The Impact of Orthodontic Treatment on Stage III / Grade C Periodontitis: A Systematic Review

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Abstract

Aims: Aggressive periodontitis evolves rapidly to severe periodontal destruction, which involves a multidisciplinary treatment for handling the periodontal infection, reconstructing defects, and realigning migrated teeth. Orthodontic treatment improves all aspects of periodontal health including aesthetics, teeth function, and oral hygiene, therefore preventing degeneration. This systematic review intends to focus on the effect of orthodontic treatment on the evolution of aggressive periodontitis. The clinical parameters are pocket depth, decrease in inflammation, attachment gain, index of dental plaque, and bone gain.

Materials and methods: This research is based on the analysis of four bibliographic databases which include PubMed, EBSCO, Science Direct, and Cochrane Library. Several search equations are used and the keywords are orthodontic treatment, aggressive periodontitis, migrated teeth, and combination treatment. Pertinent studies with a comparison of periodontal conditions before and after orthodontic treatment are selected. Several analytic parameters are used and tabulated.

Results: Two hundred and seventy-one (271) studies were found and only four (04) were selected due to inclusion criteria. The analysis shows significant improvement of all clinical parameters.

Conclusions: Although clinical parameters were improved after an orthodontic treatment, the results are irrelevant because of the insufficient studies with arguable statistical findings.
INTRODUCTION

Aggressive periodontitis (AP) or stage III / grade C periodontitis affects the entire periodontium and results in gingival recession, bone defects, odontolysis, and pathological dental migration. The dental organ may be lost. This type of periodontitis is mainly diagnosed in young adults, who occupy an important portion of orthodontic patients (1).

Recently, Paro-orthodontic treatments have been preferred for taking charge of AP. While the periodontal treatment controls microbial infection, the orthodontic ones significantly improve periodontal health, and aesthetic and dental function (realignment of the migrated teeth and the coordination of the arches).

They allow a sustainable prosthetic and/or implant work, but can easily damage the periodontal structures. With orthodontic treatment procedures, the management of teeth with reduced periodontal support needs a previous modification of biomechanics and appliance systems. To avoid further periodontal damage, active orthodontic movement must always follow an appropriate periodontal treatment procedure. Hence, initial periodontal cleaning (scaling and root debridement) is essential to avoid further periodontal disease (2, 3).

With an appropriately applied strength and permanent oral hygiene, orthodontic tooth movement is possible without any deleterious effect on teeth with reduced bone support (4).

Despite the clinical improvement of orthodontic treatment on AP patients, there is still controversy in the literature regarding the result on clinical parameters such as dental plaque index, probing bleeding, pocket depth, clinical attachment gain, and bone gain.

Through a controlled application of orthodontic forces, tooth movement can generate new alveolar bone formation by a complex, bone process of apposition/resorption (5,6).

According to some authors, orthodontic stimulation at the periodontal ligament has positive effects on the quality of periodontal wound healing, especially when orthodontic movement begins soon after the treatment.

For instance, Rathore et al. 2015 proved, through numerous studies, the benefit of orthodontic treatment for stabilizing the periodontal status after a loss of periodontal structure (7).

Shen Xiao et al. evaluated the efficacy of a combination of orthodontic and periodontal treatment to regenerate periodontium in AP patients and found improved outcomes (8).

But others think of no periodontal gain, from orthodontic treatment, according to cited clinical factors; even they think it worsens them.

Verrusio et al. 2018 discovered that the use of orthodontic appliances, especially fixed appliances, may increase the inflammatory process of periodontal tissues. Their studies included more periodontal
parameters (bleeding on probing, plaque index and gingival index) \(^{(9)}\).

Bollen et al. concluded that there is no definite evidence for the efficacy of combined treatment. It should also be remembered that gingival health is more difficult to maintain during treatment: on the one hand, access to hygiene means becomes difficult and on the other hand, the proportion of anaerobic bacteria increases in the gingival sulcus of banded teeth \(^{(10)}\).

There is very limited data on combination orthodontic and periodontal treatment with changes in periodontal clinical parameters. Currently, clinicians primarily rely on case reports and prospective and retrospective clinical case series.

The limited number of articles and the diversity of outcomes found by these different studies prompted the interest of this systematic review.

