Dental manifestations in bariatric patients – review of literature

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ABSTRACT

The rate of bariatric surgery has significantly risen in the past decade as an increasing prevalence of extreme obesity can be observed. Although bariatric surgery is an effective therapeutic modality for extreme obesity, it is associated with risk factors affecting also oral health. Based on an overview of the current literature, this paper presents a summary of dental manifestations in bariatric patients. Bariatric surgeries are associated with an increased risk for gastro-esophageal reflux which in turn might account for the higher amount of carious and erosive lesions observed in bariatric patients. As a result, also dentin hypersensitivity might be observed more frequently. The current data indicate that recommended postsurgical meal patterns and gastric reflux might increase the risk for dental lesions, particularly in the presence of other risk factors, such as consumption of sweet-tasting foods and acidic beverages. Further research is needed to evaluate the correlation of bariatric surgery and the development of dental diseases.

Key words: Bariatric patients. Dental manifestations. Gastro-esophageal reflux.

INTRODUCTION

Modern methods to reduce the weight of adipose patients, including bariatric surgery techniques, have been developed for the treatment of obesity especially in its morbid form[22,30]. Bariatric surgery is considered as effective and safe treatment for all ages, increasing in prevalence over the years[22,30]. Since the implementation, the surgical techniques have undergone great changes along the time, for both the improvements of the tools and the post-operative sequels[1].

Nowadays, there are 3 main surgery techniques reported[22]. One technique is the *Gastric Band*, in which a silicone ring is placed around the stomach, thus creating two compartments: a small one above (15-20 mL) that will store small quantities of food, thus generating a sense of satiety, and the other part is larger and placed below, which will take part of normal digestion[8]. Another option is *Gastric bypass Roux-in-Y* technique, in which a small pouch is created (15 to 30 mL) stapling the stomach itself, restricting the amount of food that can be consumed. A part of the small intestine is diverted, delaying the mixing of food with gastric juice[15]. Finally, *Misuse Biliopancreatic* is performed in a way that ¾ of the stomach are removed and the intestine is shortened, reducing the time of contact of food with the intestine, considerably reducing the absorption of the nutrients[22].

There are many factors that might influence the results. In this sense, it is worth mentioning that the elderly patients are more likely to develop post-surgical complications, attributed to lower functional reserve of this age group, in addition to the presence of other metabolic diseases such as diabetes, which led significant sequel in these patients[4,9].

Regarding this issue, post-bariatric surgery manifestations include gastro-esophageal, respiratory, cardiovascular, endocrine and psychological changes[24]. In operated patients, the most common gastrointestinal complications found are stenosis of the duodenum, gastric ulcer, diarrhea, chronic vomiting, reflux and gastro-esophageal cancer. There is also increase risk of iron, vitamin B12, vitamin D and calcium deficiencies, mainly related to poor absorption of nutrients by the stomach and intestine[24,28].

On the other hand, a reduction of almost 90% of cases of the asthma and the sleep apnea, related to weight loss, is reported[3]. For the cardiovascular high risk patients, decreasing in systolic and diastolic pressures with consequent reduced risk of hypertension and coronary artery disease are reported in the operated patients. Furthermore, reduction of total cholesterol, triglycerides and uric acid as well as increasing in HDL fraction of cholesterol is observed[26]. In patients submitted to gastroplastic surgery with reduction of weight, the rates of diabetes and risk for non-diabetics are reduced[5]. It is also reported changes in the level of plasmatic hormones related to ovulation, which are below normal due to alteration in gastro-intestinal absorption[25].

Besides the physiologic factors, psychological and emotional should also be considered regarding to postoperative consequences, as these factors might influence the effects of the treatments[18]. Along with the weight loss an increase of self-esteem is observed as well as an improvement of social relationships, reduction of anxiety and depression. On the other hand, some patient may develop a self rejection, psychotic behavior, eating disorders, returning to the initial weight[24,28]. Clinical reports suggest that patients with psychiatric complications after surgery, especially with fear of returning to the previous weight, induce vomit[18], relating at this point the psychological problem with oral manifestations.

Based on above considerations, the aim of this paper was to present a summary of dental manifestations in bariatric patients.

REVIEW OF LITERATURE AND DISCUSSION

Clinical evidence

Heling, et al.[14] (2006) conducted a study with 113 patients (around 30-50 years old), who were submitted to bariatric surgery 4-5 years ago. They examined the self-assessment of bariatric patients with regard to their dental health. 79% of the patients reported vomiting as the most frequent phenomenon after surgery; 37% reported eating more sweet foods after surgery; 20% referred to improved oral hygiene; 73% did not change their habits of oral hygiene; 34% reported to have increased their visits to dentists; 60% have not changed the frequency of queries to the dentist; 37% reported major hypersensitivity after surgery; 44% reported vomiting associated with high sensitivity; 32% are suffering from indigestion after surgery and of those 59% reported hypersensitivity; and 80% of the patients have visited the dentist due to hypersensitivity. Some clinical case reports also showed the relation between bariatric surgery and increase of tooth decay[11,13].

Bariatric surgery might affect dental health by the pH decrease caused by the high frequency of sugar ingestion as well as Gastro-esophageal reflux disease (GER). GER is a chronic condition resulting from the retrograde flow of gastroduodenal contents (mainly stomach acids, such as hydrochloric acid) to the esophagus and /or adjacent organs, such as the mouth. The pH of gastric juice is around 1.2, being a potential risk for tooth demineralization, as the critical pH for dissolution of dental apatite is around 5.5[17]. Additionally, the patients showed reduction in the production of saliva[23], in part due to the low absorption of nutrients by the intestine, which in turn can facilitate the mineral dissolution. The reduction of pH can lead or facilitate tooth demineralization (caries and erosion) and hypersensibility[14]. The main consequences of these injuries are the enamel loss and hypersensitivity due to exposure of dentinal tubules.

Dental Erosion

One of the lesions related to the demineralization is the dental erosion, which is defined as chemical dissolution of dental tissues by a chemical process (acid or chelating agents) without the bacterial involvement[21]. The etiology of erosion is multifactorial and not fully understood. The most important sources of acids are those found in the diet, such as acidic foods and drinks[20] and those originated from the stomach, like gastric acids from regurgitation and reflux disorders. Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor for the development of erosive wear[19].

The acidic attack leads to an irreversible loss of dental hard tissue, which is accompanied by a progressive softening of the surface[19]. This softened zone is more susceptible to mechanical forces, such as abrasion[27], which in turn have little or no effect on sound dental hard tissues[2].

Clinically, early enamel erosion appears as a smooth silky-shining glazed surface. Typical for erosions of the facial aspects of teeth is a ridge of enamel that separates the defect from the marginal gingival. Occlusal erosion is characterized by rounded cusps and concavities. Further progression of occlusal erosion lead to a distinct grooving of the cusps, and restorations are rising above the level of the adjacent tooth surface. In cases of severe erosion, the whole occlusal or facial morphology disappears. When the dentin is reached, it is common report of hypersensitivity to cold, heat and osmotic pressure. Other consequences of dental erosion are diastema, thin and fractured incisal edges, loss of vertical dimension, opened pseudobite and prominence of aesthetic restorations[10].

Dental caries

Dental caries is a multifactorial disease, whose aetiology is related to the presence of a dental plaque composed by cariogenic bacteria, which can metabolize sugars such as sucrose. As a result of this metabolism, organic acids are produced such as lactic acid, which in turn can induce the demineralization of dental tissues[16,29]. With time, the biofilm becomes saturated regarding minerals that are released from the dental structure, favouring the precipitation and the formation of an initial subsurface carious lesion[6,12].

The early sign of enamel lesion is characterized as white spot (known also as non-cavitated lesion) as consequence of subsurface demineralization. With time and the increase of bacterial metabolism, the intact surface layer can break down leading to formation of cavity, the spread of bacteria and progress of the lesion to dentin. Following exposure of the dentin to the masses of bacteria in the cavity, the most superficial part of dentin will soon be decomposed through the action of acids and proteolytic enzymes. This zone is referred to as the zone of destruction. Beneath this zone, tubular invasion of bacteria is frequently seen. The bacteria invasion has as consequence the pulp inflammation, which may have serious consequences as pain, pulpar necrosis and periapical lesions[7,16].

Hypersensitivity

Dentine hypersensitivity has been defined as a sharp, short pain arising from exposed dentin in response to stimuli typically thermal, evaporative tactile, osmotic, chemical and which cannot be ascribed to any other form of dental defect or pathology[31]. The short and sharp pain symptoms are thought to be derived from the hydrodynamic challenge.

The most affected patients range from 20 to 40 years-old; premolars and incisors tend to be most sensitive teeth, being the pain localized on the facial surface. Sensitive teeth have much greater numbers of open tubules per unit area and the average diameter of tubules is almost 2 times greater than tubules in nonsensitive teeth[32].

Dentine hypersensitivity represents a condition of presumable multifactorial pathology. Two processes are essential for its development: (1) dentin must be exposed through genetic disturbance, enamel defect (lamellae and spindles), loss of enamel (erosion, abrasion, attrition, abfraction), gingival recession with rapid loss of cementum and (2) the dentin tubules must be open to both the oral cavity and the pulp.

Diagnostic protocol for this condition consisted of Medical, Dental Dietary, Oral Hygiene History and Intra-oral examinations with air indexing method. The treatments in the office can be made with substances that are able to create a smear-layer on dentin surface, occluding dentinal tubules with insoluble precipitates and stimulating the production of reparative dentin and/or sclerotic. This can be achieved chemically with agents like potassium, calcium and fluoride or physically[32,33].

Clinical impact of the knowledge

Based on above considerations, medical and dentist teams need to consider potential dental problems after bariatric surgery, and to supply their patients with the appropriate information and instructions regarding oral hygiene maintenance, healthy dietary patterns and regular dental health monitoring by a dentist or dental hygienist.

CONCLUSION

The present review suggests that postsurgical meal patterns and gastric reflux might increase the risk for dental lesions, particularly in the presence of other risk factors such as consumption of sweet-tasting foods and acidic beverages. However, due to a lack of data, more research is needed to evaluate this relationship.

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Influence of preventive dental treatment on mutans streptococci counts in patients undergoing head and neck radiotherapy

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ABSTRACT

The aim of this study was to evaluate the influence of chlorhexidine gluconate, sodium fluoride and sodium iodine on mutans streptococci counts in saliva of irradiated patients. Material and Methods: Forty-five patients were separated into three experimental groups and received chlorhexidine (0.12%), sodium fluoride (0.5%) or sodium iodine (2%), which were used daily during radiotherapy and for 6 months after the conclusion of the treatment. In addition, a fourth group, composed by 15 additional oncologic patients, who did not receive the mouthwash or initial dental treatment, constituted the control group. Clinical evaluations were performed in the first visit to dental clinic, after initial dental treatment, immediately before radiotherapy, after radiotherapy and 30, 60, 90 days and 6 months after the conclusion of radiotherapy. After clinical examinations, samples of saliva were inoculated on SB_{20} selective agar and incubated under anaerobiosis, at 37°C for 48 h. Total mutans streptococci counts were also evaluated by using real-time PCR, through TaqMan system, with specific primers and probes for *S. mutans* and *S. sobrinus*. Results: All preventive protocols were able to reduce significantly mutans streptococci counts, but chlorhexidine gluconate was the most effective, and induced a significant amelioration of radiotherapy side effects, such as mucositis and candidosis. Conclusion: These results highlights the importance of the initial dental treatment for patients who will be subjected to radiotherapy for head and neck cancer treatment.

Key words: Radiotherapy. Streptococcus mutans. Dental caries.

INTRODUCTION

Treatment of head and neck cancer (HNC) consists of surgery, radiotherapy (RT), and the association between them, besides the use of chemotherapy as an adjuvant in the treatment[14]. However, radiotherapy has been associated with several side effects, such as mucositis, changes in salivary gland function, radiation caries and especially osteoradionecrosis of the jaws[2,23]. These undesirable effects may affect treatment evolution and patient compliance with treatment. The occurrence of these reactions depends on the radiation dose, volume of irradiated tissue, fraction size, fractionation scheme, type of ionizing radiation, location of the irradiated area and other concomitant treatments[23]. In addition, individual aspects including age, systemic status, oral hygiene habits, tobacco and alcohol consumption[2] also need to be considered.

The occurrence of radiation caries and mucositis is high as 40-100% of the irradiated patients[22,23], producing extreme discomfort and compromising the acceptance[15], continuity[21] and intensification of RT[7]. Salivary gland dysfunction induced by RT results in hyposalivation, which may change the oral microbiota to a highly cariogenic microbiota, decrease clearance of carbohydrates from diet and organic acids produced by microorganisms, reduce buffering capacity, and impair remineralization of the tooth structure[9,18].

In addition, patients who have xerostomia may consume a diet of soft, carbohydrate-rich foods, which may further increase the susceptibility to dental caries. Taken together, these changes may lead to rampant caries after RT[6,20]. In Brazil, three preventive schemes are followed by most of the radiotherapy centers for prevention of radiation caries and osteoradionecrosis: chlorhexidine gluconate (0.12%), sodium fluoride (0.5%, aqueous solution) and sodium iodine (2% in hydrogen peroxide 10 v/v). However, there are no microbiological evidences that these protocols are effective when associated to the oral hygiene, especially in a population composed mainly by people with low socioeconomic level.

Thus, the aim of this study was to evaluate the influence of these preventive protocols associated to the improvement of oral hygiene standards on mutans streptococci counts in 60 patients submitted to radiotherapy for treatment of head and neck cancer.

MATERIAL AND METHODS

Population

A total of 60 patients seen at the Department of Dentistry of the Barretos Cancer Hospital, SP, Brazil and the Megavoltage Radiotherapy Center, SP, Brazil, comprising 52 males and 8 females, aged 18-63 years (mean age 49.75 years), with histopathological diagnosis of malignant disease were included in this longitudinal study. Fifty patients presented squamous cell carcinoma, three with adenocarcinoma, six with Hodgkin lymphoma and one patient harbored liposarcoma.

All patients gave written informed consent to be recruited for this study, which was approved by the Research and Ethics Committee of the School of Dentistry of Araçatuba, São Paulo State University - UNESP (Proc. 136/2007). All patients had at least ten teeth after initial dental treatment (IDT) and were able to comply with the preventive clinical protocols. Patients with previous diagnosis of HIV infection, use of antibiotics 3 months before the first visit to the Cancer Hospital, uncontrolled significant cardiovascular, pulmonary, renal or hepatic disease were excluded.

Prior to radiotherapy, patients were separated randomly into four different groups:

Group I: patients were submitted to initial dental treatment (IDT), generally 3-4 weeks before RT, which consisted of extractions, restorations, scaling, and dental prophylaxis. These patients were instructed to use chlorhexidine gluconate (0.12%) once daily for additional oral biofilm control during RT and for 6 months after conclusion of the treatment. Oral hygiene instructions were reinforced at each visit;

Group II: after IDT, patients used sodium fluoride (0.5%, aqueous solution) daily and oral hygiene instructions were reinforced at each visit;

Group III: after IDT, the patients used sodium iodine (2% in hydrogen peroxide 10 v/v) once daily and oral hygiene instructions were reinforced at each visit;

Group IV: patients received no preventive dental treatment. It is important to highlight that they received no oral hygiene instructions before RT. Patients of this group were instructed by the oncologists to look for professional care in public dental clinics, but no one did it. They received medical treatment with no odontological assistance and received oral hygiene instructions only during and after RT.

The mean radiation dose received by the patients varied from 5.040 to 7.020 cGy, and the fractioning dose was 180 cGy. RT was carried out using a linear accelerator.

