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**ORIGINAL PAPERS**

# MICROBIOLOGICAL CHANGES IN ORTHODONTICALLY TREATED PATIENTS

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**Abstract.** Orthodontic appliances, both fixed and removable, impede the maintenance of proper oral hygiene and result in plaque accumulation. Many studies report that changes in the dental flora occur after starting the orthodontic treatment. **The aim** of this study was to evaluate the changes of the oral microbial flora during the orthodontic treatment. **Materials and Methods.** 24 young patients, aged 7-16, who were going to start the orthodontic treatment, have been selected. Group I was formed by the 24 patients before wearing any orthodontic appliance (T0) and group II was represented by 15 patients from the initial group, 3 months after the beginning of the treatment (T1). Coronary and subgingival plaque was collected for isolation and identification of the bacterial species involved. For the isolation of the bacteria, growth mediums were used: Todd Hewitt broth, Columbia agar, Schaedler broth and agar. The serial dilution method was used to determine the concentration of the bacteria (CFU/sample) from the aerobic and anaerobic flora. Conventional methods were used for the identification of the species and the isolated strains that are involved in pathological processes have been preserved for further study using molecular methods (PCR-based). **Results and conclusions.** The concentration of the aerobic and anaerobic bacteria has increased during the first 3 months of orthodontic treatment. The lactobacilli were isolated in a smaller proportion in group II (80% vs. 87% before the treatment). The oral streptococci and anaerobic bacteria isolation percentage increased after the beginning of the treatment. For the bacteria involved in pathogenic processes, an increase of the isolation rate has been observed for the patients wearing an orthodontic appliance, from 8.3% to 13.3% for S. mutans and from 4.2% to 6.7% for Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. These results are not statistically significant and they are preliminary. Both study groups will be extended; dental microflora will be investigated at longer intervals of wearing orthodontic appliances.
**Keywords:** orthodontic appliance, oral microbial flora, Lactobacillus, Streptococcus mutans, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis

## Introduction

Orthodontic appliances, both fixed and removable, impede the maintenance of proper oral hygiene and result in plaque accumulation. Many studies report that changes in the dental flora occur after starting the orthodontic treatment (higher concentrations of pathogen microorganisms) [1, 2], while others claim the opposite [3, 4].

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This observational study evaluates the presence of the bacterial agents involved in pathogenic processes, before and after 3 months of wearing orthodontic appliances. We hereby present partial results obtained in the doctoral research titled "Assessment of the influence of orthodontic treatment over oral micro flora", conducted by Ecaterina Ionescu, DMD, PhD.

## Aim

The aim of this study was to evaluate the changes of the oral microbial flora during the first 3 months of orthodontic treatment.

## Materials and methods

Two groups of patients have been studied. The patients presented for orthodontic treatment between April and September 2010 in the Department of Orthodontics and Dento-Facial Orthopedics of the "Carol Davila" University of Medicine and Pharmacy, Bucharest. The parents of the patients (all minor) were informed on the study and signed their consent.

Group I was represented by 24 patients (8 males and 16 females) before starting any orthodontic treatment (T0). Group II was formed by 15 of these 24 patients, 6 males and 9 females, 3 months after wearing orthodontic appliances (T1). The patients were aged 7-16 for both groups, with an average of 11.7 for group I and 11.3 for group II.

All patients were instructed on the appropriate oral prophylaxis in accordance with their individual risk factors (interdental brush for fixed orthodontic appliance, denture cleansing tablets for removable appliances, dental floss for proximal plaque accumulation etc.).

Coronary and subgingival plaque was collected according to the protocol, from the dental surface with a sterile curette followed by the rapid transfer of the sample in Amies transport media and in anaerobic bacteria culture media (Schaedler broth with vitamin $K_3$). The samples were transported to the National Institute of Research-Development for Microbiology and Immunology Cantacuzino, registered and processed according to the protocols of the national expertise centers for Bacterial Respiratory Infections and Anaerobic Infections.

Bacterial concentration (colony-forming units / sample = CFU/sample) for the aerobic and anaerobic flora was calculated by the serial dilution method of counting bacteria [5]. 0.1 ml from each diluted sample ($10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$) were plated on 2 agar growth medium plates. The colony-forming units / sample number was established using the formula: CFU/sample number = number of colonies counted on a plate X dilution factor ($10^3$, $10^4$, $10^5$, $10^6$) X correction factor for 1ml (10).

Isolation of bacteria was performed on growth media: Todd Hewitt broth, Columbia agar with 5% sheep blood, Schaedler medium containing vitamin K3 (Schaedler K3 medium, broth and agar). After 24-48 hours of incubation at 37°C for the aerobic bacteria and 2-7 days for anaerobic bacteria, the developed colonies were morphologically examined. Each colony type was subcultivated on unselective growth media to obtain pure cultures. Reference strains were used as control (ATCC).

Isolated bacterial colonies were identified based on their morpho-tinctorial characteristics (Gram stained smears), growth characteristics on media and also underwent biochemical tests. The species were identified using API-20STREP for streptococci and API-20A for anaerobic bacteria, both from bioMerieux (Fig.1).

**Figure 1.** *Streptococcus oralis* identified by API-20STREP gallery

The dental and periodontal pathology involved strains were preserved by freezing, for further investigation by molecular diagnosis (genotypic methods).

