient should be washed with water to get rid of any excess debris that may be present in the oral cavity.

Subsequently, samples were obtained from two regions: The first is the oral mucosa area adjacent to the orthodontic bracket and wire, while the second is the oral mucosa adjacent to the band area. While cell collection from non-orthodontic patients takes from buccal mucosa adjacent to the upper first premolar and first molar region.

The squamous epithelial cells were collected using cytobrush by gently scraping and rolling the mucosa region onto glass slides, which were then promptly fixed in 99.9% pure alcohol for 20 minutes. The smears were subsequently stained with the standard Papanicolaou stain and examined using Japan's Olympus Corporation light microscope. A single observer conducted the cytological examination. teen random cytological smear from each group were reexamined by the observer after period of time.

The smears were categorized using the Papanicolaou classification system{{. Class 0: Insufficient or inadequate material for analysis. Class I: The smear is normal. Class II: The smear is normal but shows signs of inflammation. Class III: The smear shows dysplastic alterations, indicating suspicion. Class IV: The smear strongly suggests malignancy but is not conclusive. Class V: The smear is malignant

Statistical analysis

In this study, the results were expressed as the mean $\pm$ SD was made using SPSS version 25.

So, the ANOVA and ,Chi sequre was used to compare between variables .when Anova analysis showed a significant different between groups we used Tukey test (Post Hoc) to determine which groups differed from other groups ,probability value of less than 0.05 and 0.01 were accepted as significant.

RESULT

The current study's results indicated statistically significant differences in the average nuclear area values across different areas in this investigation The average values and associated standard deviations for all the variables under investigation are displayed in (Table I). Table II show that the epithelial cells close to band area exhibited the most significant decrease in a nuclear area relative to the other sites examined. this decrease was statistically significant at a significance level of $p<0.05$.and the control area is more significant increase in nuclear area than other sites . Additionally, the mean values of the cytoplasmic area exhibited a statistically significant increase in the band area more than two other sites while control area was lesser significant different than other two sites. Also a statistically significant difference was observed between the mean Nuclear cytoplasmic area ratio (NA/CA) ratio values at $(p<0.05)$ so the control area was the more Nuclear cytoplasmic area ratio (NA/CA) than molar and Bracket site while the band site was the lesser one. Table III shows a higher number of nucleated cells in the superficial layer of the oral mucosa region that is in touch with the fixed orthodontic device. A nucleated superficial layer is also present near the bracket and molar band area. However, no such layer is observed in the control area.

The smears were categorized using the Papanicolaou classification system. There was a significant increase($P \leq 0.01$) in the of class I in the control area, while class II type smear (inflammatory). Show higher in contact with the molar band and less in bracket area as shown in the (Table IV). (Figure 1) shows the epithelial cell's class ll (inflammation) adjacent to the orthodontic device of neutrophils, perinuclear halo pycnotic nucleus and binucleation as characteristic of inflammation.

Table I - Cytological measurement in epithelium cells from different site

Variable	Control Mean S.D	Bracket Mean S.D	Band Mean S.D	P value
NA*	149.3 7.9	141.2 ±6.5	135.3 ±6.7	0.042
CA* *	3668 ±102.7	4396 ±137.4	6533 ±215.1	0.025
NA /CA ***	0.04 ±0.005	0.03 ±0.002	0.02 ±0.001	0.032

*nuclear area; **cytoplasmic area; ***nuclear cytoplasmic area ratio; S.D: Std. Deviation.

Table II - Comparison between difference groups in NA, CA and NA/CA by Tukey test (Post Hoc)

| Group	Control *Bracket	Control *Band	Band *Bracket
NA	0.0345*	0.026*	0.0125*
CA	0.0124*	0.0002*	0.0144*
NA/ CA	0.0507*	0.0001*	0.0419*

A significant different at * P≤0.05

Table III - Type of predominant cell from the different regions of the oral smears

Region	Enucleated superficial cells N %	Nucleated superficial cells N %	Intermediated layer cells N %	Basal layer cells N %	Total
Bracket area	3 15	9 45	8 40	0 0	20
Band area	4 20	10 50	6 30	0 0	20
Control	0 0	14 70	6 30	0 0	20
Total	7 11.6	33 55	20 33.3	0 0	60