Clinical procedures

In groups I, II or III, clinical examinations were performed at the first contact with the patient, before any dental treatment or oral hygiene instructions (stage 1), immediately after IDT (stage 2), before RT (stage 3), immediately after RT (stage 4), 30 days (stage 5), 60 days (stage 6), 90 days after RT (stage 7) and 6 months after RT (stage 8). The oral hygiene status was assessed using the plaque index (PI)[19]. In group IV, clinical examinations were performed just before RT, 3 weeks after the beginning of RT, immediately after, 30 days and 6 months after RT.

Collection of clinical samples, microbial isolation and enumeration

Whole resting saliva was collected from each patient before IDT, immediately after IDT, before RT, immediately after RT, 30, 60, 90 days and 6 months after RT, for mutans streptococci enumeration. Saliva was collected through the draining method; patients were placed in a quiet room, asked not to drink, eat or clean their mouths 1 h before saliva collection and instructed not to swallow any saliva during the collection period[3]. Test tubes containing the samples were immediately placed in a refrigerator (for culture) or liquid nitrogen (real-time PCR).

Laboratory processing was performed within 2 h. After mechanical mixing, samples were serially diluted and plated on selective SB_{20} agar, incubated anaerobically (90% N_2 + 10% CO_2), for 48 hours, at 37°C, for mutans streptococci enumeration. Bacterial identification was carried out by means of bacteria and colony morphology analyses as well as biochemical tests. After evaluation of mutans streptococci counts by culture, the caries risk of each subject was determined[24].

Quantification of mutans streptococci by real-time PCR

The presence and quantification of mutans streptococci were also confirmed by real-time PCR. After extraction of bacterial DNA from saliva by QIAamp DNA Mini Kit (Qiagen, Duisburg, Germany), real-time PCR was carried out using a Rotor Gene 6000 (Corbett Life Science, Mort Lake, New South Wales, Australia). Each PCR was performed in duplicate using a total volume of 25 µl, containing 12.5 µl 2X Taqman Universal Master Mix (Applied Biosystems), 0.2 µl each of forward and reverse primers (final concentration, 200 nM each), an appropriate concentration of Taqman probe (final concentration 100 nM), 2 µl of template DNA solution and an appropriate volume of sterilized DNase-RNase-free water. Amplification reactions were performed by an initial denaturation at 94°C for 10 min, 40 cycles at 95°C for 15 s and 60°C for 1 min. Primers were designed according to those described in literature for *S. mutans* and *S. sobrinus*[25].

Statistical analysis

Statistical analysis was performed using the software Statistical Package for the Social Sciences (SPSS Inc., v.13, Chicago, IL, USA). Quantitative variables (mean and standard deviation) were analyzed with Student's t-test. Multiple comparisons were carried out by means of Kruskall-Wallis test, while dichotomous variables were analyzed by Mann-Whitney, Chi-square or Fisher's exact tests. Difference of $P < 0.05$ was considered statistically significant.

RESULTS

Unfortunately, out of the 60 patients initially examined, 10 did not conclude RT and 11 other patients were not in physical conditions to be submitted to final intra-oral examinations. Oral manifestations associated to radiotherapy are presented in Table 1. Before RT, oral mucositis and dermatitis were not observed. Erythematous candidosis was detected in one patient of group IV and xerostomia was reported by two patients (group II and group IV). After RT, mucositis, xerostomia, and dermatitis were widely disseminated, irrespective of the experimental group, and except for candidosis, there were no statistically significant differences between groups regarding these side effects of RT.

In Group IV, immediately after RT, candidosis was diagnosed both in its pseudomembranous (two cases) and erythematous variants (six cases), while patients of the other groups presented only chronic erythematous candidosis. The occurrence of candidosis was significantly higher in group IV (P= 0.031).

The incidence of RT side effects was reduced 30 d after conclusion of the treatment, but occurrence of candidosis remained higher in group IV than in other groups. Six months after RT conclusion, xerostomia and mucositis constituted the most common alterations in the oral cavity, although their severity evidenced a mild reduction in most patients. In group IV, the reduction of mucositis and candidosis was slower than reported in other groups, and 77.78% of the patients presented this condition 6 months after RT. Moreover, in group IV, mucositis was statistically associated with xerostomia (P < 0.001), while in the other groups xerostomia was restricted to approximately 30-40% of the patients, 6 months after RT.

Xerostomia, dermatitis and mucositis were not prevented by any particular protocol, and all groups evidenced similar results (Table 1), while all preventive protocols reduced the occurrence of candidosis. It was also observed that in patients presenting clinical signs of mucositis 6 month after RT conclusion, mucositis level I and II predominated; while immediately after RT, most patients harbored mucositis level II and, especially, level III.

Initial plaque index values were very high in groups I, II, and III, ranging from 3.02 ± 0.54 (group I) to 3.46 ± 0.98 (group II), with no significant differences between groups. However, there was a gradual reduction of these values over the clinical follow-up period and no significant differences were observed between

Table 1- Oral status of the experimental groups at different periods of analysis

Clinical Condition	Occurrence of radiotherapy side effects N (%)			
	Before RT[1]	After RT[2]	30 d after RT[3]	6 months after RT[4]
Mucositis				
Group I	0 (0.0)	12 (92.31)	8 (61.54)	4 (40.0)
Group II	0 (0.0)	10 (90.91)	7 (63.63)	3 (33.33)
Group III	0 (0.0)	11 (78.57)	8 (66.67)	4 (36.36)
Group IV	0 (0.0)	10 (83.33)	8 (72.73)	7 (77.78)
Dermatitis				
Group I	0 (0.0)	12 (92.31)	8 (61.54)	1 (10.0)
Group II	0 (0.0)	11 (100.0)	8 (88.89)	2 (22.22)
Group III	0 (0.0)	11 (78.57)	7 (63.64)	0 (0.0)
Group IV	1 (6.67)	12 (100.0)	7 (77.78)	2 (22.22)
Candidosis				
Group I	0 (0.0)	3 (23.08)	1 (7.69)	0 (0.0)
Group II	0 (0.0)	3 (27.27)	2 (18.18)	1 (9.09)
Group III	0 (0.0)	4 (28.57)	1 (8.33)	0 (0.0)
Group IV	1 (6.67)	8 (66.67)	5 (45.45)	3 (33.33)
Xerostomia				
Group I	0 (0.0)	12 (92.31)	10 (76.92)	8 (80.0)
Group II	1 (6.67)	11 (100.0)	10 (90.91)	6 (75.0)
Group III	0 (0.0)	12 (85.71)	9 (75.0)	7 (63.63)
Group IV	1 (6.67)	11 (91.67)	8 (72.73)	7 (77.78)

[1]Number of patients in the different groups just before radiotherapy: Group I, N= 15; Group II, N= 15; Group III, N= 15; Group IV, N= 15. Total = 60.

[2]Number of patients in the different groups immediately after radiotherapy: Group I, N= 13; Group II, N= 11; Group III, N= 14; Group IV, N= 12. Total = 50.

[3]Number of patients in the different groups 30 days after conclusion of radiotherapy: Group I, N= 13; Group II, N= 11; Group III, N= 12; Group IV, N= 11. Total= 47.

[4]Number of patients in the different groups 6 months after conclusion of radiotherapy: Group I, N= 10; Group II, N= 9; Group III, N= 11; Group IV, N= 9. Total= 39.

Table 2- Mean counts of mutans streptococci in the experimental groups during the study

Groups	Mean mutans streptococci counts $x10^5$ ± Standard deviations $x10^5$				
	Baseline[1]	**before RT**	**after RT**	**30 d after RT**	**6 months after RT**
Group I					
Culture[2]	8.7 ± 4.6	1.9 ± 3.7	2.3 ± 1.8	2.0 ± 0.56	7.5 ± 3.1
Real-time PCR[3]	11.4 ± 5.4	2.1 ± 4.8	2.9 ± 1.9	2.6 ± 0.7	9.2 ± 3.1
Group II					
Culture	16 ± 4.9	4.4 ± 3.7	5.7 ± 1.8	5.2 ± 2.9	9.8 ± 5.1
Real-time PCR	23 ± 12.5	5.1 ± 4.7	7.0 ± 5.6	7.9 ± 6.3	14 ± 11.3
Group III					
Culture	21 ± 9.2	12 ± 3.7	5.4 ± 5.1	15.8 ± 6.2	16.2 ± 3.8
Real-time PCR	37 ± 12	9 ± 8.3	6.7 ± 4.9	15.0 ± 3.1	18.8 ± 7.1
Group IV					
Culture	—[4]	8.5 ± 4.8	18.7 ± 9.6	81 ± 41	77 ± 22.4
Real-time PCR	—	7.8 ± 5.2	15.3 ± 13.4	123 ± 45.2	83 ± 24.5

[1]Immediately before dental treatment.
[2]CFU/mL.
[3]DNA copies/mL. Total *S. mutans* + *S. sobrinus*.
[4]Group IV: patients did not receive initial dental treatment.

Table 3- Effects of preventive protocols on dental caries risk in irradiated patients. Results of mutans streptococci counts were obtained by culture

Group	Caries risk			
	[1]**low risk N(%)**	**moderate risk N(%)**	**high risk N(%)**	**plate overgrowth N(%)**
Group I				
Before IDT	0 (0.0)	2 (13.33)	7 (46.67)	6 (40.0)
Before RT	1 (6.67)	3 (20.0)	9 (60.0)	2 (13.33)
After RT	0 (0.0)	4 (30.77)	9 (69.23)	0 (0.0)
6 months after RT	0 (0.0)	3 (30.0)	4 (40.0)	3 (30.0)
Group II				
Before IDT	0 (0.0)	0 (0.0)	8 (53.33)	7 (46.67)
Before RT	2 (13.33)	3 (20.0)	9 (60.0)	1 (6.67)
After RT	0 (0.0)	3 (27.27)	8 (72.72)	0 (0.0)
6 months after RT	0 (0.0)	2 (22.22)	4 (44.44)	3 (33.33)
Group III				
Before IDT	0 (0.0)	1 (6.67)	8 (53.33)	6 (40.0)
Before RT	1 (6.67)	2 (13.33)	8 (53.33)	4 (26.67)
After RT	0 (0.0)	0 (0.0)	10 (71.43)	4 (28.57)
6 months after RT	0 (0.0)	2 (18.18)	5 (45.45)	4 (36.36)
Group IV				
Before IDT	—[a]	—	—	—
Before RT	0 (0.0)	2 (.0)	8 (53.33)	5 (33.33)
After RT	0 (0.0)	0 (0.0)	5 (41.67)	7 (41.67)
6 months after RT	0 (0.0)	0 (0.0)	3 (33.33)	6 (66.67)

[1]Low risk, mutans streptococci counts <10^4 CFU/mL of saliva; moderate risk, mutans streptococci counts 10^4 - <10^5 CFU/mL of saliva; high risk, mutans streptococci counts 10^5 - <10^6 CFU/mL of saliva; plaque overgrowth, mutans streptococci counts =10^6 CFU/mL of saliva;
[a] Group IV: patients did not receive initial dental treatment.

groups I, II and III, while group IV showed 3.25 ± 0.84 before RT and 2.86 ± 0.61, six months after RT treatment. Statistical analysis of the plaque index values revealed a significant reduction of dental plaque in groups I, II, and III (*P*= 0.0026), but in group IV the reduction was not statistically significant (*P*= 0.232). The improvement of oral hygiene standards was more pronounced just before and after RT, reinforcing the need for continuous follow-up.

In relation to mutans streptococci counts, they were very high at baseline, but initial dental treatment as well as all preventive protocols used during the study were able to reduce acidogenic cocci. The mutans streptococci counts are shown in Table 2. No statistical difference between values in each group was observed before RT, but the data 30 d after RT revealed a significant reduction of streptococci in group I (chlorhexidine) in comparison with group II (*P*= 0.03), group III (*P*=0.001) or group IV (*P*< 0.001). After the beginning of RT, group IV evidenced significantly higher counts of cariogenic cocci in relation to the other groups (P<0.001), evidencing the role of initial dental treatment in reducing cariogenic cocci. Comparison between groups II and III showed statistically non-significant differences (*P*= 0.9952).

At baseline, most patients of groups I, II and III were at high-risk for dental caries or presented microbial overgrowth on agar plates. There was a slight caries risk reduction after IDT and this phenomenon was sustained during RT, except for group IV (Table 3).

DISCUSSION

Dental caries risk is a serious problem for patients undergoing RT for head and neck cancer[9,14,23]. Carious lesions develop rapidly, and advanced destruction of the tooth structure can be observed as fast as several weeks or months after RT. Therefore, preventive measures before, during, and after RT are necessary and should include instructions regarding a noncariogenic diet, regular oral hygiene, and application of chemical compounds to prevent microbial accumulation in the biofilm or mineral loss of the dental structures[9]. However, literature[8,10] has shown that caries and cariogenic microbiota can be controlled by topical fluorides and chlorhexidine application. In the present investigation, chlorhexidine produced the most noticeable changes in the cariogenic microbiota of the patients.

In the present study, most patients with head and neck cancer are middle aged adult males who were chronic tobacco and alcohol consumers and had advanced tumors located especially in the floor of the mouth and tongue. In these patients, dental treatment before RT is necessary to avoid dental extractions and prevent osteoradionecrosis and other traumatic sequelae during and after RT[2,16]. This is particularly true for patients with low socioeconomic conditions who show poor oral hygiene status[2].

At baseline, the population evaluated in this study presented an initial very high risk for dental caries and although a significant reduction in levels of mutans streptococci was achieved especially in group I patients, the occurrence of new lesions of dental caries was observed in some patients of all groups, but especially in group IV.

Patients with high-risk for dental caries who have medium to high levels of mutans streptococci should use an antibacterial mouthrinse[11]. Currently, the most effective antibacterial mouthrinse against cariogenic bacteria is chlorhexidine. High-risk adults should rinse daily with 10 mL for 1 minute at bedtime for 1 week. This should be done for 1 week every month for up to 6 months. If used only 1 week per month, staining of the teeth and oral mucosa should be a minimal issue. Compliance is also a major issue with this product, which is why it should only be used for 1 week per month[11]. However, in the present study, all patients showed high risk for caries at baseline and this risk would obviously increase by irradiation, then the use of chlorhexidine was recommended during all the experiment.

The slight reduction in the levels of mutans streptococci observed in patients using sodium fluoride (group II) may have occurred not by the direct antimicrobial activity of the chemical agent, but due to the inhibitory activity which it carries on the enzymes related to saccharolytic metabolism. This could represent an ecological disadvantage for acidogenic cocci, as many oral microorganisms adhere better to acidic pH. Thus, the reduction of carbohydrate fermentation may have contributed to the slight reduction of mutans streptococci in the biofilm and mucositis incidence, avoiding acidification of the oral environment.

The use of topical fluoride to reduce caries has become standard practice in RT patients[9], but as all preventive protocols, this protocol is very sensitive to patients' compliance[9]. It has been estimated that patients must follow an application frequency of at least 70% to prevent decay[13]. However, compliance with fluoride application in carriers by the population with head and neck cancer is generally thought to be poor[4,13,18] and this phenomenon may be due to the inconvenient method of application. Although fluoride gel can provide additional benefits, as it maintains fluoride in the oral cavity for a longer period, our experience showed that topical aqueous fluoride solution induces a higher compliance, thus this protocol was chosen.

Other compounds used until recently in the prevention of radiation caries and osteoradionecrosis, such as sodium iodide prepared in hydrogen peroxide have been discouraged due to their suspected carcinogenicity and toxicity on fibroblasts, delaying the repair process[5].

