Changes in clinical and microbiological parameters of the periodontium during initial stages of orthodontic movement in patients with treated severe periodontitis: A longitudinal site-level analysis

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Abstract. Applying orthodontic braces makes oral hygiene difficult and increases plaque accumulation, frequently resulting in gingival inflammation. In patients with previous severe periodontitis, this inflammation overlaps with the pre-existing inflammatory challenge and can lead to further progression of periodontal attachment loss. The aim of this study was to assess longitudinal site-level changes as mirrored by clinical and microbiological parameters during the initial remodeling of alveolar bone and the periodontal ligament, produced as an effect of light orthodontic forces in adult patients with severe periodontal disease that underwent standard (non-surgical and conventional surgical) periodontal therapy. Thirteen patients with previously treated severe generalized periodontitis were given fixed orthodontic appliances for re-alignment of teeth misaligned or displaced during the course of periodontitis. Before insertion of orthodontic appliances and at 2, 4, and 6 months of treatment, periodontal clinical parameters were recorded in the same deepest residual pocket of at least 3 mm in each patient. The same pocket was sampled at baseline and after 6 months of orthodontic treatment for the frequency of positive detection of *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tanerella forsythia (Tf), Treponema denticola (Td)*. An average reduction in Pocket Depth by 0.2 mm at the end of the assessment period was identified. The only clinical parameter with statistically significant improvement was bleeding on probing. The frequency of detection of *Aa, Pg, Pi,* and *Tf* was not significantly different between baseline and 6 months of treatment, while a marginally significant increase of *Td* was found. There were no significant differences in the clinical parameters or microflora in the initial phase of orthodontic treatment in patients with reduced periodontal support. By correlating clinical and microbiological data, we concluded that the presence of periopathogens do not negatively influence periodontal health during orthodontic treatment in adult patients treated for severe periodontitis.

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Introduction

Periodontal disease is a multifactorial, polymicrobially triggered inflammatory disease whose pathogenesis is dependent on numerous host-related factors that eventually results in an individual's susceptibility to the disease (1). Although over 800 bacterial species can colonize tooth surfaces and various artificial oral appliances, the gingival margin and gingival

sulcus, periodontal disease can already be initiated by a relatively small number of pathogens present in the microbial biofilm (2-5). In order to ensure the efficacy and long-term success of the periodontal treatment, periopathogens must be drastically decreased if not completely eradicated.

In patients with a history of periodontitis resulting in displaced teeth, possible orthodontic tooth movements include changes in alignment, space redistribution, and intrusion (6). The primary aim, before orthodontic intervention might start, is to stabilize the periodontal condition. Bone loss alters the position of the tooth's center of rotation and the force required to achieve the movement; however, the orthodontist can use reduced or increased force moments to avoid excessive alveolar bone loss (6).

Orthodontic therapy has been shown to be a reliable therapy for restoring compromised dentition, closing infrabony defects, reducing gingival recessions, and improving interdental papilla levels; thus, orthodontics can be considered for the treatment of periodontal patients with tooth migration (7). Other studies have shown that orthodontic treatment can safely be used in patients with previous periodontal therapy, despite the fact that orthodontic appliances worsen conditions for oral hygiene, complicate tooth care, and, thereby, create an environment favorable to plaque accumulation (8). However, orthodontic treatment often employs the permanent or long-term use of a retainer which can complicate dental hygiene self-cleaning procedures, and can potentially harm the periodontal tissues (8). In time, periodontal parameters can deteriorate, even if some of them may improve after the removal of the orthodontic retainers (9,10). A very recent systematic review showed a deterioration of periodontal parameters after orthodontic treatment, indicating that it influences the accumulation and composition of the subgingival microbiota and subsequently induces more inflammation and higher BOP (11).

Earlier studies hypothesized that the orthodontic treatment can improve or prevent deterioration of periodontal parameters in treated periodontal patients. Significant reductions in pocket depth (PD) and clinical attachment levels (CAL), as well as radiographical improvements of periodontal bone defects were reported (12-14). Since orthodontic therapy can be safely used in patients with previous periodontal therapy, it is only coherent to use it as an additional tool in periodontitis treatment, even if the inherent risks of the therapy must be taken into consideration (15).

