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CONTENTS / *ÍNDICE*

ACTA ODONTOLÓGICA LATINOAMERICANA

Informa que a partir del Volumen 27 (2014) la revista se editará en formato digital con el *Sistema de Gestión de Revistas Electrónicas* (Open Journal System, OJS). Se utilizará el *Portal de publicaciones científicas y técnicas* (PPCT) del Centro Argentino de Información Científica y Tecnológica (CAICYT-CONICET). A partir de este volumen la revista será de acceso abierto (Open Access). Esta nueva modalidad no implicará un aumento en los costos de publicación para los autores.

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ACTA ODONTOLÓGICA LATINOAMERICANA

Wishes to inform that as of Volume 27 (2014) the journal will be published in digital format with the *Open Journal System* (OJS), employing the *Portal de publicaciones científicas y técnicas* (PPCT) of the Centro Argentino de Información Científica y Tecnológica (CAICYT-CONICET). From this volume on, the journal will be open access (Open Access). The publication fees for the authors will remain unchanged.

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MORPHOLOGICAL CHANGES RELATED TO AGE IN MESIAL ROOT CANALS OF PERMANENT MANDIBULAR FIRST MOLARS

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ABSTRACT

The aim of this study was to evaluate age-related morphological canal changes in mesial root canals of mandibular first molars of known ages. Fifty-six specimens were selected for this study and distributed into the following four age groups (n. 14): a) Group of children under 13 years, b) Group of adolescents (from 14 to 19 years), c) Group of young adults (from 20 to 39 years) and d) Group of older adults (over 40 years). The specimens were in perfect condition because after extraction they were carefully cleaned, sterilized, identified and stored in water. In order to improve the cleaning, they were placed in 1% sodium hypochlorite solution for four hours and rinsed in 10 vol. hydrogen peroxide for 8 hours. After that, a clearing technique was performed to illustrate root canal anatomy. Digitalized images of all samples were obtained by use of a stereomicroscope. Canals were noticeably simpler in older adults: they were sharply defined and narrow, sometimes too narrow. Calcification nuclei were not found and there were only a few remains of internuclear spaces. The canal system appeared cleaner, clearer and more sharply defined than in the other age groups. It may be concluded that there is a correlation between aging and morphological changes in the mesial root canals of mandibular first molars.

Keywords: *Anatomy; Molar; Morphology*

ALTERAÇÕES MORFOLÓGICAS RELACIONADAS À IDADE EM CANAIS RADICULARES MESIAL DE PRIMEIROS MOLARES INFERIORES PERMANENTES

RESUMO

O objetivo do presente estudo foi avaliar as alterações morfológicas relacionadas com a idade em canais radiculares mesial de primeiros molares inferiores. Cinqüenta e seis espécimes foram selecionados para este estudo. Os espécimes foram distribuídos em quatro grupos etários (n. 14): a) Grupo de crianças menores de 13 anos, b) grupo de adolescentes (de 14 a 19 anos), c) Grupo de jovens adultos (de 20 a 39 anos) e d) Grupo de adultos (acima de 40 anos). Após as extrações os elementos foram cuidadosamente limpos, esterilizados, identificadas e armazenadas em água. A fim de melhorar a limpeza, foram colocados numa solução de hipoclorito de sódio a 1%, durante quatro horas e enxaguados em 10 vol. peróxido de hidrogénio durante 8 horas. Depois, uma técnica de diafanização foi realizada para ilustrar a anatomia do canal radicular. As imagens digitalizadas de todas as amostras foram obtidas através da utilização de um estereoscópio. Os canais foram visivelmente mais simples em adultos mais velhos: eles foram bem definidas e estreito, por vezes, demasiado estreito. Núcleo de calcificação não foi encontrado e havia apenas alguns restos de espaços internucleares. O sistema de canal apareceu mais limpa, mais clara e mais bem definida do que nas outras faixas etárias. Pode-se concluir que há uma correlação entre as alterações do envelhecimento e morfológicas nos canais radiculares mesial de primeiros molares inferiores.

Palavras-chave: *Anatomia; Molar; Morfologia*

INTRODUCTION

The pulp dentinal complex is capable of responding to a variety of stimuli over time. These stimuli can be physiological -relating to the normal stresses to which a tooth would be exposed over a lifetime-, or pathological -due to caries, tooth surface loss, or restorative treatment[1]. One of the most obvious features of aging is a reduction in size of the pulp chamber caused by the continual secretion of dentinal matrix (physiological secondary dentinogenesis) by odontoblasts[2]. This process tends to narrow the originally wide-open root apex; with aging, the apical deposition of secondary dentin and cementum increases, and circulation and innervation are compromised. Teeth reflect the biological or physiological age of the individual, and variations caused by genetic factors and chewing habits can influence tooth anatomy[3].

The complexity of root canal systems and internal morphology has been directly correlated with endodontic treatment planning, therapy and outcome. Mandibular first molars are the first permanent teeth to erupt, often requiring endodontic care due to early caries[4]. Typically, mandibular molars have two well-defined roots: a mesial root characterized by a flattened mesiodistal surface and widened buccolingual surface, and a distal root mostly straight with a wide oval canal or two round canals[5]. However, many variations exist regarding its root and root canal anatomy, thus necessitating critical evaluation of each individual case for variations[6-8].

In this study, we evaluated age-related morphological canal changes in mesial root canals of mandibular first molars of known ages. The tested null hypothesis was that there is no difference in the anatomy of mesial root canals of mandibular first molars at different ages.

MATERIALS AND METHODS

The Ethics Committee approved this study. Fifty-six first mandibular molars were provided by the Tooth Bank of Córdoba National University. The teeth were selected for this study considering age, integrity of mesial root and complete root formation. In order to improve cleaning, teeth were placed in 1% sodium hypochlorite solution for four hours and rinsed in 10% hydrogen peroxide for 8 hours. The specimens were distributed into the following four age groups (n=14): a) Group of children under 13 years, b) Group of adolescents (from 14 to 19 years), c) Group of young adults (from 20 to 39 years) and d) Group of older adults (over 40 years). After coronal access, mesial roots were placed in a plastic tube connected to a vacuum and the tube-root junction was sealed with wax. Then, a luer-lock syringe with a 27-gauge endodontic needle was used to inject Indian ink into the mesial canals while vacuum was applied. This operation was repeated until ink emerged from the foramen. After drying, the teeth were decalcified in 5% nitric acid for 28 to 30 hours. After thorough washing of the decalcified teeth in running tap water for 4 hours, the samples were dehydrated in ascending concentrations of ethanol (70%, 80%, 95% and 100%) for 1 day, and the samples were rendered transparent by immersion in methyl salicylate for 2 days.

Digitalized images of all samples in 5 different angles were obtained by use of a stereomicroscope (Olympus Co, Tokyo, Japan) under 5X magnification in order to analyze the anatomy of each specimen of the four groups.

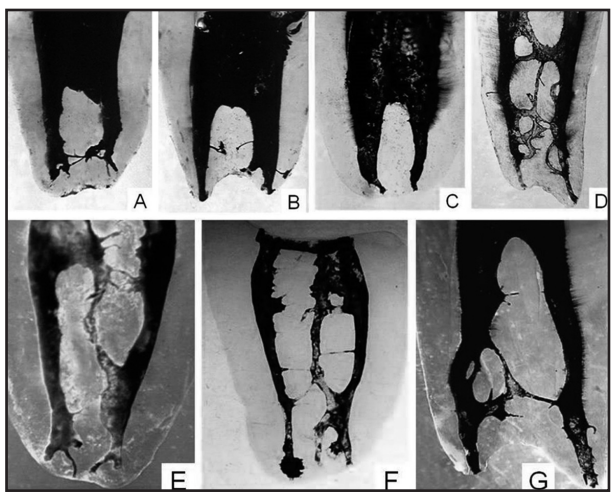

Fig. 1: Representative images from children under 13 years.

RESULTS

Group of children under 13 years

The images of the canal system in children were highly variable. Sometimes there were single, large, triangular-shaped canals with the vertex ending in a single apical foramen (Fig. 1A, B) while other times the entire canal, including the apical section, was ribbon-shaped (Fig. 1C, D). The canals often had blister-shaped dilatations, particularly in the coronal and middle sections of the roots (Figs. 1E, F, G, H, I, J). There were often unstained circular or oval-shaped areas within the canal, which were sometimes very small (Fig. 1E) and sometimes large (Fig. 1F, G, H). We call them "calcification nuclei". In most cases the calcification nucleus was single and located at the widest part of the canal, which would later allow its surface to increase, tending clearly to determining two canals. Thus, if the shape of canal tended to be triangular (Fig. 1A), the calcification nucleus developed in its coronal third (Fig. 1F, G), and if the canal was somewhat dilated in the middle third (Fig. 1B), the calcification nucleus began there and then extended in all directions, particularly towards the apex, foreshadowing the presence of two canals joining up to form a single foramen (Fig. 1H).

Ribbon-shaped canals had quite particular anatomical features. The bifurcations and trifurcations at the apical third of the canal and even the presence

of veritable apical deltas seem to be very frequent (Figs. 1C, D, I, K), more so than in triangular canals. Moreover, it should be highlighted that the calcification nucleus in these ribbon-shaped canals began at the apical third, foreshadowing the presence of the two canals ending in different foramina (Fig. 1I, K).

Group of adolescents (14 to 19 years)

The anatomical appearance of canals in adolescents was similar to that in children. The only difference between a triangular canal in adolescents (Fig. 2A) and children was that the former had a larger calcification nucleus and more clearly defined anatomy.

The presence of two, three or more calcification nuclei was fairly frequent in adolescents, whether the canals ended in one or two foramina. Nuclei were predominantly oval-shaped, and their sizes varied noticeably. Their distribution in the canal depended on their number. There were linear spaces between them, which we call internuclear spaces (Fig, 2B, C), of widely varying appearance: they could be wide or narrow; horizontal, oblique or vertical; one, two or several, according to the number of nuclei.

The development of a calcification nucleus at the apical section of the canal often led to bifurcation of the canal, ending in two individual foramina, which could either be very close to each other (Fig. 2C) or very far apart from each other (Fig. 2D, E, F, G). In the latter case, the apical bifurcations were very evident and sometimes the calcification nucleus between them was formed by the fusion of two or more smaller nuclei, with only few remains of the spaces.

In contrast, calcification nuclei were not found in the middle and coronal canal sections of the canals.

Group of young adults (20 to 39 years)

In young adults, the canals were more clearly defined than in adolescents. Nevertheless, there were still calcification nuclei and spaces separating them, often forming a complex system (Fig. 3A, B). Vertical internuclear spaces joining up with other spaces or canals after a short length were outstanding due to their clear definition.

Group of older adults (over 40 years)

Canals were noticeably simpler in older adults: they were sharply defined and narrow, sometimes too narrow. Calcification nuclei were not found and there were only a few remains of internuclear spaces. The canal system appeared cleaner, clearer and more sharply defined than in the other age groups (Fig. 4A, B, C).

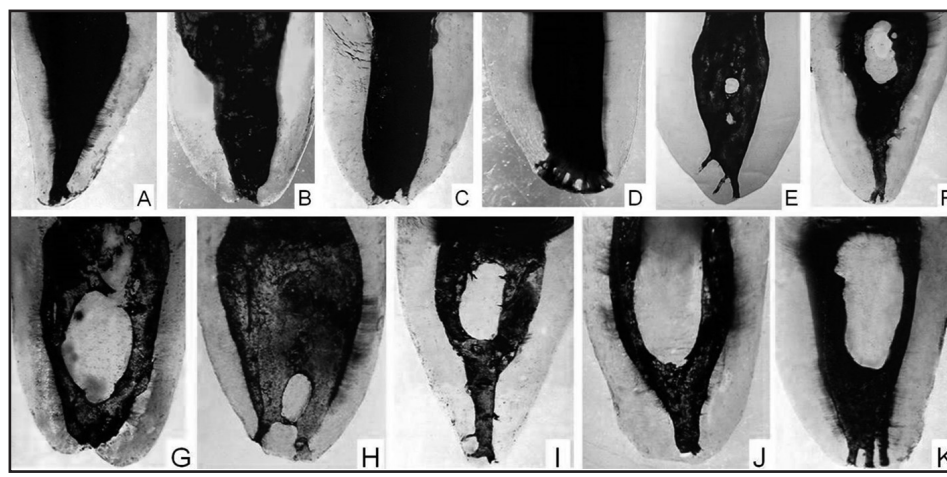

Fig. 2: Representative images from adolescents (14 to 19 years).

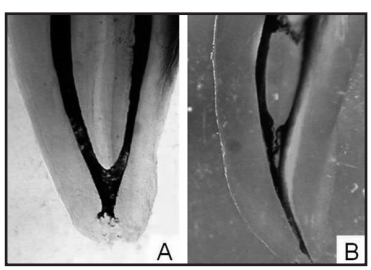

Fig. 3: Representative images from young adults (20 to 39 years).

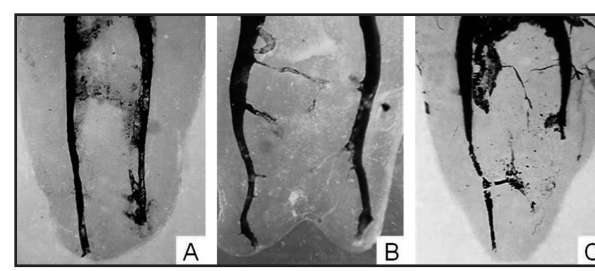

Fig. 4: Representative images from older adults (over 40 years).

DISCUSSION

Knowledge of the influence of age on root canal anatomy is needed to improve the cleaning and shaping process in endodontic treatment of patients of different ages. This study investigated age-related changes in mesial root canals of mandibular first molars. The results of this study showed a reduction of the canal, and several other differences, by comparing the canal anatomy of teeth of different ages. Therefore, the null hypothesis was rejected. Previous studies correlating patient age with root canal anatomical changes[1-3,9,10] have shown similar results, demonstrating that the canal undergoes reduction over the years.