Many head and neck cancer patients have poor oral hygiene[2,17] and patient adherence to the preventive protocols is closely correlated with follow-up visits[6,9]. Therefore, patient care must be individualized with evaluation at regular intervals to determine the caries risk and evolution, in order to preserve adequate oral health status[9]. In this study, patients were instructed to maintain a monthly visit routine to the dental office and this regimen probably interfered with patients' compliance, thus improving significantly the clinical outcome of the preventive protocols.

After RT, no single case of osteoradionecrosis was observed, probably due the time span between teeth extractions and the beginning of RT[23] in groups I, II and III, since wound healing during RT represents a high risk for the onset of osteoradionecrosis[1,12]. In group IV, due to lack of time for completion of the dental treatment, dental extractions were postponed indefinitely.

The occurrence of new caries in the experimental groups was much lower than initially expected and, in spite of the fact that no statistically significant findings were identified between caries experience and history of fluoride, iodine or chlorhexidine use, all preventive protocols were considered effective in the prevention of radiation caries and osteoradionecrosis.

CONCLUSIONS

Chlorhexidine was the most efficient mouthrinse to reduce mutans streptococci in the saliva of head and neck cancer patients undergoing radiotherapy treatment; it also ameliorated oral mucositis and eliminated oral candidosis in the experimental groups. The results evidenced the great importance of the dental team and initial dental treatment as a measure to reduce the severity and extension of radiotherapy side effects in the oral cavity.

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Resistance to tetracycline and β-lactams and distribution of resistance markers in enteric microorganisms and pseudomonads isolated from the oral cavity

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ABSTRACT

This study evaluated the occurrence of enteric bacteria and pseudomonads resistant to tetracycline and β-lactams in the oral cavity of patients exhibiting gingivitis (n=89), periodontitis (n=79), periodontally healthy (n=50) and wearing complete dentures (n=41). Microbial identification and presence of resistance markers associated with the production of β-lactamases and tetracycline resistance were performed by using biochemical tests and PCR. Susceptibility tests were carried out in 201 isolates of enteric cocci and rods. Resistance to ampicillin, amoxicillin/clavulanic acid, imipenem, meropenem and tetracycline was detected in 57.4%, 34.6%, 2.4%, 1.9% and 36.5% of the isolates, respectively. β-lactamase production was observed in 41.2% of tested microorganisms, while the most commonly found β-lactamase genetic determinant was gene bla_{TEM}. Tetracycline resistance was disseminated and a wide scope of *tet* genes were detected in all studied microbial genus.

Key words: Oral cavity. Enteric bacteria. PCR.

INTRODUCTION

The oral microbiota is composed of more than 500 different microbial species, most of them associated with oral health. However, sometimes the balance between the host's immune system and microbial virulence is lost and opportunistic infections may arise. Hence, oral infectious diseases have been frequently associated with alterations in the host' immune system, poor oral hygiene, denutrition, and alcoholism[18].

Associations between the occurrence of opportunistic and superinfecting pathogens with patients exhibiting different periodontal status[2] or wearing complete dentures[4] have been established. However, the role enteric bacteria and pseudomonads play in the etiology of periodontal disease needs further studies. In edentulous patients wearing complete dentures, the presence of enteric microorganisms may be associated with development of mucositis and usually reflects poor hygiene standards[4].

Suppression of the oral microbiota by abusive or intensive use of antibiotics may facilitate a persistent colonization of the oral cavity by these opportunistic microorganisms[18]. These microorganisms may spread to microbial populations in nosocomial infections or to the dental biofilm, acting as reservoirs for antibiotic resistance genes[7].

Tetracyclines were among the most widely used drugs in dentistry in the 80´s. Their effects on anaerobes and *Aggregatibacter actinomycetemcomitans* made these drugs the first choice in the treatment of aggressive periodontitis and necrotizing periodontitis. â-lactams, such as ampicillin, amoxicillin, cefoxitin and others constitute the basis of antimicrobial treatment of head and neck infections. However, microbial resistance to these drugs has compromised the efficacy of this therapy and the dissemination of resistance genes among oral microorganisms needs further investigation, as the oral cavity may harbor some multiresistant microorganisms, particularly enteric rods and cocci.

Thus, the aim of this study was to evaluate the presence of antimicrobial resistance genes (tetracycline and β-lactams) in enteric microorganisms isolated from the oral cavity of patients with gingivitis, periodontitis, periodontally healthy patients and patients wearing complete dentures, determining the distribution of most common β-lactamase markers and tetracycline resistance markers.

MATERIAL AND METHODS

Microorganisms and microbial identification

Enteric microorganisms were isolated from 250 patients (84 males and 166 females), mean age 43.03 years, within an 10-year follow-up period (1998-2008) at the School of Dentistry of Araçatuba, São Paulo State University (UNESP), Brazil. Forty-one patients wore complete dentures, 89 exhibited gingivitis, 70 chronic periodontitis and 50 were periodontally healthy. A written consent form was signed by all patients included in this study, which was approved by the Institutional Review Board of School of Dentistry of Araçatuba (Proc.27/2000 and 34/2006).

Microbial isolation was performed as previously described[6]. The isolates were identified by Gram staining, colony morphology on agar plates, catalase assay, and biochemical identification kits (BioMérieux, Marcy le' Etoile, France). A total of 201 enteric microorganisms and pseudomonads were submitted to susceptibility tests, as follows: *Burkholderia cenocepacia* (5 isolates), *Citrobacter freundii* (7 isolates), *Enterobacter cloacae* (18 isolates), *E. intermedius* (6 isolates), *E. sakazakii* (9 isolates), *Enterococcus* sp. (18 isolates), *E. faecalis* (31 isolates), *E. faecium* (8 isolates), *Escherichia coli* (6 isolates), *Klebsiella oxytoca* (11 isolates), *K. pneumoniae* (3 isolates), *Morganella morganii* (17 isolates), *Pantoea agglomerans* (7 isolates), *Proteus mirabilis* (5 isolates), *P. vulgaris* (7 isolates), *Providencia alcalifaciens* (6 isolates), *Pseudomonas aeruginosa* (15 isolates), *P. fluorescens* (4 isolates), *Serratia* sp. (9 isolates), and *S. liquefaciens* (9 isolates).

All isolates were examined for susceptibility to tetracycline, ampicillin, amoxicillin/clavulanic acid, cefoxitin, cephalothin, imipenem and meropenem by the agar dilution method. When the Clinical Laboratory and Standards Institute (CLSI) antimicrobial breakpoints were not established, the breakpoints adopted by the British Society for Antimicrobial Chemotherapy (BSAC) were followed. Mueller-Hinton agar (MHA) was used for all isolates.

In the susceptibility tests, five pure colonies of each bacterial strain were inoculated into 2 ml of sterile Mueller Hinton broth and incubated at 37°C for 12-24 h. Then, the turbidity was adjusted to match the 0.5 McFarland turbidity standard. The bacterial inocula were standardized in 10^5 cells and transferred to Mueller-Hinton agar plates containing the antimicrobial agent and control plates (without drugs), using a Steer's replicator (Cefar Ltda., SP, Brazil). The test and control agar plates were incubated aerobically at 37ºC, for 48 h.

Antimicrobials were tested in two-fold dilution series ranging from 0.06 µg/mL to 256 µg/mL. After incubation, the organisms were classified as sensitive or resistant, according to CLSI and BSAC guidelines. *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212 were used in the assays involving facultative anaerobes.

Detection of β-lactamases

β-Lactam-resistant isolates were also tested for β-lactamase activity by both chromogenic cephalosporin and biological method[5]. These two methods were performed because nitrocefin-based β-lactamase assays have not proven useful in detecting β-lactamase production by some microorganisms. In all tests, *S. aureus* ATCC 29213 was used as the positive control for β-lactamase production.

Distribution of antimicrobial resistance determinants

Bacterial DNA from each β-lactamase producers placed in sterile ultra-pure water was obtained by using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA concentrations were determined with a spectrophotometer at A_{260}nm (Model DU-640, Beckman Instruments, Richmond, Wash, USA).

Tetracycline-resistant isolates were screened for tetracycline resistance genes[1,16] *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(Q), *tet*(S), and *tet*(T), while β-lactam-resistant microorganisms were screened for bla_{TEM}, $bla_{CTX\text{-}M}$ and bla_{SHV} genes[3,8] using specific primer pairs. DNA amplification was performed in a DNA thermal cycler (AmpliTherm Thermal Cycler, Madison, WI, USA). The amplification conditions were 94°C (5 min) for initial denaturation, followed by 35 cycles at 94°C for 1 min, annealing temperature adequate for each primer pair for 1 min and 72°C for 1 min for extension; then 72°C for 5 min to allow final DNA extension.

RESULTS

In relation to susceptibility to antimicrobial drugs, significant levels of resistance were observed for all β-lactams, except for imipenem and meropenem, which presented 2.4% and 1.9% of resistance, respectively. Resistance to ampicillin, and cephalothin were detected in 57.4%, and 41.7% of tested bacteria, especially *Pseudomonadaceae* and *Enterobacteriaceae*. Out

Table 1- Resistance to β-lactams and tetracycline in enteric bacteria and pseudomonads

TAXON (N)	Resistance prevalence N (%)							β-lactamase production
	AM	AMC	CF	CP	IM	ME	TE	
A. bamanii (10)	6 (60.0)	1 (10.0)	2 (20.0)	3 (30.0)	0 (0.0)	0 (0.0)	2 (20.0)	6 (60.0)
B. cenocepacia (5)	4 (80.0)	2 (40.0)	1(20.0)	1 (20.0)	0 (0.0)	0 (0.0)	2 (40.0)	2 (40.0)
C. freundii (7)	4 (57.1)	2 (28.6)	3 (42.9)	3 (42.9)	0 (0.0)	0 (0.0)	2 (28.6)	4 (57.1)
E. cloacae (18)	14 (77.8)	9 (50.0)	11 (61.1)	11 (61.1)	0 (0.0)	0 (0.0)	1 (5.6)	13 (72.2)
E. intermedius (6)	2 (33.3)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	2 (33.3)
E. sakazakii (9)	4 (44.4)	1 (11.1)	2 (22.2)	9 (100.0)	0 (0.0)	0 (0.0)	1 (11.1)	4 (44.4)
Enterococcus sp. (18)	4 (22.2)	0 (0.0)	6 (33.3)	7 (38.9)	0 (0.0)	0 (0.0)	7 (38.9)	0 (0.0)
E. faecalis (31)	6 (19.4)	0 (0.0)	3 (9.7)	12 (38.7)	0 (0.0)	0 (0.0)	19 (61.3)	0 (0.0)
E. faecium (8)	4 (50.0)	0 (0.0)	4 (50.0)	4 (50.0)	2 (25.0)	1 (12.5)	3 (37.5)	0 (0.0)
E. coli (6)	4 (66.7)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	4 (66.7)
K. oxytoca (11)	7 (63.6)	5 (45.5)	1 (9.1)	3 (27.3)	0 (0.0)	0 (0.0)	0 (0.0)	6 (54.5)
K. pneumoniae (3)	3 (100.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)
M. morganii (17)	12 (70.6)	9 (52.9)	5 (29.4)	9 (52.9)	1 (5.9)	1 (5.9)	7 (41.2)	9 (52.9)
P. agglomerans (7)	6 (85.7)	6 (85.7)	3 (42.9)	3 (42.9)	0 (0.0)	0 (0.0)	2 (28.5)	6 (85.7)
P. mirabilis (5)	3 (60.0)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	2 (40.0)	4 (80.0)
P. vulgaris (7)	5 (71.4)	2 (28.6)	1 (14.3)	1 (14.3)	0 (0.0)	0 (0.0)	1 (14.3)	5 (71.4)
P. alcalifaciens (6)	4 (66.7)	4 (66.7)	1 (16.7)	2 (33.3)	0 (0.0)	0 (0.0)	4 (66.7)	4 (66.7)
P. aeruginosa (15)	13 (86.7)	13 (86.7)	8 (53.3)	9 (60.0)	2 (13.3)	2 (13.3)	11 (73.3)	3 (20.0)
P. fluorescens (4)	3 (75.8)	3 (75.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)
S. liquefaciens (9)	6 (66.7)	6 (66.7)	2 (22.2)	3 (33.3)	0 (0.0)	0 (0.0)	5 (55.6)	6 (66.7)
Serratia sp. (9)	7 (77.8)	5 (55.6)	5 (55.6)	5 (55.6)	0 (0.0)	0 (0.0)	5 (55.6)	6 (66.7)
Total (211)	121 (57.4)	73 (34.6)	58 (27.5)	88 (41.7)	5 (2.4)	4 (1.9)	77 (36.5)	87 (41.3)

AM= ampicillin; AMC= amoxicillin/clavulanic acid; CF= cefoxitin; CP= cephalotin; IM= imipenem; ME= meropenem; TE= tetracycline

Table 2- Distribution of tetracycline and β-lactam resistance genes in resistant Gram-negative isolates

Resistant microorganisms	Frequency of antimicrobial resistance determinants N(%)								
	bla$_{TEM}$	*bla*$_{CTX-M}$	*bla*$_{SHV}$	*tet*(A)	*tet*(B)	*tet*(C)	*tet*(D)	*tet*(E)	*tet*(M)
A. bamanii	3 (50.0)	0 (0.0)	3 (50.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
B. cenocepacia	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. freundii	1 (25.0)	0 (0.0)	1 (25.0)	1(50)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
E. cloacae	5 (35.7)	1 (7.1)	3 (21.5)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. intermedius	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. sakazakii	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. coli	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
K. oxytoca	1 (14.3)	1 (14.3)	1 (14.3)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
K. pneumoniae	0 (0.0)	0 (0.0)	3 (75.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
M. morganii	7 (58.3)	1 (8.3)	4 (33.3)	1 (14.3)	3 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P. agglomerans	0 (0.0)	0 (0.0)	4 (66.7)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P. mirabilis	0 (0.0)	0 (0.0)	1 (25.0)	1(50.0)	0(0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)
P. vulgaris	2 (40.0)	0 (0.0)	1 (20.0)	1(100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P. alcalifaciens	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)
P. aeruginosa	0 (0.0)	0 (0.0)	0 (0.0)	5 (54.5)	1 (9.09)	0 (0.0)	0 (0.0)	5 (45.45)	0 (0.0)
P. fluorescens	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)
S. liquefaciens	2 (40.0)	0 (0.0)	1 (20.0)	3 (60.0)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)
Serratia sp.	4 (57.1)	0 (0.0)	1 (14.3)	3 (60.0)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)
Total	32 (29.9)	3 (2.8)	25 (23.4)	12 (25.0)	10 (20.8)	3 (6.3)	5 (10.4)	6 (12.5)	2 (4.2)

Table 3- Distribution of tetracycline, ampicillin and gentamicin resistance genes in enterococci resistant to these antimicrobials

Resistant strains	Frequency of antimicrobial resistance determinants N (%)					
	bla$_{TEM}$/ *bla*$_{CTX-M}$/ *bla*$_{SHV}$	*tet* (K)	*tet* (L)	*tet* (M)	*tet* (O)	*tet* (S)
Enterococcus sp.	0 (0.0)	3 (42.9)	1 (14.3)	2 (28.6)	1 (14.3)	0 (0.0)
E. faecalis	0 (0.0)	6 (31.6)	2 (10.5)	7 (36.8)	3 (15.8)	1 (5.3)
E. faecium	0 (0.0)	3 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
Total	0 (0.0)	10 (34.5)	1 (3.5)	7 (24.1)	2 (6.9)	3 (10.3)

of 121 bacterial isolates resistant to ampicillin or amoxicillin, 87 were β-lactamase producers of (41.2% of the isolated bacteria and 72.9% of ampicillin-resistant isolates). The production of these hydrolytic enzymes seems to be the major mechanism of resistance to β-lactams, excluding most pseudomonads, and enterococci, where β-lactamases were not detected (Table 1).