Therapeutical stepbacks as 'black triagles' and reduced interdental papilla heights (16) and difficult prosthetic rehabilitation can be prevented in orthodontic patients with previous periodontal disease, together with the additional attachment loss, through strict biofilm control and periodontal maintenance. These procedures are essential during the active phase of orthodontic treatment, in order to prevent inflammation in gingival tissues (17,18).

Bone changes induced by orthodontic treatment may impact the morphology of bone defects, decrease pocket depth, and enhance connective tissue healing (16). The influence of tilting movements in the presence of intrabony pockets is further evidence that orthodontic movements may be performed in teeth with bone defects without further damaging periodontal attachment (19). Thus, orthodontic forces must be carefully applied in teeth with a reduced periodontium.

Despite the high number of published articles debating the periodontal-orthodontic interrelationship, there is a lack of good evidence on systematic treatments including both orthodontic and periodontal therapy. The periodontic-orthodontic interrelationship has been the subject of substantial investigation, yet it remains a controversial issue (20). Thus far, the literature regarding orthodontic treatment in subjects with treated periodontal disease is represented mostly by case reports on subjects already treated for chronic periodontitis (17). Of more interest for clinical studies, however, might be the relationship between aggressive periodontitis and orthodontic treatment, probably because of the significant tooth displacement related to this rapidly progressing form of periodontitis (17,21). In the early 2000's, the Cardaropoli group (13,22-24) reported that orthodontic treatment is no longer a contraindication in the therapy of severe adult periodontitis. While orthodontics has improved the ability to restore deteriorated dentition, over the last decade there has been no clinical study regarding the outcome of treated periodontium undergoing orthodontic movements. There are also relatively few clinical studies comparing the outcomes of combined periodontal-orthodontic treatment with the outcomes of periodontal treatment alone in patients with severe periodontitis (25).

The aim of the present study was to determine the longitudinal changes in clinical and microbial parameters of the periodontium at site-level during the initial remodeling processes of the alveolar bone and periodontal ligament caused by light continuous forces employed in the orthodontic treatment of adult patients with a history of severe periodontal disease treated with standard (non-surgical and conventional non-regenerative) periodontal therapy.

Patients and methods

Ethical approval, selection of patients and timeline of measurements. The study was conducted in the Department of Periodontology of the Faculty of Dental Medicine of the 'Victor Babes' University of Medicine and Pharmacy University, Timișoara, Romania, from November 2013 to November 2014, with the approval no. 14/16.09.2013 of the University Committee on Research Ethics. Before the start of the study, all patients received detailed information regarding sampling procedures, time points, and conditions to be met during the trial, as well as inclusion and exclusion criteria. Patients received guidelines for specific proper oral hygiene during the wearing of orthodontic appliances; instructions were reinforced at every subsequent appointment. After receiving all necessary information, patients signed an informed consent agreement. The study protocol was conducted in conformity with the Declaration of Helsinki.

Thirteen adult patients (8 women and 5 men, aged 23-53 years, mean age 36.5 years), with a history of severe periodontitis as described by Armitage (26) treated with standard (initial and conventional surgical) periodontal therapy received fixed orthodontic appliances. The criteria for inclusion in the study were: i) ≥21 yo; ii) good systemic health in terms of diabetes, cardiovascular diseases and other conditions that may impact the peridontal status; iii) absence of extended fixed and removable prosthetic restorations; iv) no previous orthodontic treatments; v) severe periodontitis treated by

standard (non-surgical and conventional surgical) procedures, the treatment being completed at least one year before the onset of the orthodontic treatment; vi) good compliance with a rigorous (with respect to the initial 3-months recall intervals, good personal oral hygiene), supportive periodontal therapy; vii) stable periodontal status during the previous six months (absence of inflammation and attachment loss); viii) good oral hygiene (full mouth plaque and full mouth bleeding scores under 25%); ix) teeth affected by periodontal attachment loss, misaligned or displaced following the evolution of periodontitis and x) indication for orthodontic treatment for esthetic or functional reasons. Exclusion criteria were administration of antibiotics during in the previous six months, pregnancy, lactation, smoking, allergies to the materials included in the orthodontic appliances, incapacity to read and understand the aim and nature of the study.