Table IV - Classification of smears by papanicolaou system

Papanicolaou classification	Bracket area	%	Band area	%	Control area	%	p value
Class 1	8	40	5	25	18	90	0.0001 **
Class II	12	60	15	75	2	10	0.0001 **
Class III	0	0	0	0	0	0	NS
Class IV	0	0	0	0	0	0	NS
Class V	0	0	0	0	0	0	NS
Total	20	100	20	100	20	100	

**(P≤0.01) significant different
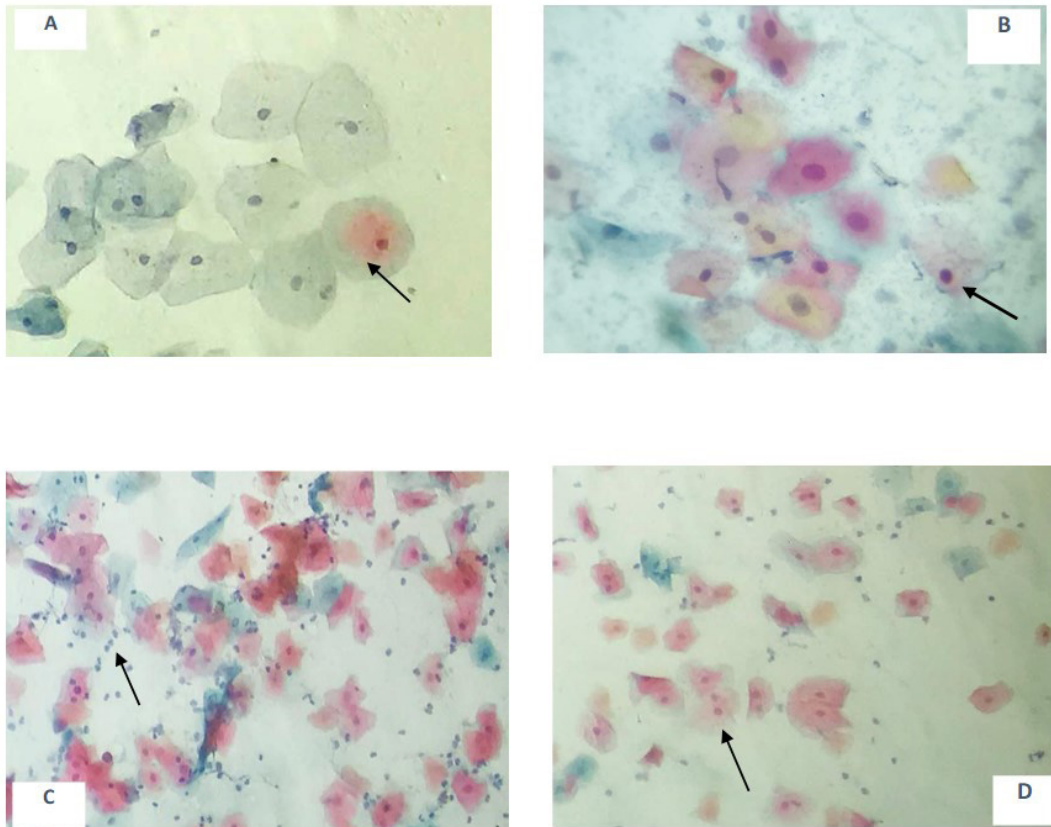
Figure 1 - High-power view showing different cellular changes in oral epithelia in class II classification from band and bracket areas. (A), show cell with perinuclear halo. (B), show Pycnotic nucleus. (C), infiltration of neutrophils. (D), Cell with Binucleation.

DISCUSSION

The current study investigated 10,250 oral epithelial cell (60 slides) from patients by mean cytological and cytomorphological analysis.

Our study findings confirmed that metallic brackets and molar bands can cause irritation to the epithelial cells and create alterations, this agree with Pereira et al. research [8]. As show in the (Tables I, II) the insertion of orthodontic brackets and band for orthodontic treatment resulted in a reduction in the size of the nucleus, an increase in the cytoplasm, and a decrease in the ratio of the nucleus to the cytoplasm of the oral epithelial cells located next to the band then

brackets area . Increase cytoplasmic area facilitate increase synthesis of proteins to adaptation of the tissue to the changes due to inflammation adjacent to orthodontic device [11].

