

# Evaluation of Changes in Oral Epithelium, Inflammation and Candida albicans Infection in Patients using Removable Orthodontic Appliances

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## Abstract

### Introduction:

Nowadays, removable orthodontic appliances are widely used in the treatment of straightening teeth and guided the growth of the jaws. These devices have acrylic parts in which methyl methacrylate monomers are used and have been the subject of discussion in recent years as a cause of epithelial lesions in the oral cavity, and they are considered as one of the factors in mucosal allergies. This study evaluated the changes in oral epithelium, inflammation, and candida albicans in patients using removable orthodontic appliances.

### Materials and methods:

In this cross-sectional study a total of 100 patients with removable orthodontic appliances was enrolled. Samples were collected from patients' mucosa using swabs and pap smear and then they were stained by the use of hematoxylin and eosin (H&E). After data collection by SPSS 14.0, they were analyzed by Fisher's exact test followed by the chi-square test at  $p < 0.05$ .

### Results:

There was no statistically significant relationship between the use of removable orthodontic appliances and candida albicans infection and inflammation. There was a significant relationship between these two in the both study group ( $p = 0.000$ ) and the control group ( $p = 0.003$ ). There was no significant relationship between candida albicans and the time of dental appliance use ( $p = 0.1$ ). A significant relationship was observed between inflammation and the length of time of dental appliance use ( $p = 0.028$ ).

### Conclusion:

The results of this study showed that the inflammation and candida albicans infection in patients using removable orthodontic appliances were minimal and there were no dysplastic epithelium changes.

### Key words:

•Oral Epithelium •Candida Albicans •Orthodontic Appliance  
•Inflammation

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## Introduction

Today, orthodontic appliances are widely used to straighten teeth or to move and guide the jaws, and a large part of orthodontic

treatments is done with the help of these devices, which have a major role in the field of dentistry.<sup>(1)</sup> The materials used in these

devices include metals and acrylics, and the acrylic part of the device remains in the vicinity of the mucosa for an average of 9 hours per day. Heat-cured acrylic releases few monomers but fits less accurately, and monomer releases in self-cured acrylic acts in reverse. Acrylics used in orthodontic treatment consist of methacrylate powder monomer.<sup>(2)</sup> One of the concerns with use of these acrylics, which are commonly available in orthodontic devices, is that the remaining monomer in the acrylics has the potential to cause pathological changes in oral mucosa and can cause complications in oral mucosa, such as stomatitis, allergic reactions, and cheilitis in the patients.<sup>(3)</sup>

Another substance in this particular acrylic is formaldehyde, which can lead to potential changes that also cause allergies. Formaldehyde is the oxidized product of the remaining methyl methacrylate that is formed in low polymerized polyethylenes.<sup>(4)</sup> In a study by, Jesani et al., they showed that the acrylics commonly used 2%-3% of monomers initially released, and of 1%-2% after three years.<sup>(5)</sup> Javed et al. showed that methyl methacrylate monomer was the cause of dermatitis among the patients who were in a constant contact with the monomer.<sup>(6)</sup> Sataloff proved that the methyl methacrylate monomer is found under the skin after using resins.<sup>(7)</sup> In general, many reports showed side effects of the monomer in resins, such as dermatitis/ stomatitis allergy, showing that the monomer is harmful to the oral epithelium.<sup>(5, 6)</sup> Regarding the increasing use of orthodontic appliances in treatment and the importance of epithelial changes in progression of precancerous and cancerous lesions, and examining epithelial changes in relation to the amount of time in which patients use acrylic orthodontic devices, the device seems to be important. There are several ways to assess the oral mucosa, of which incisional and excisional biopsy are two of the most common methods. However, biopsies are of no use in this study since they are both invasive and can cause psychological problems for patients.<sup>(7)</sup>

Exfoliative cytology is the least invasive assessment, with a lower cost and no damage to the mouth tissues, and it can be used for the early diagnosis and prognosis of both precancerous and other lesions.<sup>(8)</sup> In this study, epithelial changes in patients using orthodontic appliances are

described in relation to the degree of patient compliance in order to obtain a clear relationship between the rate of the appliance usage and epithelial changes in the patient population.