Clinical measurements and subgingival plaque sampling were performed for each individual patient on the same tooth and site by the same intra-examiner calibrated investigator (AJ). The selection criteria for experimental periodontal sites were: i) residual PD ≥3 mm; ii) situated on single-rooted teeth; iii) at the site where the periodontal ligament underwent compression. Experimental teeth underwent orthodontic corporeal movements predominantly in mesial direction (12 teeth) and distal direction (one tooth). In each experimental tooth, at the periodontal site that displayed the deepest residual pocket at the beginning of the orthodontic treatment, the periodontal clinical status was evaluated at baseline and at 2, 4, and 6 month intervals; microbiological status was evaluated at baseline and again after 6 months. Gingival crevicular fluid (GCF) sampling for determining enzymatic and inflammatory changes during the orthodontic treatment was performed on the same experimental sites (data to be published elsewhere). Orthodontic treatment was initiated 12 months after the completion of the planned active periodontal therapy, even if a small number of pockets deeper than 3 mm persisted, within a well-controlled periodontal maintenance program. The timeline of measurements is displayed as a flow chart in Fig. 1.

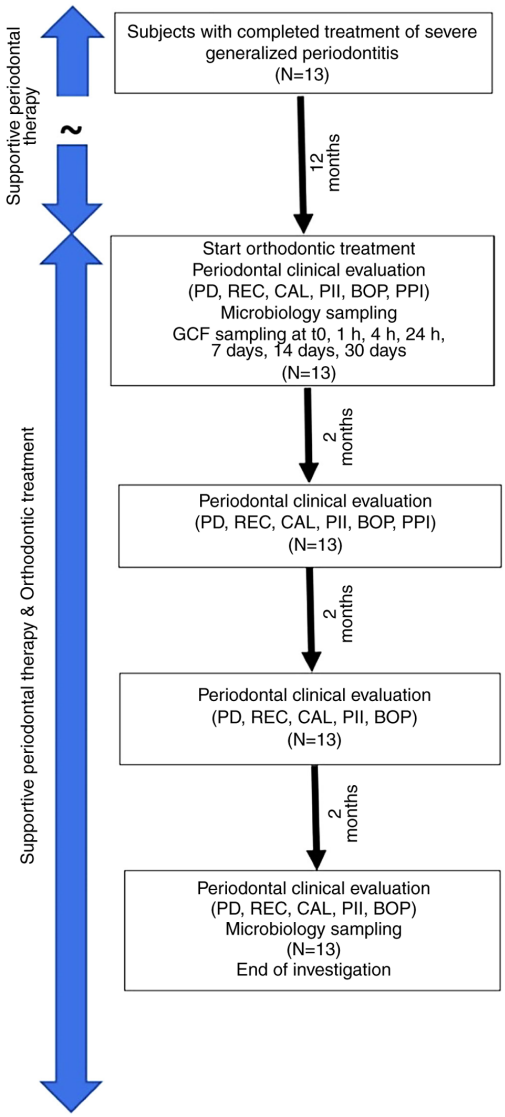

Figure 1. Flow chart of the study protocol. PD, Periodontal Pocket Depth; REC, Gingival Recession; CAL, Clinical Attachment Level; PII, Plaque Index; BOP, Bleeding On Probing; PPI, Papilla Presence Index; GCF, Gingival Crevicular Fluid; N, number of subjects.

Orthodontic treatment. Twelve months after the end of periodontal treatment, orthodontic brackets (Omniarch®, Dentsply GAC), slot 0.018 inch and Sentalloy Superelastic® (Dentsply GAC), size 0.014 inch wires were applied to each patient with a Roth Rx prescription.

