Microbiological analysis of gingivitis in pediatric patients under orthodontic treatment

ABSTRACT

**Aim** The aim of this study was to determine the relationship between gingival inflammation and changes in bacteria of the gingival sulcus in children in orthodontic treatment with brackets.

**Materials and methods** Study design: this prospective study assessed gingival and plaque index of two groups: children with brackets (Group 1) and without brackets (Group 2). The sample was selected from patients treated at the Faculty of Dentistry, Complutense University of Madrid, Spain. Microbiological assessment was performed in every child and all data were statistically analysed.

**Results** Group 1 showed significantly higher microbiological values and the difference was greater in lower teeth. Comparing the total plaque percentage, it was significantly higher in Group 1. Statistics: there was no significant correlation between gingival and plaque indexes in any group. No significant correlation was found between plaque index and bacteria.

**Conclusion** Children using brackets showed significantly higher gingival and plaque indices than children without brackets. No direct relationship was found between the increase in gingival and plaque indices and the presence and quantity of bacteria; therefore it was not possible to identify specific bacteria as responsible for the high gingival index in patients with brackets.

**Keywords:** Crevicular fluid; Gingivitis; Gingival index; Metal allergies; Microbiological analysis; Orthodontics; Plaque index.

**Introduction**

Orthodontic treatment involves applying forces on the dental structures and supporting tissues of the tooth. It is accepted that this poses a risk to the teeth and periodontal tissues and, if the oral ecosystem conditions are unfavorable, an excessive growth of microorganisms that cause caries or periodontal disease occurs [Gafan et al., 2004; Türkkahraman et al., 2005; Ristic et al., 2007; Sallum et al., 2004]. Moreover, these treatments are started at ages in which it is very difficult to make children understand the need for a rigorous oral hygiene.

The presence of gingival inflammation in young patients wearing fixed appliances is very common, leading sometimes to very striking clinical conditions [Türkkahraman et al., 2005; Ristic et al., 2007; Naranjo et al., 2006]. However, gingival inflammation in these patients does not always present a linear cause-effect relationship with dental plaque as dental caries. In fact, gingival inflammation visible in some children does not seem to be justified by the amount of dental plaque. Other variables which might be involved are the inflammatory response during dental movement, which results from force application, and the inflammation due to an allergic response like contact stomatitis due to hypersensitivity to nickel or cobalt present in the wires and metal devices [Meikle, 2006; Studen-Pavlovich and Ranalli, 2006; Krishnan and Davidovitch, 2006; Rams et al., 1993].

Therefore, it is difficult but important to highlight the role which each one of these variables play in the gingival inflammation that can be observed in some children, since the therapeutic approach is completely different. In case of bacterial plaque accumulation, the treatment should be targeted towards the elimination of dental plaque with proper oral hygiene measures and microbiological control [Gafan et al., 2004; Naranjo et al., 2006; Darby and Curtis 2001; Baldwin et al., 1999; Albhandar and Rams, 2001].

The aim of this investigation is to study the relationship between gingival inflammation and changes in the bacterial flora of the gingival sulcus in children in orthodontic treatment with fixed appliances.

**Material and methods**

The sample of this study was selected from the patients attending the clinic of the Department of Pediatric Dentistry, Faculty of Dentistry, Complutense University of Madrid, Spain, and the study was conducted between 2008 and 2010. Prior to the beginning of the study, approval from the ethics committee of the Clinical Hospital of San Carlos, Madrid, Spain (E-08/344) and parental informed consent were obtained.

The inclusion criteria in the study were: subjects with no systemic pathology with manifestations in the oral cavity;
subjects with no physical or psychological disabilities; subjects presenting 1st phase mixed dentition (complete), or permanent dentition; right-handed subjects; subjects whose parents voluntarily accepted to participate in the study.

The sample was formed by two groups: Group 1- consisting of 30 children from both genders, wearing brackets (all from the same brand name and composition) for more than 3 months and Group 2 - Control group, consisting of 30 children of both genders, who were not undergoing orthodontic treatment. Every child included in the study was trained in oral health maintenance procedures.

Gingival index of teeth 21, 22, 41 and 42 was calculated through visual and tactile exploration, identifying values at the buccal (mesial, central and distal) and palatal/lingual (mesial, central and distal) surfaces according to Ramfjord criteria but, in this case, measuring teeth 21, 22, 41 and 42 only. Teeth 16 and 36, also proposed in Ramfjord index, presented orthodontic bands and, therefore, were excluded in order to eliminate possible uncontrolled variables.