Most of β-lactamase Gram-negative producers harbored β-lactamases. The detection of antimicrobial resistance determinants evidenced that 29.9% of Gram-negative isolates resistant to ampicillin harbored *bla*$_{TEM}$ genes, while *bla*$_{SHV}$ and *bla*$_{CTX-M}$ were detected in 23.4% and 2.8% of the resistant isolates, respectively (Table 2). These genes were not detected in enterococci (Table 3).

Resistance to tetracycline was also widely disseminated in the microbial enteric strains and 36.5% of tested microorganisms were resistant. The presence of tetracycline resistance determinants was widely disseminated among resistant Gram-negative isolates and enterococci. *Tet*(A) and *tet*(B) were the most common in Gram-negative bacteria; while *tet*(K), *tet*(M) and *tet*(O) were predominant in resistant enterococci. *Tet*(G), *tet*(Q) and *tet*(T) were not detected.

DISCUSSION

Enteric bacteria and pseudomonads have been involved in many oral and extra-oral infections, and some studies have evidenced that the oral cavity may act as a reservoir of enteric microorganisms and their virulence genes[1,6,7].

In spite of the small participation of enteric bacteria and pseudomonads in the total microbial load present in the gingival sulcus, supragingival biofilm, saliva and other sites of the oral cavity, the occurrence of these pathogens should not be neglected[7]. Antimicrobial resistance surveillance programs have provided sufficient data about antimicrobial susceptibility of clinically relevant enteric bacteria and pseudomonads from nosocomial infections and environment[14,17], although few reports describe the antimicrobial susceptibility of these organisms isolated from the oral cavity[2]. In addition, information about the genetic determinants associated with this resistance is not clarified yet and most available data regards nosocomial infections, as mentioned above.

β-Lactam agents such as penicillins, cephalosporins, monobactams and carbapenems are among the most frequently prescribed antibiotics worldwide. In Gram-negative pathogens, β-lactamases remain the most important factor to β-lactam resistance, and their increasing prevalence, as well as their alarming evolution seems to be directly linked to their clinical use[14].

In the present study, the genetic bases of β-lactamase production in enteric Gram-negative rods were mainly associated with bla_{TEM} gene, which evidenced a noticeable dissemination among Gram-negative enteric bacteria[10,19]. Presence of β-lactamase genetic markers was significantly more pronounced in our study than previously reported in literature, even though the distribution of particular determinants in β-lactamase-producer strains was similar[10,19].

However, the introduction of new β-lactams with different activity spectra has led to a selection of different genes and mutations that confer resistance to these drugs, especially β-lactamase-producers, mainly in members of family *Enterobacteriaceae*. In this family, most β-lactamase producers harbor bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ resistance determinants[14]. Thus, the distribution of these resistance markers in enteric microorganisms distributed in the dental biofilm and mucosal surfaces remains unclear.

Therapeutic options for infections caused by Gram-negative organisms expressing β-lactamases are limited because these organisms are usually resistant to all β-lactam antibiotics, except the carbapenems. Several families of β-lactamases from Gram-negative organisms were identified, but no phenotypic test can differentiate them, impairing surveillance and epidemiological studies[13].

The genes screened in the β-lactamase family represent only a small part of the cellular defense mechanisms that prokaryotes developed to avoid the action of β-lactams. *Enterobacteriaceae* isolates that exhibited uncertain identification by PCR were later classified as *K. oxytoca*, *Enterobacter* spp. and *C. freundii* due to detection markers of β-lactam resistance[8]. Moreover, *K. oxytoca* strains are known to express specific class A β-lactamases that were not considered in this study; while the resistance to β-lactams in *Enterobacter* sp. and *C. freundii* is generally attributed to the expression of chromosomal AmpC β-lactamases, as also described to some pseudomonads[9]. Possibly, these lactamases may be responsible for the β-lactam resistance phenotype, specifically to penicillins and narrow-spectrum cephalosporins, registered in some isolates affiliated to these genera.

Enterococci in general and *E. faecium* in particular, are intrinsically more resistant to penicillin and ampicillin than the other streptococci. Ampicillin resistance in *E. faecium* is due to expression of the low-affinity class B penicillin-binding protein 5 (PBP5). Early studies suggested that higher levels of ampicillin resistance in *E. faecium* were achieved by increasing levels of PBP 5 expression. More commonly, mutations that are presumed to lower the affinity for β-lactam antibiotics have been identified within *pbp5* genes of highly resistant clinical isolates[15]. The results of the present investigation also suggested that enterococcal resistance to β-lactams, especially ampicillin, is not related to gene bla_{TEM}, as this gene and β-lactamase activities were not detected.

Tetracycline resistance was also often observed. The most common genetic determinants of tetracycline resistance are represented by genes *tet*, which can be separated into genes that encode efflux proteins, especially genes *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *tet*(I), *tet*(K), and *tet*(L); those that protect the ribosomes from the action of tetracycline, genes *tet*(M) *tet*(O) *tet*(Q); and gene *tet*(X) that encodes a protein able to inactivate the antibiotic drug[16]. In Gram-positive cocci, the concomitant presence of two or more genes *tet* is common but this peculiarity was not confirmed in the present study, since only 5 isolates (17.2%) of enterococci expressed simultaneously *tet*(K) and *tet*(M) determinants.

In *Enterobacteriaceae*, the most common tetracycline resistance markers were *tet*(A) and *tet*(B), which were present in 45.8% of the tetracycline resistant isolates, according to previous studies[1,11,12,16]. In enterococci, genes *tet*(K) and *tet*(M) represented 58.6% of the detected resistance markers.

Heterogeneity of tetracycline resistance genes in Gram-negative enteric rods and enterococci was significant, as also previously reported[11], although these genes were not detected in 18 enteric resistant isolates. There are several possible explanations for the non-detection of *tet* genes in 23.4% of our resistant isolates. The most probable possibility is that we screened only 12 of the 38 recognized *tet* genes and some isolates present an inherent resistance to tetracycline as opposed to acquired resistance.

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Influence of voids in the hybrid layer based on self-etching adhesive systems: a 3-D FE analysis

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ABSTRACT

The presence of porosities at the dentin/adhesive interface has been observed with the use of new generation dentin bonding systems. These porosities tend to contradict the concept that etching and hybridization processes occur equally and simultaneously. Therefore, the aim of this study was to evaluate the micromechanical behavior of the hybrid layer (HL) with voids based on a self-etching adhesive system using 3-D finite element (FE) analysis. Material and Methods: Three FE models (Mr) were built: Mr, dentin specimen (41x41x82 μm) with a regular and perfect (i.e. pore-free) HL based on a self-etching adhesive system, restored with composite resin; Mp, similar to M, but containing 25% (v/v) voids in the HL; Mpp, similar to Mr, but containing 50% (v/v) voids in the HL. A tensile load (0.03N) was applied on top of the composite resin. The stress field was obtained by using Ansys Workbench 10.0. The nodes of the base of the specimen were constrained in the x, y and z axes. The maximum principal stress (σ_{max}) was obtained for all structures at the dentin/adhesive interface. Results: The Mpp showed the highest peak of σ_{max} in the HL (32.2 MPa), followed by Mp (30 MPa) and Mr (28.4 MPa). The stress concentration in the peritubular dentin was high in all models (120 MPa). All other structures positioned far from voids showed similar increase of stress. Conclusion: Voids incorporated into the HL raised the σ_{max} in this region by 13.5%. This behavior might be responsible for lower bond strengths of self-etching and single-bottle adhesives, as reported in the literature.

Key words: Hybrid layer. Voids. Dentin. Finite element analysis.

INTRODUCTION

The structural behavior of the adhesive layer plays an important role in maintaining the integrity of the dentin-resin bond over time[1]. Self-etching adhesive systems have been introduced to allow dry bonding based on shallower demineralization with the formation of thinner hybridization of dentin. Consequently, a more homogenous dentin/adhesive interface is expected to be recreated[5]. Self-etching adhesives also reduce the steps necessary for bonding in comparison with etch-and-rinse adhesives. Because many self-etching adhesives leave the bottom of smear plugs intact, they tend to create resin-dentin bonds that exhibit less dentin sensitivity[5,6].

Although self-etching adhesives are used in order to form a stable and strong biopolymer[18], lower bond strength has been reported[4] mainly in wet enviroments[14]. Unfortunately, the benefit of saving time by using self-etching adhesives may impair the quality of resin–dentin bonds (e.g. incomplete sealing)[6].

The complex environment of hybrid layers (HL) created by self-etching adhesives may explain their reduced performance[30]. For instance, nano and micro analysis of one-bottle adhesives show the presence of high concentrations of hydrophilic acid monomers as being responsible for the incorporation of voids in these hybrid layers that, in turn, increase their permeability[24,29]. In these zones, water is incompletely removed, resulting in regions of imperfect polymerization and/or hydrogel formationbetween the remaining water and the HEMA present in the adhesive systems[7,25].Therefore, partial hybridization of dentin may occur with more aggressive self-etching adhesives (lower pH). The presence of these defects may act as stress raisers[19] in resin-dentin interfaces, reducing adhesion over time.

A previous study provided data about bond strength and the characteristics of the adhesive interface obtained using scanning electron microscope (SEM) and nanoleakage[9-]. However, little information is available about the mechanical behavior of the dentin/adhesive interface based on hybrid layer quality[2,15,19].

The aim of this study was to evaluate the mechanic behavior of the hybrid layer containing voids, using 3-D finite element analysis. The voids were incorporated in different proportions (25% and 50% by volume). The null hypothesis is that voids have no effect on stresses within the hybrid layer.

MATERIAL AND METHODS

In order to perform the micromechanical analysis of dentin/adhesive interfaces, a virtual dentin specimen restored with composite resin[18] (41 x 41 x 82 μm) was built using SolidWorks software (SolidWorks Corporation, Concord, MA, USA)[2]. The dimensions of each structure of the model and their mechanical properties were based on previous data, assuming a linear, isotropic and linearly elastic study (Table 1).

Considering the dimensions reported in Table 1[8,15,17,18,26], three models were built by varying their void content in the HL by 0%, 25% or 50% (Mr, Mp, Mpp, respectively) (Table 2, Figure 1). Mr represents a perfect HL, without voids and completely infiltrated. Mp and Mpp represent a HL containing 25% and 50% of the volume with voids, respectively.

It was assumed that the adhesive that infiltrated through the collagen fibrils in these models bonded to adjacent and subjacent

Table 1- Dimensions (μm) and mechanical properties of the materials (E and ν)

Structures		**Dimension (μm)**	***E* (GPa)[18]**	**ν[15]**
Specimen	Width (base)	41x 41		
	Length	82		
Composite resin		41	30	0.3
Adhesive layer[18]		2 (Length)	5	0.28
Hybrid layer[26]		4 (Length)	4	
			3	0.28
			2	
			1	
Intertubular dentin close to HL[2,18]		3 (Length)	13	0.3
Intact intertubular dentin[18]		36 (Width)	20	0.3
Diameter of Peritubular dentin[18]		0.75 (Width)	28.6	0.3
Pulp[17]			0.0002	0.45
Resin Tag[26]		17 (Length)	5	0.28
Number of dentinal tubules[8]		16 (deep dentin)		
Diameter of dentinal tubules[20]		1 (deep dentin)		
Diameter of spherical voids		3.75		

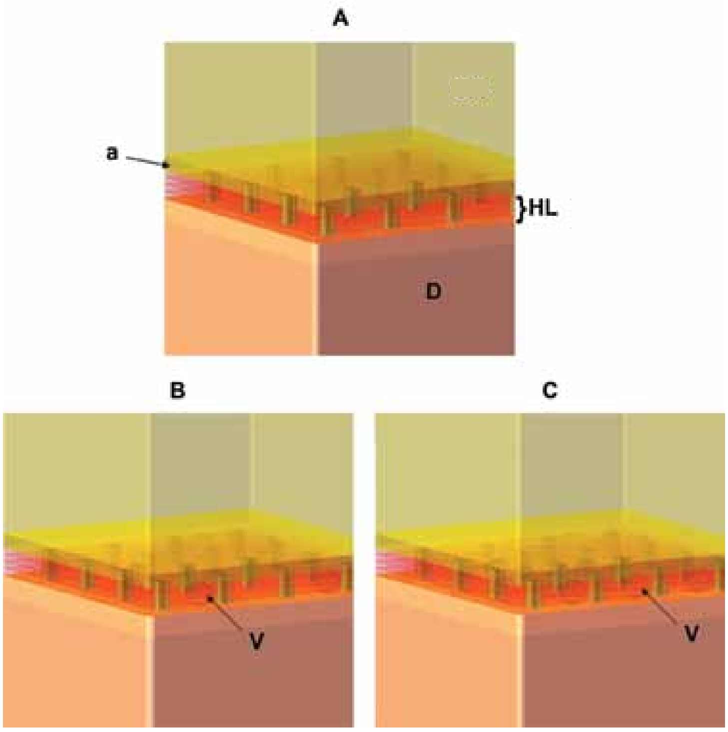

Figure 1- A - Model (Mr) with perfect bond between the hybrid layer (HL) and dentin (D); B – Model (Mp) with 25% of voids (V) in volume in the HL; C – Model (Mpp) with 50% of voids in volume in the HL. (Diameter of voids = 3.75 μm). HL (hybrid layer); V (voids); D (dentin); a (adhesive layer)

Table 2- Percentage of voids in the hybrid layer

Models	% of voids in the HL
Mr	0 (ideal bond)
Mp	25
Mpp	50

structures. Regarding the rigidity of the bonded structures, Misra, et al.[18] (2004) established that the elastic modulus of peritubular dentin and intertubular dentin show values of 28.6 GPa and 20 GPa respectively. However, Katz, et al.[12] (2001) reported that the elastic modulus of the intertubular dentin, adjacent to the HL, has a value of 13 GPa, due to effect of previous etching required by the conventional adhesive system. Unfortunately, these data have not been reported for aggressive self-etching adhesive systems.

In their study, Misra, et al.[18] (2004) varied the HL thickness between 2 and 10 μm. These authors considered the HL to be constituted of several sublayers of equal thickness. Thus, the elastic modulus was graded from 4 GPa for the most superficial sublayer in contact with the adhesive layer, to 1 GPa for the deepest sublayer in contact with the mineralized dentin base. According to these authors, due to the lower dimethacrylate adhesive infiltration of deep demineralized dentin, hybrid layers may be built with different elastic modulus in order to reproduce *in vivo* conditions. In the present study, a 4 μm thick HL was stratified in four 1 μm thick layers. A 4 GPa elastic modulus was used for the layer closest to the adhesive material; 3 GPa for the second layer, 2 GPa for the third layer and 1 GPa for the deepest layer in contact with the adjacent mineralized intertubular dentin[18].

The convergence criterion was applied to reach optimal mesh quality. All models showed up to 38.497 tetragonal elements and 102.580 nodes. The nodes at the base of the specimen were fixed on the x, y and z axes (x=y=z=0) to set up the border line.

In order to determine the loading value, it was observed that in a dentin macro-specimen (5x2x2 mm) restored with composite resin, in an hourglass shape (sectional area of 1.1 mm^2) there was an equivalent tensile force of 18 MPa after 20 N of tensile loading at the top of the resin surface, exactly as previously described[22].