In children, there were cases in which the canal was single throughout its length, wide at the coronal section, narrowing gradually towards the apex and ending in a single foramen. In other cases, the width and ribbon shape were even along the entire length of the canal, even in the apical section. The results in this group are probably related to the dentin deposition process, which, over time, reduces the main canal lumen and significantly changes the shape of the root canal, including a partial separation with the presence of an isthmus[10]. Isthmuses cause great difficulty in the cleaning and shaping process, as they have been shown to be inaccessible to conventional hand and rotary instrumentation[11,12].

Similar anatomy was observed in adolescents, although the size and number of calcification nuclei was greater. In certain cases, the internuclear spaces formed a real systems or plexus whose complexity depended on the number of calcification nuclei. In this group, there were frequently calcification nuclei dividing the canals in the apical section, some of which seemed to be formed by the fusion of two or more nuclei. On the other hand, nuclei in the middle and coronal sections of the canal were noticeably absent. Nevertheless, to compensate, it would appear that the apical calcification nucleus develops upward, increasing the division between the canals. The ending of both canals in independent foramina contributes to an interesting anatomical feature, because despite the lack of endodontic maturation still evident in adolescence, the apical portion of the canals showed some degree of anatomical definition and above all, a narrowing of both canals enabling better quality in instrumentation and canal filling aspects, which have been reported previously[13]. These observations allow us to state that in the mesial root of mandibular molars, a certain degree of endodontic maturation can only be spoken of in late adolescence, and not in all cases, because sometimes the process continues into the young adult stage.

In young adults, there is no doubt that the signs of endodontic maturation are clearer. Nevertheless, there are still calcification nuclei and spaces separating them, although these spaces are smaller and sometimes fewer. Figún & Garino[14], referring to them as inter-canal communications and disregarding age, report that there may be 4 or 5 in a single canal and their sizes may range in diameter from filiform to 1mm. They describe their shape and direction as straight, arched or italic-S-shaped, and according to the communicating canals, classify them as primary, secondary or tertiary. In contrast, Peiris et al.[15] reported, without providing details, that the prevalence of inter-canal communications is low at young and old ages and high at intermediate ages, which, in very general terms, is what our study found. Vertical internuclear spaces are particularly outstanding in young adults, and have not been reported by the abovementioned authors, although they have sometimes been considered as additional canals[16], when in fact they simply show immaturity, since they will soon be vestigial or will have disappeared entirely.

In older adults, we did not find any calcification nuclei or internuclear spaces, although there were a few remains. The anatomical features usually found in canals of older adults are those which could already be foreseen in children, began to take shape in adolescents and particularly in young adults, to reach full maturity in older adults. These observations are similar to those in a previous study[15], which reported that in lower molars, the canals are large up to the age of 11 to 15 years, and the internal shape is defined between 30 and 40 years. The success of endodontic treatment in adults, particularly in older adults, might be due to the fact that the pulp cavity becomes narrower with age, enabling better shaping and filling procedures[17]. Therefore, successful endodontics can be achieved in older adults with special attention to diagnosis, good quality radiographs and an adequate technique to overcome the challenges posed by anatomy changes of the root canal system.

Under the present experimental framework and with the limited sample size, a correlation between aging and morphological changes was observed in the mesial root canals of mandibular first molars.

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MICROBIAL DIVERSITY IN DENTAL UNIT WATERLINES

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ABSTRACT
Dental health care providers and patients are exposed during ongoing work to contamination by the water used in the dental units, due to accidental swallowing or aspiration of the sprays generated by the high-speed handpiece and the three-way syringe. This study evaluated the quality of water in dental units in the public dental care system of Maceió, Alagoas, Brazil, by conducting analyses of contamination by total coliforms, E.Coli, heterotrophic bacteria and filamentous fungi. We collected 200 mL of water at 5 sites in 6 dental offices of the Department of Health located in different parts of the city. A total 212 isolates and 16 genera of filamentous fungi were identified in the water collected from the dental units. Total coliforms indicated that the water used in dental units was not appropriate for human consumption. The high levels of contamination found in this study showed that water was a potential source of cross-infection.

Key words: *Biofilms; Dental service Infection control; Water quality*

DIVERSIDADE MICROBIANA NAS LINHAS D'ÁGUA DAS UNIDADES DENTAIS

RESUMO
Durante a rotina de trabalho, a equipe odontológica e os pacientes ficam expostos a possível contaminação pela água utilizada nas unidades dentais, devido à ingestão acidental da água ou pela aspiração dos sprays gerados pela caneta de alta rotação e seringa tríplice. Este estudo avaliou a qualidade da água usada em consultórios odontológicos da rede pública estadual de Maceió, Alagoas, Brasil, através da análise de contaminação por coliformes totais e E.coli, bactérias heterotróficas e fungos filamentosos. Foram coletados 200 mL de água, em cinco pontos de seis consultórios odontológicos pertencentes à Secretaria Estadual de Saúde, localizados em diferentes bairros da cidade. Um total de 212 isolados e 16 gêneros de fungos filamentosos foram identificados. A presença de coliformes totais indicou que a água utilizada nas unidades dentais era imprópria para consumo humano. O alto índice de contaminação mostrou que as águas estudadas eram uma fonte potencial de infecções cruzadas.

Palavras-chave: *Biofilme; Controle de infecção; Qualidade da água; Serviços odontológicos.*

INTRODUCTION

The quality of dental unit water is of considerable importance to patients and dental health care providers because they are exposed to water and aerosols generated from the dental unit during routine practice[1,2].

Microbial concentrations in dental unit waterlines were first reported by Murray and Slack in 1957[3]. Today, the presence of high concentrations of microorganisms in the water of dental units is recognized by the scientific community[4]. This contamination has been an important problem in dentistry for over 50 years[5,6].

In Brazil, there is no specific standard for the microbial quality of water used in dental units, but the Ministry of Health issued Directive # 2914 in December 2011[7], establishing that the quality of potable water supplied to the population by the public distribution systems should be evaluated through monthly bacteriological analyses assessing total coliforms and *Escherichia coli*. Heterotrophic bacteria should be counted in 20% of the samples and the total should not be greater than 500 colony forming units (CFU) per milliliter of water. Similar standards are used in Japan <100 CFU/mL, Europe <200 CFU/mL and the United States <500 CFU/mL for drinking water. Coliform count is also used internationally as an indicator of unsafe drinking water[5,8,9].

This study evaluated the quality of water in dental units in the public dental care system of Maceió, Alagoas, Brazil, by conducting a quantitative analysis of contamination by total coliforms, *E.coli,* heterotrophic bacteria and filamentous fungi.

MATERIALS AND METHODS

Water samples were collected from six dental clinics of the Department of Health located in different parts of the city, in hermetically closed, sterilized graded wide-mouth bottles containing 0.1mL of 10% sodium thiosulfate solution to neutralize residual chlorine, following the protocol recommended by Standard Methods for the Examination of Water and Wastewater[10]. Water was collected from the following sites: three-way syringe – SYRINGE; high-speed handpiece coupled to tubing – HANDPIECE; tubing of high-speed handpiece without handpiece coupled - TUBING; water reservoir – RESERVOIR; and the site supplying the reservoir – SOURCE.

To disinfect these sites before collection, they were wiped quickly with a piece of gauze soaked in 70% ethyl alcohol. The three-way syringe and high-speed handpiece were turned on and the water allowed to run for 10 seconds before collecting. 200mL of water from each site at the six dental units. The samples were kept cool in ice boxes and processed within four hours of collection.

Sample Inoculation and Culture

Total coliforms and E. coli

An enzyme substrate test (Colilert®, IDEXX Laboratories, Westbrook, ME) was used. The water containers were cleaned with a piece of gauze soaked in 70% ethyl alcohol. Then, 100 mL of the collected water were measured with a sterile pipette and placed in a sterile Erlenmeyer flask, and the reagent was added. It was incubated at 35 ± 0.5 °C for 24 hours. Results were read using ultraviolet light. The test was positive for total coliforms if the water was yellow, and for *E.coli*, if it was blue under ultraviolet light. The test was negative if there was no color.

Heterotrophic bacteria

The methodology used was adapted from the protocol suggested by Mayo et al.[11]. Each sample was diluted to 10^{-1}, and 0.1 mL of the original sample and of the dilution were plated in duplicate onto Petri dishes containing plate count agar (PCA), to which 50 $mg.L^{-1}$ of ketoconazole was added. The dishes were incubated at 37 °C for 24 to 72 hours.

Filamentous Fungi

The samples were diluted to 10^{-1}, and 0.1 mL of the original sample and the dilution were plated in duplicate onto Petri dishes containing Sabouraud dextrose agar, to which 50 $mg.L^{-1}$ of chloramphenicol and 50 $mg.L^{-1}$ ampicillin were added. The dishes were incubated at 28° C for four to six days.

Identification of Filamentous Fungi

Filamentous fungi were identified according to genus based on macroscopic and microscopic features. Microscopic analysis was conducted using the microculture technique in Lactrimel medium (14 g wheat meal, 14 g dried milk, 7 g honey and 0.4 g chloramphenicol per liter)[12].

RESULTS

E. coli was not detected in any of the water samples analyzed. However, nine of the thirty samples (30%) showed total coliforms (Table 1).

All the dental units had at least three sites at which heterotrophic bacteria exceeded the 500 CFU/mL limit (Table 2).

Filamentous fungi were isolated from 70% of the samples (21/30), totaling 212 isolates grouped in 16 genera. The most frequent genera were *Acremonium* (46.7%), *Exophiala* (14.7%), *Penicillium* (9.4%), *Aspergillus* (8.9%). Other genera had fre-

Table 1: Total coliforms, quantitative results in 100 mL, according to collection site.

Unit	Source	Reservoir	Tubing	Handpiece	Syringe
1	N	N	N	N	N
2	N	P	P	P	P
3	N	N	N	N	N
4	N	N	N	N	N
5	P	P	P	P	P
6	N	N	N	N	N

N = Negative sample P = Positive sample

Table 2: Heterotrophic bacteria counts in CFU/mL according to collection site.

Unit	Source	Reservoir	Tubing	Handpiece	Syringe
1	2.8×10^4	3.1×10^4	$2.5x\ 10^3$	6.5×10^2	3.2×10^4
2	3.5×10^2	7.0×10^3	2.9×10^3	8.6×10^2	6.5×10^2
3	2.2×10^4	2.3×10^2	1.0×10^3	4.7×10^2	5.3×10^2
4	1.5×10^2	7.0×10^2	1.1×10^3	$2.3x\ 10^2$	8.6×10^3
5	9.3×10^4	8.9×10^4	8.9×10^4	8.6×10^4	1.2×10^5
6	NG	2.3×10^3	2.4×10^3	1.6×10^3	3.0×10^4

NG = no growth

Table 3: Number of fungi according to genus isolated and identified in 30 samples from 6 dental units.

Isolated fungi	(n°)	(%)
Acremonium spp	99	46.7
Aspergillus spp	19	8.9
Bipolaris spp	6	2.9
Cladophialophora spp	10	4.7
Cladosporium spp	6	2.9
Chrysosporium sp	1	0.4
Curvularia spp	5	2.3
Exophiala spp	31	14.7
Fusarium spp	2	1
Mycelia Sterilia	6	2.9
Paecilomyces sp	1	0.4
Penicillium spp	20	9.4
Phoma spp	1	0.4
Rhinocladiella spp	2	1
Scopulariopri sp	1	0.4
Verticilium spp	2	1
Total	212	100

n = total number of isolates
% = percentage according to number of isolates

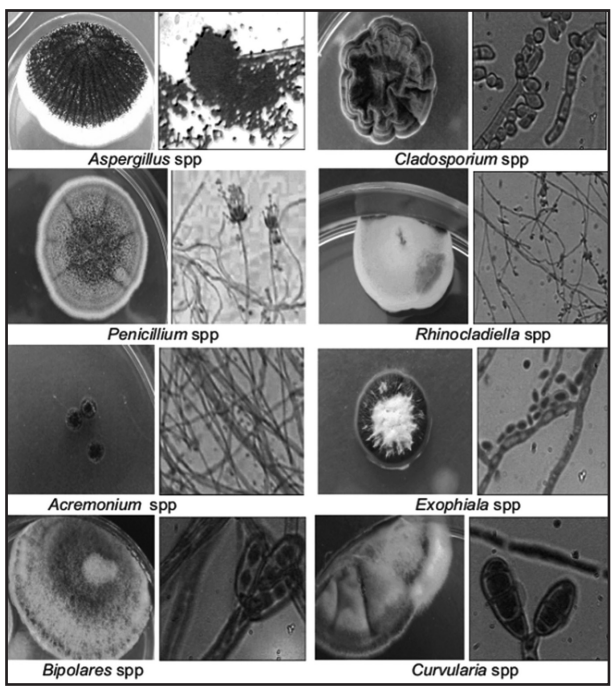

Fig. 1: Macroscopic and microscopic image of eight of the sixteen genera isolated and identified in 30 samples from 6 dental units.

quencies below 5%. All the genera isolated include potentially pathogenic species (Table 3 and Fig.1). The highest number of fungi was isolated and identified from tubing, with 108 isolates (50.9%), followed by reservoir, with 43 isolates (20.3%), and handpiece, with 39 isolates (18.4%). The percentages at the remaining sites did not exceed 7% (Table 4).

DISCUSSION

Although contamination of dental unit water systems was identified over 50 years ago, many dentists nowadays are still unaware of microbiological contamination or its health risk for dental care providers and patients[13].

According to Standard Methods for the Examination of Water and Wastewater, sodium thiosulfate is an adequate dechlorinating agent that neutralizes any residual chlorine and prevents continuation of bactericidal action during sample transport. Thus, the exam will indicate more precisely the true microbial content of the water at the time of sampling[10].

The minimum contamination level of heterotrophic bacteria detected in the water samples collected from the high-speed handpiece was 2.3 x 10^2 CFU/mL, in agreement with Souza-Gugelmin et al.[14], who found contamination levels of 1.9 x 10^2 CFU/mL for the same collection site.

