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Mesio-marginal findings at tilted molars. A histological–histomorphometric study

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SUMMARY The aim of this study was to investigate the mesio-marginal findings at tilted molars (TM) by means of histological–histomorphometric evaluation. Eight lateral tooth bone segments with TM (six mandibular, two maxillary) from males aged 20–32 years were compared with those of eight samples with non-tilted molars (NTM) in males aged 18–35 years. In comparison with the NTM samples, the TM revealed a higher amount of supra- and subgingival plaque, a significantly higher total number of inflammatory cells ($P < 0.05$) and blood vessels ($P < 0.05$) in the connective tissue adjacent to the junctional epithelium, and a lower density and corono-apical width of gingival fibres. No significant differences ($P > 0.05$) were found between the mesio-marginal bone level of the TM (mean: 978 μm) and that of the NTM (mean: 1222 μm). In contrast, indications were found that TM may affect the disto-marginal bone level of the mesial tooth.

Introduction

Evaluation of marginal conditions at tilted molars (TM) by means of clinical and radiological methods has been the subject of many articles. Some authors have reported varying degrees of soft tissue inflammation mesial to mesially tilted than to non-tilted molars (NTM) (Brown, 1973; Norton, 1981; Becker *et al*., 1982). However, these findings were not corroborated by Lundgren *et al*. (1992) whose comprehensive intra-individual study compared the periodontal conditions around TM with those at the contralateral upright molars but found no significant differences between the two groups with respect to plaque, gingivitis and probing depth of 4 mm or more.

In clinical–radiological investigations examining periodontal conditions before and after orthodontic uprighting of TM, Lang (1977) and Wehrbein and Diedrich (1992) found that periodontal parameters such as plaque index, sulcus bleeding index and probing depth resulted in more favourable conditions after uprighting.

Besides assessment of soft tissue conditions, marginal bone mesial to mesially TM has been analysed by means of dental radiographs. Brown (1973), Norton (1981) and Becker *et al.* (1982) reported that infrabony pockets are more commonly associated with TM, while Lundgren *et al.* (1992) found no such infrabony lesions. According to Geiger and Wasserman (1980) and to Diedrich (1986) a radiologically detectable acute angle between the alveolar crest and the tooth surface mesial to a mesially TM cannot invariably be interpreted as an infrabony pocket but may be a sign of adaptation of the bone to the tilted tooth position.

However, clinical and radiological recordings involve some inherent limitations. The degree of correlation between clinical assessment of gingivitis and the microscopic appearance of the tissue is generally somewhat weak (Oliver *et al*., 1969; Orban *et al*., 1970; Zachrisson, 1972). Furthermore, assessment of the marginal bone level on dental radiographs, e.g. by the 'standard bisecting-the-angle technique' may also be associated with some limitations (Sjølien and Zachrisson, 1973a,b). Due to the technical procedure, radiological assessment may not necessarily reflect the true anatomical situation within the midline between the teeth but probably in a more or less lateral area of the interdental crestal bone.

It was therefore considered of interest to perform a histological study on the hard and soft tissue conditions associated with TM, with the aim of obtaining more objective data on the status of the epithelium and connective tissue and on the crestal bone–tooth relationship at mesially TM. The density, location and type of inflammatory cells present could also be investigated.

The aim of the present study was, therefore, to analyse the mesio-marginal histological findings at mesially TM and to compare them with those at corresponding NTM.

Material and methods

The material comprised eight lateral tooth–bone segments including at least the TM plus the tooth mesial to the TM. Eight specimens from males aged 20–32 years were available: six from the mandible and two from the maxilla. The first molars were absent from six specimens, and the second premolars from the remaining two. Accordingly, six tilted second and two tilted first molars were available for histological analysis. Whether the maxillary TM were not only tilted but also rotated cannot be proved retrospectively.