The cross-sectional area of the micro-specimen model in the present study was equal to 1681 μm^2. To apply an 18 MPa tensile force to the adhesive interface, a tensile load equal to 0.03N was perpendicularly applied to the composite resin surface (Figure 2).

As developed by Misra, et al.[18] (2004), the maximum principal stress (σ_{max}) was utilized for identifying failures that could start out of small flaws, and it is an adequate criterion for brittle structures, such as dentin. All structures at the interface, i.e. peritubular dentin, intertubular dentin, adhesive layer and HL, were individually analyzed.

The numerical analysis was performed using ANSYS Workbench 10.0 (Swanson Analysis

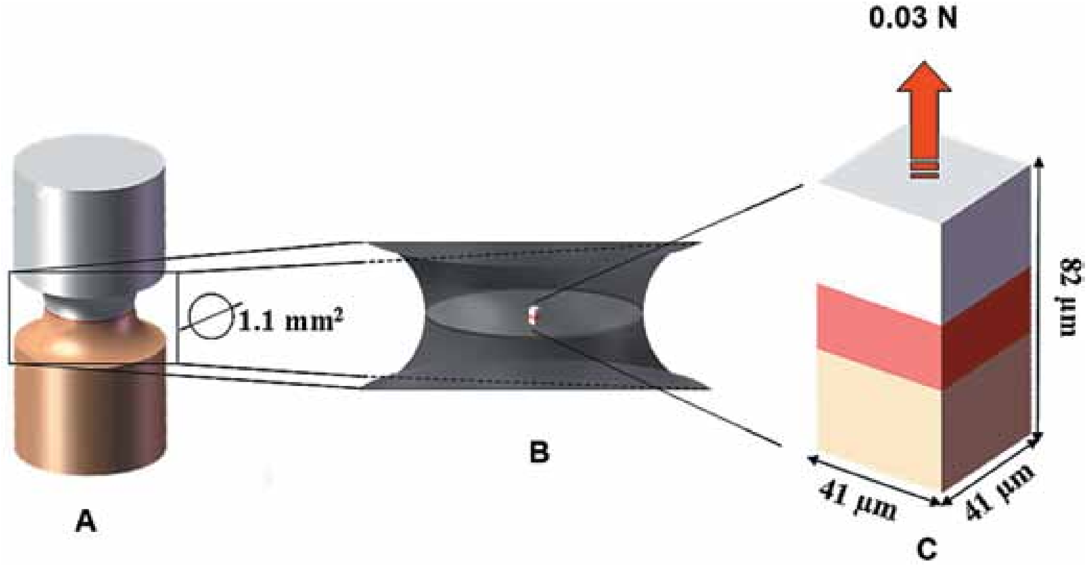

Figure 2 - A - Hourglass-shaped specimen with 1.1mm^2 of sectional area; B - Visualization of the model inside the hourglass-shaped specimen; C - High magnification of the model. Model dimensions (41x41x82 μm) and loading condition (tensile load, perpendicular to the top of the composite resin, with 0.03 N)

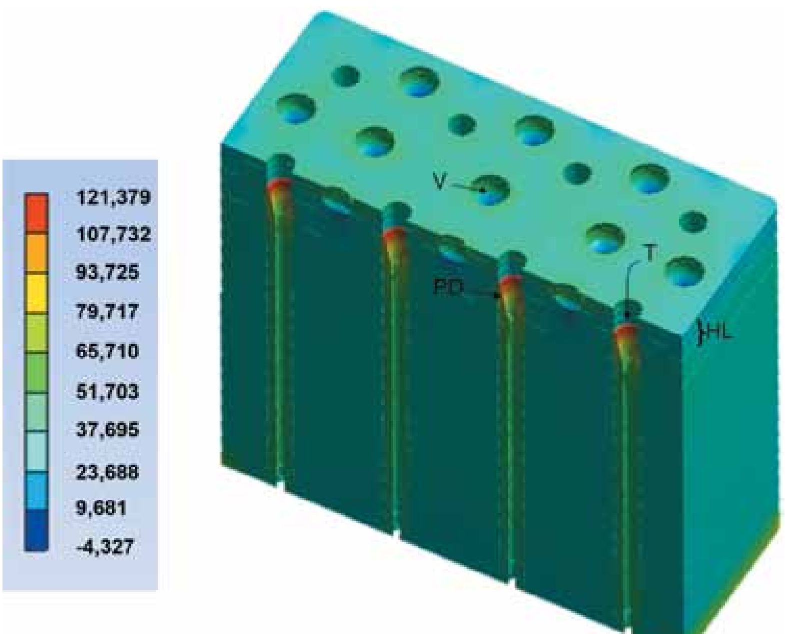

Figure 3- Maximum principal stress (σ_{max}) in the peritubular dentin (PD) and void (V) for the Mpp. Stress concentration in the three deepest layers of the hybrid layer (HL), voids (V), peritubular dentin (PD) and dentinal tubules (T) with no resin tags

System, Canonsburg, PA, USA).

RESULTS

The peak of σ_{max} was observed in the peritubular dentin (Figure 3) for all models (Figure 4).This behavior was similar in all other structures of the dentin/adhesive interface that showed an increase of σ_{max} in Mp when compared with Mr and in Mpp when compared with Mp (Figure 4 and 5). Figure 4 shows the σ_{max} for the adhesive layer, peritubular dentin, resin tags and intertubular dentin.

The σ_{max} in the peritubular dentin was observed in its upper border, exactly where the resin tags start (Figure 3). The σ_{max} in the tags occurred in their upper border (top of tags), in contact with the dentinal tubule walls (Figure 6).

The increase in the percentage of porosities within the HL, like in other structures, negatively influenced its mechanical behavior, increasing the stress concentration (Figure 5). The increase in stress in the HL was of 6% in Mp when compared with Mr and of 6.5% in Mpp when compared

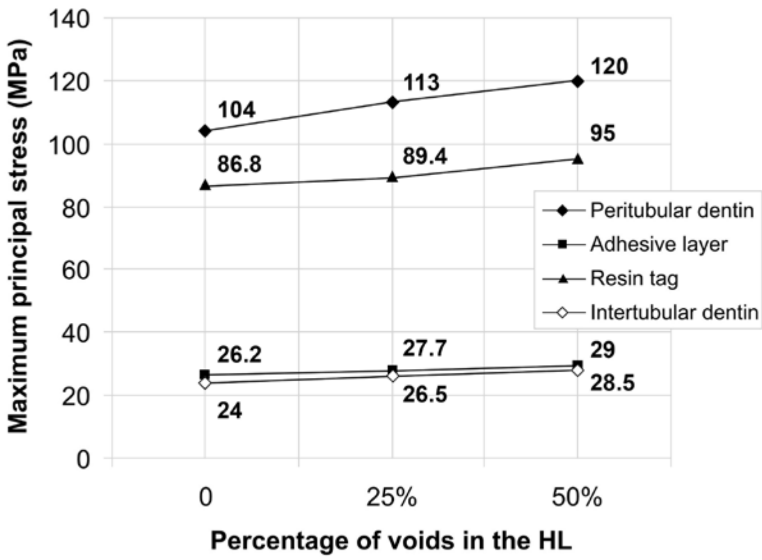

Figure 4- Maximum principal stress (MPa) in the peritubular dentin, adhesive layer, resin tags and intertubular dentin according to the percentage of voids (0, 25% and 50%) in the hybrid layer

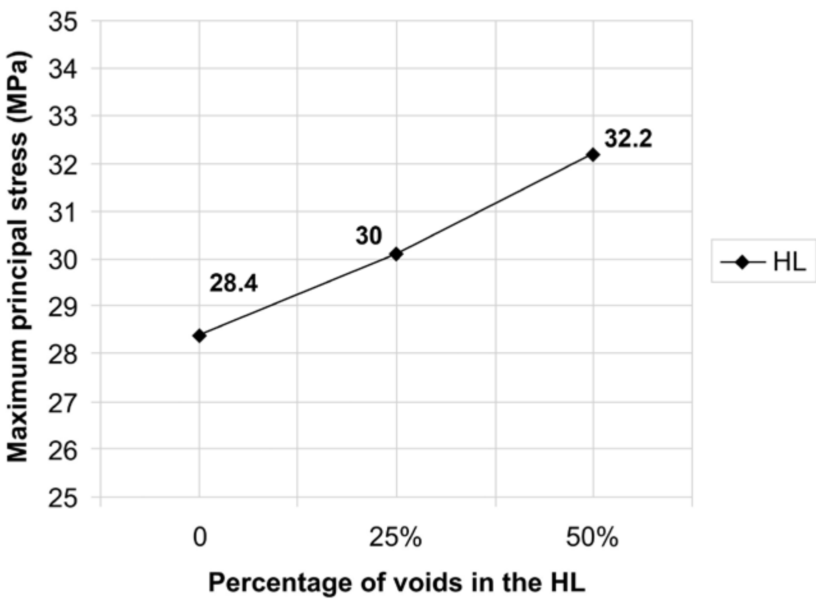

Figure 5- Maximum principal stress (MPa) in the hybrid layer according to the percentage of voids (0, 25% and 50%) for Mr, Mp and Mpp, respectively

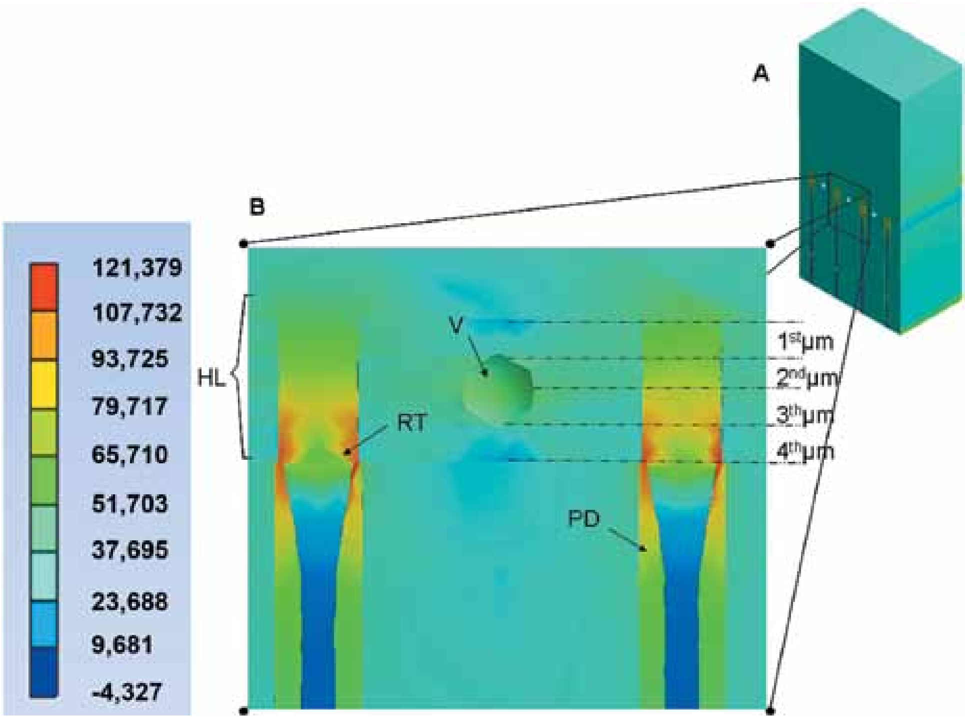

Figure 6- Maximum principal stress (MPa) for the Mpp. A – Cross-section view of the model Mpp to observe stress distribution (high magnification in B). B - Stress concentration on top of resin tags (RT), peritubular dentin (PD) and in lower intensity in the porosities (V). Note that the sectioning was done to visualize half of the dentinal tubules. This allowed partial visualization of porosities

with Mp.

The σ_{max} in the HL occurred near the porosities and between the second and third micrometers deep in the Mp and Mpp (Figure 6).

DISCUSSION

While a durable seal between several current bonding systems and enamel has been achieved, it is still a challenge to seal the resin-dentin interface, due to the heterogeneous characteristic of the dentin structure, surface morphology[10], and/or intrinsic shortcomings of the design of these modern adhesives[21].

Conventional thought is that a perfect seal along the resin-dentin interface can be achieved within the demineralized collagen matrix when it is completely infiltrated by adhesive resins in permanent and primary teeth[11]. This concept is based on the assumption that the polymerized resins used for bonding are nonporous and impermeable to fluids[23]. However, adhesive phase separation into hybrid and adhesive layers do not create an impervious collagen/polymer network but instead produce a porous web, and at the same time, affecting the chemical and mechanical properties of the adhesive layer[27].

Consequently, in order to evaluate the influence of the HL voids on the stress distribution at dentin/adhesive interfaces in this study, the bond established between the adhesive system and dentin was either considered ideal or incorporated with voids in different contents (25% and 50%).

It was observed that the peritubular dentin showed the highest stresses in all models, in accordance with previous studies[2,3]. The next structures to bear high stress were the resin tags, HL, adhesive layer and intertubular dentin.

The increased stress concentration observed in the top of tags can be related to the dentinal tubule diameter (1.0 μm) and closeness to the peritubular dentin which shows the highest elasticity modulus among the structures simulated at the dentin/adhesive interface[2,18].

The voids in the HL (Mp and Mpp) increased the σ_{max} to values 13.5% higher in comparison with the void-free perfect HL (Mr). Nevertheless, the σ_{max} in the 3 models were below the failure load established for the adhesives[28]. This indicates that the bottom of the adhesive layer might not be the site to start up failure when the bond between dentin and HL is porous. The peak of σ_{max} in the Mr was in the HL close to the peritubular dentin, very similar to that previously found in a perfect bond scenario[2,3].

Mollica, et al.[19] found that the presence of voids raised the σ_{max} by 3.7 times. In our study, the influence of voids was able to raise the σ_{max} only 13.5% (Figure 5). The study of Mollica, et al.[19] (2004) showed a generic model with no refinement of structures (i.e. peritubular and intertubular dentin, adhesive layer based on multiple layers, and resin tags) and mesh (only 5,000 elements and 10 nodes). This might justify the differences observed on the influence of voids.

When voids were incorporated into the HL (Mp and Mpp), the peak of σ_{max} moved from the contact with the peritubular dentin to the vicinity of the voids, 2 μm above the base of the HL (Figure 6). This behavior is very similar to that found in studies with micro-tensile loading analyzing the fracture pattern in specimens of dentin sticks[24]. In these, the failure was commonly observed at the base of the HL, close to areas with poor hybridization.

Some authors[13,27] showed that σ_{max} in the HL with poor hybridization was higher than the ultimate tensile strength of the self-etching adhesive system, even with a homogenous HL in contact with the dentin base. This issue is very important for the integrity of resin-dentin bonds over time. Lohbauer, et al.[16] (2008) showed that resin tags do not contribute to dentin adhesion in self-etching adhesive systems, in conditions of low bond strength values. In such circumstances, this means that the adhesion of the self-etching system is solely based on the quality of the HL and on its capacity to remain bonded to adjacent structures.

The present 3-D FE measurements showed that tensile stresses are restricted to two main sites in the dentin/adhesive interface: concentrated inside the HL, near the voids (Figure 6); and concentrated at the top of resin tags, in contact with dentinal tubule walls (Figure 6). Thus, our hypothesis can be accepted, as the σ_{max} was higher in the presence of voids. Further studies on the porous dentin/adhesive interface should be carried out, considering different degrees of bonding between the HL and the adhesive layer, as well as the intertubular dentin.