## Results

Table I presents the results obtained for aerobic and anaerobic bacteria counted in samples collected from the two studied groups. An increased aerobic and anaerobic CFU/sample number was observed after 3 months of wearing orthodontic appliances.

| CFU/sample | Aerobic bacteria | | | | Anaerobic bacteria | | | |
|---|---|---|---|---|---|---|---|---|
| | Group I | | Group II | | Group I | | Group II | |
| | No | % | No | % | No | % | No | % |
| $<10^3$ | - | - | - | - | - | - | - | - |
| $\geq 10^3 < 10^4$ | 5 | 20.8 | 1 | 6.6 | 2 | 8.4 | - | - |
| $\geq 10^4 < 10^5$ | 10 | 41.6 | 7 | 46.7 | 11 | 45.8 | 7 | 46.6 |
| $\geq 10^5 < 10^6$ | 8 | 33.3 | 5 | 33.3 | 9 | 37.5 | 6 | 40 |
| $\geq 10^6$ | 1 | 4.2 | 2 | 13.3 | 2 | 8.2 | 2 | 13.3 |

**Table I.** The aerobic and anaerobic bacteria concentration in dental plaque

The aerobic bacteria percentage, with a concentration between $10^5$ and $10^6$, remained at the same level after 3 months. Regarding the anaerobic bacteria, the percentage increased from 37.5% to 40%.

Concerning the bacteria detected as more than $10^6$ CFU/sample, a higher isolation rate was noticed, from 4.2% to 13.3% for aerobic bacteria, while the anaerobic bacteria increased from 8.2% to 13.3%.

Many strains that are involved in dental and periodontal pathology were isolated from the dental plaque (Table II). The lactobacilli and oral streptococci species were dominant. 21 strains of lactobacilli (87.5%) were isolated from group I and 12 strains (80%) for group II. Oral streptococci (17 strains isolated in group I and 13 in group II) represented 70.8% and 86.6% of the isolated strains.

Most of the isolated bacterial strains' percentage from the dental plaque increased after starting the orthodontic treatment. A different situation was noted for lactobacilli that had a lower isolation rate in group II, compared to group I (80% vs. 87%).

The pathogen processes involved a bacteria isolation rate which increased from 8.3% to 13.3% for *Streptococcus mutans* and from 4.2% to 6.7% for *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

| Species | Group I (24 patients) | | Group II (15 patients) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| *S.oralis* | 5 | **20.8** | 4 | **26.6** |
| *S mutans* | 2 | **8.3** | 2 | **13.3** |
| *S sanguis* | 3 | **12.5** | 2 | **13.3** |
| *S salivarius* | 7 | **29.2** | 5 | **33.3** |
| *Lactobacillus* | 21 | **87.5** | 12 | **80** |
| *Corynebacterium spp* | 2 | **8.3** | - | - |
| *Moraxella spp* | 11 | **45.8** | 6 | **53.3** |
| *Escherichia coli* | 1 | **4.2** | - | - |
| *Staphylococcus spp* | 4 | **16.7** | 2 | **13.3** |
| *Agreggatibacter actinomycetemcomitans* | 1 | **4.2** | 1 | **6.7** |
| *Porphyromonas gingivalis* | 1 | **4.2** | 1 | **6.7** |

**Table II.** The most important bacterial species isolated from oral plaque

Similar to other studies [9, 10], most of the isolated pathology-involved bacterial strains' percentage from the dental plaque increased after starting the orthodontic treatment.

A small number of anaerobic strains were isolated (one *Aggregatibacter actinomycetemcomitans* and one *Porphyromonas gingivalis* strains from each group). This could be explained by the young age of the patients, but also by the difficulty of the anaerobic bacteria phenotypic diagnosis: difficult and time-consuming (2-7 days) isolation and identification, morpho-tinctorial variability and growth characteristics. These inconveniences will be eliminated by using the genotypic methods (PCR-based techniques) in order to improve the accuracy of the diagnosis [11 - 13]. A PCR method was developed by the Molecular Biology Laboratory from the National Institute of Research-Development for Microbiology and Immunology Cantacuzino, Bucharest. Species-specific regions within the genes coding for glucosyltransferases (*gtf* genes) were targeted for PCR identification of *S. mutans*.

Statistical significance of the obtained data was evaluated by applying chi-square test. The results showed that the obtained differences are not statistically significant.

## Discussions

The results concerning the aerobic and anaerobic CFU/sample number are in concordance with the results published by most other authors [1, 2, 7, 8] that sustain that the presence of orthodontic appliances may produce an increase of the oral bacterial concentration.

## Conclusions

The concentration of the aerobic and anaerobic bacteria (CFU/sample) has increased during the first 3 months of orthodontic treatment.

An increase of the isolation percentage for oral streptococcus and anaerobic bacteria, that are involved in dental and periodontal pathological

processes, was observed after starting a treatment with orthodontic appliances: from 8.3% to 13.3% for *Streptococcus mutans* and from 4.2% to 6.7% for *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Results are not statistically significant (p=0.5192 for aerobic bacteria, p=0.6843 for anaerobic bacteria, according to chi-square test with a 90% confidence interval). The lactobacilli were isolated in a smaller proportion in group II (80% vs. 87% before the treatment).

These results are preliminary, as part of an extended study. More patients will be investigated; dental microflora will be investigated at longer intervals of wearing orthodontic appliances; the bacterial strains that were isolated and preserved by freezing will be analysed by PCR.

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