Periodontal clinical parameter measurements. In each selected site of the experimental teeth, the following parameters were evaluated: PD (pocket depth), REC (gingival recession), CAL (clinical attachment level), BOP (bleeding on probing), and PPI (papilla presence index). The PII (plaque index) was evaluated in each patient in the experimental tooth only. The measurements were made before applying the orthodontic braces (T0) and again at 2, 4, and 6 months after orthodontic treatment with the exception of PPI, which was assessed at baseline and after 2 and 4 months of orthodontic treatment. PPI is useful in evaluating the aesthetic success of periodontal-orthodontic treatment (25) and was measured as scores (PPI-1 to PPI-4) to quantify the loss of height of interdental papilla following periodontitis. The last PPI assessment took place at 4 months, as it was considered that the orthodontic movement was completed at that time. Before measurement, teeth were isolated using dental cotton rolls and dried with warm air. In order to measure the PII, each experimental tooth was stained using a plaque disclosing solution (micropellets soaked with a disclosing agent) (Rondells Blue, Directa AB). Digital photos were taken at each visit after staining for documentation. PD, REC, and CAL were measured on the same tooth with a periodontal probe (PCP-UNC15); measurements were rounded up by 0.5 mm if necessary. BOP was recorded 20 sec after probing and scores were attributed (score 0, absent, score 1, present).

Microbiological parameters sampling and evaluation. The presence of five main periodontopathic bacteria in the gingival sulcus was evaluated at baseline and after 6 months: *Aggregatibacter acinomycetemcomitans (Aa), Porphyromonas gingivals (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf),* and *Treponema denticola (Td)*.

Table I. Descriptive statistics for PD, REC, CAL, and PlI measured at baseline and at 2, 4, and 6 months of orthodontic treatment (mean ± SD, range specified in parentheses).[a]

Parameter	Baseline	2 months	4 months	6 months	P-value
PD	4.23±1.09 (3-7)	3.77±1.24 (2-7)	3.92±0.86 (2-5)	4.00±0.82 (2-5)	0.412
REC	0.77±1.01 (0-3)	0.77±1.01 (0-3)	0.85±0.99 (0-3)	0.69±0.95 (0-3)	0.494
CAL	4.92±1.50 (3-8)	4.54±1.66 (2-8)	4.77±1.42 (3-8)	4.69±1.11 (3-7)	0.559
PlI	1.04±0.43 (0-2)	1.31±0.43 (1-2)	1.23±0.44 (0.5-2)	1.19±0.43 (0.5-2)	0.019

[a]P-values correspond to Friedman tests for comparison of responses at successive time points.

Sampling was performed after complete removal of the supragingival plaque and isolation of the tooth with cotton rolls. The tooth was dried with gentle air flow in order to avoid contamination of the samples with saliva. At each site, two #30 0.04 sterile paper points (Roeko GmbH) were inserted and held in place for 30 sec until soaked. After sampling, the cones were transferred to Eppendorf tubes containing 700 μl PBS solution and kept refrigerated in a special thermoisolated box during the transport to the laboratory. Enzymatic and microbiological testing was performed in the laboratories of the Department of Biochemistry of the 'Victor Babes' University of Medicine and Pharmacy (Timișoara, Romania). Each tube containing plaque sample received a code. It was vortexed for 30 sec at room temperature. The points were removed and the eluates clarified by centrifugation for 5 min at 3,000 x g at 4˚C. Samples were stored for one day at -20˚C and then at -80˚C until microbiological analysis (no longer than a month). The processing of the samples included DNA extraction, amplification, and hybridization. For DNA extraction the QIAamp DNA Micro kit (Qiagen GmbH) was used, in accordance with the manufacturer's instructions. Sample DNA hybridization was performed with a micro-IDent plus® kit (Hain Lifescience GmbH). For amplification, a HotStar Taq Polymerase kit (Qiagen GmbH) was employed; this is an inactive polymerase that offers high specificity for PCR and facilitates the amplification process by eliminating several reaction steps. Amplification was performed using a thermocycler (Thermo Fisher Scientific Inc.) for 32 cycles. After hybridization, the reading strips were submerged in the sample tube and were incubated at 45˚C for 30 min. Extracted DNA was quantified by spectrophotometry (230 nm), using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc.). Accordingly, semi-quantitative data were recorded.