The NTM material also comprised eight specimens, six from the mandible and two from the maxilla, but with no missing or tilted teeth, from males aged between 18 and 35 years. From this material the same number and type of tooth as well as marginal areas (six second molars and two first molars) as in the TM sample were selected for histological comparison.

The specimens originated from the Pathologic Institute and the Institute of Forensic Medicine of the University of Aachen, Germany. In all cases the required authorization for autopsy and sample gaining for scientific purposes had been given by the legal person responsible.

Directly after removal, the specimens were placed in a neutral buffered formaline solution. Within the following 24 hours they were sectioned in the sagittal plane and again put into formaline solution for further fixation. Thereafter, the parallel sections were prepared according to the microsection technique (Donath, 1988) and stained with toluidine blue. Due to the polychromatics of this staining the different hard and soft tissue components as well as the cellular elements are clearly identifiable. From five to seven sequentially ground sections, with a thickness of 6–10 μm, of the most central section of the alveolar bone were selected for microscopic evaluation.

Comparative histological analysis criteria were presence of calculus and plaque, appearance of the interdental dentogingival and junctional epithelium, including density and location of inflammatory cells, as well as of the connective tissue of the interpapillary fibres and the transseptal fibre system.

Morphometric analysis covered the marginal bone level (MBL) and the number of inflammatory cells and capillaries within the connective tissue adjacent to the papillary epithelium. Qualitative analysis was carried out using a microscope type DMRX (Leica, Wetzlar, Germany) connected to an adapted colour video camera (Sony, Tokyo, Japan) interfaced with a computerized morphometric unit (Quantimed 600 S, Leica, Cambridge, UK).

The MBL was measured mesially at the tilted and corresponding NTM as well as distally at teeth located mesial to the tilted and corresponding non-tilted teeth. The MBL was measured as the distance in microns between the cemento–enamel junction (CEJ) and the most coronal level along the root surface at which periodontal ligament fibres inserted into the cementum. Measurements at each site were carried out twice at a magnification of ×25.

The total number and individual types of inflammatory cells as well as the number of capillaries present within the connective tissue adjacent to the junctional epithelium mesio-marginal to the tilted and corresponding NTM were analysed within 10 randomly selected regions each with an area of 7500 μm^2 (100 × 75 μm). This procedure was repeated and the mean value was then calculated for each tooth. Measurements for this parameter were carried out at a magnification of ×500.

Statistical evaluation of the histomorphometric results was performed to compare the corresponding parameters and sites of the NTM with the TM samples. A paired *t*-test at a level of 0.05 was used to determine significant differences.

To determine the measurement error, double measurements were carried out in all sections by the same operator and calculated according to the formula $s = \sqrt{\Sigma(x_1 - x_2)^2/2n}$. The error percentage was ± 2.61 per cent for the CEJ–MBL distance and 5.37 per cent for the number of inflammatory cells.

Results

At none of the tilted or corresponding NTM was an absolute plaque- and calculus-free mesial enamel surface found. In comparison with the NTM sample, however, most of the mesial enamel surfaces of the TM revealed a markedly higher amount of supra- and subgingival plaque and calculus.

In the NTM sample, inflammatory cell infiltrates of a low to moderate density were present in the connective tissue mesial to the teeth. The inflammatory cell density decreased from coronal to apical. The inflammatory cells adjacent to the junctional epithelium comprised predominantly lymphocytes and a smaller number of plasma cells. In the more central areas between the teeth, the plasma cell fraction was dominant. The junctional epithelium revealed no or only slight lateral projections. The connective tissue of the interpapillary fibres located coronal to the transseptal fibres was clearly identifiable in all sections. The transseptal fibre system was intact in all teeth. It ran horizontally, was in general relatively dense, and revealed a corono-apical thickness of approximately 1000 μm. Figure 1a, b and c illustrates some findings of the NTM.