## Materials and Methods

This was a cross-sectional study that was conducted at the Urmia University of Medical Sciences. Laboratory techniques, questionnaires, observations, and examinations were used in this study. Fifty patients (25 male and 25 female) were selected. The control group was 50 cases (25 male and 25 female) consisted of people without removable orthodontic appliances with good to excellent oral hygiene, and the age of 8–16 years.

The patients group included patients aged 8 to 16 years who had removable orthodontic appliances as well as good to excellent oral health and hygiene. A questionnaire with a validity confirmation using focus groups with respect to clinical judgment of cooperation or non-compliance was prepared. The questionnaire was designed based on the severity of pain (low, medium, high), day length (8–12 in the morning, 12–4 in the afternoon, 4–8 in the night, 8–12 in the night, 12–4 in the morning).

The patients were asked to rinse their mouths several times to remove food debris and dead cells from the oral cavity before inserting the removable acrylic device, then a gas was slowly set on the areas to ensure there were no food particles, after which a smear was prepared on the patient palate (an area that was in contact with the acrylic pad of the device) using two disposable cytology brushes (Cytobrush, Iran, Aria Company). Each cytology brush was rotated 10–15 times in each place with no bleeding in any of the sampling, then each cytology brush was spread on clean, dry glass lam that had been labeled and numbered. Cells on the surface of the microscopic glass slide were immediately sprayed using pathofix spray (containing 95% ethanol, Tehran-Iran Pad Tan Teb) from a distance of 25 cm with a maximum of 2 bars of pressure to fix. Fixed smears were stained through three methods of hematoxylin and eosin (H&E) for examination of cellular morphology and inflammation, and periodic acid-Schiff

(PAS) method for examination of candida organisms and papanicolao (PAP staining) for examination of dysplastic changes of nucleous and cytoplasm of cellules. To determine the degree of dysplasia, average size of the nucleus and cytoplasm and the size of the nucleus to the cytoplasm, binary or multiple nucleoli were considered.<sup>(8)</sup> To assess the presence of inflammation, it is necessary to describe inflammatory cells (macrophages, mast cells, neutrophils, and lymphocytes). The candida albicans organism can be seen microscopically in cytologic preparation on staining with the PAS method. The hyphae and yeast form of candida albicans can be identified by fungal cell walls that approximately 2µm in diameter.<sup>(1)</sup>

Smear from the infected area comprising epithelial cells creates opportunities for detection of the yeasts and inflammatory cells.

Microscopic glass slides were examined by oral pathologists. Microscopic glass slides were examined by oral pathologists. Percentage of inflammatory cells was calculated in 10 successive microscopic fields and their means was recorded. Percentage of inflammatory cells was categorized as following 0= no inflammation, 1-30% of inflammatory cells = mild inflammation, 31-60% of inflammatory cells= moderate inflammation, 61-100% of inflammatory cells= severe inflammation. Percentage of candida organism was calculated in 10 successive microscopic fields and their means was recorded. Percentage of candida was categorized as following 0= absence of candida, 1-30% = mild candidiasis, 31-60%= moderat candidiasis, 61-100% = severe candidiasis.

The data were analyzed with Fisher's exact test and Chi-square statistical test using SPSS14.

According to the results, there is no association between the case and control groups and the p value is not significant.(P=0.87)

Only a significant correlation is the association of candida an inflammation. (P=0.025)

## Results

In this study, 100 patients were enrolled;50 were included in the study group and 50 were included in the control group. There was no statistically significant relationship between the use of removable orthodontic appliances with candida infection and inflammation (Figures 1&2).