The Index per tooth (sum of the values obtained in six locations) and the Total index (sum of the average values obtained in the four teeth and divided by 4) were calculated. The recorded values could vary between 0 and 24. Plaque index was obtained for teeth 12, 11, 21, 22, 32, 31, 41, 42, according to the formula: PI = (number of surfaces presenting dental plaque/total number of surfaces) x 100.

For the microbiological assessment, samples were collected in the buccal sulcus of teeth 21, 22, 41 and 42 of each patient according to Ristic et al. (3): isolation with cotton rolls, buccal and palatal/lingual, and placement of a saliva ejector to prevent contamination; cleaning of the gingival sulcus margin in which the sample was to be taken with a cotton pellet; careful air drying; specimen collection introducing a paper point (ISO 30) 1 mm into the sulcus for 30 seconds. The paper point was introduced in the mesiobuccal surface and in the same direction vertical axis of the tooth; introduction of the paper point with the sample in a vial containing 1.5 ml of reduced transport fluid brain-heart infusion.

Samples were transported to the Laboratory of Microbiology, Faculty of Dentistry, Complutense University of Madrid (UCM), and inoculated for a maximum period of 24 hours after collecting the sample.

Statistical analysis of data was completed at the UCM Research Support Service (RSS), using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Student t and Kruskal-Wallis tests were completed to evaluate gingival and plaque indexes. All variables identified in the study were correlated using a Pearson's test. Prevalence within bacteria was compared by Chi-square test, Student t test and Kruskal-Wallis test. Statistical significance was established at p <0.05.

Results

The final sample of this study comprised 58 children. In Group 1, 30 children wearing brackets were included. In Group 2, initially, 30 children without brackets were included; however, two microbiological samples in this group suffered deterioration during the laboratory processing, leading to a total of 28 children in this group.

In Group 1 (children with brackets), the tooth presenting a higher average gingival index was the 42, followed by 41, 21 and 22, on average. The lower teeth (41 and 42) presented a higher gingival index on average than the upper ones (21 and 22). 

In Group 2 (children without brackets), the tooth presenting a higher gingival index on average was the 42, followed by 22, 41 and 21. In this group, the upper teeth (21 and 22) presented a gingival index similar to that of the lower ones (41 and 42).

Comparing the data obtained for each tooth from both groups, significant differences were obtained in the gingival index for teeth 41 and 42, which presented a higher value in Group 1. Similarly, significant differences were obtained when comparing the gingival index of the upper and lower teeth between groups; in both cases, Group 1 had significantly higher values. This difference was greater in the lower teeth (Table 1).

In Group 1, the plaque index showed higher values in the mandible (33, 31, 41 and 42) than in the maxilla (11, 12, 21 and 22) (Table 2). For Group 2, this value was higher in the upper arch. In both groups the difference was significant when comparing both arches (Table 1).

When comparing the total plaque percentage, a statistically significant difference (P<0.05) was obtained between the two groups, significantly higher in the group of children with brackets (Table 1).

There was no significant correlation between the values obtained for the gingival and plaque index in any of the two groups studied (Table 2).

In both groups, the bacterium Fusobacterium nucleatum was predominant, followed by Prevotella intermedia, Eikenella corrodens and Porphyromonas gingivalis, and there were no statistically significant differences between the presence of bacteria in either group (Table 3).

 Tannerella forsythia, Eubacterium and Aggregatibacter actinomycetemcomitans were not present in any patient in both groups.

In Group 1 no statistically significant positive correlation

<table>
<thead>
<tr>
<th>Teeth</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
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<tbody>
<tr>
<td>GI</td>
<td>21</td>
<td>13.39</td>
<td>12.00</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>13.35</td>
<td>12.64</td>
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<tr>
<td></td>
<td>41</td>
<td>14.42</td>
<td>12.43</td>
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<td></td>
<td>42</td>
<td>14.71</td>
<td>12.93</td>
</tr>
<tr>
<td></td>
<td>21+22</td>
<td>13.37</td>
<td>12.32</td>
</tr>
<tr>
<td></td>
<td>41+42</td>
<td>14.56</td>
<td>12.68</td>
</tr>
<tr>
<td></td>
<td>21+22+41+42</td>
<td>13.97</td>
<td>12.50</td>
</tr>
<tr>
<td>PI</td>
<td>11+12+21+22</td>
<td>58.75%</td>
<td>40.25%</td>
</tr>
<tr>
<td></td>
<td>32+31+41+42</td>
<td>67.25%</td>
<td>38%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>62.88%</td>
<td>42.38%</td>
</tr>
</tbody>
</table>

* (NS)* = not statistically significant

TABLE 1 - Average of gingival index (GI) and plaque index (PI) values.
was found between gingival index and bacteria. However, a significant negative correlation between the gingival index and the bacteria Eikenella corrodens was noted (Table 4). In Group 2 no statistically significant correlation was found between the gingival index and the quantified bacteria (Table 4).