The mean CEJ–MBL distance was 1222 μm mesial to the NTM and 1293 μm within the distal area of the teeth located mesial to the NTM (Table 1). The mean density of the total inflammatory cell population and the single cell fractions in the connective tissue adjacent to the junctional epithelium of NTM is shown in Table 2. The mean number of capillaries counted in the same area units was 0.5 (SD 0.4, maximum

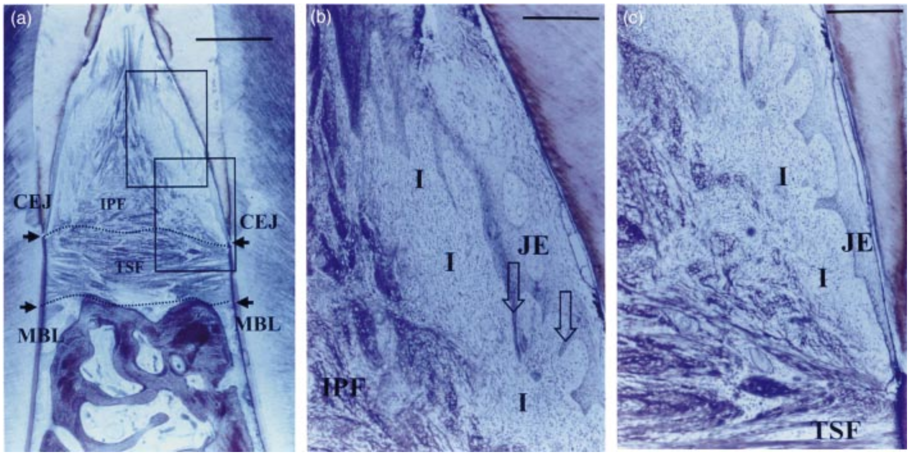

Figure 1 (a) Interdental marginal area between non-tilted molars. IPF, interpapillary fibres; CEJ (arrows), cemento-enamel junction; MBL (arrows), marginal bone level; TSF, transseptal fibres. Horizontal course of the dense supracrestal transseptal fibre system which is approximately 1000 μm thick (between dotted lines). Staining, toluidine blue; bar, 1000 μm. (b) Inserted rectangle (above) of (a). Slight to moderate infiltrations (I) of inflammatory cells in the loose connective tissue between the junctional epithelium (JE) and the tough connective tissue of the interpapillary fibres (IPF); slight lateral projections (arrows) of the junctional epithelium. Staining, toluidine blue; bar, 250 μm. (c) Inserted rectangle (below) of (a). Less dense infiltrations (I) of inflammatory cells as compared with (b) in the loose connective tissue above the supracrestal transseptal fibres (TSF) and adjacent to the apical part of the junctional epithelium (JE). Nearly normal appearance of the JE directly above the transseptal fibres (TSF) inserting into the supracrestal cementum. Staining, toluidine blue; bar: 250 μm.

Table 1 Distance from the cemento–enamel junction (CEJ) to the marginal bone level (MBL).[a]

Distance CEJ–MBL (μm)	Distal areas of teeth mesial to non-tilted teeth ($n = 8$)	Mesial areas of non-tilted molars ($n = 8$)	Distal areas of teeth mesial to tilted teeth ($n = 8$)	Mesial areas of tilted molars ($n = 8$)
Mean	1293	1222	1767	978
SD	186	224	910	453
Max	1621	1534	3572	1295
Min	1072	973	871	782

[a]The mean differences between the corresponding areas of the non-tilted and tilted sample were not significant ($P > 0.05$).

Table 2 Number of inflammatory cells in the connective tissue adjacent to the junctional epithelium mesial to non-tilted molars ($n = 8$).[a]

Number (n)	Inflammatory cells (total)	Granulocytes	Lymphocytes	Plasma cells
Mean	21.1	0.3	12.3	8.5
SD	4.1	0.4	4.0	2.7
Max	27.1	1.3	19.7	12.7
Min	14.7	0	7.3	5.7

[a]In each tooth 10 areas measuring 7500 μm^2 were assessed and the mean value per tooth calculated.