There was a significant relationship between inflammation and candida in both the study group (p =0.000) and the control group (p = 0.003). There was no significant relationship between candida and the time of dental appliance use (p=0.1). A significant relationship was observed between inflammation and the time of dental appliance use (p = 0.028). The results of this descriptive study and the frequency of inflammation and candida are shown in Tables 1 and 2, respectively. No malignancy including atypical cells and nucleus and also atypical mitotic figures was observed among samples.

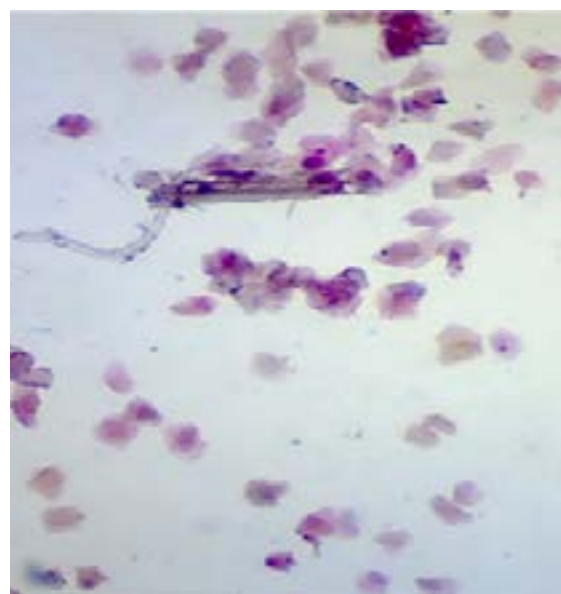


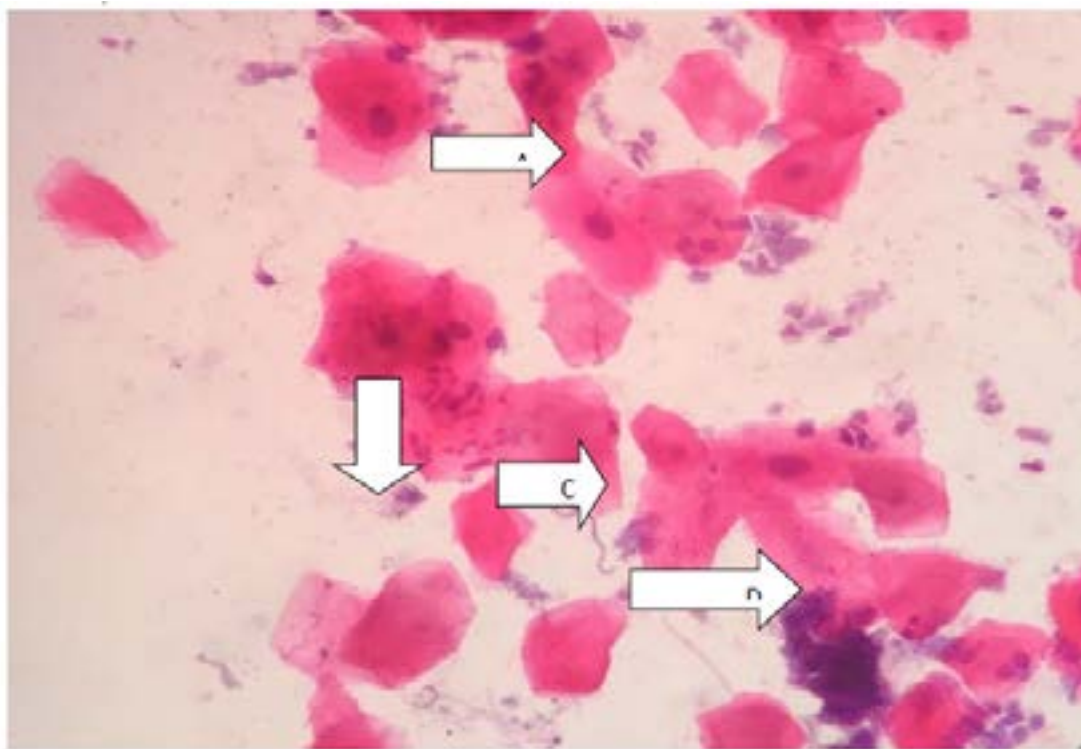
Figure 1. Papanicolaou stain, Matured and keratinized squamous cells (magnification ×100)