No statistically significant correlation was found, in any of the studied groups, between plaque index and the quantified bacteria (Table 4).

Discussion

For the selection of teeth used in the assessment of the gingival index, Ramfjord criteria were followed, but in this study only teeth 21, 22, 41 and 42 were used. Teeth 16 and 36, as proposed in Ramfjord index, had orthodontic bands and were excluded to eliminate possible uncontrolled variables. According to the results obtained by Ristic et al., in a study evaluating clinical and microbiological effects of fixed appliances on the periodontium of adolescents, gingival and plaque index showed a higher value at the upper and lower incisors. Following these results, in the present study only those teeth were used for this determination [Ristic et al., 2007]. In the same study the authors compared the same subjects before and after placement of the appliances. In contrast, in our investigation, comparisons were made between patients with brackets and a group control similar to the study group in all other variables except the fact that they did not have brackets [Ristic et al., 2007].

When comparing the gingival index obtained in both groups, Group 1 showed significantly higher values than Group 2 (Table 1). This result coincides with the result obtained by Rego et al. who also evaluated gingival index in children with fixed or removable orthodontic appliances [Rego et al., 2010].

For determining the plaque index, all four upper and four lower incisors were used. Children from Group 1 had significantly higher values of plaque index than children in Group 2 (Table 1). This can be attributed to the presence of brackets and wires on these teeth making it difficult to eliminate the dental plaque, resulting in increased levels of this index. The results agree with those of Ristic et al. (3), who compared the plaque index in a group of children before and after placing fixed appliances; they concluded that children presented the highest values of plaque index 3 months after placement of the device, followed by a slight decrease 6 months after beginning treatment. They also state that the other parameters they had measured (gingival index, gingival bleeding and pocket depth) presented similar changes and that these values only reached the baseline values at the end of the orthodontic treatment, when the device was removed. These authors concluded that orthodontic treatment with fixed appliances can temporarily increase the values of periodontal index and stimulate the growth of periodontal pathogens, but without destructive effects on periodontal tissues [Ristic et al., 2007].

In the present study no significant correlation was found between gingival and plaque indices, in any of the studied groups (Table 2). These results agree with Peretz et al. [1993], who followed 78 children for 3 years, assessing periodontal characteristics, and no relationship between the values of these two indices was found. This may show that other variables may lead to the increased rate of gingivitis, as in the case of children in Group 1 submitted to orthodontic treatment.

In tooth movement, resulting from force application, there is an inflammatory response in the tissues involved. Usually this response is temporary, limited and accepted as part of treatment [Ren et al., 2003; Vande et al., 2006]; however, in some children, tooth movement can enhance the gingival inflammatory process, even when good oral hygiene levels are maintained. Another possible cause of inflammation, not associated with dental plaque, may be the occurrence of an allergic response like contact stomatitis due to hypersensitivity to nickel or cobalt present in the wires and metal devices, which can lead to the presence of signs such as oedema and generalised erythema in the gingiva [Rams et al., 1993].

The microbiological method of determination used in this study was the cultivation method. Aggregatibacter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival index</td>
<td>13.97</td>
<td>12.50</td>
</tr>
<tr>
<td>Plaque index</td>
<td>62.88%</td>
<td>42.38%</td>
</tr>
<tr>
<td>Pearson's Correlation</td>
<td>0.210 (NS)</td>
<td>*0.122 (NS)</td>
</tr>
</tbody>
</table>

*(NS) indicates Not statistically significant

**TABLE 2** - Pearson correlation between gingival and plaque indices in each group.

- **TABLE 3** - Percentage of individuals presenting bacteria specimens evaluated in both groups.

