

Periodontal aspects in orthodontics

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Abstract

Introduction

The demand for orthodontic treatment has been growing in the past few years, due to growing awareness and interest in general population for improving dental aesthetics and functionality. Adult orthodontics is increasing in popularity as it is becoming more feasible to move and improve teeth alignment, irrespective of the technique. Although a comprehensive orthodontic treatment cannot preclude the possibility of periodontal disease developing later, periodontal diagnosis and treatment can be a useful part of the overall treatment plan for a patient who could have periodontal involvement. The patient's ability to achieve and maintain good overall oral hygiene and prevent periodontal disease is fundamental while undergoing orthodontic treatment. The aim of our study was to demonstrate the presence of red complex bacteria in orthodontic patients and the usefulness of new diagnostic tests to control periodontal disease during orthodontic treatment.

Materials and methods

A total of 146 individuals participated to the study, 72 of them were adult orthodontic patients (age range: 16–45 years, mean age: 27.3 years), and 74 constituted the control group homogeneous for age and sex. A single species of each patient is quantitatively analyzed with the real-time polymerase chain reaction, using the LABtest (LAB® s.r.l, Ferrara, Italy).

Results

Prevalence of each red complex species is different among groups of patients with or without orthodontics appliances and has a high degree of statistical significance (*Porphyromonas gingivalis*, $p = 5 \times 10^{-6}$; *Tannerella forsythia*, $p = 8 \times 10^{-5}$; and *Treponema denticola*, $p = 3 \times 10^{-3}$).

Conclusion

Three bacterial species that constitute the red complex group—*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*—are considered the main pathogens involved in periodontitis related to orthodontic therapy.

Introduction

Lately, the demand to provide orthodontic treatment (OT) for enhancing aesthetics and functional ability is increasing because of the development and availability of innovative materials and technologies. The treatment scenario shows that adult patients' compliance considerably improved during OT and the time spent on therapy has considerably reduced. Indeed, OT can also be suggested for patients with slight and moderate periodontal disease in order to improve occlusal balance.

Periodontal health is essential to achieving successful OT, in the short as well as in the long term: orthodontists must have a good knowledge about the clinical manifestations, therapies of periodontal diseases, and the effects of tooth movement on the dental and periodontal support structure.

Therefore, it is clear that OT cannot be planned for patients presenting with active periodontal disease, and in patients with positive case history, the therapeutic program must consider controls required to stop disease relapse. An accurate examination of

periodontal conditions and evaluation of oral hygiene must always precede the planning for OT.

The two most important parameters to be evaluated during OT in a periodontal patient are as follows:

1. Recurring periodontal examination and periodical oral hygiene
2. Application of light and constant forces during therapy.

Indeed, accumulation of bacterial plaque increases after fixing an orthodontic brace. This results in generalized gingivitis, which does not however evolve into periodontal disease with loss of attachment.

Advantages of OT given to periodontal patients

In different situations, OT can prove quite advantageous for teeth and periodontal apparatus.

Alignment and levelling

Dental alignment and levelling assure gingival regular profile and reduce alimantal deposits. An irregular dental arch and tooth overlapping promotes accumulation of bacterial plaque, preventing a thorough cleaning of dental elements.

Tooth crown clinical extension

Slow orthodontic extrusion of a tooth improves not only the periodontal situation (deep periodontal attachment follows the tooth) but also the aesthetics, due to improved cervical margin alignment.

Progressive periodontal disease with horizontal bone reabsorption

Excessive loss of bone implies a dental movement caused by occlusional forces exceeding the physiological

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level. As a result, some diastemas are generated in the frontal zones, leading to worsening of overjet and overbite: the dental elements, no longer contained by the occlusional contact, extrude and come out (vestibularization). In these cases, an exclusive periodontal therapy does not lead to therapeutic success or restoration of morpho-functional stability.

Protesic and implanting molar uprighting

In order to plan a correct protesic or implantar-protesic treatment, it is sometimes necessary in pre-protesic and orthodontic therapy to carry out molar uprighting, as doing so will get the protesic pillars correctly positioned into the dental arch.