Table 1. The frequency of the inflammation

Inflammation	Frequency	Percent	Valid Percent	Cumulative Percent
No inflammation	63	63.0	63.0	63.0
Mild	28	28.0	28.0	91.0
Moderate	8	8.0	8.0	99.0
Severe	1	1.0	1.0	100.0
total	100	100.0	100.0	

**Table 2.** The frequency of the candidia albicans

candidia	Frequency	Percent	Valid Percent	Cumulative Percent
No candidia	48	48.0	48.0	48.0
Mild	26	26.0	26.0	74.0
Moderate	20	20.0	20.0	94.0
Severe	6	6.0	6.0	100.0
total	100	100.0	100.0	



**Figure 2.** (A); Mononucleated Squamous cells with normal N/C. (B); Normal keratinization. (C); Hypha and yeasts of candida. (D); Colony of bacteria. (H&E staining, magnification  $\times 100$ )

## Discussion

The results of this study showed a significant relationship between the two variables (control group and patient group, using removable acrylic orthodontic devices and fungal pollution or candida albicans colonies, which was similar to the results of the study by Williams et al. showing that there was a fungal infection in 58% of new dental prosthodontics, and the results of the study by Khanpayeh et al. in which the number of candida colonies in fixed devices was higher than those found in removable ones.<sup>(9, 10)</sup>

The results of Arikan and Hägg indicated candida infection rates in both fixed and removable devices, which is consistent with the results of the present study.<sup>(11, 12)</sup> The results of the study

by Katharina et al. demonstrated that the use of orthodontic devices did not cause candida infections, which is inconsistent with the results of this study.<sup>(13)</sup>

The results of this study showed there was a significant relationship between candida infection and inflammation. Some sources indicate that the aspartic protease enzyme of candida can cause changes in pro-inflammatory gene expression pattern, such as TNF- $\alpha$ , through the impact on the NF-KB transcription factor.<sup>(14)</sup>

The results of a study conducted by Elcy Pinto de Arruda showed that inflammation and hyperkeratinization in areas close to orthodontic wires were higher than in other parts of the

mouth, which is compatible with the results of this study.<sup>(15)</sup> The results of the study by Zhu et al. showed that candida had the ability to attach to epithelial cells and was capable of causing inflammation in the epithelial cells, similar to the findings of the current study.<sup>(16)</sup> Heat-cured acrylic releases little monomer, but has less accuracy of fit, and monomer release in self-cured acrylic is reversed. Cell death is exacerbated after oxidative stress and inflammation.<sup>(17)</sup>

The materials were used in vivo after curing in this study. Therefore, inflammation can not be attributed to the curing of the materials used. On the other hand, it is expected that the body compensates for damage by various kinds of immune responses and adapts to acrylic materials through exposure to them.<sup>(18, 19)</sup> Goncalves et al., showed in their study that increasing the duration of acrylic resin in cell cultures reduced their toxicity<sup>(20)</sup>. The results of several studies have indicated that the trachea and lung of patients involved in histopathological changes increases through exposure to monomers. In addition, skin and neuropathy damage and allergic stomatitis can also occur due to exposure to methyl acrylate.<sup>(21-23)</sup>

This study showed that removable orthodontic devices increase oral inflammation. However, candida species were only seen in patients with removable acrylic devices and thus are probably unrelated to the release or lack of release of methyl methacrylate as a mutagen or as a known allergen. In this regard, the lack of any type of dysplasia in both study and control groups also confirms this. According to the explanation of Puj's hypothesis, the orthodontic acrylic plate has no effect on oral epithelium.

## Conclusion

The results of this study show that the inflammation and candida albicans infections in patients using orthodontic removable appliances are minimal and there are no dysplastic epithelium changes.

## Conflict of Interest

Authors declare no conflicts of interest.

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