CONCLUSIONS

Within the limitations of the present study, we can conclude that:

- The presence of voids in the hybrid layer raised the maximum principal stress in all structures of the dentin/adhesive interface;
- The increase of void content has some influence on stress. The 50% void content was able to raise the stress by 13.5% inside the HL;
- In the presence of voids, the maximum stress moved from the peritubular dentin to the HL in contact with the voids.

ACKNOWLEDGEMENTS

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Comparative analysis between mandibular positions in centric relation and maximum intercuspation by cone beam computed tomography (CONE-BEAM)

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ABSTRACT

This research consisted of a quantitative assessment, and aimed to measure the possible discrepancies between the maxillomandibular positions for centric relation (CR) and maximum intercuspation (MI), using computed tomography volumetric cone beam (cone beam method). The sample of the study consisted of 10 asymptomatic young adult patients divided into two types of standard occlusion: normal occlusion and Angle Class I occlusion. In order to obtain the centric relation, a JIG device and mandible manipulation were used to deprogram the habitual conditions of the jaw. The evaluations were conducted in both frontal and lateral tomographic images, showing the condyle/articular fossa relation. The images were processed in the software included in the NewTom 3G device (QR NNT software version 2.00), and 8 tomographic images were obtained per patient, four laterally and four frontally exhibiting the TMA's (in CR and MI, on both sides, right and left). By means of tools included in another software, linear and angular measurements were performed and statistically analyzed by student t test. According to the methodology and the analysis performed in asymptomatic patients, it was not possible to detect statistically significant differences between the positions of centric relation and maximum intercuspation. However, the resources of cone beam tomography are of extreme relevance to the completion of further studies that use heterogeneous groups of samples in order to compare the results.

Key words: Computed tomography. Centric relation. Occlusion.

INTRODUCTION

Centric Relation (CR) is the maxillomandibular relationship in which the condyles articulate with the medial portion of their respective disks, being this complex (disk-condyle) in an antero-superior position against the surface of the articular eminence[1]. The position of maximum intercuspation is defined as the complete intercuspation teeth independent of condylar position.

Numerous studies have been elaborated in order to highlight the possible differences between the position of CR and MI[9,13,14,21,24, where different methods have been employed, among these it is possible to highlight studies by imaging. Great emphasis has been given by the scientific community to the Cone Beam Computed Tomography (CBCT)[10,11,12,19].

Cone Beam Computed Tomography (CBCT) systems have been designed for imaging hard tissues of the maxillofacial region. CBCT is capable of providing sub-millimeter resolution in images of high diagnostic quality, with short scanning times (10–70 seconds) and radiation dosages reportedly up to 15 times lower than those of conventional CT scans. Increasing availability of this technology provides the dental clinician with an imaging modality capable of providing a three-dimensional representation of the maxillofacial skeleton with minimal distortion[12].

The objective of this study was to assess, quantitatively, in frontal and lateral diagrams, the existence of possible discrepancies in the relation condyle/articulate fossa on the positioning of Centric Relation and on Maximum Intercuspation using the Computed Volumetric Tomography Cone Beam, in asymptomatic young adults presenting normal occlusion and Angle Class I malocclusion.

SUBJECTS AND METHODS

Subjects

Ten patients (2 male, 8 female) aged 18 to 25 years were recruited for this study after signing a written informed consent form approved by the Ethics in Research Committee of the Federal University of Uberlândia (n° 479/2008). Within the group of 10 patients, 5 presented normal occlusion (standard) and 5 presented malocclusion, classified as Angle Class I[3].

The inclusion criteria were: complete natural teething, except for third molars and presence of occlusal contacts with the related antagonist teeth in both arcades. Exclusion criteria were: previous orthodontic handling, presence of non sound teeth, signs of significant periodontal illness, absence of teeth, except for the third molars, symptoms related to prior occlusal adjustment, patients who did not fit the required age pattern for this study (18 to 25 years), patients who wore dental prostheses, patients who presented signs and/or symptoms of temporomandibular dysfunction and facial traumas.

Procedures

Initially, the clinical survey of patients was carried out, in order to identify the occlusal features of each patient. To register the centic relation, it was performed a manipulation of the mandible and an anterior deprogramming device (JIG) was used. This device was confectioned using acrylic resin chemically activated (Duralay – Reliance Dental Mfg. Co)[16]. In order to maintain a CR position during the tomographic examination, the first contact was identified between superior and inferior arcades in each patient, coinciding with the temporomandibular articulations position, also in CR. Afterwards, a precise wear was performed over the palatal acclivity of the JIG until the obtainment of that first contact which produced a dental stability for the maintenance of both articulations in CR.

- Computed Tomography

The device utilized in this research was NewTom 3G tomograph (Quantitative Radiology, Summer, Italy) and some parameters were taken into account.

In order to standardize the tomographic image's inclination orientation on the sagittal view of the primary reconstructions (both in CR and MI), 2 spheres, 5 mm each, were affixed with adhesive tape on each patient. Both spheres were placed following the orientation line of the Frankfurt diagram: the first was placed over the anterior portion of this diagram, that is, on the most inferior point of the left orbital margin (located by means of palpation); the second was placed 3.5 cm posteriorly to the first sphere, bearing the same orientation mentioned on the Frankfurt diagram specifications.

Initially, the first scanning was performed, in which the patient was instructed to keep his/her tooth in maximum habitual intercuspation. This scanning was obtained by means of the device's specific software (QRNNT, Version 2.00). Afterwards, the second tomographic session reconstruction area was standardized superiorly over the fronto-nasal suture, corresponding to the nasion point, and inferiorly, over the most inferior point of the mandible basis. On the frontal view, the inclination was oriented by means of a vertical line that passed internally to the nasal sept until the anterior nasal spine. There were used 0.2 mm tomographic slices, which made possible the secondary reconstructions of the images.

The measurements on the sagittal and frontal norms were carried out bilaterally on both mandibular positions in Centric Relation and Maximum Intercuspation, according to the following images:

1- Greater medium-lateral dimension of the condyles: axial image in which the condyle has shown the greatest medium-lateral linear dimension, measured until the tangency of its external cortical walls.

2- TMA lateral image selection: image obtained by means of an angle tool to orientate perpendicular cuttings to the greatest medium-lateral dimension of the condyle head.

1- TMA frontal image selection: image obtained by means of an application of a cut coincident to the condylar dimension on an axial view.

On the lateral cuttings, there were assessed the distances of the relations condyles/articular fossa, over the posterior, superior and anterior senses, according to the following mensuration standards (Figure 1):

- Line 1: Reference line, joining the most inferior points of the posterior region of the fossa and of the articular eminence, in order to measure the lateral cuttings.
- Line 2: Extension on which the Line 1 overlapped the condylar process, on the antero-posterior sense.
- Line 3: Line regarding the demarcation of the medium point on Line 2.
- Line 4: From the adaptation of the 90º tool, positioned exactly on the medium point of the reference, an extension of the vertical rod of this tool was performed in order to obtain a linear measurement, that means the distance between the medium point of the Line 2 and the most superior point of the cortical wall of the condyle head bone.

By means of the vertical rod of the angle tool included into the device's software, there were established the desired posterior, superior and anterior measurements on the lateral images.

On the frontal cuttings, the measurements comprised the distances between the condyles and the external surface of the articular fossa, on the medial, superior and lateral senses. On the methodology of the frontal cuttings, the initial references were found directly over the condyles head, where the most lateral and medial points were identified, by means of the angle tool. Since then, there were standardized the following reference lines (Figure 2):

- Line Alpha: identifying the most medial and lateral point over the condyle head
- Line Beta: drawn overlapping the alpha line, until reaching its middle length, determining the medium point of reference
- Line Gamma: extending from the medium point of reference until the most superior point of the external cortical wall of the condyle head, aiming to certificate the encounter of this same medium point of reference on the frontal right cuttings in RC and MI.

By means of the movement of the vertical rod of the angle tool, there were established the superior, medial and lateral measurements. On the same manner, the identification of the lines alpha, beta, gamma and the medium point of reference was performed.

The non-coincidence of the values of the standardized lines at this present study was interpreted as two different medium points of reference on 2 positions. If any coincidence was noticed, all the methodology had to be restarted.

Statistical Analysis

After the obtainment of all the measurements, the average and standard deviation were established for each one of them, following their respective positioning and corresponding cuttings, for the application of the statistical tests: the method’s Error Test, student t test and Tukey tests at a 5% level of significance.

was taken when the device of CR maintenance was inserted. At the end of the scannings, data processing was obtained by means of the NewTom 3G's software in order to achieve the desired images.

On the lateral view of the tomographic images, the inclination of these images was oriented by means of a horizontal line, in tangency with the spheres' extremity that could be seen, following the Frankfurt diagram specifications. The

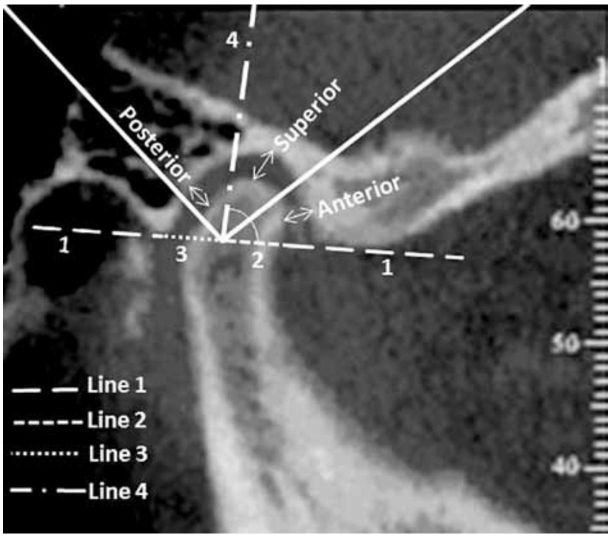

Figure 1 - Lateral view of the distances of the condyle/ articular fossa, over the posterior, superior and anterior senses

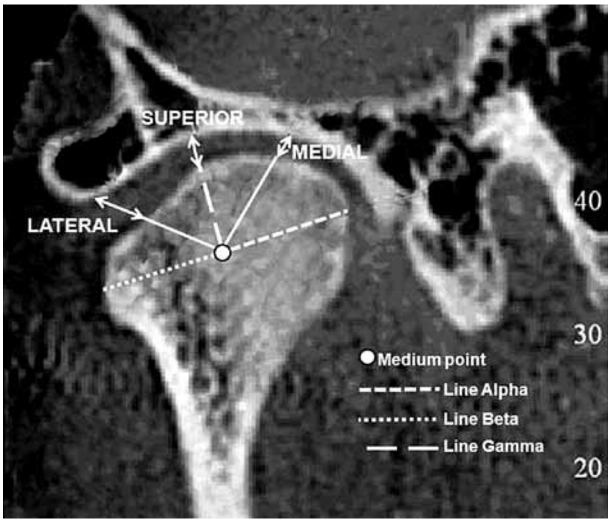

Figure 2 - Frontal view of the distances of the condyle/ articular fossa, over the posterior, superior and anterior senses

Table 1- Comparisons among the means obtained by the student t test, for evaluation of eventual differences between maximal intercuspation (MI) and centric relation (CR)

Variable		Mean	Standard deviation	t-Student	p-value
Lat right POST	MI	1.88	0.529	1.303	0.209
	CR	1.61	0.387		
lat right ANT	MI	1.76	0.744	-0.892	0.384
	CR	2.07	0.808		
lat right UPPER	MI	2.41	0.810	0.093	0.927
	CR	2.38	0.625		
fron right LAT	MI	2.32	0.789	-0.307	0.763
	CR	2.43	0.815		
fron right UPPER	MI	2.81	0.736	0.244	0.810
	CR	2.73	0.729		
fron right MED	MI	2.75	0.940	0.189	0.852
	CR	2.67	0.955		
lat left POST	MI	1.89	0.536	0.940	0.360
	CR	1.67	0.510		
lat left ANT	MI	1.88	0.721	0.034	0.973
	CR	1.87	0.581		
lat left UPPER	MI	2.69	0.828	0.749	0.464
	CR	2.43	0.721		
fron left LAT	MI	2.47	0.648	0.247	0.808
	CR	2.40	0.620		
fron left UPPER	MI	3.01	0.722	0.318	0.754
	CR	2.90	0.821		
fron left MED	MI	2.85	0.905	0.142	0.889
	CR	2.79	0.987		

P-value > 0.05: not statistically significant differences between the mean of MI and CR, accomplished by the student t test.

Table 2- Comparisons between the means obtained by the student t test, for evaluation of the eventual differences within Group I, regarding maximal intercuspation (MI) and centric relation (CR)

Variable		Mean	Standard deviation	t-Student	p-values
Lat right POST	MI	1.820	0.5718	0.524	0.614
	CR	1.640	0.5128		
lat right ANT	MI	1.680	0.6458	-0.762	0.468
	CR	2.000	0.6819		
lat right UPPER	MI	2.360	0.7403	-0.269	0.795
	CR	2.480	0.6686		
fron right LAT	MI	2.080	0.7981	-0.121	0.907
	CR	2.140	0.7701		
fron right UPPER	MI	2.660	0.5505	0.367	0.723
	CR	2.540	0.4827		
fron right MED	MI	2.860	0.9397	0.155	0.881
	CR	2.760	1.0922		
lat left POST	MI	1.960	0.6269	0.202	0.845
	CR	1.880	0.6261		
lat left ANT	MI	1.540	0.8473	-0.128	0.902
	CR	1.600	0.6205		
lat left UPPER	MI	2.84	0.727	0.581	0.577
	CR	2.60	0.570		
fron left LAT	MI	2.56	0.456	0.553	0.595
	CR	2.42	0.335		
fron left UPPER	MI	3.060	0.6427	0.414	0.690
	CR	2.880	0.7294		
fron left MED	MI	2.880	1.2215	0.026	0.980
	CR	2.860	1.1929		

P-value > 0.05: not statistically significant differences between the mean of MI and CR obtained by the student t test.

Table 3- Comparisons among the means obtained by the student t test, for evaluation of the eventual differences within the Normal Group regarding maximal intercuspation (MI) and centric relation (CR)

Variable		Mean	Standard deviation	t-Student	p-value
Lat right POST	MI	1.940	0.5413	1.332	0.219
	CR	1.580	0.2683		
lat right ANT	MI	1.840	0.9017	-0.499	0.631
	CR	2.140	0.9965		
lat right UPPER	MI	2.460	0.9607	0.349	0.736
	CR	2.280	0.6380		
fron right LAT	MI	2.560	0.7861	-0.313	0.763
	CR	2.720	0.8319		
fron right UPPER	MI	2.960	0.9263	0.068	0.947
	CR	2.920	0.9338		
fron right MED	MI	2.64	1.036	0.097	0.925
	CR	2.58	0.915		
lat left POST	MI	1.82	0.492	1.412	0.196
	CR	1.46	0.288		
lat left ANT	MI	2.220	0.4025	0.300	0.772
	CR	2.140	0.4393		
lat left UPPER	MI	2.54	0.979	0.476	0.647
	CR	2.26	0.879		
fron left LAT	MI	2.380	0.8468	0.000	1.000
	CR	2.380	0.8672		
fron left UPPER	MI	2.960	0.8678	0.068	0.948
	CR	2.920	0.9910		
fron left MED	MI	2.820	0.5891	0.213	0.837
	CR	2.720	0.8701		

P-value> 0.05: not statistically significant differences between the means of MI and CR by the student t test.