Periodontal remodelling during OT

The forces applied during OT leads to periodontal tissue remodelling. The correct application of orthodontic forces does not determine periodontal damages, since a non-controlled orthodontic therapy could trigger worsening of existing periodontal disease.

Orthodontic therapy and periodontal disease

The literature data regarding the efficacy of orthodontic therapies effects does not show any consensus among specialists.

Davies *et al.*¹ evaluated plaque, bleeding indexes and malocclusion conditions in a group of patients including both children treated orthodontically and not treated and reported that the level of bacterial plaque, bleeding and oral hygiene observed in treated patients is similar to that of non-treated persons.

In a test carried out using bonded patients and a control group not subjected to OT, Diamante-Kipiotti *et al.*² demonstrated an increase of dark bacterial pigments but not of gingival index (GI) and plaque index (PI).

Other tests demonstrated that the use of fixed braces for long periods could foster subgingival

microbic shift and periodontal disease development.

Farronato³ describes some of the main periodontal risk factors that are likely to arise during OT. Presence of orthodontic bands, poor oral hygiene and intrusive movements of dental elements can lead to the movement of deeper bacterial plaque into the gingival site and subsequent variations in the microflora and development of gingivitis into full-blown periodontal disease. For this reason, the authors advise preventive screening that will help detect the presence of periodontal-pathogenical bacteria or possible genetic predisposition to periodontal diseases in each treated patient.

In a longitudinal research, Sadowsky *et al.*⁴ described the conditions associated with the onset of periodontal diseases in adults orthodontically treated and carried out an analysis comparing conditions between the treated group and a homogeneous control group.

Petti *et al.*⁵ evaluated the influence of permanent and removable braces on the internal and external periodontal pocket; their data showed that although there were no signs of no gingivitis or parodontitis observed during the first 6 months of therapy, in the next 6 months the amount of periodontal-pathogen bacteria increased, with the consequent onset of gingivitis and periodontal diseases.

Lee *et al.*⁶ demonstrated meaningful differences in the subgingival plaque of patients equipped with fix structures and reported that *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*) and *Prevotella nigrescens* (*P. nigrescens*) were the most common conditions. These results suggested that, following the implantation of orthodontic devices, an increase of periodontal-pathogen micro-organisms and gingivitis does occur but there was no substantial loss of attachments observed in the tissues.

Huser *et al.*⁷ performed clinical and bacteriological tests at the beginning of the treatment, and 90 days

after the bonding was made, detected in treated patients an increase in plaque and bleeding in the bonded dental elements compared to those in control group. Furthermore, there was no increase in pocket depth.

The composition of bacterial plaque showed significant growth in spirochaetes percentage, fusiform rods with motility and filaments in post-bonded sites, but no relevant differences were found in the flora of the control group.

Naranjo *et al.*⁸ observed a transformation of microorganisms populating the sub-gingival plaque, after the positioning of brackets, and a considerable increase of gingivitis in the test group.

The level of *Porphyromonas gingivalis* (*P. gingivalis*), *Prevotella intermedia* (*P. intermedia*)/*P. nigrescens*, *T. forsythia* and *Fusobacterium* species increased after the bracket positioning in patients treated, compared to that observed in the non-treated control group.

Super-infectant microorganisms such as *Enterobacter cloacae* (*E. cloacae*), *Klebsiella oxytoca* (*K. oxytoca*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Serratia marcescens* (*S. marcescens*) were detected in the treated group.

A recent test carried out by Nelson-Filho⁹ analysed the presence of gram-negative bacteria in 33 orthodontic patients aged between 11 and 33 years. A bracket correspondent to three premolars was applied to each patient; 16 patients were given a wash with clorexidina (0.12%) and 17 with a placebo twice a week. After 30 days, brackets were removed and analysed: those of the first group had a significantly lower 'red complex' bacterial charge ($p = 0.01$), compared to the second group.

In a study conducted with 69 patients (age range: 6 to 17 years), Topaloglu-Ak¹⁰ reported the effect of orthodontic devices in terms of increase in the presence of *Streptococcus mutans* (*S. mutans*), *Lactobacillus sp.* and *Candida albicans*

in saliva after 6 months of therapy and observed a growth in oral microflora and, in particular, *Candida albicans* after 3 months.