Statistical analysis. The statistical unit was the patient, as one orthodontically moved tooth per periodontal patient was included in the study. Statistical analysis of the data was performed using the software R 3.1.3. (R Core Group 2015). The data distribution was checked for normality and the Friedman test was used for analyzing continuous or ordinal clinical parameters, for which at least three successive measurements were made (PD, CAL, REC, PlI, and PPI), in order to describe the mean value variation during the observation period. The Q Cochran test was employed for BOP. For P-values lower than the significance level of 0.05, the null hypothesis stating no difference between the samples was rejected, concluding that the mean parameter values (or proportions in case of BOP) differ significantly between at least two time points. For these cases, post hoc tests (Conover and Holm adjustments for Friedman test; Benjamini-Hochberg adjustment for Cochran test) were employed in order to determine the exact point at which these differences occurred. The microbiological results were recorded as the following categories (scores) of detectability: 0=nondetectable, 1=10^4 (10^3 for *Aa*), 2=10^4-10^5 (10^3-10^4 for *Aa*), 3=10^5-10^6 (10^4-10^5 for *Aa*), and 4=>10^7 (10^6 for *Aa*). The Wilcoxon signed-rank test was used for analysis of microbial detectability variations between baseline and the final timepoint. The differences were considered significant at P<0.1.

Results

The changes in mean PD, REC, and CAL between the measurements at different timepoints are given in Table I. There were no statistically significant differences between the measurements at different timepoints.

For PPI, there were no significant differences between successive time points (Friedman test, P=0.36). At each time point, PPI values ranged from 1 to 3 (median=2). For 12 out of 13 patients, no PPI changes occurred between the three observation time points. For one patient, PPI increased from 2 to 3 in the first interval, but remained unchanged afterwards (Fig. 2).

For the PlI, the Friedman test revealed significant differences between the time points analyzed (P=0.019). Post-hoc Conover tests showed that these differences are due to the fact that the mean PlI at baseline was significantly lower than at later time points. Furthermore, the PlI at 6 months also shows a significant decrease as compared with 2 months (P<0.05 in each case) (Table II).

The prevalence of BOP at the four analyzed time points is shown in Fig. 3.

The Cochran Q test showed that there are significant differences between the proportions at various time points

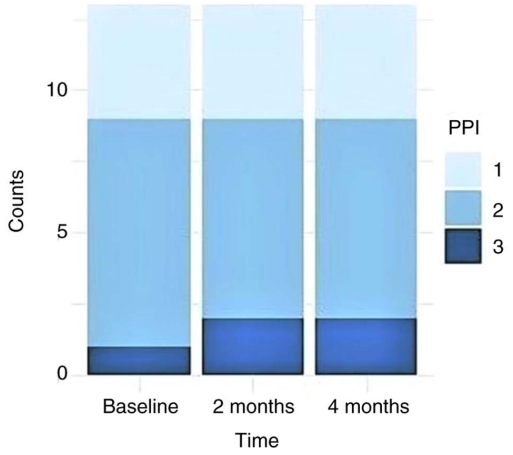

Figure 2. Distribution of PPI at baseline, 2 and 4 months of orthodontic treatment.

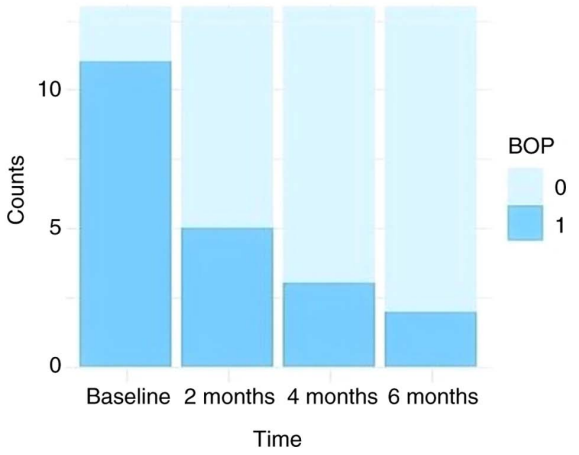

Figure 3. BOP prevalence at baseline, 2, 4 and 6 months of orthodontic treatment.