The level of bacterial growth from all the water samples collected from the high-speed handpiece, either connected to the tubing (HANDPIECE) or not (TUBING), exceeded acceptable levels, except at dental units 3 and 4 for the HANDPIECE site, for which the results were within the acceptable limits. This finding indicates that bacterial contamination was greater in the tubing than in the handpieces.

According to Watanabe et al[15], water reservoirs should be cleaned regularly with mechanical and

Table 4: Number of fungi isolated and identified according to collection site.

Collection site	Positive Samples	Isolates (n)
Syringe	3	7
Reservoir	6	43
Handpiece	3	39
Tubing	5	108
Source	4	15
Total	21	212

n = number of isolates

chemical methods to remove the biofilm. Our study found that the highest concentration in the reservoirs was 8.9×10^4 CFU/mL, which is lower than the result detected in a previous study, in which the concentration was found to be 1.1×10^5 CFU/mL[16].

The results of studies conducted by Aprea et al.[17] and Watanabe et al.[15] were negative for bacteria of the coliform group and *E.coli*. However, in our study, nine samples were contaminated with total coliforms.

Opportunistic fungal pathogens, such as *Candida* spp., *Cryptococcus neoformans* and *Aspergillus* spp., usually only cause infections when there are breaks in the protective skin and mucosal barriers or when immune system defects allow their penetration, colonization and reproduction in the host[18]. *Candida* yeasts mixed with traces of saliva may be present in water and aerosols produced by dental handpieces mainly because of dysfunction of anti-retraction valves[19]. Thus, sprays contaminated with yeasts and fungi generated during routine work may be a threat to the health of patients and dental care providers[20].

Acremonium spp., *Bipolares* spp., *Cladosporium* spp., *Penicillium* spp., *Paecilomyces* spp. and *Verticillium* spp. may cause corneal infections and be a problem for contact lens wearers[20-26]. In our study the most frequent genus was *Acremonium* spp., which differed from the study conducted by Szymanska[27], in which the most frequent filamentous fungus was *Aspergillus* spp.

Aspergillus spp. and some other species of fungi, such as *Curvularia* spp., Mycelia Sterilia, *Phoma* spp. and *Scopulariopsis* spp., have a pathogenic potential to cause allergic reactions and hypersensitivity[27-29]. Infections resulting from inhaling *Aspergillus* spp. spores may cause asthma in patients who are allergic to it[20]. This study showed rates of 8.9%, 2.9%, 2.3%, 0.4% and 0.4% of *Aspergillus* spp., Mycelia Sterilia, *Curvularia* spp., *Phoma* spp. and *Scopulariopsis* spp., respectively.

Exophiala spp. and *Chrysosporium* spp. may cause skin lesions and endocarditis[30]. Porteus et al.[31] isolated *Exophiala* sp, which they claim was not often found in dental unit waterlines. *Exophiala* spp. was also found in this study, being the second most frequent genus.

Our study also found *Cladophialophora* spp. (4.7%), *Rhinocladiella* spp. (1.0%) and *Fusarium* spp. (1.0%). The two former are an additional source of concern because they may cause chromoblastomycosis, a chronic, granulomatous infection characterized by verrucous, occasionally ulcerated nodules[32,33]. The latter has been associated to infections in patients after trauma and surgeries, because some of these fungal species may cause ocular and systemic infections, sinusitis, and skin and nail infections[20,34].

According to Mungara et al[35], to maintain the sterility of dental unit waterlines it is essential to have a good water source and an effective disinfectant. In this study, the water delivered to most patients was poor of quality and was considered a potential source of cross infection.

Regular microbiological evaluation of the water used in dental units is extremely important to prevent infections in patients and dental care providers. Standardized procedures to evaluate the water used in dental units should therefore be established. Water should be monitored not only for number of total coliforms, *E.coli* and heterotrophic bacteria, but also for the presence of filamentous fungi.

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MAXIMUM BITE FORCE IN ELDERLY INDIGENOUS AND NON-INDIGENOUS DENTURE WEARERS

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ABSTRACT

The aim of this study was to compare the measures of maximum bite force (MBF) in elderly edentulous indigenous (Mapuche) and non-indigenous individuals with new complete dentures at two different measuring times. A sample of 100 elderly subjects was divided into two groups: 50 indigenous and 50 non-indigenous, each including 25 females and 25 males. All individuals were totally edentulous, with new maxillary and mandibular removable complete dentures. Measurements were taken at the time of new prosthesis placement and after 1 month of use. Subjects were asked to perform with maximum effort three bites per side at maximum intercuspidation, with a rest time of 2 minutes in between. Statistics were analyzed with Student´s t-test. The MBF values were significantly higher in indigenous than non-indigenous subjects. Force after 1 month of wearing the new prosthesis was significantly higher than at the time of new prosthesis placement. No significant difference was found between sides. Elderly indigenous complete denture wearers had the greatest MBF values. Denture wearers were observed to undergo an adaptation process to the new prosthesis, with MBF increasing considerably after one month of use.

Key words: *Bite Force; Aged; Dentures; Health Services, Indigenous.*

FUERZA MÁXIMA MASTICATORIA EN PACIENTES ADULTOS MAYORES PORTADORES DE PRÓTESIS TOTALES INDÍGENAS Y NO INDÍGENAS

RESUMEN

El objetivo de este estudio fue comparar las medidas de fuerza máxima de mordida (MBF) en pacientes desdentados adultos mayores indígenas (Mapuches) y no indígenas, en el momento de recibir sus prótesis totales y un mes posterior a la inserción. Una muestra de 100 sujetos adultos mayores fue dividida en dos grupos: 50 indígenas y 50 no indígenas, cada uno de ellos con 25 pacientes de sexo femenino y 25 masculino. Todos los individuos estudiados eran completamente edéntulos, quienes recibieron prótesis removibles totales nuevas tanto superior como inferior. Las medidas fueron realizadas en el momento de la inserción de ambas prótesis y posterior a un mes de uso. Se les solicitó a los sujetos que realizaran un esfuerzo máximo con tres mordidas por lado en máxima intercuspidación, con un tiempo de descanso de 2 minutos entre cada medición. El análisis estadístico fue realizado por medio del test t-Student´s. Los valores de fuerza máxima observados en los sujetos indígenas fueron significativamente mayores que en los individuos no indígenas. Además, los valores de fuerza posterior al mes de uso de la prótesis nueva fueron significativamente mas altos que los obtenidos al momento de la inserción de la prótesis. Por otro lado, no se identificaron diferencias significativas en los valores entre los lados izquierdo y derecho. Así, los pacientes indígenas mostraron valores mayores de fuerza masticatoria máxima. También, se pudo observar que los pacientes sufrieron un proceso de adaptación a las prótesis nuevas, en los cuales la fuerza máxima masticatoria posterior a un mes aumentó considerablemente.

Palabras clave: *Fuerza masticatoria; Anciano; Prótesis; Servicio de salud, Indígenas.*

INTRODUCTION

Considering the constant increase in elderly people in the world population, it has become important to evaluate muscle changes associated with age[1,2]. When people age, their muscles undergo functional changes, mainly through atrophy and tooth loss[1,3]. Maximum bite force (MBF) is directly related to chewing, and is determined in elderly subjects by the loss of muscle mass expressed as a reduction in the number and size of muscle fibers during the natural aging process[4-7]. In addition to influencing the chewing function, MBF also influences diet choice, which

has an important role in the maintenance of musculoskeletal function[7-9]. This is because elderly people with fewer or no molars avoid fibrous foods, crisp foods, and dry solids, showing reduced food intake ability and leaving out many sources of proteins, fibers, minerals and vitamins[10]. Even more serious is the fact that urban human diet is usually mainly based on soft foods which are rich in carbohydrates and poor in proteins and fibers[11]. In contrast, some indigenous groups eat roots and dried fruits, and food that is less cooked and rudimentarily roasted[11].

The Mapuche race is the most predominant indigenous group in Chile and one of the largest groups in the continent[12]. In Chile, 19.7% of the population belongs to the Mapuche ethnic group, though a lower percentage is limited to living in rural areas[13]. The general physiological changes that members of this group undergo in old age do not differ from those of non-indigenous people[13]. The Mapuche staple diet is based on fruits and forest species, mainly nuts, grains, fruits, and dried meats, with less cooking preparation and harder consistency[12,14], possibly affecting the force exerted by the masticatory muscles when chewing, although chewing and its components have not been studied in Mapuches.

The aim of this study was to compare MBF in elderly edentulous indigenous and non-indigenous individuals with new complete dentures at two different times.

MATERIALS AND METHODS

Subject selection

This study was approved by the Ethics Committee at Universidad de La Frontera, Temuco, Chile (Protocol Nº138/13). Data were collected from 100 subjects whose average age was 60-80 years (mean age 69 years) and who provided informed consent after an explanation of the methodology. All the patients studied were autonomous, edentulous, with no psychiatric or movement disorders, had received new maxillary and mandibular complete dentures, with stable occlusion and free from discomfort. The subjects were divided into two groups according to race: the Mapuche indigenous group and the non-indigenous group. Each group consisted of 50 individuals (25 females and 25 males). The indigenous subjects belonged to a Mapuche community, and all of them had both surnames of indigenous origin. All patients belonged to a government prosthesis program.

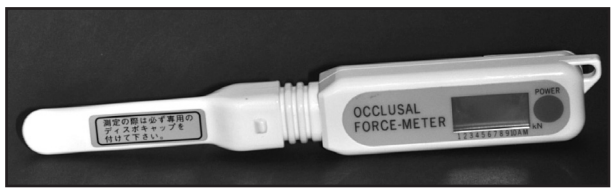

Fig. 1: Occlusal force meter GM10 used in the study.

Bite force recordings

Prior to recording maximum occlusal force, two operators were calibrated to bilaterally measure only in the first molars region using an occlusal force meter (Fig.1) (GM10, Nagano Keiki, Tokyo, Japan). The instrument consisted of a hydraulic pressure device with a disposable polyvinyl cap for biting on (17 mm in width and 5.4 mm in height). The measuring range of the instrument was 0 to 1000 N with an accuracy of ± 1 N. Measurements were made with the subject in upright position, with head in natural posture and the maxillary jaw approximately parallel to the floor, at the time of new prosthesis placement and after 1 month of use. The transducer was positioned such that all bite forces were directed to the center. The subjects were instructed to bite as forcefully as possible three times per side at maximum intercuspidation, with a rest time of 2 min in between. The maximum occlusal force recorded on screen of the device in Newtons (N) was used to analyze the results. The highest of the three measurements was considered to be the subject's MBF. Statistical analyses were carried out using SPSS software v.15.0 with Student´s t-test.

RESULTS

No statistical difference was found between MBF values on right and left sides ($p>0.01$) between races (indigenous and non-indigenous) at the time of new prostheses placement and after one month (Table 1).

Regarding gender, statistical differences were found ($p<0.05$) between the time of new prostheses placement and after one month for both races (Table 2). In addition, in relation to race, higher MBF values were found in the indigenous group than in the non-indigenous group ($p<0.01$). Regarding time of measurement, there were statistically significant differences for both genders, with lower values at the time of new prostheses placement (female= 58.42±16.1N; male=60.28±17.8N) than after one-month (female= 68.04±13.9N; male=70.34±18.8N).

Table 1: Comparison by side between the new prostheses placement time and after 1 month of use.

	NPP				1 month			
Mean	**Non-indigenous**		**Indigenous**		**Non-indigenous**		**Indigenous**	
	Right	Left	Right	Left	Right	Left	Right	Left
Female	51.6(±16.6)	54.8(±16.1)	67.4(±15.2)	63.2 (±17.0)	60.3(±15.8)	64.7(±13.1)	74.0(±14.1)	70.6(±15.0)
Male	53.3(±19.9)	57.6(±19.9)	66.6(±15.2)	67.6(±14.3)	64.4(±21.2)	60.0(±20.9)	74.3(±13.3)	79.0(±15.3)

Table 2: Gender comparison between indigenous and non-indigenous individuals at new prosthesis placement time and after one month of use.

	NPP			1 month		
t-test	**Total**	**Female**	**Male**	**Total**	**Female**	**Male**
t-value	4.52	4.2	3.18	4.85	3.92	3.36
DF	98	48	48	98	48	48
Mean indig.*	66.74(±14.5)	67.64(±15)	68.24(±14.3)	74.96(±13.7)	73.72(±13.5)	77.40(±13.8)
Mean non-indig.*	53.36(±17.1)	52.00(±14.9)	54.32(±19.2)	60.92(±16.9)	61.36(±12.8)	60.48(±20.4)
Prob. H0	0.001%	0.02%	0.28%	0.001%	0.06%	0.1%
Significance	(α<0.01)	(α<0.01)	(α<0.01)	(α<0.01)	(α<0.01)	(α<0.01)

* Values in Newton

DISCUSSION

MBF has been considered as an important variable to assess the function of the masticatory system from the action of jaw elevator muscles modified by craniofacial biomechanics[6,8]. Bite force varies in different regions of the oral cavity and is greatest in the first molar area, because almost 80% of the total bite force is distributed in that area[15,16], and it is easier and faster to measure. Multiple recordings are more reliable than a single recording[17].

MBF also plays an essential role in the choice of diet. Patients with diminished bite force values have been observed to select predominantly less nutritious food – higher in calories, lower in protein and fiber, and therefore softer – increasing the risk of malnutrition and consequently the risk of cardiovascular disease and cancer[10,18,19].

The differences in MBF we observed between males and females are in accordance with some studies[3,8,20] and may be explained by the masseter muscle in males having larger diameter fibers and greater cross-sectional areas than that of females[6].

The significant differences observed in MBF between indigenous and non-indigenous groups are not in agreement with the findings of Regalo *et al.*[6] who reported no statistically significant difference between Brazilian indigenous and white population groups, despite having noted a higher tendency of MBF in the molar region of the indigenous group.

The higher values observed in elderly indigenous individuals may be directly related to indigenous diet, which consists principally of nuts, grains, fruits and dried meats, with less cooking preparation and harder consistency[12-14]; in other words, food that requires high force to shred, and thus more bite force, exercising and toning the masticatory muscles. Conversely, current urban human diet is mostly based on soft foods, rich in carbohydrates and poor in proteins and fibers[11]. Some authors have concluded that different races may have different biting forces, attributable to different eating habits[11,21]. For instance, Corrucini et al.[22] reported higher bite force among rural youths, who had more forceful chewing habits, which is in agreement with the results observed among the indigenous (rural) subjects.