Corbacho De Melo¹¹ analysed and compared the GI of second bonded molars in 100 adult patients compared to a homogeneous non-treated group, determining that the test group had a higher GI.

A Brazilian study¹² evaluated PI, GI and decayed, missing and filled teeth index (DMFT index) in 30 patients treated with fixation of permanent devices compared to a homogeneous non-treated control group. Same parameters were measured on 18 patients treated with removable devices. The results showed a prevalence of PI and GI in patients with both types of devices and no significant differences in DMFT index between the study group and the control group.

Attin *et al.*¹³ evaluated the recolonization of streptococcus mutans after the professional application of 40% clorexidina gel onto the dental elements with or without orthodontic attachments. Statistical analyses showed that after 8 weeks of application, the recolonization of streptococcus mutans showed an increase ($p < 0.05$) in the test group compared to the control group.

Ristic¹⁴ performed a prospectical test on 32 adolescents and observed that orthodontic device applications induce an increase in the number of patients testing positive to *P. intermedia* and other anaerobic periodontal-pathogen bacteria. Furthermore, other behaviours deduced were a general increase in the presence of microorganisms and pocket depth from T0 before therapy to T2 two months later. These parameters decrease 6 months after therapy; the authors stated that device applications cause a temporary increase in the presence of periodontal-pathogen bacteria, which can be correlated to inflammatory conditions caused by gingival tissues, but without any damage to the deepened periodontal pockets.

By contrast, a test led by Hamp and others¹⁵ showed that during a long-term OT provided to 53 patients, the preventive oral hygiene actions taken before and during the therapy caused a slight but significant loss of periodontal support. At the end of the therapy, which lasted about 24 months, the average loss of vestibular attachment was about 0.28 mm but it was only 0.22 mm at the tongue. A follow-up over 20 to 30 months did not show any further losses.

Avvantaggiato and others¹⁶ performed a test on 26 adult patients subjected to orthodontic therapy and found reabsorption of crestal bone in 45.2% of the patients, even after carrying out preventive protocols and application of light orthodontic forces.

Boyer¹⁷ studied the effects of periodontal therapy in 15 adult patients (11 women and 4 men, age range: 22–61 years) affected by heavy periodontal disease studied for 16 years (age range: 11–32 years). The first group consisted of patients provided with periodontal-orthodontic treatment, and the second periodontal group was included for comparison purposes only. The height of the alveolar bone was measured at T0 before treatment, T1 post-operation and T2 long-term post-operation via digital radiography. The results highlighted a neobone apposition at T1 and T2 without meaningful differences between the two groups. These authors stated that the combined periodontal-orthodontic treatment had no negative impacts on crestal reabsorption.

Another highly debated argument is the effect of different modern orthodontic materials on the periodontal health.

Turkkakraman and others¹⁸ evaluated the influence of different arch ligatures used (steel vs elastomeric rings) on the intra-oral bacterial flora and the periodontal health, stating that the rings led to a higher increase in bacterial plaque than steel wires did.

Forsberg¹⁹ evaluated elastomeric rings and metallic wires in 12 patients subjected to a fix therapy and reported on the association with *S. mutans* and *lactobacilli* colonizations. Elastomeric rings on one semi-arch were fitted on one side and steel wires on the other; in the elastomeric side, the presence of microorganisms in the plaque was markedly higher compared to the second side. The application of fixed devices led to a higher presence of salivary *S. mutans* e *lactobacilli*.

Sukontapatipark²⁰ conducted a test with patients treated with the extraction of 2 to 4 premolars; brackets were applied on these teeth using elastic ligatures on one side and metallic ones on the other. The dental elements were extracted 1, 2 and 3 weeks later; the electronic microscope showed that the bracket area had a bacterial plaque accumulation.

In a clinical randomized trial with a split-mouth design, Van Gastel and others²¹ compared the presence of plaque, periodontal parameter and crevicular fluid flow between patients with teeth bonded with two different types of brackets and those with non-bonded teeth, and their study confirmed a significant increase in the presence of aerobic and anaerobic bacteria in orthodontic patients.