Table 4 - Estimates of means of variables lateral right POST, lateral right ANT, lateral right SUP, frontal right LAT, frontal right SUP, frontal right MED, lateral left POST, lateral left ANT, lateral left SUP, frontal left LAT, frontal left SUP, frontal left MED, cutting in the maximal intercuspation and centric relation

Variable	Grupos	
	Class I Angle	Normal
Lat right POST	1.73 a	1.76 a
	1.84 a	1.99 a
lat right ANT	2.42 a	2.37 a
	2.11 a	2.64 a
lat right UPPER	2.60 a	2.94 a
	2.81 a	2.61 a
fron right LAT	1.92 a	1.64 a
	1.57 a	2.18 b
fron right UPPER	2.72 a	2.40 a
	2.49 a	2.38 a
fron right MED	2.97 a	2.94 a
	2.87 a	2.77 a

Proportions followed by the same letter are not statistically different by the test Tukey, considering a level of significance of 0.05.

Intra-examining error test:

A unique user, previously calibrated, carried out the trial selection and measurement of the images of interest for this work. This user applied the Test of Intra-Examining Error after 20 past days of all the measurement carried out by him. 5 patients and 3 measures of the research were randomly chosen for the statistical accomplishment of the Error Test. The new measurements were remade using the software Basic 3G, without the knowledge of the values of the measures initially found (annex 5). Of possession of the values of the measures that were remade, comparisons between the average of the initial measurements with those performed 20 days after the first ones were carried out. Therefore, the T of Student test shows the results on Table 1.

RESULTS

Tables 1, 2 and 3 show the results of comparison between two positions: RC and MI. The standard deviation of each position for every patient was calculated considering, independently, the left and right sides. The averages between the standard deviation for CR and MI standard deviation for both left and right sides were calculated. The results of the test error of intra-examiner has not shown significant differences.

DISCUSSION

A mandibular position that determines occlusal, muscular and articular balance is indispensable to plan and execute oral rehabilitation, in concordance to the stomatognathic system. There is little consensus on the contours of concept of Centric Relation as a reference position for oral rehabilitation[5,7,16,17,23,25,26]. A fundamental question in dentistry is what is the optimal position of the condyle in the articular fossa when teeth are in maximum intercuspation. Despite the way the teeth come together in occlusion can be observed directly in the patient's mouth, the condylar position into the articular fossa is impossible to be seen by the clinician's naked eye.

In order to estimate the condylar positions, several methodologies have been proposed at the current literature. However, all those methodologies have shown many controversies. Within the most used methods to measure different mandibular positions, it can be taken into account the use of semi-adjustable articulators and imaging examinations[2,5,7,8,9,13,14,21,24]. According to the semi-adjustable articulators use, some drawbacks can be highlighted when treating and/or studying the condylar positions, i.e.: the fact of the articulators not considerate the presence and anatomical variability of the present tissues at the temporomandibular articulation and possible distortions during the assemblage on these apparatuses.

Various radiographic modalities have been used to visualize the condylar positions. In spite of that, the radiographies obtainment represents a non-precise method for this analysis, for the various magnificence radiographic degrees and, also, because there is a restriction related to the two-dimensional diagram. Another important imaging resource is the magnetic resonance, a very applied method along the clinical studies, which refers to the articular disc positioning. Despite this study has not employed the magnetic resonance method, attention can be called to the association of magnetic resonance technology and computerized tomography - techniques of great contribution to a better comprehension of the temporomandibular area.

Within the imaging resources, this study employed the cone beam computed tomography technology, considering that the spatial variations of the condyles related to the fossa on RC and MI positions are predominantly very small. This method allows a three-dimensional assessment of the temporomandibular articulation, presenting higher precision over images delineation. Within the imaging exams, TCBT presents more substantial details when compared to other exams, allowing greater trustworthiness from the acquired data. CTCB presents lower radiation levels and lower costs when compared to the conventional computed tomographs of the medical area[11,20,21,22].

Several researchers have related that the majority of toothed patients exhibit a discrepancy between the CO and MI positions[9,13,14,21,24]. According to the results of this study, the analyzed patients presented differences between these two positions, however, when submitted to a statistic analysis, those differences were not significant. Within the possible explanations for this result, the features of the selected sample can be highlighted, constituted by young and asymptomatic adult individuals. The use of a reduced sample of 10 individuals could be explained for the pilot character of this type of study, employing the cone beam method, and because it is a research which deals with x-radiation directly over human beings.

Most of the studies that demonstrated a significant discrepancy between RC and MI used heterogeneous samples, in which were included patients of different ages, with symptoms of TMD and absence of occlusal stability[9,13,14,21,24]. Because of these differences, it could be presumed that, in spite of the samples presented various occlusal arrangements, these were in relative balance or were not yet capable of generating alterations that could create significant changes over the condyle/fossa relation.

The results obtained in this present study are of clinical relevance. It is indispensable to carefully examine the status of the condyles and discs while performing three-dimensional occlusal reconstruction by orthodontic, prosthodontic or other modalities in dentistry. In spite of the great importance of differentiation of these two condylar positions in any dentistry modality and of the results achieved in this study, attention must be given to the fact that each patient has unique features that should be carefully examined in order to obtain a suitable result.

Further studies are needed in this research area. Studies which involve a greater amount of patients (samples) with normal patterns of occlusion, without parafunctional habits, asymptomatic vs. symptomatic, young patients vs. old patients, dentulous vs. edentulous and many more variables that could reveal information for the establishment of some parameters, yet obscure, taking into account the worthiness of the cone beam method.

CONCLUSION

Based on the limitations of the present study, it was concluded that there are not statistically significant differences between centric relation and maximum intercuspation in the group of individuals without any symptom or sign of temporomandibular disorder.

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Analysis of the dentin-pulp complex in teeth submitted to orthodontic movement in rats

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ABSTRACT

In order to microscopically analyze the pulpal effects of orthodontic movement, 49 maxillary first molars of rats were submitted to orthodontic appliance composed of a closed coil spring anchored to the maxillary incisors, placed for the achievement of mesial movement. Material and Methods: Ten animals were used as the control group and were not submitted to orthodontic force; the other animals were divided into groups according to the study period of tooth movement, namely 1, 2, 3, 4, 5, 6 and 7 days. The investigation of pulp and periodontal changes included hyalinization, fibrosis, reactive dentin and vascular congestion. Statistical evaluation was performed between control and experimental groups and between periods of observation using non-parametric chi-square, Kruskal-Wallis and Dunn tests. Results: There was no statistically significant difference concerning pulpal changes between control and experimental groups nor between periods of observation. The control group, at 3 and 5 days, revealed greater hyalinization of the periodontal ligament ($p<0.05$), whereas root resorption was significantly greater at 5 and 7 days ($p<0.05$). Conclusion: No morphological change from the effect of induced tooth movement could be found in the dentin-pulp complex. In addition, no inflammatory or pulp degeneration, detectable in optical microscopy, was found in experimental groups.

Key words: Dental pulp. Rat. Tooth movement. Root resorption.

INTRODUCTION

The pulp and periodontal tissues are richly cellularized, and their metabolic rates are adapted to their functional needs. The structural and functional normality of these tissues seem to be influenced by local and systemic factors[7]. Clinical detection of periodontal and pulp changes induced by local and systemic factors probably depend on the type, duration and intensity of the stimulus applied.

Early studies have suggested that vascular changes promoted by orthodontic movement might cause pulp necrosis. Oppenheim[20-22], in 1936, 1937 and 1942, affirmed that teeth submitted to orthodontic movement presented occasional atresia of the pulp and root canal, observed by radiographic examination. Microscopically, there was an increase in collagen fibers and inflammatory cells and a reduction in blood vessels, indicating probable pulp degeneration.

Strang[29] (1943) suggested that abrupt forces on teeth, especially intrusion, might damage the blood vessels in the pulp, causing blood vessel congestion and pulp necrosis. Butcher and

Taylor[3,4], in 1951 and 1952, applied retraction forces to incisors of monkeys and reported the occurrence of pulp necrosis.

In their study, Mjör and Stenvik[19] (1969) applied intrusion forces to human teeth and did not observe any significant differences in the pulp tissue of experimental and control groups; the observation of vacuolization in the pulp tissue was considered to be an artifact. Anstendig and Kronman1 conducted an investigation on dogs and also observed vacuolization of the odontoblast layer as the main pulp changed after orthodontic movement.

In the 1980s, Hamersky, et al.[12] and Unsterseher, et al.[31] (1987) investigated biochemically the effect of orthodontic forces on the respiratory metabolism of the pulp of human teeth, with the aid of radioactive carbon dioxide. The authors concluded that pulp respiration is reduced in orthodontically moved teeth.

Studies using laser doppler flowmetry conducted by McDonald and Pitt Ford[18] (1994), Ikawa, et al.[14] (2001) and Sano, et al.[26] (2002) revealed that application of forces on human teeth reduced the initial blood flow to the pulp, in disagreement with the findings of Barwick and Ransay[2] (1996), which did not reveal any changes.

Encouraged by the initial flowmetry studies, Derriger, et al.[9,10] (1996, 1998) analyzed the secretion of angiogenic factors on human teeth and observed that these factors were increased in orthodontically moved teeth. Santamaria, et al.[28] (2006), detected a slight increase in the volume of blood vessels only during the first 6 hours of induced tooth movement in molars of rats. Ramazanzadeh, et al.[25] (2009), described similar results in human teeth submitted to intrusive and extrusive forces.

The related literature presents variable models and criteria for analysis, as well as results and conclusions. Thus, the following doubts remain: 1. May induced tooth movement promote early pulp aging, even if only microscopically observable? 2. May it promote pulpitis, pulp necrosis or any other change? These questions seem to remain without answers and were the initial premises of this study.

MATERIAL AND METHODS

Appliances were placed on the maxillary left first molars of 49 Wistar albino rats aged 120 days, divided into 7 experimental groups, according to the period of tooth movement (1 to 7 days)[13,17]. Three animals were analyzed at each period, yet nine animals were evaluated at 3, 5 and 7 days, which are the most representative periods of the biological effects of induced tooth movement. Other 10 animals were taken apart as the control group and were killed without accomplishment of any orthodontic movement. The device was placed as suggested by Heller and Nanda[13] (1979) and improved by Martins-Ortiz[17] (2005), and was composed of a stainless steel coil spring tied to the maxillary first molar and anchored to the maxillary incisors, which delivered 75 g of force (Figure 1). To place the device for tooth movement, the animals were anesthetized with a mixture of equal parts of ketamine hydrochloride 100 mg/mL (Dopalen – Vetbrands) and muscle relaxant xylazine hydrochloride 20 mg/mL (Anasedan – Vetbrands), at a dose of 1 mL/Kg. The solution was applied with a 1-mL syringe and 12.7-mm sterile needle by intramuscular injection.

The rats were killed by an overdose of anesthetics (ketamine and xylazine), according to the periods of tooth movement of each group. After that, the maxillae were removed with surgical scissors and the specimens were fixed in 10% buffered formalin. After fixation, the specimens were decalcified in Morse's solution for one week, embedded in paraffin and longitudinally sectioned of 6-μm thickness. The sections were placed on glass slabs and stained with hematoxylin-eosin. Ten slabs with 5 sections each were prepared for each animal.

The mesiobuccal and distobuccal roots were analyzed on longitudinal sections. The presence of hyaline areas of periodontal ligament, frontal bone resorption and undermining bone resorption was registered for both roots of each animal. When there were extensive hyaline areas involving segments of periodontal ligament and undermining resorption, the microscopic findings were interpreted as resulting from intense force.

Small hyaline areas and frontal bone resorption were interpreted as the result of biologically acceptable forces.

At the apical third, more specifically at the apical periodontal ligament, microscopic evaluation addressed the detection of circulatory changes such as blood vessel congestion, hemorrhage and thrombosis.

Figure 1- Orthodontic appliance used to achieve mesial inclination of the maxillary left first molar, anchored on the maxillary incisors, delivering a force of 75 g

The main changes investigated in the dental pulp comprised cell vacuolization, tubules with nuclei, reduced cellularity, fibrosis, pulp hyalinization, pulp nodules, dystrophic calcifications, reactive dentin, blood vessel congestion, areas of hemorrhage and thrombosis. The analysis also addressed the disorganization of the odontoblast layer and areas of pulp necrosis.

Statistical analysis

Statistical evaluation was performed between control and experimental groups and between periods of observation using non-parametric chi-square, Kruskal-Wallis and Dunn tests.

At 30 days after the first microscopic analysis, 30 specimens were randomly selected and re-evaluated; the results of the first and second analyses were compared for investigation of intra-examiner agreement by *Kappa* analysis, considering values above 0.80 as good level of agreement.

RESULTS

Evaluation of intra-examiner agreement revealed a *kappa* value of 0.83, demonstrating the effectiveness of the analysis of microscopic sections conducted by the examiner.

Concerning the evaluation of periodontal microscopic phenomena, statistically significant differences were found in the Experimental

Table 1- Frequency of periodontal phenomena microscopically observed in the control and experimental groups, with induced tooth movement (ITM) on the maxillary first molars of rats

Periodontal changes	Groups							
	No ITM	1 day	2 days	3 days	4 days	5 days	6 days	7 days
	n=10	n=3	n=3	n=9	n=3	n=9	n=3	n=9
Focal hyalinization	0	2	0	2	0	2	0	3
Segmental hyalinization	0	1	3	7*	3	7*	3	6
Frontal bone resorption	0	0	1	3	1	2	0	3
Undermining resorption	0	0	0	6**	2	7*	3	6**
Root resorption	0	0	0	0	1	8*	3	9*

* Significant at the 5% level in relation to the Control group without ITM

** Significant at the 10% level in relation to the Control group without ITM

Table 2- Frequency of pulp phenomena microscopically observed in the control and experimental groups, with induced tooth movement (ITM) on the maxillary first molars of rats

Pulp changes	Groups							
	No ITM	**1 day**	**2 days**	**3 days**	**4 days**	**5 days**	**6 days**	**7 days**
	n=10	n=3	n=3	n=9	n=3	n=9	n=3	n=9
Cell vacuolization	2	0	1	2	0	0	0	0
Tubules with nuclei	0	0	0	0	0	0	0	0
Reduced cellularity	0	0	0	0	0	0	0	0
Increased fibrosis	0	0	0	0	0	0	0	0
Hyalinization	0	0	0	0	0	0	0	0
Pulp nodules	1	0	0	0	0	0	0	0
Diffuse calcification	0	0	0	0	0	0	0	0
Reactive dentin	0	0	0	0	0	0	0	0
Blood vessel congestion	10	3	2	8	3	9	2	9
Hemorrhage	0	0	0	0	0	0	0	0
Thrombosis	0	0	0	0	0	0	0	0