Table II. Results (P-values) of post hoc comparisons between PII values at baseline and after 2, 4, and 6 months of orthodontic treatment (Holm adjustment).

Items	Baseline	2 months	4 months
2 months	$<10^{-5}$	-	-
4 months	<0.001	0.165	-
6 months	0.021	0.021	0.241

Table III. Results (P-values) of post hoc comparisons between BOP prevalence ratios at baseline and after 2, 4, and 6 months of orthodontic treatment (Benjamini-Hochberg adjustment).

Items	Baseline	2 months	4 months
2 months	0.063	-	-
4 months	0.023	0.824	-
6 months	0.023	0.562	1

(P=0.001). Post-hoc comparisons indicate that significant differences occurred between baseline and after 4 and 6 months (Fig. 3). BOP frequency of occurrence decreased from 84.61 to 38.46%, 23.08, and 15.38% at 2, 4, and 6 months after orthodontic treatment, respectively (Table III).

Assessing the presence of the periopathogens *Aa, Pg, Pi,* and *Tf,* the Wilcoxon signed-rank test did not reveal statistically significant differences between the values recorded at baseline and after 6 months of treatment. For *Td*, the only periopathogen that exhibited an increase throughout the observation period, the differences were only marginally significant (P<0.1) (Table IV).

Discussion

To our knowledge, this is the first clinical study to describe at site-level the evolution of clinical and microbiological parameters in orthodontic patients previously treated for severe periodontitis.

Naranjo *et al* noted a shift in the microflora populating the subgingival plaque after orthodontic bracket placement as well as a considerable increase of gingivitis in the test group (27). Another study found that levels of *Pg, Pi, P. nigrescens, Tf,* and *Fusobacterium spp.* increased after bracket placement in treated patients when compared with patients in the untreated control group. Super-infectant microorganisms such as *Enterobacter cloacae, Klebsiella oxytoca, Klebsiella pneumoniae,* and *Serratia marcescens* were detected by the authors in the treated group (28). In an earlier study, clinical and bacteriological evaluations at baseline and at 90 days after orthodontic bonding treatment; the authors detected an increase in plaque and bleeding in periodontal sites of bonded teeth in patients undergoing orthodontic treatment as compared with the control group. Furthermore, there was no increase in pocket depth (29). Surprisingly, a microbiologic study from 2004 attributed the marked improvement in the periodontopathogenic bacterial spectrum under fixed appliance therapy with metal brackets, NiTi archwires, and stainless steel wires to metal corrosion, which entailed the release of nickel ions that are thought to be exclusively toxic to periopathogenic bacteria (30).

In the present study, clinical and microbiological evaluations were performed on each selected tooth at the site with the deepest residual PD (≥3 mm) to allow for potential changes of the respective surrogate parameters. All analyzed sites were chosen on the aspect of the root that underwent compression so that GCF sampling and analysis could also include inflammatory resorptive activity on the alveolar bone induced by the orthodontic movements (31,32).

The Friedman test for the mean values of PD, REC, and CAL variations did not indicate changes between the timepoints. The evolution of individual PDs revealed that, at 2 months, all 13 patients scored values equal to or lower than baseline.

The main periodontal objective of orthodontic treatment in patients with treated periodontitis is to maintain or improve attachment level. In our study, we concluded that PD did not change significantly following orthodontic movement,

Table IV. Comparative detection scores at baseline and after 6 months of orthodontic treatment for periopathogens *Aa, Pg, Pi, Tf,* and *Td*, and P-values for the corresponding Wilcoxon signed-rank tests.