1.3, minimum 0.3), meaning that approximately one blood vessel was found in each second area unit analysed.

In the TM sample ($n = 8$) two different topographical types were observed: TM relatively far from the mesial tooth (Figure 2) and TM relatively close to the mesial tooth (Figure 3a).

Compared with the NTM sample, qualitative histological analysis of inflammatory cells revealed the following findings in both topographical types of TM: the density, distribution and cell types present in connective tissue mesial to the TM seemed largely to correspond to those observed in the NTM group. The junctional epithelium (JE) also showed only slight lateral projections.

At the TM relatively far from the mesial tooth, the interdental epithelium had a normal to slightly hyperkeratinized appearance and a thick layer of dense connective tissue fibres covering the edentulous alveolar bone section.

At the TM relatively close to the mesial tooth, some different histological findings were recorded. In some sections the coronal parts of the epithelium showed signs of lysis. Only a few gingival fibres were found. In some areas the corono-apical thickness was less than 500 μm. An absence of supracrestal fibres inserting into the cementum was not observed in any of the sections. Figure 3a, b and c exemplifies some

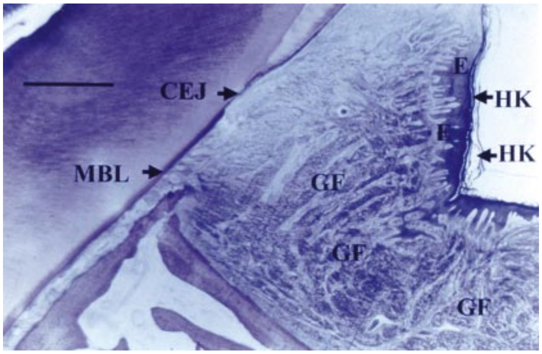

Figure 2 Tilted molar at some distance from the mesial tooth. E, epithilium; HK, hyperkeratinization. Note the thick layer (>1000 μm) of the gingival fibres (GF) running in different directions between the epithelium and the crestal bone. Staining, toluidine blue; bar, 1000 μm.