* Significant at the 5% level in relation to the Control group without ITM

** Significant at the 10% level in relation to the Control group without ITM

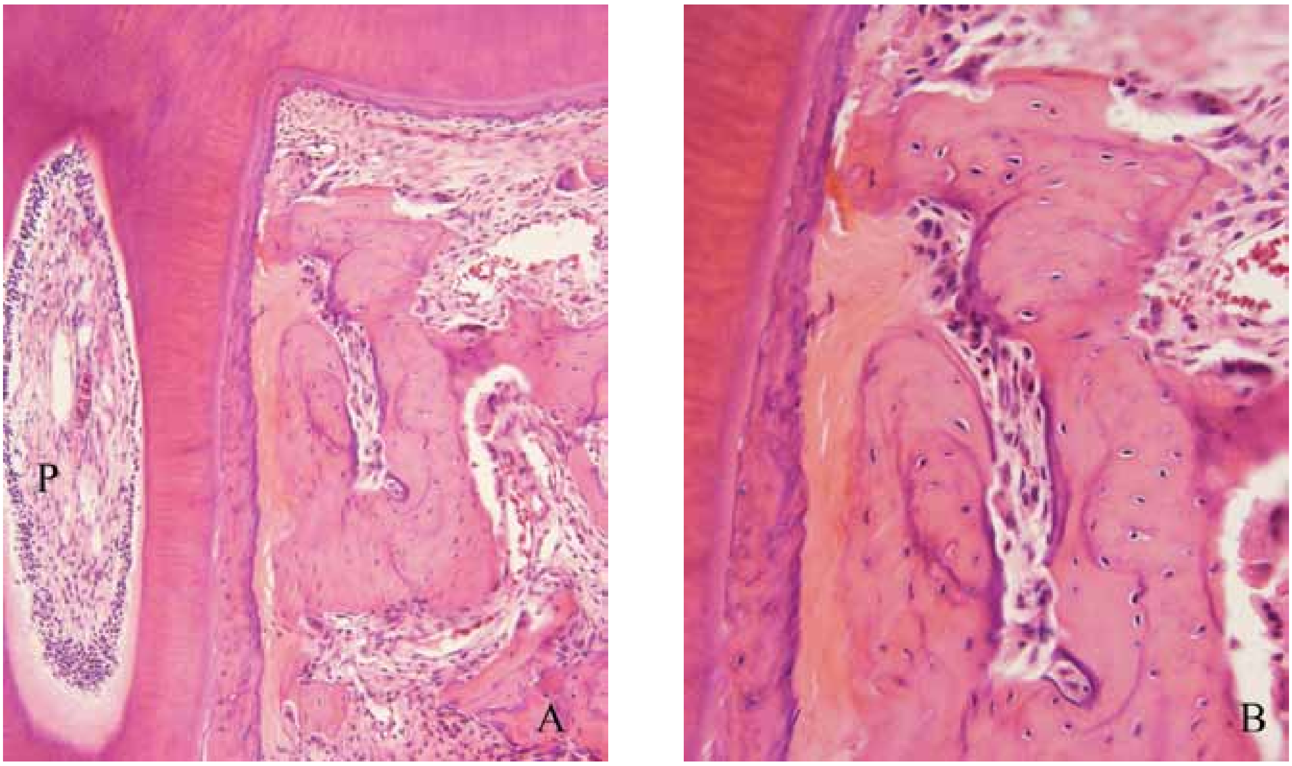

Figure 2- Periodontal morphological changes induced on the cervical region of the distobuccal root submitted to intense forces (A and B). Experimental group, after 3 days of induced tooth movement on the maxillary first molar of rats. (A) Note the normal aspect of the pulp (P). A: 25 x magnification; B: 40 x magnification

groups compared to the Control group. This difference was found for the presence of segmental hyalinization at 3 and 5 days, at the 5% level, between control and experimental groups. The analysis of frequency of frontal bone resorption did not reveal significant differences among groups; however, undermining resorption was significantly more frequent at 5 days of induced tooth movement. Considering a lower significance level of 10%, undermining resorption

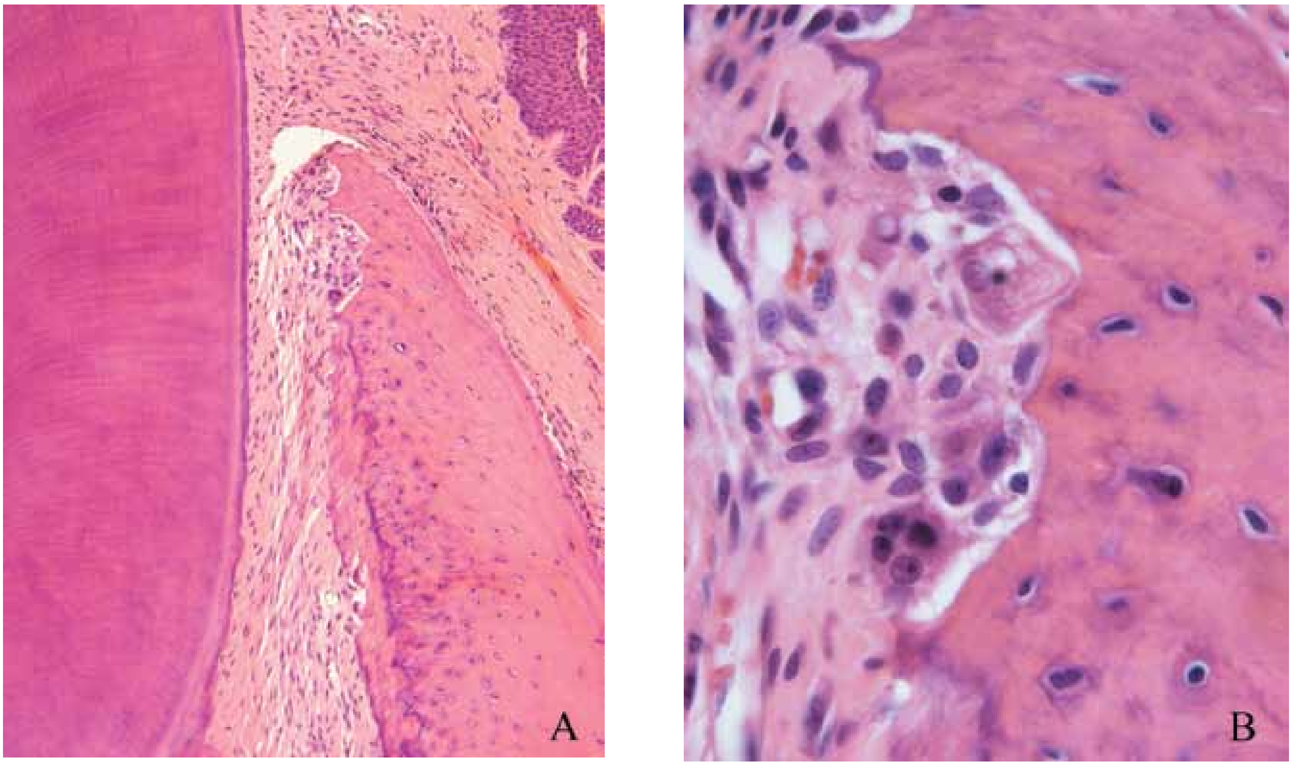

Figure 3- Periodontal morphological changes induced on the cervical region of the mesiobuccal root (A and B) submitted to moderate forces with frontal bone resorption. Experimental group, after 3 days of induced tooth movement on the maxillary first molar of rats. A: 25 x magnification; B: 40 x magnification

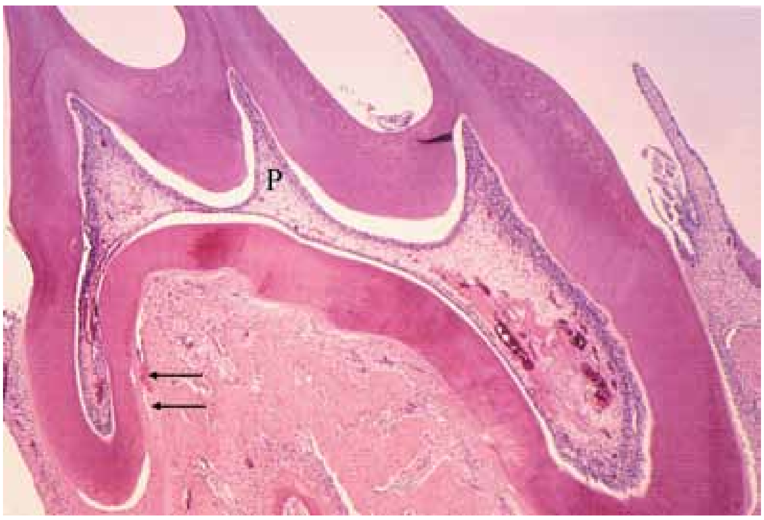

Figure 4- Microscopic aspects of the pulp and periodontal morphological changes in the periodontal ligament, after 4 days of induced tooth movement on the maxillary first molar of rats. Note the segmental hyalinization of periodontal ligament on the distal root (arrows) and the normal aspect of the pulp (P). Pulp vascular congestion was due to operatory procedures during material collecting and histotechnical process. 10 x magnification

was greater at 3, 5 and 7 days compared to the Control group. The most significant results were observed for the presence of root resorption at 5 and 7 days compared to the Control group (Table 1).

Evaluation of the Control and Experimental groups at the different periods revealed uniform morphology of the dental pulp. In both groups, the blood vessels were usually congested and filled with blood components, predominantly erythrocytes. The odontoblast layer occasionally presented cell vacuolization and loss of continuity,

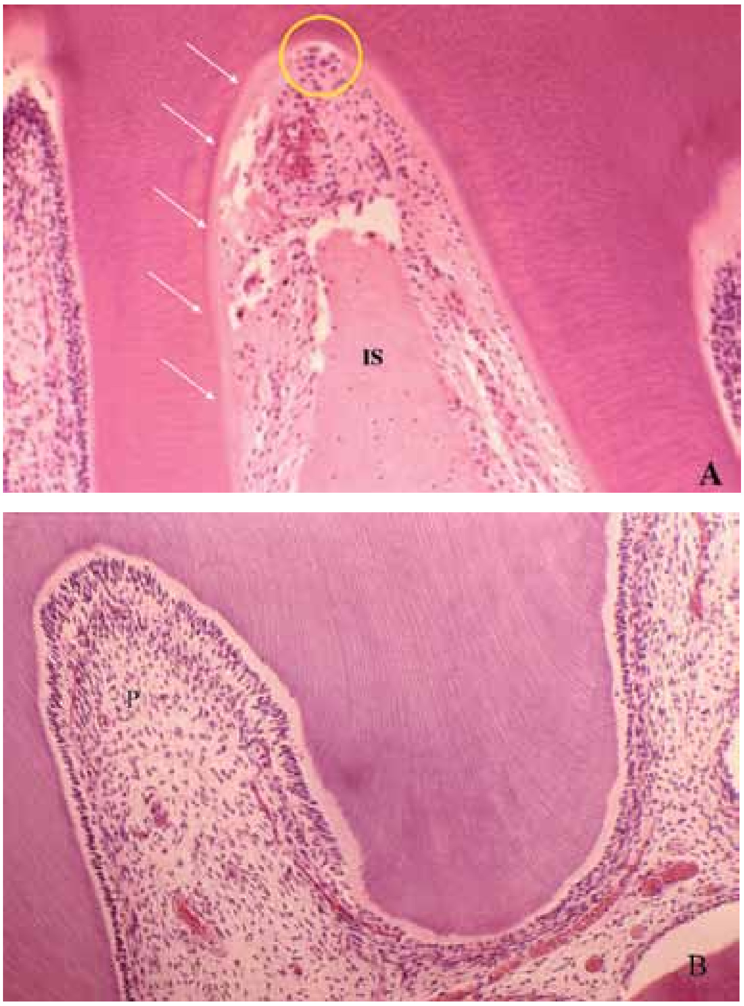

Figure 5- Microscopic aspects of the root pulp (P) in A, and coronal pulp in B, compatible with normal morphology of maxillary first molar of rat submitted to induced tooth movement for 7 days. On the interseptal surface (IS) of the distobuccal root, the cementoblast layer was extensively lost (arrows), with presence of several clasts and areas of root resorption (circle). A: 25 x magnification; B: 25 x magnification

yet associated with artifacts. These observations on morphological normality were valid for the entire dental pulp, from the coronal to the apical region. In the internal dentinal wall, turned toward the dental pulp, there was neither thickening of the pre-dentin layer nor morphological signs of reactive dentin that could indicate any pulpal effect from the force applied throughout this experiment of induced tooth movement in rats (Table 2).

DISCUSSION

The forces applied to the maxillary first molars of rats were intense on the mesial interseptal surface of the distobuccal root (Figure 2) and moderate on the mesial region of the mesiobuccal root, both analyzed on the cervical third (Figure 3).

Analysis of the distobuccal root microscopically demonstrated the biological effects of application of intense forces, with presence of hyaline areas of periodontal ligament at 3 to 5 days of induced tooth movement, especially on the cervical region at the pressure side (Figure 2 and 5). At 5 days and especially at 7 days, on the same region, there was higher frequency of root resorption (Figure 5). The forces applied to the mesiobuccal root had moderate intensity, with absence of hyalinization of the periodontal ligament and root resorption at the most advanced periods of induced tooth movement (Figure 3).

The results revealed that the forces generated during experimental induced tooth movement were not able to cause changes in the pulp tissue even if intense, as observed in the initial periods up to 7 days (Table 2, Figure 2, 4 and 5).

The experimental model of induced tooth movement adequately reproduces the biological situation found in humans. However, the metabolic rate of connective tissues of rats should be considered when using this experimental model. A period of 21 days is required for the observation of all stages of induced tooth movement in humans, whereas in rats this period is reduced to 7 days[11].

Previous studies in the literature[1,4,19,25,27,28] indicate that orthodontic movement does not induce pulp changes such as increased number of pulp modules, calcific metamorphosis of the pulp, permanent vascular changes, or early pulp aging represented by hyalinization of the extracellular matrix and fibrosis. These changes are generically called regressive and/or degenerative[8,24].

Investigations reveal the occurrence of several biological reactions during induced tooth movement, including increased[15] or reduced[14,18] blood flow; reduced[12,31] or increased[16] cell respiration; and increased angiogenesis[9,10]. However, changes in the local level of enzymes and mediators observed in cultures and laboratory biochemical tests on the pulp tissue[23,32] indicate possible changes in the metabolic rates of the pulp tissue, rather than loss of biological viability or vitality. These metabolic changes did not morphologically changes the pulp tissue.

Clinical cases[8,30] should properly include all records, from photographs to periapical radiographs, before and during orthodontic treatment, or rule out the possibility of dental trauma[5,6,19].

For the endodontist and orthodontist, the pulp status should be precisely and safely determined before the onset of orthodontic treatment. After diagnosis of pulp status of teeth which will be submitted to orthodontic movement, treatment may be performed in a safe manner and with good prognosis. In teeth without caries, restorations or periodontal disease, trauma should be investigated as the main suspected cause of pulp changes during and after orthodontic treatment.

Pulp necrosis induced by trauma may be diagnosed during orthodontic treatment. The tissue changes inherent to tooth movement may exacerbate the clinical and radiographic signs of chronic pericementitis and periapical granuloma, not diagnosed on the initial orthodontic records. The initial correct diagnosis of the teeth should be done with periapical radiographic.

When compared to traumatisms or mechanic forces of different kinds, orthodontic forces can be considered little or small, even when called heavy or intense[5,6]. Studies comprising experimental intrusion and tooth movement with forces considered intense did not produce pulp necrosis in a similar context as the orthodontic treatment[4,25].

In the present study, the effectiveness of the force applied was demonstrated by the microscopically observable changes in the periodontal tissue, including significant loss of segmental hyalinization at 3 to 5 days, undermining resorption at 5 days and root resorption at 7 days; however, the pulps of moved teeth did not present any degenerative or inflammatory change (Figures 2, 4 and 5). The odontoblast layer occasionally presented cell vacuolization and loss of continuity, yet associated to artifacts.

Therefore, according to the present results and previous reports in the literature, it is suggested that clinical cases of pulp necrosis occurring during and after orthodontic treatment should be managed with greater emphasis on the dental history of the patient and possibility of trauma, rather than on the orthodontic force applied.

CONCLUSION

Based on the present results and considering the inherent limitations of this experimental model, it may be concluded that induced tooth movement does not promote microscopically observable degenerative or inflammatory morphological changes in the dental pulp.

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