**MATERIALS AND METHODS**

2-1 Search strategy
A systematic search was performed based on an electronic search of several databases (Pub Med, Science Direct, Cochrane Library) covering publications from January 2010 to February 2020. The search was made regarding the acronym PICOS, and limited to the following keywords in English and French with several equations: *Orthodontic treatment/stage III / grade C periodontitis /aggressive periodontitis; / dental migration/combination treatment / PICOS:*

<table>
<thead>
<tr>
<th>Population:</th>
<th>stage III / grade C periodontitis/ aggressive periodontitis in adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention:</td>
<td>orthodontic treatment</td>
</tr>
<tr>
<td>Comparison:</td>
<td>periodontal parameters before and after orthodontic treatment</td>
</tr>
<tr>
<td>Outcomes:</td>
<td>changes in periodontal pocket depth (PPD), bleeding on probing (BOP), loss of attachment (CAL), alveolar bone level (DW), and plaque retention (PI).</td>
</tr>
<tr>
<td>Comparative studies:</td>
<td>clinical trials, retrospective studies, cohort</td>
</tr>
</tbody>
</table>

### 2.2. Selection criteria
The selection of studies was based on well-defined criteria, only randomized trials, prospective and retrospective studies, cohorts, case controls and comparative studies published between 2011 and 2022, in French or English were selected. Only items reporting changes in the following variables were evaluated: changes in PPD, BOP, CAL, DW, and PI. But all the patients with AP who performed regenerative surgery prior to orthodontic treatment, all the cases treating BOP alone, and the non-comparative and animal studies were excluded. The inclusion and exclusion criteria are listed in Table (1).

### Table 1: Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Randomized controlled trials (RCTs), -Randomized clinical trials, -Prospective and retrospective clinical studies, -English and French articles.</td>
<td>-Editorials, personal opinions, -Case reports or case series reports, -Articles without reported samples, -Reviews and articles dealing with BOP alone, -Articles comparing only aggressive periodontitis, aggressive periodontitis treatments with regenerative surgery before orthodontic treatments -Non-comparative and animal studies -Patients with systemic disorders, congenital malformations, smokers</td>
</tr>
</tbody>
</table>
3. Data Collection
To select appropriate articles, the focus was on the title, the abstract and certain headings like objectives and methodology. Two independent reviewers analyzed them separately. If the analysis of the title and the abstract left any doubt about the eligibility of the bibliographic reference, the entire document had to be read before inclusion or exclusion. Studies that did not respect the inclusion criteria were rejected. Any disagreement in article selection was resolved by consensus between the two operators. After the eligibility assessment, only four articles were included (Table 2).

Table 2: characteristics of the 04 articles included in the systematic review:

<table>
<thead>
<tr>
<th>Title /Type</th>
<th>Plaque index Variation (T0-T1)</th>
<th>Pocket depth Reduction (To-T1)</th>
<th>Reduction of Bleeding on probing (To-T1)</th>
<th>Attachment Gain (To-T1)</th>
<th>Bone gain (To-T1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cao T et al. (2015) Combined orthodontic-periodontal treatment in periodontal patients with anteriorly displaced incisors: Prospective study</td>
<td>0.07 (p= 0.304)</td>
<td></td>
<td>0.29 (p= 0.004)</td>
<td>0.66 (P= 0.001)</td>
<td></td>
</tr>
<tr>
<td>Carvalho CV et al (2018) Orthodontic treatment in patients with aggressive periodontitis Prospective study</td>
<td>0.09 (p= 0.02)</td>
<td>0.18 (p=0.03)</td>
<td>0.04 (p=0.03)</td>
<td>0.27 (p= 0.001)</td>
<td></td>
</tr>
<tr>
<td>Khorsand A et al. (2013) Periodontal parameters following orthodontic treatment in patients with aggressive periodontiti: a before-after clinical study uncontrolled clinical trials</td>
<td>T0 et T3 : 0.87 (P = 0.032)</td>
<td>T0 et T6 : 0.45 (P = 0.022)</td>
<td>T3 et T6 : 0.85 (P = 0.047)</td>
<td>T0 et T3 : 0.44 (P = 0.042)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T0 et T6 : 0.87 (P = 0.039)</td>
<td>T3 et T6 : 0.33 (P = 0.932)</td>
<td></td>
<td>T0 et T3 : 0.54 (P = 0.042)</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS
Selection process: Figure 1 shows the research strategy for PubMed Science Direct and Cochrane search engines. Manual search: The reference lists of the selected articles were studied.