Detection score	*Aa*		*Pg*		*Pi*		*Tf*		*Td*	
	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months
Median	0	0	0	0	0	0	0	1	0	1
Range (min-max)	0-4	0-4	0-1	0 - 4	0-2	0-2	0-3	0-4	0-1	0-2
P-value	0.1814		0.3447		0.8241		0.7252		0.0649	

demonstrating that orthodontic treatment does not adversely affect the periodontal condition in patients with a history of periodontal disease. The results obtained are consistent with other clinical studies in the literature. It was previously shown that orthodontic treatment in patients with treated periodontal disease resulted in a 0.7 mm decrease in PD after one year of treatment (33). Other authors, in a similar clinical trial, concluded that orthodontic treatment has no negative impact on PD variations. Although both studies used regenerative surgical techniques, the effects of orthodontic treatment on PD between the beginning of therapy and at the end of the assessment period are relevant (34). Other studies have reported significant reductions in PD values following orthodontic treatment, concluding that orthodontic treatment can actually improve periodontal conditions (21,22,35,36). Differently to the aforementioned studies, in our study the reduced magnitude of the PD changes could be potentially attributed to the severity of the treated periodontitis.

REC and PD did not exhibit statistically significant changes at the time points analyzed. The evolution of REC at successive time points suggests that no change in the parameter occurred during the first 2 month interval. After 4 months of orthodontic treatment, there was a slight increase in mean values, while at 6 months there was a decrease. One important observation is that for 7 out of 13 patients, REC values did not undergo any change during the study period. One patient experienced a 1 mm increase over the 4 month interval that remained constant until the end, and two patients experienced a 1 mm reduction in REC over the 6 month period. The maximum recorded value was 3 mm for the first three intervals, and remained unchanged until the end of the assessment period. Similar to PD, a slight reduction in mean REC was observed in our study. These results provide some evidence for possible beneficial effects on periodontal status following orthodontic treatment. In the literature, the incidence of gingival recession following orthodontic therapy is disputed and the results are largely contradictory (37).

In our study, the mean CAL did not yield statistically significant variations (P=0.559). However, a 0.2 mm gingival attachment gain was noted at the end of the assessment period, when compared with baseline. These results are consistent with other clinical studies that did not identify statistically significant differences (31) or found no differences in CAL following orthodontic treatment (38).

The only clinical parameter that recorded statistically significant differences was BOP. Its incidence was 90% at baseline, 40% at 2 months, and 20% at 4 and 6 months of orthodontic treatment. These results are somewhat surprising, given the positive relationship between gingival inflammation and the occurrence of BOP, as well as the pro-inflammatory effect of the orthodontic appliances on the gums. However, BOP, as a clinical symptom of inflammation, is largely influenced by plenty of other host-related and external parameters, than the presence of periopathogenic bacteria and subgingival calculus deposits (39).

Although PlI showed a significant increase between baseline and two months, reaching the maximum mean value of 1.31±0.43, it recorded a subsequent continuous decrease until the six month timepoint (all differences were statistically significant, P=0.019), reaching 1.19±0.43. Initial growth can be explained by the deterioration of oral hygiene due to the insertion of the orthodontic appliance, followed by a continuous decrease, possibly due to continuous re-inforced instruction in oral hygiene that was specific for patients undergoing supportive periodontal therapy. Generally, these scores indicate good oral hygiene throughout the study interval. However, there is no clear correlation between the evolution of this parameter and the BOP. While BOP scores exhibited statistically significant decreases between baseline and 2 months, PlI increased statistically significantly over the same interval and was the second largest change in this parameter over the observation period. The reduction in BOP could also be explained by the particular supportive therapy program, including monthly visits with a particular emphasis on preventing plaque accumulation in both supragingival and subgingival areas.

The results from the present study related to the evolution of BOP, PD, and PlI values are in line with those found in a study from 2009 (39), which focused on the correlation of clinical parameters with the presence of subgingival plaque deposits identified by endoscopy. The results demonstrated a linear correlation between the presence of subgingival plaque and the proportional increase of BOP and PD. As in the present study, the authors concluded that differences in the efficacy of oral hygiene among patients make this correlation difficult, and failed to find a concrete link between PlI and the evolution of BOP.

The variations in frequency of detection of the main periopathogens *Aa, Pg, Pi,* and *Tf* did not reveal statistically significant differences between the mean values recorded at baseline and those at six months of orthodontic treatment. The only periopathogen more frequently detected at the end of the

observation period was *Td*, but was only marginally significant (P=0.0649). When analyzing the comparative detection scores at baseline and after 6 months of treatment, a very low but detectable presence of *Aa*, *Pg,* and *Pi* was observed, with the median being 0 both at baseline and after six months of treatment. Although the median was 1 at baseline for *Tf*, the value remained unchanged after 6 months of orthodontic treatment. Even in the case of *Td*, which exhibited a slight increase at the 6 month time point as compared with baseline, the highest score was 2, the lowest score to ascertain detection.