Oral microflora and orthodontic

Periodontal disease is a polymicrobial infection caused by pathogenic bacteria, which are organized in biofilm; they cooperate with colonization's strategies in complex ways, as recently reported by Socransky²². The inflammatory reaction created by the deposit of bacterial plaque causes the destruction of periodontal tissues^{23,24}. Therefore, it is necessary to identify, estimate and classify²⁵ the most periodontal pathogenic bacteria as part of regular diagnosis and therapeutic planning. Indeed, it has been demonstrated that a strict correlation exists between gingival inflammation²⁶ and microbiological spectrum.

Red complex

- *Aggregatibacter actinomycetemcomitans*
- *T. forsythia*
- *P. gingivalis*
- *T. denticola*

The presence of these bacteria is mainly linked with severe periodontal diseases (deep pocket ≥ 6 mm). Clinically, these bacteria are related to pocket depth and bleeding during probing. A greater concentration is related to a progressive deepening of the pocket, wherein the three main bacteria act together²⁷.

The aim of our study is to demonstrate the presence of red complex bacteria in orthodontic patients, and the usefulness of a new diagnostic test to control periodontal disease in patients undergoing OT.

Material and methods

This work conforms to the values laid down in the Declaration of Helsinki (1964). The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed. All subjects gave full informed consent to participate in this study.

In order to study the clinical manifestation of different promoting factors, our research team evaluated²⁸ the type and quantity of 'red complex' bacteria found in the crevicular fluid collected from 146 individuals participated in the study, out of whom 72 were adult orthodontic patients (age range: 16–45 years, mean age: 27.3 years; Table 1), and 74 patients constituted the control group homogeneous for age and sex.

A sample of the gingival microbiota was obtained from a single site (sulcus of first superior molar). A single species of each patient was quantitatively analyzed with the real-time polymerase chain reaction (RT-PCR), using the LABtest (LAB® s.r.l, Ferrara, Italy; see Figure 1). Using a paper probe, samples of bacterial plaque

Sample characteristics	Total	Control group	Orthodontic patients
Number of participants	146	74	72
Male	63	31	32
Female	83	42	41
Age (mean years \pm SD)	27.03		



Figure 1: Kit for the 'red complex' bacterial test used in our study (LAB® s.r.l, Codigoro, Ferrara, Italia).

composed of *P. gingivalis*, *T. forsythia* and *T. denticola* were collected from both the study group and control group. DNA was extracted and purified using standard protocols that included two consecutive incubations with lysozyme and proteinase K, followed by spin-column purification.

RT-PCR

Primers and probes of oligonucleotides were designed on the basis of 16S ribosomal RNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq, Version 10.1) consisting of 845 entries. All the sequences were

aligned to find either consensus sequence or less conservative spots. Two RT-PCR runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conservative sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria, that is, *P. gingivalis*, *T. forsythia* and *T. denticola*, in a multiplex PCR. This reaction included a total of six primers and three probes that were highly specific for each species. Oligonucleotide concentrations and PCR conditions

were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10-minute incubation period at 95° C to activate polymerase, followed by a two-step amplification for 15 seconds at 95° C and for 60 seconds at 57° C over 40 cycles. All these experiments were performed using non-template controls to avoid contamination of reagents.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg, Germany) were used for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial dilutions ranging from 10^1 to 10^7 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number and the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations were estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative amount of red complex species. To prevent contamination of samples subjected to polymerase chain reaction, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

Statistical analysis

A descriptive statistical information was prepared using Microsoft Excel spreadsheets. The Freeman–Halton extension of Fisher's exact test to compute the (two-tailed) probability of obtaining a distribution of values in a 2 x 3 contingency table, based on the number of observations in each cell. Odds ratio calculation was measured online using the tools available on the website of OpenEpi.

Results

The incidence and quantity of red complex bacteria in crevicular fluid were evaluated in 146 individuals. A single specimen from each patient was analyzed using PCR-IT, in order to measure total bacteria load and the amount of three bacteria in the periodontitis of orthodontics patients, that is, *P. gingivalis*, *T. forsythia* and *T. denticola*. Ours is a preliminary study that focused mainly on the prevalence of these three bacteria among two specific groups of patients, one with orthodontic appliances and one without, in order to understand whether the presence of red complex species may be considered predictive of developing or onset of periodontitis in orthodontics patients.