A flow chart is established to summarize the different stages of article selection (Figure 1). A total number of 271 articles were identified as follows: 100 articles in PubMed, 139 articles in the Cochrane database, and 32 articles were found through the Science Direct search. The exclusion of irrelevant articles was like this: 236 were excluded by title as irrelevant to the subject of the study; of the remaining 35 articles, 10 were found to be duplicates through all databases and 14 were excluded after reading the abstracts. References of retained articles were manually searched for relevant studies that may have been missed. Eligibility was determined by reading the reports identified by this search.

![Flow chart of the study selection process.](image-url)

**Figure 1:** Flow chart of the study selection process.
At this stage, of the 11 articles selected, 07 were excluded because they did not meet the inclusion criteria (Table 3). Finally, 04 articles were included in the study. Of these, 01 was a retrospective analysis, 01 was an uncontrolled clinical trial, and 02 were prospective cohort studies. (Table 4)

The different characteristics of the 04 articles included in the systematic review are as follows (Table 4)
- Authors and year
- Titles of the articles and type of study
- The evaluation criteria for each study.
- And finally, the results for these same criteria.

**Table 3: Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Was the research question or objective in this paper clearly stated?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Was the study population clearly specified and defined?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Was the participation rate of eligible persons at least 50%?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Was a sample size justification, power description, or variance and effect estimates provided?</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>10. Was the exposure(s) assessed more than once over time?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>12. Were the outcome assessors blinded to the exposure status of participants?</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13. Was loss to follow up after baseline 20% or less?</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Results: 11/14 11/14 11/14

Criteria: Yes, NO, Other (CD, cannot determine; NA, not applicable; NR, not reported).
Table 4: Guidance for Assessing the Quality of Before-After (Pre-Post) Studies with No Control Group

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Khorsand A et al. (2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Was the study question or objective clearly stated?</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Were eligibility/selection criteria for the study population prespecified and clearly described?</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?</td>
<td>No</td>
</tr>
<tr>
<td>4. Were all eligible participants that met the prespecified entry criteria enrolled?</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Was the sample size sufficiently large to provide confidence in the findings?</td>
<td>No</td>
</tr>
<tr>
<td>6. Was the test/service/intervention clearly described and delivered consistently across the study population?</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?</td>
<td>Yes</td>
</tr>
<tr>
<td>8. Were the people assessing the outcomes blinded to the participants' exposures/interventions?</td>
<td>NR</td>
</tr>
<tr>
<td>9. Was the loss to follow up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?</td>
<td>No</td>
</tr>
<tr>
<td>10. Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?</td>
<td>Yes</td>
</tr>
<tr>
<td>11. Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?</td>
<td>Yes</td>
</tr>
<tr>
<td>12. If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?</td>
<td>No</td>
</tr>
</tbody>
</table>

Results: 7/12

Criteria: Yes, NO, Other (CD, cannot be determined; NA, not applicable; NR, not reported).

5. Quality assessment

The selected articles were graded upon the criteria proposed by the National Institutes of Health, Department of Health and Human Services, USA (11). The risk of bias in the studies was assessed independently by both authors and the bias between studies by an independent reviewer. Any disagreement was resolved by discussion with the reviewer. The appraisal criteria included: randomization of the sample, comparison of intervention effects, validation of measures, definition of inclusion and exclusion criteria, and statistical analysis.

The risk of bias assessment for each article is as follows: if the number of its concorded criteria is between 9-14, the article is rated as "low risk"; when the number is within 6-8, then the article is considered as "medium risk", and "high
risk “has this number equal or less than 5 concorded criteria. The risk of bias was considered low in three studies (Cao T et al., Carvalho CV et al, and Jiao et al.) and considered medium in one study (Khorsand A et al). The following methodological criteria were not assessed: sample justification, blinded assessment, and measurement validation. Studies that were not met were: sample justification, blinded assessment, and measurement validation.

DISCUSSION
Due to the rapid evolution of the destruction of the periodontium in AP, several therapeutic approaches, such as tissue regenerative surgery, prostheses, implants, or orthodontics, are implemented for remodeling periodontal tissue. Currently, orthodontic treatment is preferred for taking charge of AP, the resulting remodeling tissue is the icing on the cake of periodontal treatment.