The results of this study are consistent with the values reported by Bilhan et al.[23] and Müller et al.[4], demonstrating that values lower than 100 N are generally observed in non-indigenous removable complete denture wearers.

Moreover, significant differences were observed between the measurements at the time of new prosthesis placement and after 1 month. This matches the findings of some authors[24], and may be explained by the adaptation period of the stomatognathic system to the new prosthesis[4,25]. It is important to highlight that although there was only one month between measurements, significant differences were observed.

There is a direct relationship between quality of life, tooth loss, and complete dentures[26,27]. It is also known that the ideal treatment for edentulous patients is the implant-supported overdenture, because of the huge differences reported in MBF, its advantages, and the greater satisfaction level of patients, when compared to complete dentures[1,28,29]. Unfortunately, low socioeconomic status and inability to pay for such treatment, in addition to the possible risk of implant surgery in aged patients, have resulted in the government prosthetic program offering only treatment with removable complete dentures[2,30].

Even though retention, mucoperiosteum sensibility, and alveolar ridge height, which could all influence the results, were not evaluated, this is one of the few studies on elderly removable complete denture wearers, measuring MBF at the time of new prostheses placement and after 1 month, and the only study on elderly indigenous denture wearers. The study is significant because of the difference in the staple diets in each group, which has an influence on maximum occlusal force.

CONCLUSION

Indigenous elderly complete denture wearers had the highest MBF values with the test used. In addition, denture wearers were found to undergo an adaptation process to the new prosthesis, during which MBF was found to increase considerably after 1 month of use.

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GINGIVAL RESPONSE IN ORTHODONTIC PATIENTS. COMPARATIVE STUDY BETWEEN SELF-LIGATING AND CONVENTIONAL BRACKETS

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ABSTRACT

Orthodontic brackets contribute to the accumulation of bacterial plaque on tooth surfaces because they hinder oral hygiene. In contrast to conventional brackets, self-ligating brackets do not require additional parts to support the arches, thus improving dental hygiene. The aim of this study was to compare the gingival response in orthodontic patients wearing self-ligating or conventional brackets. A sample of 22 patients aged 16 to 30 years was divided into two groups: Group A, treated with self-ligating brackets (Damon system) and Group B, treated with conventional brackets (Roth technique). The following were assessed during the treatment: Plaque Index (PI), Gingival Index (GI) and Probing Depth (PD), and sub-gingival samples were taken from teeth 14/24 for microbiological observation. No statistically significant difference was found between Groups A and B; p> 0.05 (sign-ranked) or between PI, GI and PD at the different times (Friedman's Analysis of Variance), even though the indices were found to increase at 14 days, particularly for self-ligating brackets. The quantity and quality of microorganisms present were compatible with health on days 0, 28 and 56. As from day 14 there is a predominance of microbiota compatible with gingivitis in both groups. In the samples studied, orthodontic treatment increases bacterial plaque and inflammatory gingival response, but gingival-periodontal health can be maintained with adequate basic therapy. Self-ligating and conventional brackets produced similar gingival response.

Key words: *Gingival disease; Orthodontic bracket; Biofilm*

RESPUESTA GINGIVAL EN PACIENTES ORTODÓNCICOS. ESTUDIO COMPARATIVO ENTRE BRACKETS AUTOLIGABLES Y CONVENCIONALES.

RESUMEN

Los brackets ortodóncicos contribuyen al acúmulo de placa bacteriana en las superficies dentarias, debido a que dificultan la higiene oral. A diferencia de los brackets convencionales, los brackets autoligables no requieren elementos adicionales para sujetar los arcos, lo cual favorecería la higiene dentaria. El objetivo del presente trabajo fue comparar la respuesta gingival en pacientes ortodóncicos, utilizando brackets autoligables y brackets convencionales. Se estudiaron 22 pacientes, entre 16 y 30 años, divididos en dos grupos: A, tratado con brackets autoligables, Sistema Damon) y B, tratado con brackets convencionales, Técnica de Roth. Durante el tratamiento, se evaluaron los Índices de Placa (IP), Índice Gingival (IG) y Profundidad de Sondaje (PS) y se tomaron muestras subgingivales de las piezas 14/24 para su observación microbiológica. En la evaluación estadística no se encontraron diferencias estadísticamente significativa entre los grupos A y B; p> 0.05 (de los rangos con signo) y ni en los IP, IG y PS en los diferentes tiempos evaluados (Análisis de varianza de Friedman), sin embargo se observa un aumento en los índices a los 14 días, sobre todo en autoligables. Los microorganismos se presentaron en cantidad y calidad compatible con salud el día 0, 28 y 56; a partir del día 14 predomina microbiota compatible con gingivitis para ambos grupos. En las muestras estudiadas el tratamiento ortodóncicos produce incremento de placa bacteriana y respuesta gingival inflamatoria, pero con terapia básica adecuada puede mantenerse la salud gingivo-lperiodontal. Los brackets autoligables y los o convencionales, produjeron respuesta gingival similar.

Palabras clave: *Enfermedad gingival; Brackets ortodóncicos; Biofilm*

INTRODUCTION

Orthodontic attachments are known to contribute to the accumulation of bacterial plaque and make it difficult to maintain appropriate hygiene. Mateu et al.[1] studied clinical indices and conducted microbiological evaluation, finding that patients visited the clinic with initial indices compatible with gingival-periodontal disease, but after receiving instruction on how to perform proper oral hygiene and undergoing plaque control before beginning their ortho-

dontic treatment, they achieved indices compatible with periodontal health, as is needed in order to attach the brackets.

The advent of orthodontic treatment for adults gave rise to the increasing popularity of esthetic brackets, and with them, new questions about the adhesion of microorganisms and their organization in the biofilm[2]. Adhesion of microorganisms to tooth surfaces is the result of specific reactions, electrostatic interactions and Van der Waals forces, but depends on the type of surface and its capacity to retain microorganisms[3,4]. Numerous studies have demonstrated the viability of *Candida albicans* and *S. mutans* on removable orthopedic devices, but little is known about their survival on fixed orthodontic braces[3,5].

The use of orthodontic appliances contributes to gingival-periodontal and cariogenic alterations because it modifies the oral microbiota[6,7]. Numerous clinical studies have demonstrated that patients undergoing orthodontic treatment are more susceptible to periodontal disease and white spots[8-12]. Some authors claim that this is because orthodontic braces act as a "trap" which retains microorganisms, and thus serve as an ideal niche for normal microbiota which may become established and act as an opportunist, inducing imbalance and subsequent disease[13].

There is controversy regarding whether self-ligating brackets can retain less or more bacterial plaque than conventional brackets. Comparisons of the two systems have produced varying results, possibly because different bracket designs within each system determine how much plaque is trapped and the response induced in the tissues. This study compares gingival responses induced by self-ligating Damon brackets and conventional Synthesis brackets in terms of quantity and quality of accumulated bacterial plaque and gingival-periodontal lesion indices.

MATERIALS AND METHODS

The study included 22 patients of both sexes, aged 16 to 30 years, with cast discrepancy less than 7mm, and with comparable relative crowding. They signed informed consent to participate in the clinical trial.

Inclusion criteria: Patients aged 16 to 30 years, of either sex, with permanent dentition, in whom bone-tooth discrepancy allowed alignment without the need for prior extractions, interproximal reduction or rapid expansion.

Exclusion criteria: Patients with joint disorders, periodontal disease or untreated caries on the day the brackets were attached; mixed or deciduous dentition; retained or impacted canines or missing permanent teeth.

Experimental design: Participants were divided into two groups of 11 and treated by 4 calibrated professionals. Patients were randomized for assignment to each group and each professional.

Group A was treated with low-friction, self-ligating system Damon III brackets (A Company) and .014 Copper Ni-Ti Damon arches.

Group B was treated with Roth straight arch technique system with friction, Synthesis brackets (A Company) and elastomers which were replaced every 14 days whose function was to attach the arches to the bracket slot. Ni-Ti .012, .014 and .016 arches were used.

The following routine diagnoses were performed on the patients.

Operational definition of variables

All patients were given the same information about oral care, oral and appliance hygiene, in writing, before the brackets were bonded (day 0) and by means of practical explanation at each visit. Basic periodontal therapy was provided to patients who needed it. Patients were instructed to brush their teeth using Bass's technique[15]. To supplement hygiene, they were instructed on how to use the inter-tooth brush vertically, placing it behind the arch and between the brackets.

A specialist in periodontology calibrated the professionals to take bacteriological samples and clinical indicators of gingival-periodontal status. The following indicators were taken on each tooth present in the mouth: Silness and Löe plaque index, Löe and Silness gingival index[14], probing depth and bleeding on probing, using a pressure-sensitive probe, at 0, 14, 28 and 56 days after bonding the appliances. At the same times, subgingival samples were taken from teeth 14 and 24 for microbiological analysis.

Clinical indicators were evaluated as follows

Plaque index (PI):

0. No plaque
1. Plaque when the probe is passed along the gingival margin
2. Visible plaque
3. Abundance of plaque

Gingival index (GI):

0. Complete absence of visual signs of inflammation
1. Slight change in color and texture
2. Visible inflammation and tendency to bleeding when the probe is passed lightly along the gingival margin
3. Clear inflammation with tendency to spontaneous bleeding

Probing Depth (PD)

Normal:

0 to 1 millimeter on free surfaces and up to 3 millimeters on proximal surfaces.

Microbiological analysis (MA)

The zones of teeth 14 and 24 were relatively isolated with cotton rolls. One of the active parts of Gracey 7/8 curettes was used eliminate supragingival plaque, and the other was used to take subgingival samples which were placed in Eppendorf tubes with 0.5 milliliters of saline physiological and VMGA lll transport medium. At the same time, smears were prepared from the material collected from teeth 14 and 24 (first upper premolars) for Gram and Giemsa staining. Samples of microbiota were taken from the brackets on the same teeth, because they are difficult sites to reach with a toothbrush.

The samples from the VMGA lll were processed individually after homogenizing by sonication in a water bath and spinning in microcentrifuge at 12,500 rpm. An aliquot of the sample was diluted 1/100 in recently recovered anaerobic broth, 20µl were seeded on Anaerobe Laked Blood Agar, NAM and PY broth. 20 µl aliquots of the pure sample were seeded in TSB, VK medium. The plates for anaerobes were incubated in a jar with controlled atmosphere for 7 days at 36ºC±1ºC. The samples from the physiological saline were plated on CHROMagar Candida in anaerobic conditions. Micromorphology was observed on milk 1%-Tween 80 agar, urease production, and carbohydrate assimilation profile were determined using commercial systems Api ID 32D (âBio-Mérieux, France). In addition, species which developed green color in chromogenic medium were tested for Xilose assimilation, mircromorphology on Staib's medium, growth at 45° to confirm the species.

Brackets were bonded on the same day that the initial samples were taken (day 0). Clinical records and microbiological studies were taken at baseline and repeated at 14, 28 and 56 days.

Statistical analysis

Sigmaplot v.11 software was used for the statistical analysis. Each parameter (gingival index, plaque index and probing depth) was analyzed separately. There were some times at which it was not possible to measure the indices in all the patients, thus groups of 10, 11 and 12 data were obtained. In order to make the tests symmetrical, only the first ten data from each group were used.

Wilcoxon's signed-rank test was used to compare the two groups of brackets (self-ligating vs. conventional), a non-parametric test that pairs the values of days (0, 14, 28 and 56) for each type. Friedman's two-way analysis of variance by ranks for more than two dependent samples was used to compare the four groups of days (0, 14, 28 and 56), a non-parametric test which pairs bracket values (self-ligating vs. conventional) for each group.

RESULTS

Figs 1, 2 and 3 show values of gingival parameters from both groups under study. There was no statistically significant difference for any of the cases (Friedman's analysis of variance) although indices tend to increase after 14 days, particularly in self-ligating brackets.

Although self-ligating brackets tend to have higher indices, the comparison of techniques using Wilcoxon's Signed-Rank Test shows no statistically significant difference between groups at any of the evaluation times ($P = 0.250$). The comparison between days by Friedman's Analysis shows no statistically significant difference ($P = 0.458$) although the indices tend to increase on day 14.

Microbiological analysis. The following were found in the day 0 sample: *Actinomyces, Candida albicans, Cocci* with prevalence of Gram (+). As from day 14, the predominant microorganisms were: *Prevotella intermedia, Candida dubliniensis, Porphyromona gingivalis,* spirochaetes*, Candida* spp, rods*,* with prevalence of Gram (-), in quantities compatible with gingivitis. The same microorganisms were found in the samples taken on days 28 and 56, as on day 14, although in lower quantity and quality, compatible with gingival health.

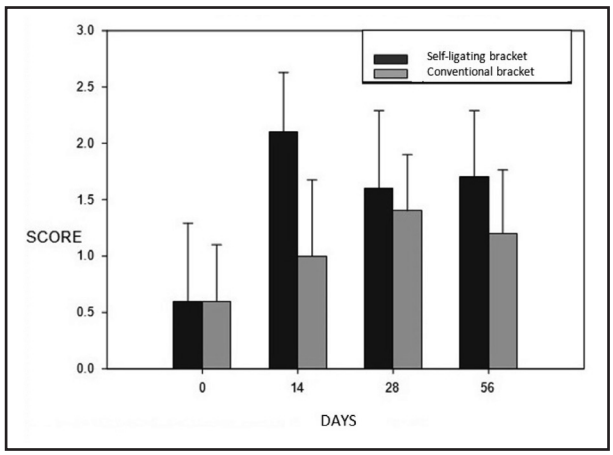

Fig. 1: Plaque index, Means and 95% confidence interval (upper half).