These results demonstrate that the level of subgingival pathogenic bacteria was very low, close to zero, during the first months of orthodontic treatment. The low levels of detection correlate with the results obtained for clinical parameters, especially for BOP, demonstrating once again the effectiveness of systematic periodontal therapy prior to orthodontic therapy, and the role of properly performed oral hygiene during the maintenance phase. Similar results were obtained in a study on the incidence of *Aa* and *Pg* during orthodontic treatment with lingual brackets (40) and in earlier study of *Aa, Tf,* and *Pi* (41). The presence of the main periopathogens in the gingival sulcus during classical orthodontic treatment was investigated as well (42). In this study, *Aa, Pg, Pi,* and *Td* did not show statistically significant differences between baseline and the endpoint; *Td* exhibited only marginally significant differences. Thus, the authors concluded that patients with healthy periodontium prior to orthodontic treatment have decreased risk of further periodontal deterioration during orthodontic treatment. Although these results are not in line with the data from our present study, they show that orthodontic treatment does not have a negative effect on microbial flora as long as oral hygiene is properly carried out and the level of bacterial plaque accumulation is reduced to a minimum. Nevertheless, other studies have reported significant increases in pathogenic bacteria during orthodontic treatment (43,44).

Although in the mentioned studies the detectable amount of periopathogens during orthodontic treatment increased significantly, the changes did not appear to have negative clinical effects; they returned to baseline with the removal of orthodontic appliances. Of note, studies that reported increased levels of periopathogens at the end of the treatment also showed significantly higher frequencies of detection at baseline, in contrast to studies that did not report significant differences and where the frequency of detection was absent or slightly positive. Thus, it can be argued that orthodontic treatment could have a negative effect on patients with high levels of bacteria before insertion of orthodontic devices, but does not cause them to occur in a healthy periodontium. Orthodontic treatment had no detrimental effect on the clinical parameters studied; however, we cannot draw a conclusion that there were improvement in periodontal conditions following orthodontic treatment, despite several studies that reported such outcomes (13,14,23).

One limitation of the present study is the relatively small number of investigated patients. This can be explained by the high number of inclusion criteria (both for the subjects and for the experimental sites), and by the numerous time points at which evaluations occurred. On the contrary, similar studies in the literature employed groups with a similar or a lower number of patients (25,30,33).

As in the daily practice the periodontal status of orthodontic patients previously treated for severe periodontitis is being routinely monitored using articulated supportive therapy sessions, there is clear need for further clinical studies to identify the periodontal sites at risk of deterioration and the measures necessary to mitigate the recurrence of the disease.

Within the limits of the present study, we concluded that there were no significant changes in the clinical parameters and microflora during the initial phase of orthodontic treatment in patients with periodontal support reduced by severe periodontitis, once the primary disease is systematically treated and the residual inflammation controlled. By correlating the clinical parameters with the microbiological ones, we inferred that residual levels of periopathogens did not negatively influence the periodontal health during orthodontic treatment in adult patients who underwent therapy for severe periodontitis.

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Availability of data and materials

The datasets used/analyzed in the present study are available from the corresponding author upon reasonable request.

Authors' contributions

SIS, MT, AA, DR and AJ participated in the treatment of the patients, the sample collection and data acquisition. HC, LN, AO, AMR and DR participated in the study design. AMR drafted the manuscript and critically revised it for important intellectual content. SB, SM, MB, AR, PS and LS drafted the manuscript and critically revised it for important intellectual content, and were also involved in the conception of the study. AD and SS performed the statistical analysis. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the 'Victor Babes' University of Medicine and Pharmacy (approval no. 14/16.09.2013). All subjects were informed about the nature and purpose of the study, and each subject signed an informed consent document giving permission for the dental procedures and sampling of biological material.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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