- **TABLE 4** - Correlation between gingival (Gl) and plaque (Pl) indices of each group and the quantified bacteria.
actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Eubacterium and Eikenella corrodens were quantified. Comparing the percentages in which different bacteria were present, there were no significant differences between the two groups (Table 3). Comparing the most common bacteria in each group, the same frequency was obtained in both groups. The highest frequency corresponded to Fusobacterium nucleatum, followed by Prevotella intermedia, Eikenella corrodens and, finally, Porphyromonas gingivalis (Table 3). In Group 2, children without brackets, the same order of frequency of bacteria was found and, paradoxically for us, in a greater number of children (Table 3). Although statistically there were no significant differences, this is a result for which we have no explanation. Ristic et al. [2007] also obtained that Prevotella intermedia was more frequent in dental plaque samples in patients undergoing fixed orthodontic treatment than Aggregatibacter actinomycetemcomitans, a highly specific periodontopathic microorganism.

The results from our study differ from those reported by Okada et al. who, although using PCR technique, quantified Prevotella intermedia, P. nigenscens, Tannerella forsythia, Treponema denticola and Campylobacter rectus in a group of 119 children, collecting samples from their toothbrushes. In their study the results showed that Tannerella forsythia presented a moderate prevalence, while in our study it was not present in any child of either group [Okada et al., 2001]. A possible explanation for this difference could be the different sampling technique and sampling site; in our study samples were collected from the gingival sulcus and in the Okada et al. [2001] study, from children’s toothbrushes. Maybe the presence of bacteria and their percentage differs between different locations of sample collection. Tanaka et al. [2006] achieved consistent results with this possible explanation in an investigation where they evaluated the distribution of three periodontal pathogens (Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomyctemcomitans) in different locations of the mouth: teeth, tongue and buccal mucosa. These authors concluded that the three studied bacteria had a different frequency according to their location: the frequency of the three bacteria was higher in teeth than on the tongue or oral mucosa and the frequency of Porphyromonas gingivalis and Prevotella intermedia was significantly higher in supragingival plaque than on the tongue or in the buccal mucosa [Tanaka et al., 2006].

Regarding the presence of the bacterium Prevotella intermedia, which in our study was the second most common bacteria in both groups, Conrads et al. [1996] did not find it in any child from 3 to 10 years with healthy periodontium. Kulecki G et al., studying the presence in saliva of bacteria Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola and Prevotella nigescens in children with healthy gingival tissues, concluded that Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis may be present in saliva with no clinical signs of gingival disease but the same cannot be stated for Prevotella intermedia and Tannerella forsythia, suggesting that the presence of these bacteria is associated with gum disease [Kulecki et al., 2008]. In Group 1 a negative correlation between gingival index and presence of bacteria Eikenella corrodens was found, a result for which we have no justification.

Bacteria Tannerella forsythia, Eubacterium and Aggregatibacter actinomycetemcomitans were not found in any sample in our study. Ristic et al. were able to identify Aggregatibacter actinomycetemcomitans in one patient [Ristic et al., 2007]. It is possible that microbiological results are dependent on the population that is evaluated, as our study was performed in Spain and the study of Ristic performed in Serbia, and this could be a possible explanation for some differences on the microbiological results between both studies.

Yang et al., in 2004, found a much higher prevalence of Tannerella forsythia in subgingival plaque of a group of children with periodontal disease than in children with healthy periodontium [Yang et al., 2004]. Comparing this parameter, both groups in our study had more similarities with the children with the healthy periodontium group of their study. Suzuki et al, using a standard culture technique in a case of localised aggressive periodontitis in a 5-year-old boy, also failed to identify the presence of Aggregatibacter actinomycetemcomitans. However, when evaluating the same sample with PCR technique, they found positive results [Suzuki et al., 2003].

In the present study it was not possible to demonstrate a statistically significant relationship between the results obtained for gingival and plaque indices and bacteria (Table 4). Similarly, Sakai et al., in a study that assessed the prevalence of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella nigescens and Treponema denticola in the saliva of children with mixed dentition, using PCR technique, also could not find a statistically significant relationship between the presence of bacteria and gingival index values [Sakai et al., 2007].

From the present study it can be concluded that children wearing fixed appliances showed significantly higher gingival and plaque indices than children without fixed appliances.

No direct relationship was found between the increase in gingival and plaque indices and the presence and quantity of bacteria. In this study it was not possible to indentify a specific bacterial species as one responsible for the high gingival index obtained in patients with brackets.

The results obtained in this study justify further investigations to determine the relationship between gingival inflammation in children undergoing orthodontic treatment with brackets, with other factors which were not evaluated in this study and that may be involved.

References

Conrads G, Mutters R, Fischer J et al. PCR reaction and dot-blot hybridization