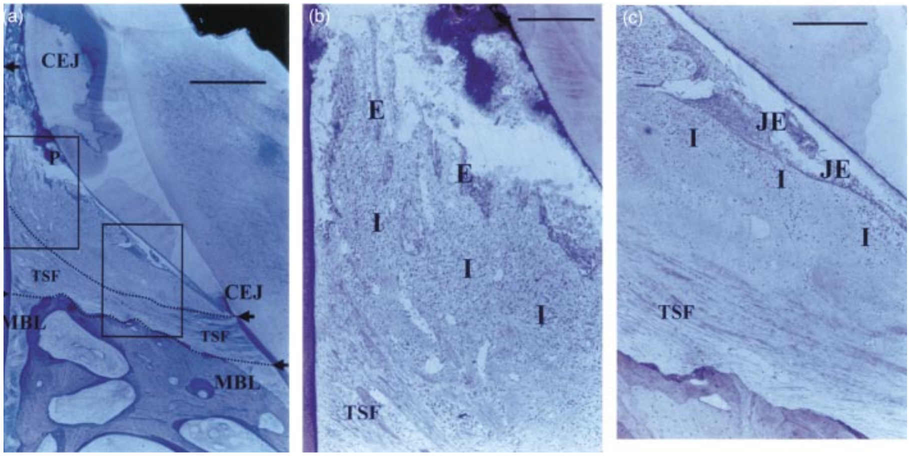

Figure 3 (a) Interdental area of a tilted molar close to the mesial tooth. CEJ, cemento–enamel junction; MBL, marginal bone level; TSF, transseptal fibres. In comparison to Figure 1: higher amount of calculus and plaque (P), markedly higher CEJ–MBL distance within the distal area of the tooth located mesial to the tilted molar (>3000 μm). Lower density and corono-apical width of the gingival fibres (between dotted lines) running from mesio-coronal to disto-apical. Note the normal appearance of the apical part of the junctional epithelium and the transseptal fibres inserting into the supracrestal cementum of the tilted molar; CEJ–MBL distance <1000 μm. Staining, toluidine blue; bar, 1000 μm. (b) Inserted rectangle (above) of Figure 3a. Loss of integrity of the epithelium (E), slight to moderate infiltrations (I) of inflammatory cells in the loose connective tissue. Staining, toluidine blue; bar, 250 μm. (c) Inserted rectangle (below) of Figure 3a. Only slight infiltrations (I) of inflammatory cells directly adjacent to the junctional epithelium (JE) which shows signs of disintegration. Sparse transseptal fibres (TSF). Staining, toluidine blue; bar, 250 μm.

findings at a TM relatively close to the mesial tooth.

The mean CEJ–MBL distance was 978 μm mesial to the TM and 1765 μm in the distal area of the teeth located mesial to the TM (Table 1).

The histomorphometrically assessed mean density of inflammatory cells in the connective tissue mesial to the TM is shown in Table 3. The mean number of capillaries counted in the same areas was 1.8 (SD 0.7, maximum 2.3, minimum 0.7). The mean number of capillaries present was thus approximately four-fold higher than in the NTM sample ($P < 0.05$).

Discussion

The main emphasis of the present study was on the mesio-marginal histological findings associated with TM. As no relevant histological studies exist to our knowledge, the present

Table 3 Number of inflammatory cells in the the connective tissue adjacent to the junctional epithelium mesial to the tilted molars ($n = 8$).[a]

Number (n)	Inflammatory cells (total)	Granulocytes	Lymphocytes	Plasma cells
Mean	26.9	0.9	17.9	8.1
SD	4.3	1.0	4.3	2.4
Max	32.7	1.8	24.3	12.0
Min	22.3	0	4.3	5.3

[a]In each tooth 10 areas measuring 7500 μm^2 were assessed. Compared with the non-tilted sample (Table 2) only the mean differences between the total number of inflammatory cells as well as between the lymphocytes were significant ($P < 0.05$).

results cannot be directly compared with those of earlier investigations.

Qualitative histological analysis of the density and type of inflammatory cells present in the connective tissue mesial to the teeth yielded relatively similar findings, i.e. mild to moderate chronic gingivitis, at the tilted and the non-tilted molars. To this extent the present study seems to support the results of the clinical–radiological study of Lundgren *et al*. (1992). As the degree of correlation between the clinical and the histological assessment of gingivitis from individual specimens has to be classified as moderate in non-orthodontic as well as in orthodontic patients (Oliver *et al*., 1969; Orban *et al*., 1970; Zachrisson, 1972), comparison of qualitative histological data with clinical findings has to be made with caution.

Molar tilting, potential plaque accumulation and associated soft and hard tissue reactions are dynamic processes which depend on many factors: healing after tooth extraction, time span after tooth extraction, occlusal loading conditions and oral hygiene (Lang, 1977; Geiger and Wasserman, 1980; Wehrbein and Diedrich, 1992). Although no anamnestic or clinical data were available for this study, the histological results for the two different topographical types of tilted teeth may at least provide some information on potential soft and hard tissue findings associated with different topographical situations of mesially tilted molars.

As long as the TM was relatively distant from the mesial tooth, which may well represent a more initial stage of tilting, an interdental epithelium with a relatively normal appearance and a thick layer of dense connective tissue fibres were observed. In the group in which the TM was close to the mesial tooth, signs of lysis of the whole coronal part of the dentogingival epithelium and only a few gingival fibres were found. Whether these differing findings between the two topographical types of TM are a result of sustained gingival inflammation or the TM being in a closer relationship to the mesial tooth, or possibly both factors, is difficult to evaluate. The fact is that if the molar is approaching the mesial tooth due to tilting, the interdental fibres are compressed out of a disto-coronal direction and are subject to change. If these soft tissue changes are accompanied by inflammation, lysis of the epithelium and rarification of gingival fibres may occur.