Shabana et al. [12], agree this phenomenon as well, confirming that cells exposed to orthodontic devices had an enlargement of unaffected cells. The smears obtained from exfoliative cytology, from (Table III), typically show a deficiency of basal cell layer of intact oral epithelium. This shows that this method does not eradicate cells from the underlying layers of the epithelium.

The quantitative investigation revealed that a majority of cells in the superficial layer of the

oral mucosa were affected by friction occur with the site of band tube. This phenomenon may arise due to the thickening of the cornea layer, resulting in a higher prevalence of superficial cells. This is agree with the findings of Kwon et al. [13]. Additionally, it was discovered that prolonged irritation leads to increased cells of the superficial layer due to the process of keratinization and the excessive growth of the oral mucosa.

Regarding the Papanicolaou's classification [14], in Table IV, the classification in the bracket area was as follows: 40% class I and 60% class II; in the area of orthodontic bands: 25% class I and 75% class II. For the control group, 90% were class I and only 10% were class II. Therefore, inflammation was associated, in decreasing order, with the orthodontic band area, followed by the bracket area and, more discreetly, in the mucosa of patients in the control group.

The presence of brackets and molar tubes can cause detrimental effect to the epithelial cells. These physical agents can hurt the epithelial cell and the neighboring epithelial cell near the molar band [9]. This is may be due to the challenges ineffectively cleaning of this particular location in band area, leading to plaque buildup. Consequently, the presence of plaque bacteria might trigger inflammation (due to Prescence of acidic *streptococcus mutans* in the plaque[10].

Ramaesh *et al.*, [15], demonstrated through cytomorphometric analysis that dysplastic lesions and spinocellular carcinomas display a gradual reduction in cytoplasm width when compared to normal cells, accompanied by a gradual loss in nucleus size. Our investigation found no changes in the morphological features of smear cells collected from the mucosa neighboring the orthodontic device. The findings of this study are consistent with previous research [8,16] that has proven that cellular alterations in the oral mucosa resulting from friction with orthodontic devices do not lead to malignant transformation (with a 0% occurrence of class III, , and V). Studies have demonstrated that attaching orthodontic appliances to the oral mucosa cannot produce cytotoxicity or genotoxicity [17-19].

Berstein and Miller [20], shown normal cell morphology is characterized by copious cytoplasm and a small, concentrated singular nucleus. However, in this study, the area where the orthodontic device is placed, specifically the band area, exhibits some degree of dysplasia and morphological changes as show in (Figure 1 A, B, C, D).

Biopsy is widely regarded as the gold-standard method of evaluating mucosal diseases. However, the noninvasive technique of exfoliative cytology is sensitive for detecting cellular alterations in healthy mucosa. Exfoliative cytology evaluations can serve as a para-clinical method for assessing cellular damage before the manifestation of clinical symptoms. An orthodontic device is a physical agent that irritates the oral mucosa. A more significant number of keratinized cells and inflammatory cell in the mucosa can be attributed to the friction experienced in the area close to the orthodontic device [9]. The ions liberated by stainless steel orthodontic device have a genotoxic effect due to corrosion of the metal in the orthodontic appliance on oral mucosa cells. However, this impact can be undone due to the presence of cellular DNA repair mechanisms [21,22]. So, the orthodontist should emphasize of the important oral hygiene to the patient to prevent further inflammation which occur due to plaque accumulation associated with device [21] and check any signs of inflammation or sharp edge and make adjustments. And the orthodontic device should be fabricated with least alteration to the epithelium tissue as much as possible.

CONCLUSION

Metal brackets and molar bands cause cytomorphometric and cytomorphological changes in tissue adjacent to orthodontic devices, especially adjacent molar bands, which show inflammatory change in type II inflammation smears according to the Papanicolaau classification. The orthodontic appliance induces inflammation, however malignant change was not observed. As a result, the orthodontist must pay attention to the patient's careful cleanliness of their teeth and orthodontic device to prevent any inflammation that could worsen the condition.

Author's Contributions

ZHA: Conceptualization, Methodology, writing- original draft, preparation, Visualization and supervision. ATB: Sample collection, Validation and investigation. LHAAS: Writing -Review and Editing, Software, Data curation and formal analysis. BZK: Cytological study.

Conflict of Interest

No conflict of interest of this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: University Of Anbar, Ethical Approval Committee.

The approval code for this study is: 116 at 8/11/2023.

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