This work aims to show the contribution of orthodontics combined with periodontal treatment, in aggressive periodontitis, to improve clinical periodontal parameters such as variation of the dental plaque index (PI), bleeding on probing, gain bone, attachment gain, and decrease in pocket depth. The pertinence of this systematic review consists in the fact that there are limited number of articles dealing with, and the lack of comparative studies of all clinical periodontal parameters together.

Orthodontic treatment along with its different biomechanics leads to odonto-periodontal reactions likely to modify the occlusal constraints encountered in each type of pathology (12). Thus, with any fragile reduced periodontium, any biomechanics (retraction, recoil, etc.) will be used with low strength during treatment, due to the stress on the suffering periodontium to promote tissue reorganization or regeneration (13).

The present review focuses on periodontal changes during orthodontic treatment of AP patients. The selected studies assessed changes in the clinical periodontal parameters for more than 6 months, going from the initial and maintenance phase of periodontal treatment to the removal of orthodontic appliances.

Thus, in the 04 articles retained, all the authors were able to observe an improvement in the periodontal state after orthodontic treatment.

Here, clinical periodontal parameters were assessed in the periods of T0 (1 to 3 months after conventional periodontal treatment) to T1, T2, T3, T6 (corresponding to 0 to 6 months after orthodontic treatment). The results of this review show that orthodontics with dental movement lead not only to periodontal tissue stability, but also slight and pertinent improvement in periodontal conditions.
Plaque index and orthodontic treatment (PI)

C.V. Carvalho et al. (2018) and Khorsand A. al (2013) were able to assess and conclude, after a certain period of observation, a clear significant improvement in the plaque index, despite the methodological difference between the 2 studies.\(^{19,20}\)

Carvalho et al (2018) began orthodontic treatment 45 days after periodontal cleaning and quantitatively evaluated the plaque index at 6 sites per tooth on all dentitions except for wisdom teeth. The period of T0 corresponds to the time after periodontal treatment and before orthodontic treatment, and T1 to the end of orthodontic treatment\(^{19}\).

From T0 to T1, they performed a session of monthly maintenance and control of dental plaque by periodontal cleaning. They found a significant decrease in PI with \(p=0.02<0.05\); \((p= 0.05 \text{ is considered the reference value})\)\(^{19}\).

Khorsand A et al (2013), proceeded with the qualitative method by evaluating the O’Leary PI using the revealing tablets on the mesial, distal, vestibular, and lingual surfaces in 3 periods: T0 and T3, T0, and T6, and from T3 to T6. T0 corresponds to the end of periodontal treatment, T3 to the 3rd month after the end of orthodontic treatment, and finally T6 to the 6th month following treatment. Repeated root planning and oral hygiene instruction were performed once every 3 months. They found a significant decrease in PI with the \(p < 0.05\).\(^{20}\) These results are concordant with those of some authors:

According to Castellanos-Cosano et al., before any orthodontic treatment, a periodontal treatment plays a crucial role. Periodontal maintenance and follow-up should take place throughout treatment and even afterward, to maintain the stability of the results\(^{17}\).

For Levin et al., in patients who do not comply with the required oral hygiene, active orthodontic treatment should be postponed until satisfactory plaque control is achieved. Periodontal recall appointments once every three months are advised all along active orthodontic treatment, and this should be done at a separate dedicated visit\(^{18}\).

Pocket depth and orthodontic treatment (PP)

The 04 studies were able to evaluate the variation of the pocket depth after orthodontic treatment using the same classic probing techniques. However, the authors are not unanimous in the results due to the variation in the evaluation methods.

Jiao J et al., (2019) reported no significant improvement between T0 -T1 (corresponding to 1 month after periodontal treatment and 1 month after orthodontic treatment).\(^{8}\)

For Cao T et al., (2015), PP was assessed before and after incisor intrusion which corresponded to T0-T1 (T0= during incisor
retraction); there was an improvement but the difference was not statistically significant with p= 0.304 ≥0.05 (9).

Carvalho CV et al., (2018) proceeded, after periodontal treatment, on a combination of anti-microbial (amoxicillin 500mg + metronidazole 250mg, 3 times a day for 15 days) and a full-thickness flap surgery without regeneration, then measured 6 sites per tooth (V, L, ML, MV, DL, and DV) with the Williams probe from T0-T1 (corresponding after periodontal treatment and immediately after orthodontic treatment) to assess the decrease in pocket depth (19).