Furthermore it has to be assumed that if the degree of tilting is sufficiently large, potential plaque and inflammatory cell infiltrations will be in a closer relationship to the adjacent gingival fibres and crestal bone (Figure 3a). Even under the precondition of similar inflammatory activity at tilted and non-tilted teeth, these changed morphological conditions as compared with those at upright molars might make TM more susceptible to inflammatory induced changes in the soft and hard tissues mesial to the teeth. To this extent orthodontic uprighting of TM may create more favourable periodontal conditions, as reported in clinical–radiological studies (Lang, 1977; Wehrbein and Diedrich, 1992).

Morphometric analysis of the samples used in this study revealed that the mean number of total inflammatory cells, lymphocytes and capillaries counted mesial to the TM was significantly higher than in NTM (Tables 2 and 3). Consideration of the mean values of the stated cell numbers reveals, however, that the absolute differences were not very great. On the other hand, the fact that the mean number of capillaries counted in the same area was approximately 4-fold higher indicates a tendency towards more pronounced inflammatory activity mesial to the tilted teeth. This may well be due to the higher amount of plaque accumulation as observed at the mesial enamel surfaces of the TM.

The course of the alveolar crest mesial to the TM most frequently ran from mesio-coronal to disto-apical and formed an acute angle with the tilted teeth. As far as the results of histomorphometric analysis of the material available for this study are concerned, these findings may not be interpreted as infrabony pockets because the mean CEJ–MBL distance measured mesial to the TM was even somewhat smaller (mean: 0.978 mm) than in the corresponding areas of the NTM (mean: 1.222 mm). That does not necessarily imply, however, that TM may not develop an infrabony pocket. If the host defence mechanism cannot successfully resist plaque attacks, periodontal bone loss may occur and infrabony pockets as described by Brown (1973) may develop.

No intra-individual comparison was possible with the material available for this study. The upright as well as the TM samples in this study, however, originated from individuals of a comparable age group, all of them aged 35 years or less. In a comprehensive cross-sectional radiographic study Papapanou *et al.* (1988) investigated the CEJ–MBL distance in relation to age and tooth type and found that marginal bone loss was non-existent in subjects aged below 35 years. Therefore the results of Papapanou *et al.* (1988) may at least put the need for intra-individual comparison into perspective as far as the above-stated age group is concerned.

The results of the present study suggest that a TM may affect the disto-marginal bone level of the tooth located mesial to the tilted tooth. In these areas a mean CEJ–MBL distance of 1.8 mm was measured, compared with only 1.3 mm in the corresponding areas of the non-tilted sample. Although this difference was not significant, the maximum values measured within this area indicate that disto-marginal bone loss may occur at the tooth located mesial to the TM. Possible causative factors are inflammatory influences, tilting of the molar and bone changes following earlier tooth extraction. Depending on the degree of tipping or the individual morphological situation, the plaque-induced inflammatory infiltrates may be in a closer relationship not only to the crestal bone adjacent to the mesially TM but also to the distal area of the tooth located mesial to the tilted tooth (Figure 3a). Orthodontic uprighting may improve such conditions.

Tilting causes the mesial area of the tilted tooth with its attached supracrestal fibre system to migrate apically and may induce an apically directed traction to the supracrestal fibre system of the tooth mesial to the TM. Tooth extractions may also cause slight marginal bone loss. Zachrisson and Alnæs (1974) reported slight alveolar bone loss of approximately 0.25 mm mostly at closed extraction sites. Those authors speculated that slight crestal bone loss may be due to extraction, tooth movement, or both.

Finally, it has to be borne in mind that molar tilting and associated reactions are dynamic processes involving a series of ultrastructural changes. The findings reported in the present study, however, reflect only the individual situation. On the other hand, histological data provide information which cannot be obtained even by longitudinal and cross-sectional clinical and radiological investigations.

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