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ORIGINAL ARTICLE

Influence of fixed orthodontic appliances on the change in oral *Candida* strains among adolescents

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Abstract *Background/purpose:* The aim of this study was to explore the presence and variability of oral *Candida* in adolescents before and during treatment with fixed orthodontic appliances.
Materials and methods: A total of 50 patients aged 10—18 years old were randomly selected for this study. Microorganism samples were obtained prior to and after orthodontic treatment and identified by culture methods. Molecular biology techniques were used to investigate the samples further and the effect of the orthodontic appliance on oral pathogenic yeasts was studied longitudinally.
Results: The percentage of patients with candidiasis and the total number of colony-forming units significantly increased 2 months after orthodontic treatment. Changes in the type of oral candidiasis prior to and after treatment were significant.
Conclusion: Fixed orthodontic appliances can influence the growth of oral pathogenic yeasts among adolescents.

Introduction

Candida is a pathogenic fungus. The pathogenicity of *Candida* isolated from human mouths can be classified into eight strains: *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida kefyr*, *Candida stellatoidea*, and *Candida dubliniensis*. *C. albicans* accounts for 45—75% of the total incidence of candidiasis, whereas *C. tropicalis* and *C. parapsilosis* account for about 7% of all cases.[1—5] Many internal and

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external factors, such as systemic disease and impaired immune function, can result in environmental changes in the oral cavity. These changes affect the kinds of microorganisms found in the oral cavity as well as their metabolic and pathogenic activities.[6] *Candida* is often detected in the oral cavity of patients with denture stomatitis, especially middle-aged and elderly people with false teeth.[7]

Fixed orthodontic appliances (FOAs) are artificial devices in the mouth that can greatly affect oral health and allow plaque and food scraps to accumulate. FOAs can also bring about an increased number of microorganisms and amalgamated infections in the mouth,[8] including caries of the teeth, lips, buccal surfaces, and tongue. FOAs can also cause an increase in the number of Gram-positive bacteria in the mouth.[9,10] Increased levels of dental plaque are related to the development of gingivitis.[11] Patients with gingivitis are prone to periodontal disease[12] and loss of periodontal support.[13]

Few studies have been published about fungal colonization in patients with FOAs. Among 60 patients treated with an FOA, oral *Candida* flora were found in 15 (25%) patients, 14 of whom were aged between 16 years and 18 years. Removable orthodontic appliances can temporarily affect *Candida* colonization.[14–16] No study on the type, number, and pathogenic changes in oral *Candida* caused by orthodontic appliances has yet been published. The aim of this study was to explore changes in oral *Candida* strains among healthy adolescents prior to and after treatment with FOAs.

Materials and methods

Patients and samples

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Lanzhou University, Lanzhou, China. Written informed consent was obtained from all participants. Fifty patients with FOAs were randomly selected. Of these patients, 23 were male adolescents and 27 were female adolescents. The average age was 13.6 years. No patient had a systemic, oral mucosal, or periodontal disease. The administration of antibiotic drugs to patients was stopped 2 weeks prior to sampling. The participants carefully brushed their teeth after breakfast and samples were taken 2 hours after food consumption. Microbiological samples were obtained via the gargle method 1 month, 2 months, 3 months, and 6 months prior to and after installation of the FOAs. The patients were required to gargle with 10 mL of sterile Phosphate Buffered Saline (PBS) for 1 minute. The resulting gargle was sent to the laboratory within 2 hours.

Identification and culture

Microbiological samples were centrifuged and 50 μL of the supernatant were cultured in CHROMagar *Candida* identification Petri dishes (CHROMagar, Paris, France) at 37°C for 36–48 hours.

Different *Candida* strains were identified based on the color of the colonies. *C. albicans* exhibits green coloration, smooth *Candida* is purple, tropical *Candida* is blue, *C. krusei* is pink, and other unidentified fungi in the culture medium are white. Colonies that did not grow within 7 days were not included in the count.

Polymerase chain reaction identification

DNA was extracted according to the instructions provided in the kit used (Tiangen Biotech, Beijing, China). A specific sequence of a wild strain of phage M13 microsatellites was used as a single primer for polymerase chain reaction (PCR) amplification. The primer sequence was 5′-GAGGGTGGCGGTTCT-3′.[16] For the PCR reaction, 1 μL of DNA, 2 μL of the primer, and 10 μL of 2 × PCR Master Mix (Tiangen Biotech, Beijing, China) were added to 20 μL of double-distilled H_2O. The reaction conditions were as follows: 95°C denaturation for 1 minute, 95°C denaturation for 30 seconds, 60°C annealing for 30 seconds, extension at 68°C for 90 seconds, 25 cycles, and finally extension at 68°C for 10 minutes. PCR products were detected using 1.5% agarose gel electrophoresis and scanned by a gel imaging camera. International standards for *C. albicans* ATCC90028 were used as positive controls and sterilized PBS buffer was used as a negative control.

Results

Identification and culture results

As shown in Fig. 1, several patients were carriers of pure strains of bacteria, which appeared as monochromatic colonies. The strains could be identified by colony color. Other patients were carriers of mixed bacteria, where the colonies had two or three different colors.

PCR results

The PCR results showed that green colonies have bands similar to those of *C. albicans* ATCC90028, thus these colonies may be identified as *C. albicans*. This result further confirmed the accuracy of the CHROMagar *Candida* color culture. *C. albicans* was also detected by PCR using the international standard for *C. dubliniensis* ATCC6258 as the control. The results confirmed that the green colonies in the clinical samples were *C. dubliniensis* (Fig. 2).

Rate of carrying bacteria and strain analyses

Tables 1 and 2 show that the number of total and mixed carriers was higher 2–3 months after treatment than prior to treatment. After 6 months these changes were comparable with the levels prior to treatment, suggesting that the most significant changes in the number and type of *Candida* strains in the mouth may be found 2–3 months after fitting an FOA.

Total number of colonies

The results were analyzed using two-factor variance analysis, which revealed that the total number of colonies in different Petri dishes were not significantly different ($P = 0.928$). This finding suggests that the cultivation