The Freeman–Halton extension of Fisher's exact test indicated that the prevalence of each red complex species is different between groups of patients and has a high degree of statistical significance (*P. gingivalis*, $p = 5 \times 10^{-6}$; *T. forsythia*, $p = 8 \times 10^{-5}$; and *T. denticola*, $p = 3 \times 10^{-3}$). The higher level of association with periodontitis was observed for *T. forsythia*; indeed, the observed odds ratio was 8.6 (95% confidence interval [CI]: 3.0–27) when healthy individuals were compared to periodontitis patients and was 5.8 (95% CI: 2.3–15) when healthy and gingivitis groups were combined and compared to orthodontic patients.

Discussion

The polymerase chain reaction (PCR) is a more sensitive and faster method for detecting microbial pathogens in clinical specimens. In particular, the diagnostic value of PCR is significantly higher when specific pathogens that are difficult to culture in vitro or require a long cultivation period, such as anaerobic bacteria species involved in the onset of periodontitis. A recent improvement of this technique is the RT-PCR, which allows for quantification of DNA target using

fluorogenic probes in a close setup. In addition to the ability of quantifying the target, another advantage to using PCR is that the assay can be performed in a closed system wherein the reaction tube is never opened after amplification. This is of great value in preventing laboratory contamination and false-positive results. Also, using a probe in addition to the two PCR primers further enhances the specificity of reaction.

In the present investigation, we designed and tested the performance of a RT-PCR-based assay to detect and quantify the red complex bacteria involved in orthodontic patients. In particular, we found that *P. gingivalis*, *T. forsythia* and *T. denticola* were strongly related to orthodontics appliances because their prevalence was higher among orthodontic patients. The presence of these bacterial species can significantly increase the risk of periodontitis, given the fact that the odds ratio measured was between 8.6 (*T. forsythia*) and 4.4 (*T. denticola*).

Molecular analysis of periodontal pocket microflora by RT-PCR is an effective and inexpensive method and can rapidly detect and quantify red complex bacterial species in orthodontic patients also.

Our preliminary study is focused especially on the prevalence of these three bacterial species among groups of orthodontic patients, in order to determine the correlation between the quantity and the presence of 'red complex' bacteria and periodontitis as predictive factor of periodontal disease in these patients.

In our research, we have drawn and tested the performances of a trial in PCR-RT to identify and quantify the red 'complex bacteria' related to orthodontic therapy. Above all, we emphasize the deep association between *P. gingivalis*, *T. forsythia* and *T. denticola* and the evolution of periodontitis in orthodontic patients, since the prevalence of these species was greater in orthodontic patients.

Clinical relevance of the microbiological test in OT (LABtest, LAB® s.r.l, Ferrara, Italy)

The periodontal test is a fundamental tool for the diagnosis and therapeutic planning in periodontal treatments. Specific advantages of this test are as follows:

- Identification and quantification of main pathogens during the diagnostic phase of OT
- Measurement of the 'periodontal bacteria's presence', to draw up a detailed profile of risks likely to surface during OT
- Objective control over therapeutic surgery and orthodontic-periodontal upkeeping protocol
- Improving patient compliance who, having been made aware of his correct microbiological profile, will consciously follow the therapeutic plan
- Accurate diagnosis of patients and/or the recidivous sites against the therapy
- Innovation in orthodontic research

The presence or absence of different bacteria in different proportions and the presence of highly pathogen sub-types define the level of intensity of periodontal disease during OT.

These parameters are fundamental to

- define a therapeutic procedure
- plan follow-up intervals
- promote patient involvement in therapeutic plan
- improve patient compliance with domiciliary and professional oral hygiene
- certification of the actual healing or of possible further activities post-therapy
- monitoring the sustainability of results via periodical checks.

Conclusion

The molecular analyses of micro-flora in periodontal pockets via RT-PCR is a valid, rapid and inexpensive method to identify and quantify the 'red complex' bacteria in orthodontic patients. This test has been carried out with a large sample of patients and results indicate that RT-PCR can improve the accuracy of periodontal disease diagnosis.

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