Khorsand A et al (2013) after the stability of periodontal status and reduction of PI to 15% or less, performed root planning and repeated oral hygiene instructions every 3 months throughout orthodontic treatment, and loads were applied equally at 10-15 g on each tooth depending on their specific periodontal situations (verified with Correx™ measuring device, HAAG-STREIT Holding AG). The measurement was taken on 06 sites (V, L, ML, MV, DL, and DV) per tooth to assess the decrease in pocket depth (20).

Carvalho CV et al (2018) and Khorsand A et al (2013) were able to conclude that there was a statistically significant decrease in pocket depth between T0 -T1; T0-T3; T0-T6 and T3-T6 with values of p<0.05 (19).

**Bleeding on probing and orthodontic treatment**

If inflammation is not fully controlled before starting orthodontic treatment, it can be exacerbated thus accelerating the periodontal destruction, even in patients with good oral hygiene (21).

Only 02 studies have assessed this parameter, and the results are concordant: Carvalho CV et al. (2018) concluded that there was a statistically significant reduction in bleeding on probing between T0 and T1 (p<0.05) and up to 4 months after orthodontic treatment (T2). The result was stable. And Jiao J et al (2019) found a non-statistically significant decrease in bleeding on probing between T0 and T1 with p<0.05 (19.8).

This difference can be explained by the fact that the time of the index evaluation, the methodology, and the size of the sample differ between the 2 studies.

**Attachment Gain and Orthodontic Treatment**

Three studies evaluated this clinical parameter and were able to observe a clear improvement in attachment loss, but it was not statistically significant. The measurements were taken at 6 sites per tooth in the entire dentition (excluding third molars) (Florida Probe System; Florida Probe, Gainesville, Florida). The variables were evaluated after periodontal treatment and before orthodontic treatment (baseline, T0), immediately after orthodontic treatment (T1), T3 at the 3rd month after
the end of orthodontic treatment and finally T6 at the 6th month following treatment according to each study.

According to Carvalho CV et al. (2018), the attachment gain is 0.27mm (p= 0.004); Cao T et al. (2015) found an attachment gain of 0.29mm after incisor intrusion (P= 0.001). So, for the 2 studies the attachment gain is statistically significant between T0 and T1. But with Khorsand A et al. study, there was no statistically significant difference with p > 0.05 (19, 20). This heterogeneity of the results could be due to the difference in the time of evaluation of this parameter.

**Bone gain and orthodontic treatment**

Three studies were able to evaluate this clinical parameter, but two of them (Cao T et al. and Khorsand A. et al.) concluded statistically significant results in favor of bone gain. The measurements were repeated at three and 6 months after the end of orthodontic treatments and the data were statistically analyzed using repeated ANOVA measures and post hoc Bonferroni tests at 0, 3, and 6 months after orthodontic treatments. (9, 20 & 8).

These specific long-term clinical and radiographic findings support the fact that there is a significant improvement in bone gain in AP after orthodontic-periodontal treatment for both studies. This could be related to the movement of intrusion and orthodontic displacement of teeth during treatment.

Combined periodontal treatments and intrusion of crumpled and displaced anterior teeth with controlled oral hygiene is the best treatment approach for the correction of dental relationships in aggressive periodontitis (21).

**LIMITATION**

The limitations encountered in this review are the limited number of articles, the difficulty in interpreting the results, and the heterogeneity of the methodology in the selected studies, especially with the evaluation criteria of clinical parameters. This makes it impossible to perform a quantitative meta-analysis.

**CONCLUSION**

This systematic review suggests that orthodontic treatment yields positive outcomes for aggressive periodontitis, as evidenced by favorable clinical parameters such as the plaque index, bone gain, attachment gain, and pocket depth. Consequently, there appears to be no contraindication to orthodontic treatment for patients with aggressive periodontal disease. In certain cases, orthodontics may even be deemed necessary to enhance the possibilities of dentition restoration.

Nevertheless, it would be prudent to advocate for well-designed large-scale studies on this topic to gain a more nuanced understanding of the impact of orthodontic-
periodontal combination treatment on aggressive periodontitis.

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Ethical statement
All authors confirmed that the manuscript meets the ethical standards.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES