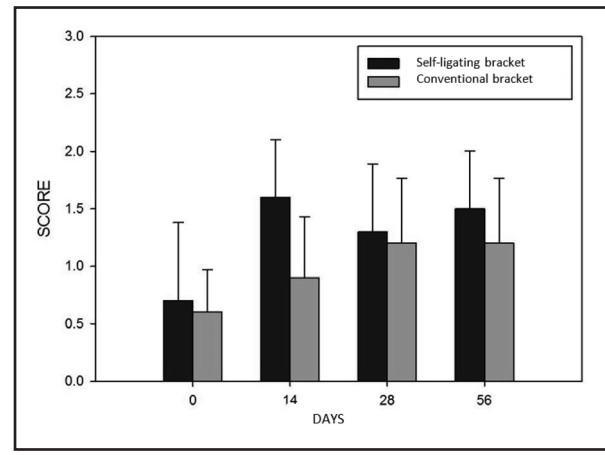

Fig. 2: Gingival index. Means and 95% confidence interval (upper half).

DISCUSSION

The results of our study agree with those in Ristic et al.[5], who report that probing depth, total number of microorganisms and number of patients with positive results for *Prevotella intermedia* and other periodontal pathogenic anaerobes increased from the time before the braces were attached, attaining maximum values after 3 months. Both the clinical and the microbiological values declined 6 months after the beginning of orthodontic treatment. Therapy with fixed appliances may temporarily increase bacterial growth, producing gingival inflammatory response, although with no destructive effect on deep periodontal tissues.

According to the literature, bracket design may influence the gingival response in orthodontic patients. A study comparing self-ligating brackets (Speed) to conventional brackets (GAC)[16] found statistically significant differences between the values obtained by counting microorganism colonies and the anaerobe/aerobe ratio shortly after cementing brackets (0, 3 and 7 days), with more colonization at sites cemented with Speed brackets. This study found more hypertrophy at sites near self-ligating brackets, although the gingival bleeding response was similar in both groups. These results agree with the tendency to increasing values found in our experience for self-ligating brackets when assessed at 14 days; although subsequent evaluations at 28 and 56 days showed that the indices approached values compatible with health for both groups. This suggests that if the dental care provider insists on the instructions for hygiene and reinforces

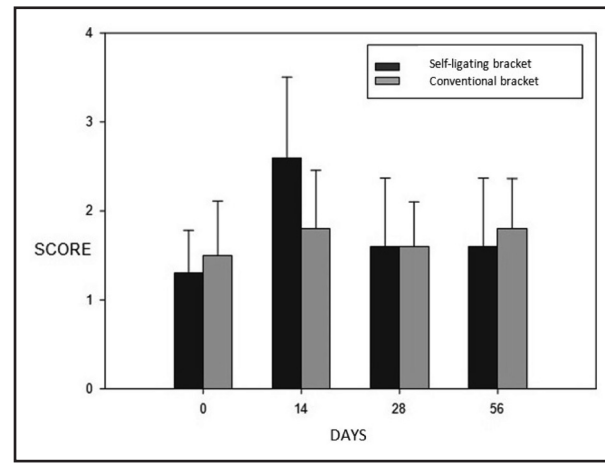

Fig. 3: Probing depth. Means and 95% confidence interval (upper half).

instruction of oral hygiene at successive visits, patient cooperation, improved hygiene, and thus, better clinical indices may be achieved.

Pellegrini et al.[17] evaluated bioluminiscence 1 and 5 weeks after placing the appliances and found that self-ligating brackets had less bacterial adhesion than conventional brackets. However, the findings of Pandis et al. (2010)[18] suggest that *S. mutans* levels in complete saliva from patients treated with orthodoncy do not differ significantly between conventional and self-ligating brackets, based on total bacteria and *Streptococcus mutans* counts in saliva samples taken at time 0 and 2 or 3 months after cementing.

Our study was performed on a relatively small sample of patients with a wide range of ages. Preliminary data reported contribute to supporting the

hypothesis that the inflammatory response to orthodontic treatments depend on multiple factors. The data reported to date are not conclusive regarding whether the choice of brackets has a direct influence on gingival response. On the contrary, professional care and patient cooperation seem to be more decisive in maintaining periodontal health.

CONCLUSIONS

Damon self-ligating brackets and Synthesis conventional brackets seem to produce similar responses regarding bacterial plaque indices and gingival clinical responses.

Wearing brackets involves a change in the quantity and quality of bacterial plaque in patients, with gingivitis as a response in some of them in the early stages of treatment. After the first control and reinforcing learning of proper tooth brushing, gingival conditions improve and are compatible with health. It is important to control bacterial plaque in orthodontic patients, to enable them to maintain gingival-periodontal health status.

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ASSOCIATION BETWEEN POST-ORTHODONTIC TREATMENT GINGIVAL MARGIN ALTERATIONS AND SYMPHYSIS DIMENSIONS

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ABSTRACT

Orthodontic therapy is known to be associated with the development of gingival recession. Several factors may be involved in the causal chain of this outcome, including anatomical and behavioral aspects. Among the anatomical aspects, the dimensions of the mandibular symphysis could play a predisposing role. This study evaluated the relationship between the mandibular symphysis dimensions prior to orthodontic therapy and the development of gingival recessions on the lower incisors and cuspids. Records from 189 orthodontically treated adolescents were evaluated, including radiographs, casts and intra-oral photographs. Symphysis dimensions were assessed by cephalograms. Gingival margin alterations were determined in photographs and cast models. Association between gingival margin alterations and symphysis dimensions was tested by chi-square (α=0.05). Occurrence of gingival recession increased after orthodontic therapy. No association was observed on average of symphysis dimensions and the occurrence of gingival recessions. It may be concluded that pretreatment symphysis dimensions may not be used as predictors of gingival recession after orthodontic therapy.

Key words: *Gingival Recession; Orthodontics; Adolescent*

ASSOCIAÇÃO ENTRE ALTERAÇÕES DA MARGEM GENGIVAL PÓS-TRATAMENTO ORTODÔNTICO E DIMENSÕES DA SÍNFISE

RESUMO

Sabe-se que o tratamento ortodôntico está associado com o desenvolvimento de recessão gengival. Vários fatores podem estar envolvidos na cadeia causal deste desfecho, incluindo aspectos anatômicos e comportamentais. Dentre os aspectos anatômicos, as dimensões da sínfise mandibular poderia ter um papel predisponente. O presente estudo avaliou a relação entre as dimensões da sínfise mandibular prévias ao tratamento ortodôntico e o desenvolvimento de recessões gengivais nos incisivos e caninos inferiores. Prontuários de 189 adolescentes tratados ortodonticamente foram avaliados, incluindo radiografias, modelos e fotografias intra-orais. Dimensões da sínfise foram determinadas em cefalogramas. Alterações da margem gengival foram observadas em fotografias e modelos. Associações entre alterações da margem gengival e dimensões da sínfise foram testadas por qui-quadrado (α=0.05). A ocorrência de recessão gengival aumentou após tratamento ortodôntico. Não foi observada associação entre as medidas médias de sínfise e a ocorrência de recessões gengivais. Pode-se concluir que as dimensões da sínfise pré-tratamento ortodôntico não podem ser usadas como preditores de recessão gengival pós-tratamento ortodôntico.

Palavras-chave: *Recessão gengival; Ortodontia; Adolescente*

INTRODUCTION

Gingival recession is a common undesirable side-effect that can occur during orthodontic treatment. Although some studies have shown the association between incisor proclination, bone dehiscences and the subsequent gingival recession,[1-6] it is a controversial subject with a number of studies that have not been able to find such association[7-10].

Årtun *et al*.,[11] and Furhrmann[12] concluded that small alveolar process and thin bone plates are anatomic risks for the development of gingival recession. Furthermore, Wennström *et al.*[8] and Engelking and Zachrisson[13] in studies with monkeys observed that as long as the tooth movement occurred exclusively within the alveolar bone, no apical shift of the gingival margin is likely to take place. Also, bone

repositioning can occur in a coronal direction once teeth that are in extreme labial position are moved to a more ideal position.

The possibility that recessions can be caused by tooth roots moving through the alveolar plate suggests the need to evaluate the thickness of the alveolar process in order to assess and reduce the risk of bone dehiscences and recessions.

Since the use of computerized tomography involves unnecessary exposure of the patient to radiation and additional expense, the lateral cephalogram became the simplest option to evaluate symphysis morphology. However, the precise measurement of the alveolar process is nearly impossible to achieve due to the superimposition of the anterior teeth and the bone structures.

Aki *et al.*[14], created a symphysis ratio based on lateral cephalograms, and found an association between the amount of mandibular anterior growth with short height and large depth of the symphysis. It may therefore be presumed that individuals with a higher anterior growth have a larger alveolar bone and may be the least susceptible to having bone fenestrations.

The purpose of this study was to answer the question: is symphysis morphology a predisposing factor to post-orthodontic treatment gingival recession in lower anterior teeth? The hypothesis underpinning the study is that recession could be facilitated by different anatomical characteristics of the symphysis.

MATERIALS AND METHODS

The sample consisted of records (pretreatment and post-treatment) of Caucasian adolescents that completed orthodontic therapy with fixed appliances at two private practices with experienced orthodontists. The Institutional Review Board of the Lutheran University of Brazil approved this study protocol. From a total of 209 records, 189 were selected (82 males and 107 females) with mean ages of 11.2 years (SD: 1.9 years) and 14.7 years (SD: 1.8 years) when the initial and end of treatment records were taken, respectively. The inclusion criteria were:

- Angle Class I or a Class II malocclusion, Angle Class III were excluded due to their tendency of incisor retroclination;
- With or without transverse and/or vertical discrepancy;
- Treated without extractions but allowed enamel stripping, since extraction requires a subsequent space closure that may result in tooth retroinclination that can generate unreliable data;
- Spacing or crowding not exceeding 4 mm. This limit was established because greater crowding may be treated with extraction or enamel stripping associated with proclination, while larger spaces require similar space closure of the cases treated with extractions;
- The lower permanent incisors were fully erupted to allow recession evaluation;
- Apparently good periodontal health, as gingivitis may produce edema that could mask existing recession;
- Final records (study models and intra-oral photographs) taken 28 days or more after removal of the appliances.

The average duration of the active treatment in the lower arch was 1.99 ± 0.89 years. All patients in the study received oral hygiene instruction and/or periodontal treatment based on their individual needs. Patients were excluded if they had a pre-existing systemic condition that could interfere in gingival or orthodontic outcomes, were taking medication associated with gingival changes, and if the pre- and/or post-treatment records could not be measured.

Main outcome

Dependent Variable

The dependent variable in this study is gingival recession on the lower incisors and canines, which were evaluated by means of visual inspection of the models and intra-oral photographs. Gingival recession was considered present when the cementoenamel junction was visible at the buccal gingival margin. The positions of the gingival margins relative to the maximum curvature of the labial surfaces of the lower canines and incisors were measured with a digital caliper (Digimatic®, Mitutoyo,UK) on pre- and post-treatment 3.54 x 5.12 inch intra-oral photographs (anterior and lateral views) and study models. The amount of the recession was measured to the nearest tenth of a millimeter.

To determine the errors of the method, gingival margin measurements were performed twice on 20 randomly selected photographs, with an interval of at least one week. Kappa statistics were used to evaluate intra-examiner agreement, and a Kappa value of 1.0 was obtained for the presence/absence of gingival recession.

Since the records used in this study included photographs, it was necessary to use a multiplication factor, as suggested by Djeu *et al.*[15], to reduce the unknown magnification present on the photographs as a measurement distortion. The enlargement correction for the photographs was achieved by comparing the crown length of the upper incisor, recorded in the same photograph, with the same tooth as recorded on the cast. The following equation was then used: actual recession is equal to photographic measurement of the recession multiplied by the actual cast crown length and divided by the photographic measured crown length.

Independent Variables

Lateral cephalograms were scanned and cephalometric landmarks were marked and digitized using Radioceph® program (Radiomemory, Belo Horizonte, Brazil) by an experienced examiner who was not informed of the objectives of the study.

In order to assess the dimensions of the symphysis, its height and width (depth) were measured as described by Aki *et al.*[14] A ratio was created by dividing height by depth:

Height was measured by a vertical line tangent to point B, from point B to a line tangent to the inferior limit of the symphysis.

The symphysis larger depth was considered to be the distance from the projection of the most anterior point to the most posterior point of the symphysis on the horizontal plane.

Other attempts to evaluate the symphysis smaller depth were made: in the lower incisor, a 15-millimeter measurement from the tooth edge towards the root apex was used to create a cephalometric landmark Sli (symphysis lower incisor). Over this point, a plane was established parallel to the mandibular plane that cuts the symphysis at SyA (Symphysis Anterior) and SyP (Symphysis Posterior) and a measurement across SyA-SyP was used to determine a depth closer to the gingival margin and to the bone crest.

The inclination of the lower incisors was obtained through IMPA (Incisor Mandibular Plane Angle - lower incisor to mandibular plane). Initial and final measurements were evaluated and divided into three groups: patients with teeth that were proclined, patients with teeth that were retroclined and patients without alteration in the labio-lingual position of the lower incisors.

Statistical Analysis

Absolute and relative frequencies of gingival margin alterations as well as measurements from the symphysis and indexes were obtained. Chi square analysis was utilized to verify the association between recession and tooth inclination. The data collected was submitted to one-way ANOVA and Student's t test, depending on the number of groups, at 5% level of significance.

RESULTS

After treatment, 91.6 % of the evaluated teeth remained without recession, 6.3 % developed recession, in 1% there was coronal migration masking a previous recession and 1.1 % of the pre-existing recessions remained the same after treatment ($p<0.001$). The chi square analysis revealed no significant association ($p=0.277$) between the alteration of tooth inclination and the presence of new gingival recessions (Table 1).

Although it was observed that at the end of treatment patients with greater symphysis heights and therefore higher ratio values were more frequently associated with recessions; these associations were not statistically significant (Table 2).

Ratio values changed upon comparing the initial and final means, as observed in Table 3. This table also shows that different ratio values and their association to the gingival margin position at the beginning and at the end of the treatment.

No statistically significant difference was observed between the mean ratio values (at the beginning and the end of the treatment) that could be related to gender (Table 4).

Table 1: Occurrence of gingival recession in relation to the experimental times (before and after orthodontic treatment) – Site/tooth level analysis.

Before	After				Total	
	Absent		Present			
	N	%	*N*	%	*N*	%
Absent	1074	91.6	74	6.3	1148	97.9
Present	12	1.0	13	1.1	25	2.1
Total	1086	92.6	87	7.4	1173	100.0

Statistically significant differences observed comparing before and after treatment (Mc Nemar test; $p<0.001$)

Table 2: Association of the gingival margin position and the values for symphysis height and depth before and after orthodontic treatment.

	Gingival Margin Position						P
	Coronal Migration		Unaltered		Recession		
	Mean	*Standard Deviation*	*Mean*	*Standard Deviation*	*Mean*	*Standard Deviation*	
Initial Height	20.12 mm	2.20 mm	19.83 mm	2.50 mm	19.67 mm	2.28 mm	0.842
Initial Depth	15.11mm	1.96 mm	14.37 mm	1.76 mm	14.53 mm	1.84 mm	0.419
Final Height	22.13 mm	2.51 mm	22.56 mm	2.74 mm	23.53 mm	2.88 mm	0.073
Final Depth	15.32 mm	1.86 mm	15.12 mm	1.93 mm	15.35 mm	2.23 mm	0.767

p= minimum level of significance from one-way ANOVA

Table 3: Association of the gingival margin position and the average ratios at the beginning and end of the treatment.

	Classification						P
	Coronal Migration		Unaltered		Recession		
	Mean	*Standard Deviation*	*Mean*	*Standard Deviation*	*Mean*	*Standard Deviation*	
Initial Index	1.34	0.17	1.38	0.22	1.34	0.23	0.496
Final Index	1.46	0.19	1.50	0.23	1.55	0.23	0.265

p= minimum level of significance from ANOVA

Table 4: Symphysis ratio prior to orthodontic therapy (Initial Index) and after treatment (Final Index), by gender.

	Sex				P
	Male		Female		
	Mean	*Standard Deviation*	*Mean*	*Standard Deviation*	
Initial Index	1.35	0.20	1.38	0.24	0.287
Final Index	1.52	0.25	1.51	0.22	0.834

p= minimum level of significance from T Test

DISCUSSION

Even though the amount, size and shape of the symphysis are expected to be important in achieving adequate proclination of the lower incisors, in this study, different symphysis dimensions were not related to higher degrees of gingival margin recession development. The balance between esthetics, health and stability has been the subject of a great number of studies[2, 3, 14].

Considering only relative numbers, 6.3% of new recessions evaluated on the post treatment lie within the expected range of new recessions described in the literature, from 3% of development or aggravation of preexisting ones[16] to 10% and improved in 5% of those previously existing[5].

Since the sample was taken from adolescent records, differential growth of the symphysis can be expected, and as a consequence, the ratios could change over the treatment, as observed on Table 2. Although there was no statistically significant difference between the symphysis ratio in the three groups (coronal migration of the gingival margin, unaltered and recession) it was possible to observe that each group had different symphysis depth/height ratios. Another consequence of this ratio was described by Aki et al.[14] as having a correlation with the direction of mandibular growth. Individuals with mandibles with an anterior growth direction were associated with a small height, large depth and a small ratio. As

a result, individuals with small ratios are expected to have not only more anterior bone and better facial esthetics when teeth are proclined, but also more space to solve dental crowding.

Conversely, when an increase in facial height occurs, it is apparently related to the occurrence of an increase in alveolar height and a decrease in thickness of the alveolar bone[17]. Årtun et al., [9] showed similar results relating alveolar process to facial proportions. Furthermore, the results suggested that the risk of gingival recession was higher in patients with thin alveolar processes.

Since this was a restrospective study conducted on growing patients who sought treatment at two private practices, there was no control group. Considering the multi-causality associated to periodontal diseases in general, from plaque control to genetics, aging, changes in the oral microbiota associated with fixed appliances, among others, it is virtually impossible to isolate all the variables and to obtain a perfect untreated control group of similar age, with all the records needed. In another study using a similar age group representative of the same city, it was observed that among 14- to 29-year-olds, the mandibular central and lateral incisors showed the highest prevalence of gingival recession with 32.8% and 24.5 % of these teeth affected, respectively[18].

It should also be considered that plaque control plays an important role, especially taking into consideration that plaque can be shifted into a subgingival position resulting in infrabony cell infiltrate, and infrabony pockets[19]. This loss of attachment might appear clinically as recessions. In this study, plaque control was checked once a month on average, but it may not reflect the quality of everyday oral hygiene.

There is also evidence in dogs and monkeys that bone fenestrations caused by tipping the teeth against the facial cortical plate may not necessarily be accompanied by loss of connective tissue attachment[7,8]. Orthodontic therapy may result in recession of the gingival margin and loss of connective tissue attachment in areas with gingivitis and in situations where teeth were moved through the alveolar process[8]. Some previous studies concluded that the degree of proclination of mandibular central incisors during fixed appliance therapy was not correlated to gingival recession[15,20] and that the gingival marginal thickness was more important than proclination in causing recession[6].

The results of this study are also in agreement with previous studies, where more recessions were found after orthodontic therapy, although without statistically significant differences[5]. It is also acknowledged that if biomechanical and periodontal conditions are controlled, the risk of periodontal damage secondary to protrusion of incisors is low[21]. We believe that in order to identify those patients with higher chance of gingival recession at least the quality of plaque control and thickness of the gingival margin should also be evaluated. It may be concluded that different symphysis dimensions alone are not related to greater susceptibility to developing gingival margin recession, according to this study. The evaluation of symphysis height and depth seems to be one of the several factors that may explain and contribute to bone dehiscence and gingival margin alteration.

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INFLUENCE OF CANTILEVER LENGTH AND TYPE OF ARCH ANTAGONIST ON BONE LOSS IN TOTAL IMPLANT-SUPPORTED PROSTHESES

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ABSTRACT

This study selected forty-two implants with full arch implant-supported fixed prostheses (with and without a cantilever) with at least five years' loading. Radiographic measurements were performed using Digimizer software (MedCalc Software, Belgium). Bone loss was measured on the distal side of the implant, from the surface of the platform to the edge of the bone crest, and the extent of the cantilever was measured from the distal surface of the last abutment to the end of the metal structure. Three groups were formed according the length of the cantilever: G1: cantilever ≤ 15 mm; G2: cantilever >15 mm; G3: no cantilever. Types of antagonists were grouped as: RP = removable complete denture; FP = fixed implant-supported prosthesis; ND = natural dentition. Data were analyzed according to the length of the cantilever and type of antagonist using Person's test to analyze normality and Student's t-test with P ≦ 0.05. No statistically significant difference was found between G1 and G2; however, increased bone loss was observed in both cantilever groups (G1 and G2) compared to G3 (P> 0.05). The antagonist showed no significant difference in bone loss (P ≦ 0.05). Cantilevers showed increases in marginal bone loss. The type of antagonist did not influence bone loss.

Key words: *Rehabilitation; Alveolar Bone Loss; Implant-supported dental prosthesis.*

INFLUÊNCIA DA EXTENSÃO DO CANTILEVER E DO ARCO ANTAGONISTA SOBRE A PERDA ÓSSEA EM PRÓTESES TOTAIS IMPLANTOSUPORTADAS

RESUMO

O estudo selecionou quarenta e dois implantes que possuíam próteses totais implantosuportadas (com e sem cantilever) instaladas a pelo menos cinco anos. As radiografias foram mensuradas através do software Digimizer (MedCalc Software, Bélgica). A mensuração da perda óssea foi realizada na distal dos implantes, a partir da superfície da plataforma do implante até a crista óssea marginal; e a extensão do cantilever foi mensurada a partir da superfície do implante distal até a extremidade da estrutura da prótese. Três grupos foram formados de acordo com comprimento do cantilever: G1 = ≤ 15 mm, G2 = > 15 mm; G3 = sem cantilever. O tipo de antagonista foi agrupado em: RP = prótese removível; FP = prótese fixa implanto-suportada; ND = dentição natural. A análise dos dados foi realizada de acordo com a extensão do cantilever e o tipo de arco antagonista. Utilizou-se o teste estatístico de Person's para verificação da normalidade e Test t de Student com valor de P (P ≦ 0.05). Ao comparar G1 e G2, não houve diferença estatisticamente significativa, no entanto, uma perda óssea aumentada foi observada em ambos os grupos com cantilever (G1 e G2) quando comparados ao G3. Extensões de cantilever irão causar um aumento na perda óssea marginal, porém o tipo de antagonista não irá influenciar esta perda óssea.

Palavras-chave: *Reabilitação; Perda óssea Alveolar; prótese total implantosuportada.*

INTRODUCTION

Although the choice of complex dental treatment may be limited by its expense and the morbidity involved in certain clinical situations such as bone deficiencies and/or the presence of anatomical structures[1], the number of treatments with fixed implant-supported prostheses has increased considerably.

One alternative is a complete fixed implant supported prosthesis (CFISP), which usually requires an extension of prosthetic structures bilaterally from the most distal implant, called a cantilever[2-4]. The obvious clinical advantages of CFISP include shorter treatment time, lower cost, and the fact that it does not require complex reconstructive surgeries[4,5].

The definition of a cantilever, according to the Glossary of Prosthodontics terms[6], is a fixed bridge with a free end that is supported and retained only on one end by one or more abutments. Initially, it was suggested that the length of the cantilever should be limited to the size of to two teeth after the last implant in the mandible, and only one tooth in the maxilla, in order to minimize the potential torque transmitted to the implants and the surrounding bone[7]. Some authors[2-7] suggestthat excessively long cantilevers increase the risk of complications. Shackleton et al. [3] evaluated two cantilever lengths (≤ 15 mm and > 15 mm) for fixed prostheses on implants, and concluded that short cantilevers had better clinical performance than long cantilevers.

Sertgöz et al. [2] evaluated the distribution of stress at the implant/bone interface and found that increased length of the cantilever resulted in higher values of stress at the interface. The incorporation of the cantilever in CFISPs has resulted in an increased magnitude of forces on the crestal bone around the implants, and this overload is proportional to the length of the cantilever[4-8].

However, in a prospective study[9], the performance of mandibular implant-supported complete dentures was evaluated clinically. The success rates for implants and prostheses were 98.9 and 95.6% respectively. The most common complication is the loosening of the prosthetic retaining screws and bone loss around the most distal implant[10], specifically on the distal surface closest to the extension where the greatest stress occurs[10-11]. Another factor that contributes to an increased rate of complications is the type of antagonist[3].

According to Naert et al.[12], several factors contribute to prosthesis-related increased bone loss in the peri-implant region, including the height of the abutment, the type of material used on the occlusal surface and the type of antagonist. An experimental animal model showed that excessive static load on implants did not result in marginal bone loss or loss of osseointegration, but that the bone adjacent to the implants loaded showed a higher density compared with unloaded implants[13].

Naert et al.[14] reported data of 91 jaws that were treated with complete fixed prostheses supported by Brånemark implants (n = 589). The authors concluded that for three years, the length of the cantilever had a significant impact on the amount of marginal bone loss around implants.

The aim of this study is to evaluate marginal bone loss over the most distal implants in full arch fixed implant supported dentures with cantilevers longer than 15 mm, shorter than or equal to 15 mm and without cantilevers, and the influence of the type of antagonist on peri-implant bone loss. The null-hypothesis is that the opposing arch and cantilever length do not influence bone loss around the distal implants of full arch implant supported fixed dentures.

Table 1: The Patients divided according to gender, age and implant location.

Gender	Age (mean)	Implant Location	Total
Female	50	Maxilla	12
Female	47	Mandible	10
Male	55	Maxilla	10
Male	66	Mandible	10

METHODS

The patients were selected from the Center for Teaching and Research in Dental Implants (CEPID), Department of Dentistry, Federal University of Santa Catarina - UFSC (Florianopolis, Santa Catarina, Brazil), who were treated with dental implants between 2002 and 2012 (a 10-year period). Forty-two implants were analyzed from a database, including 22 female patients (12 maxilla and 10 mandibles) and 20 male patients (10 maxilla and 10 mandibles), aged 43-87 years (Table 1), with full arch fixed implant-supported prostheses (with and without cantilever) installed at least 5 years prior to the study evaluation.

Inclusion criteria

All edentulous patients included had worn their prosthesis for at least five years and were referred for evaluation or re-evaluation of their implant-supported fixed prostheses. They signed an informed consent as provided by the Ethics Committee of Research in Humans of the Federal University of Santa Catarina – UFSC (protocol number: 128/2006). All patients demonstrated adequate oral hygiene and absence of any local inflammation. In addition, neither residual roots nor mucosal diseases were present and there was adequate bone height for the placement of dental implants.

Exclusion criteria

Excluded from this study were patients that used tobacco, alcohol or other drugs, those who had severe bruxism or clenching habits, poor general health, were pregnant, had a history of radiation to the head and neck, previous grafting at the surgical sites, lack of motivation or physical handicaps that would prevent proper oral hygiene.

Implant placement and prosthodontic treatment

Patients received prophylactic antibiotic regime before surgery (Amoxicillin 500mg) and oral rinse with 0.12% chlorhexidine gluconate for 1 minute for local disinfection. The peri-oral skin was washed with a skin disinfectant. Rough surface one-piece implants were placed under local anesthesia and aseptic conditions with a surgical handpiece with a maximum drilling speed of 1200 rpm and plentiful cooling with sterile saline. External hex implants with regular platform of two commercial brands (Conexão Sistemas de Prótese, São Paulo, SP, Brazil and Neodent, Curitiba, PR, Brazil) were placed. Healing caps were placed and flaps were sutured. After implant placement, the old dentures were relieved completely from direct implant contact and adjusted with soft reline material (GC America Inc. - Leuven, Belgium). All patients were instructed on post-operative home care. Three to six months after implant placement, the patients returned to receive the final implant-supported denture. All prostheses were screw-retained.

Radiographic Examinations

A General Electric model 1000x-ray machine (General Electric Co., Milwaukee, WI, USA) operating at 65kVp and 10 mA, with an aluminum filter 1.5 mm thick was used to take standardized periapical radiographs (Kodak Insight film, Carestream, INC., New York, EUA) of each distal implant using the paralleling technique with an occlusal bite index prepared with a repositioning jig. The bite index was saved to be used at all visits.

The radiographs were digitized using a slide scanner (SprintScan 35,CS-2700, Polaroid Scanner, Cambridge, MA, USA), 600 d.p.i. resolution, and 256 grey levels. The images were coded so that they could be blinded and stored in JPEG File Format without compression.

The radiographs were then measured using Digimizer software (MedCalc Software, Belgium). Bone loss was measured on the most distal portion of the implant, from the surface of the platform to the edge of the crystal bone, and the length of the cantilever was measured from the distal surface of the last abutment to the end of the metal structure (Fig. 1)[3].The patients were then divided into three groups according to the length of the cantilever and type of antagonist.

Data were analyzed using Stata 9 (*Stata* Corp., College Station, TX, EUA)with Person's to analyze normality and Student's t-test. The significance level was set at $P \leqq 0.05$.

RESULTS

A total 42 distal implants were measured out of 110 implants placed to support complete fixed dentures. These implants were then divided into 3 groups according to the cantilever length: 22 with cantilever ≤ 15 mm, 10 with cantilever > 15 mm and 10 without a cantilever (total 42) (Table 2). The length of the distal cantilever extension (i.e.> 15 mm and ≤ 15mm) was based on previous research that demonstrated the influence of cantilever length on the survival rate of complete fixed prostheses[3].

According to the type of antagonist, there were 8 patients with traditional complete dentures, 17 with fixed prostheses (implant-supported prosthesis) and 17 with natural teeth (Table 3).

No statistically significant difference was found between groups 1 and 2, but groups 1 and 2 differed significantly from group 3 ($p \leq 0.05$) (Figs 2 and 3). There was no statistical significance between types of antagonist (Fig. 4).

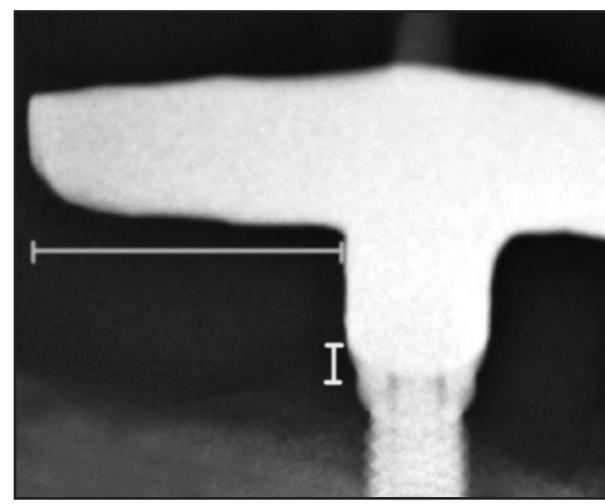

Fig. 1: X-ray showing the measurements of bone loss related to cantilever length.

Table 2: The groups divided according to length of their cantilevers.

Group	Total
Cantilever ≤ 15 mm	22
Cantilever > 15 mm	10
Absence of cantilever	10

Table 3: The groups divided according to type of antagonist.

Antagonist	Total
Removable complete denture	8
Fixed Prosthesis	17
Natural Teeth	17

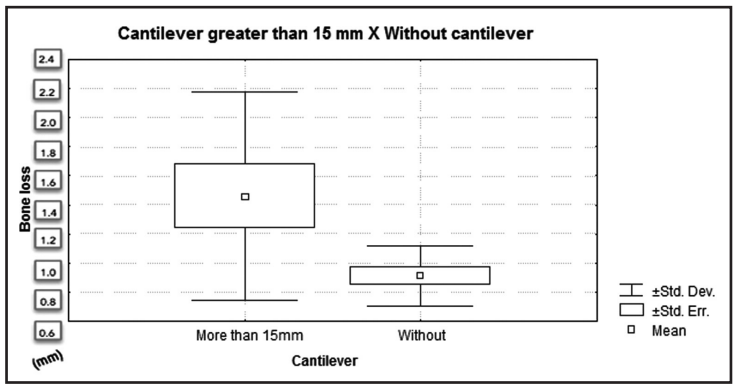

Fig. 2: Bone loss with cantilever longer than 15mm or without cantilever.

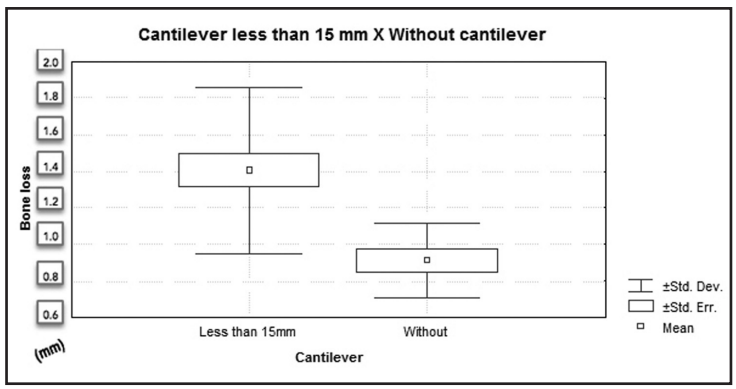

Fig. 3: Bone loss with cantilever shorter than 15mm or without cantilever.

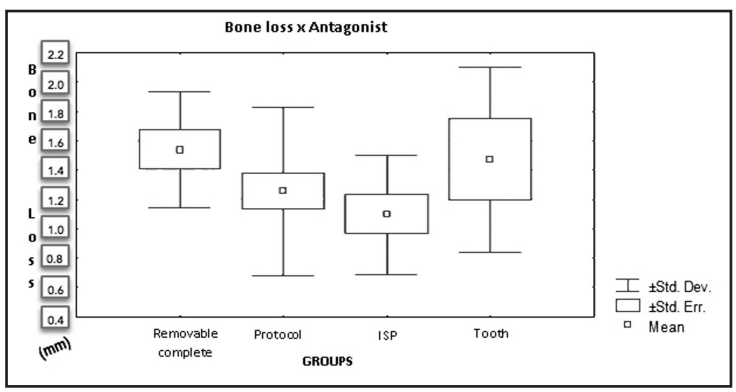

Fig. 4: Comparison of bone loss related to the type of antagonist.

DISCUSSION

The implant supported fixed complete denture with a cantilever extension is a simple restoration often used for the rehabilitation of edentulous patients[4,11]. In this study we used a comparative model following Shackleton et al. [3], which compares cantilever lengths shorter than or equal to 15 mm and longer than 15 mm[3,15]. Other authors advocate the use of 6 implants with cantilever lengths ≤ 10 mm in the maxilla. However, for the mandible, five implants are recommended according to the Branemark protocols[12].

To allow an even distribution of functional forces to the bone without overloading the implant/bone interface, the length of the cantilever should not exceed 15 mm in the mandible[3] and 10 mm in the maxilla[11]. Cantilever length must be less than 10 mm in the maxilla due to the poor bone quality in this region. The inclusion of cantilevers in fixed prosthodontics is considered an important risk factor. In the present study, when comparing bone loss in relation to the length of the cantilever (G1: cantilever ≤ 15mm and G2: cantilever > 15mm), there was no statistical difference between groups.

The literature reports the benefit of more posterior support, which minimizes mechanical stress on the prosthesis in an 'all-on-four' situation[16-18]. In this study there was a statistically significant difference between the groups with and without cantilevers, with greater bone loss around implants with cantilevers.

Bone loss was reported in two others studies in which radiographic bone level

changes around implant supporting prostheses with cantilever extensions were compared to implant-supported FPDs without cantilever extensions (IFDPs). The radiographic bone level showed slightly greater bone loss around implants close to the cantilever extensions. However, no statistically significant difference was found, with a summary estimate of difference in bone loss per year of 0.033 (95% CI: 0.02–0.087; p>0.05)[19,20].

Romeo et al.[21] reported that after an average of three years, the amount of bone loss in the most distal implant, adjacent to the cantilever, was totally correlated with the cantilever extension. In a clinical follow-up study conducted by Ekelund et al.[9] with over 15 years of observations, patients rehabilitated with implant supported fixed dentures had a success rate exceeding 90% in relation to bone loss. This study revealed a similar success rate of 93%, suggesting that removable prosthesis, fixed prosthesis and natural teeth as cantilever antagonists did not influence bone loss.

Romeo et al.[21] also reported no significant difference in peri-implant bone resorption among different types of antagonists. On the contrary, the frequency of prosthetic complication was significantly higher for the prostheses with cantilever extensions opposite implant-supported restorations (58.3% VS. 38.3%). More evidence is needed to confirm these results.

Pjetursson et al. (2004)[22] in a systematic review, found a survival rate of 95% (95% CI 92.2 to 96.8) after 5 years on implant-supported prostheses with cantilevers. Other authors[21-25] found that peri-implant bone loss is more pronounced in the maxilla than the mandible.

The most frequent technical complications in implant supported prostheses with cantilevers included veneer fractures, followed by screw loosening and loss of retention[22]. No detrimental effects on bone levels were observed around implants close to the cantilever extensions. However, there is as yet little evidence of the effects of various prosthetic designs (e.g. distal or mesial cantilever extension), number of implants and occlusal concepts on the incidence of complications in a complete implant supported denture.

The null hypothesis was partially confirmed. According to the methodology used and the limitations of this study, it was concluded that implant supported fixed dentures with a cantilever extension show greater bone loss than those without cantilevers, but the lengths of the cantilevers (shorter than 15 mm and longer than 15 mm) revealed no significant difference. The opposing antagonist arch had no direct influence on bone loss.

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OCCURRENCE OF *PORPHYROMONAS GINGIVALIS* AND ITS ANTIBACTERIAL SUSCEPTIBILITY TO METRONIDAZOLE AND TETRACYCLINE IN PATIENTS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Chronic periodontitis is a multifactorial infectious disease associated with Gram-negative strict anaerobes which are immersed in the subgingival biofilm. Porphyromonas gingivalis, an important periodontal pathogen, is frequently detected in patients with chronic periodontitis. Although isolates of P. gingivalis tend to be susceptible to most antimicrobial agents, relatively little information is available on its in vitro antimicrobial susceptibility. The aim of this study was to determine the frequency of P. gingivalis in patients with chronic periodontitis and to assess antimicrobial susceptibility in terms of minimum inhibitory concentration (MIC) of clinical isolates to metronidazole and tetracycline. A descriptive, observational study was performed including 87 patients with chronic periodontitis. Samples were taken from the periodontal pocket using paper points, which were placed in thioglycollate broth. Samples were incubated for 4 hours at 37°C in anaerobic conditions and finally replated on Wilkins-Chalgren anaerobic agar (Oxoid). Bacteria were identified using the RapID™ANAII system (Remel) and antimicrobial susceptibility was determined with the M.I.C. Evaluator test (MICE, Oxoid). P. gingivalis was identified in 30 of the 87 patients with chronic periodontitis, which represents a frequency of 34.5%. All 30 isolates (100%) were sensitive to metronidazole, with MIC values ranging from 0015-4ug/ml. Regarding tetracycline, 27 isolates (90%) were sensitive, with MIC values ranging from <0.015 to 4 ug /ml, the remaining three isolates (10%) were resistant to tetracycline with MIC values of 8ug/ ml. There was no statistically significant difference in age, gender, pocket depth, clinical attachment level and severity of periodontitis between the group of patients with chronic periodontitis and P. gingivalis and the group of patients with chronic periodontitis without P. gingivalis. In conclusion, P. gingivalis was found at a frequency of 34.5% in patients with chronic periodontitis and clinical isolates were highly sensitive to metronidazole and tetracycline.

Key words: *Porphyromonas gingivalilis; Microbial Sensitivity Test; Chronic Periodontitis*

PRESENCIA Y SUSCEPTIBILIDAD ANTIBACTERIANA DE *PORPHYROMONAS GINGIVALIS* A METRONIDAZOL Y TETRACICLINA EN PACIENTES CON PERIODONTITIS CRÓNICA

RESUMEN

La periodontitis crónica es una enfermedad infecciosa multifactorial asociada a bacilos Gram-negativos anaeróbicos estrictos que se encuentran inmersos en la biopelícula subgingival. Porphyromonas gingivalis, importante patógeno periodontal, es frecuentemente detectado en pacientes con periodontitis crónica. Los aislamientos clínicos de P. gingivalis tienden a ser susceptibles a la mayoría de agentes antimicrobianos; sin embargo, se tiene poca información sobre la susceptibilidad antimicrobiana in vitro. El objetivo de este estudio fue determinar la frecuencia de P. gingivalis en pacientes con periodontitis crónica y determinar la susceptibilidad antimicrobiana en términos de concentración inhibitoria mínima (CIM) de los aislamientos clínicos a metronidazol y tetraciclina. Se realizó un estudio observacional descriptivo en el que se incluyeron 87 pacientes con periodontitis crónica. Las muestras tomadas con conos de papel de la bolsa periodontal se depositaron en caldo tioglicolato, se incubaron durante 4 horas a 37 °C en anaerobiosis y se resembraron en agar anaeróbico Wilkins-Chalgren (Oxoid). La identitficación de los aislamientos se realizó con el sistema RapID™ ANA II (Remel) y la susceptibilidad antibiótica para metronidazol y tetraciclina se evaluó mediante la técnica M.I.C.Evaluator (M.I.C.E., Oxoid). En 30 de los 87 pacientes con periodontitis crónica se identificó P. gingivalis, lo que representa una frecuencia de 34.5%. Todos los 30 aislamientos (100%) fueron sensibles al metronidazol con valores de CIM desde 0.015 hasta 4 ug/ml. En cuanto a tetraciclina, 27 aislamientos (90%) fueron sensibles con valores de CIM desde <0.015 hasta 4 ug/ml; los restantes 3 aislamientos (10%) fueron resistentes a tetraciclina con valores de CIM de 8 ug/ml. En cuanto a edad, género, profundidad de bolsa, nivel de inserción clínico y severidad de la periodontitis no se presentaron diferencias estadísticamente significativas entre el grupo de pacientes con periodontitis crónica y P. gingivalis y el grupo de pacientes con periodontitis crónica sin P. gingivalis. En conclusión, P. gingivalis se encontró en una frecuencia de 34.5% en pacientes con periodontitis crónica y los aislamientos clínicos fueron altamente sensibles a metronidazol y tetraciclina.

Palabras clave: *Porphyromonas gingivalis; test de sensibilidad microbiana; periodontitis crónica*

INTRODUCTION

Periodontal disease is an infectious oral disease which affects many people in the world[1-5]. Measured by clinical attachment loss, it affects 50.2% of the population in Colombia[6].

Periodontitis is defined as an inflammation compromising the whole tooth supporting apparatus and classified as chronic, aggressive and associated to systemic diseases[1,2]. Chronic periodontitis is the most frequent form of periodontal disease, and because of its insidious, asymptomatic behavior, is nearly always diagnosed at an advanced age and even in the terminal stages of the disease[1,2,7,8]. It leads to progressive attachment loss and bone loss and is characterized by the formation of pockets which can affect a variable number of teeth in different stages of progression[1,2,7]. Factors inherent to the host, smoking and environmental factors are important and determinant in its evolution and severity[1,2,7,8].

Chronic periodontitis is a multifactorial infectious disease and several microorganisms are involved in its etiology, among which *Porphyromonas gingivalis* is of vital importance due to its virulence factors and the role it plays in the development of periodontal pathology[1,2, 7-10].

P. gingivalis is a Gram-negative, obligate anaerobe rod, which produces black-brown colonies on anaerobic blood agar, and in the oral cavity is found mainly immersed in the subgingival microflora[1,2,7]. It meets the criteria to be considered a pathogen: it stimulates the host's immune response, evades defense mechanisms and destroys host tissues by secreting its own substances [1,10,11].

Different studies have shown that the frequency and distribution of periodontal microorganisms in the subgingival microflora, in particular *P. gingivalis,* is variable according to factors such as geographic region, race, diet, development level and living conditions, among others [1-3,9-13].

When antimicrobial therapy is needed in patients with chronic periodontitis for the eradication of *P. gingivalis,* its susceptibility or resistance profile to antibiotics needs to be known [11,14,15]. Various different susceptibility patterns have been found for *P. gingivalis*. [16-21].

In vitro antimicrobial susceptibility tests can be used to determine microorganism profiles and changes in behavior in response to different periodontal therapies, with the aim of contributing to developing adequate antibiotic management policies and delaying the appearance of antimicrobial resistance[11,20-22].

The aim of this study was to determine the frequency of *P. gingivalis* in patients with chronic periodontitis and to determine its antimicrobial susceptibility to metronidazole and tetracycline.

MATERIALS AND METHODS

Study characteristics

This was an observational, descriptive study of 87 patients diagnosed with untreated chronic periodontitis (localized chronic periodontitis and generalized chronic periodontitis) who visited the pre-graduate and post-graduate Periodontal Clinics at the School of Dentistry of Pontificia Universidad Javeriana, from May 2011 to July 2012.

Clinical study

A previously calibrated researcher performed the clinical periodontal evaluation (full mouth) on all patients using a Williams probe (Williams color-coded probe PQW, Hu-Friedy, Chicago-Illinois, USA), including gingival margin, bleeding on probing, pocket depth and clinical attachment level, and patients were classified following the recommendations of the 1999 International Consensus of the American Academy of Periodontology[23]. Periodontal probing was performed and 6 surfaces of all teeth were measured to select the one which would be included in the sample (mesial-buccal, mesial-lingual/palatal and buccal/palatal interproximal surfaces).

Inclusion criteria were: patients diagnosed with chronic periodontitis, with at least 10 teeth, without systemic compromise, over 18 years old, who had not received previous periodontal therapy (for at least 6 months). Exclusion criteria were: patients who had taken antibiotics, corticoids or non-steroid analgesics within three months prior to the sampling, pregnant or lactating women, and smokers. The study was approved by the Ethics and Research Committee at the School of Dentistry of Pontificia Universidad Javeriana. All patients signed informed consent which described the nature of the project and associated benefits. A survey was conducted to determine each patient's systemic condition, which also provided information on whether the inclusion and exclusion criteria were met.

Microbiological study

Samples were taken by selecting 5 sites with pocket depth ≥4mm and clinical attachment level ≥ 2 mm. The supragingival biofilm was removed with sterile gauze, the zone was isolated with sterile cotton, and paper points (New Stetic®) were placed in the periodontal pocket for 1 minute. The paper points were removed and placed in Eppendorf tubes with 900 ul thioglycollate broth (BBL™ Fluid, Becton Dickinson and Company) supplemented with hemin and menadione[24-27], and placed in jars with anaerobiosis generating envelopes (Anaerogen, Oxoid) until they arrived at the laboratory.

Isolation and identification of P. gingivalis

In the laboratory, the samples in the anaerobiosis jars were incubated for 4 hours at 37°C in order to enrich and thus multiply the anaerobes [26,27]. After incubation, they were centrifuged (Eppendorf® centrifuge) at 4000 rpm for 10 minutes. Of the centrifuged product, 300µl were removed and the remaining 600µl were vortexed (Maxi mix II Thermolyne®) to produce a homogeneous mixture of the sample. Then the rest of the thioglycollate broth was used to make a series of five dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) in thioglycollate broth, to isolate *P. gingivalis.* Fifty ul of the three latter dilutions only (10^{-3}, 10^{-4}, 10^{-5}) were re-plated en masse on Wilkins-Chalgren (Oxoid) anaerobic agar supplemented with 1% (v/v) hemin and menadione and 5% (v/v) lamb`s blood, and incubated at 37°C for 8 days in anaerobic atmosphere. After the incubation period, types of colony present in the culture medium were observed (black-brown pigmented and non-pigmented), Gram stained and exposed to long-wave ultraviolet light to show presence or absence of fluorescence. Absence of fluorescence is considered to be a quick test to distinguish between *P. gingivalis* and other Gram-negative anaerobe rods with black-brown pigmentation[28-30]. Colonies presumed to be *P. gingivalis* were plated again for an air tolerance test in Wilkins-Chalgren agar (Oxoid) supplemented with 1% (v/v) hemin and menadione, and 5% (v/v) lamb's blood, and incubated at 37°C for at least 8 days. Finally, the obligate anaerobic colonies were selected and their purity was confirmed by Gram stain. Pure isolates of obligate anaerobes were identified with the RapID™ ANA II system (Remel). The respective quality controls were included in order to ensure that tests were performed correctly.

Antibacterial susceptibility test

After isolation and identification of *P. gingivalis,* its antibiotic susceptibility (minimum inhibitory concentration - MIC) to metronidazole and tetracycline was evaluated using the M.I.C-Evaluator technique (M.I.C.E., Oxoid). All 30 strains of *P. gingivalis* were isolated again on anaerobic Wilkins Chalgren agar (Oxoid) supplemented with 1% (v/v) hemin and menadione and 5% (v/v) lamb's blood. All isolates were incubated at 37°C for 5 days in anaerobic atmosphere in order to produce fresh colonies for subsequent susceptibility tests, following CLSI M100-S22 standards and the recommendations for evaluating susceptibility with M.I.C-Evaluator. After 5 days' incubation, the purity of the isolates was examined again, and suspensions of each of the 30 isolates were prepared in isotonic sterile saline solution and adjusted to 1 on the Mc Farland scale. A brush was used to plate all suspensions en masse on Wilkins-Chalgren anaerobic agar (Oxoid) supplemented with 1% (v/v) hemin and y menadione, and 5% (v/v) lamb's blood, and incubated at 37°C for 5 days in anaerobic atmosphere. Finally, the MIC was read, following the manufacturer's instructions and taking into account the cut-off points for the antibiotics evaluated: values ≤ 8ug/ml for metronidazole and ≤ 4ug/ml for tetracycline were considered sensitive.

Statistical analysis

Descriptive univariate and bivariate statistical analysis was performed (distribution of frequencies of categorical variables, mean and standard deviation of continuous variables). A U-Mann Whitney test was used to determine whether there are differences between presence and absence of *P. gingivalis* according to pocket depth, level of attachment, severity of periodontitis and age. The Chi square test was used to determine differences between presence and absence of *P. gingivalis* according to the variables sex and bleeding on probing. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Table 1 shows the demographic and clinical characteristics of patients with chronic periodontitis with or without presence of *P. gingivalis*. *P. gingivalis* was identified in 30 of the 87 patients with chronic periodontitis, representing a frequency of 34.5%.

Table 1: Principal demographic and clinical findings in the 87 patients with chronic periodontitis included in the study, according to presence or absence of *P. gingivalis*.

Caracteristics		*P. gingivalis* Present	*P. gingivalis* Absent	Statistics
Number		30 (34.5%)	57 (65.5%)	
Age (years)		45.63 ± 12	46.73 ± 12	P > 0.834*
Sex	Female	15	33	P = 0.482**
	Male	15	24	
Sites with Bleeding on Probing (%) [a]		97.3 ± 14	95.9 ± 12	P > 0.672**
Pocket depth (mm) [a]		5.62 ± 1.4	5.77 ± 1.6	P > 0.6514*
Attachment level (mm) [a]		5.77 ± 3.1	5.56 ± 1.33	P > 0.8687*
Severity of periodontitis (mm) [a]		5.56 ± 2.6	5.43 ± 1.18	P > 0.7511*

[a] Values correspond to mean ± standard deviation
* U-Mann Whitney test, **Chi squared test

Of the 87 study patients diagnosed with chronic periodontitis, 48 (55.2%) were female and 39 (44.8%) were male; and regarding age, 10 (11.5%) were 18-30 years old, 42 (48.3%) were 31-50 years old and 35 (40.2%) were 51-70 years old.

Age (mean ± standard deviation) of patients with and without *P. gingivalis* respectively, was 45.63±12 and 46.73±12 years, with no statistically significant difference (P > 0.834, Table 1). The 30 *P. gingivalis* isolates were distributed as follows: 15 isolates (50%) from the 18-30 year range, 11 (36.7%) from the 31-50 year range, and 4 (13.3%) from the 51-70 year range. There was no statistically significant difference regarding sex for patients with or without presence of *P. gingivalis* (P>0.05, Table 1).

With regard to clinical parameters, the values (mean ± standard deviation) for pocket depth in patients with and without *P. gingivalis* were, respectively, 5.62± 1.4 mm and 5.77±1.6 mm, with no statistically significant difference (P > 0.6514, Table 1). Among the 30 patients with chronic periodontitis and presence of *P. gingivalis*, pocket depth was 4-5 mm in 13 patients (43.3%), 5-7 mm in 12 (40%) and greater than 7 mm in 5 (16.7%). Loss of attachment level (mean ± standard deviation) was 5.77 ± 3.1 in patients with *P. gingivalis,* and 5.56±2.6mm in patients without *P. gingivalis*. The differences were not statistically significant (P > 0.8687, Table 1). Severity of the periodontitis did not differ significantly (P > 0.7511) between patients with *P. gingivalis* (5.56 ± 1.33) and without *P. gingivalis* (5.43 ± 1.18) (Table 1).

Percentages of sites with bleeding on probing in patients with chronic periodontitis with and without *P. gingivalis*, respectively, were (mean± standard deviation) 97.3±14 and 95.9±12, with P > 0.672 by Chi squared test, showing that the differences are not statistically significant (Table 1).

With relation to the extension of periodontal destruction, there was localized chronic periodontitis in 71.3% of patients. Of the 87 patients (48 female and 39 male) with chronic periodontitis, 40 (46%) had moderate chronic periodontitis and 47 (54%) had severe chronic periodontitis. Of the 48 females in the study, 26 had moderate chronic periodontitis and 22 had severe chronic periodontitis. Of the 39 males, 14 had moderate chronic periodontitis and 25 had severe chronic periodontitis. Of the 30 patients with chronic periodontitis in whom *P. gingivalis* was found, 14 had moderate chronic periodontitis and 16 had severe chronic periodontitis; and of the 57 patients with chronic periodontitis in whom *P. gingivalis* was not found, 26 had moderate chronic periodontitis and 31 had severe chronic periodontitis. The U-Mann Whitney statistical analysis showed no statistically significant difference between these two groups (P >0.5063).

Table 2 shows the MIC results for the 30 *P. gingivalis* clinical isolates to metronidazole and tetracycline. The strains showed widely differing susceptibility two the two antimicrobial agents. All 30 isolates (100%) were sensitive to metronidazole with MIC values ranging from 0.015 to 4 ug/ml; the highest frequency to sensitivity (n=3)

was at MIC values of 0.015, 0.03 and 0.06 ug/ml. For tetracycline, 27 isolates (90%) were sensitive, with MIC values ranging from <0.015 to 4 ug/ml; the highest frequency to sensitivity (n=3) was at MIC values of 0.015, 0.03 and 0.06 ug/ml. The remaining 3 isolates (10%) were resistant to tetracycline, with MIC values of 8 ug/ml.

Table 2: Values for antimicrobial susceptibility to metronidazole and tetracycline at minimum inhibitory concentration for the 30 *P. gingivalis* isolates, found using the M.I.C.Evaluator system (MICE, Oxoid).

Isolates	Metronidazole ug/ml	Tetracycline ug/ml
P. gingivalis (n=3)	0.015	0.015
P. gingivalis (n=2)	0.015	4
P. gingivalis (n=3)	0.03	0.06
P. gingivalis (n=2)	0.03	< 0.015
P. gingivalis (n=1)	0.03	0.12
P. gingivalis (n=2)	0.03	2
P. gingivalis (n=3)	0.06	0.03
P. gingivalis (n=1)	0.06	0.06
P. gingivalis (n=1)	0.06	0.12
P. gingivalis (n=1)	0.12	4
P. gingivalis (n=2)	0.15	2
P. gingivalis (n=2)	0.25	0.06
P. gingivalis (n=2)	0.3	< 0.015
P. gingivalis (n=2)	0.15	8
P. gingivalis (n=1)	2	0.25
P. gingivalis (n=1)	4	0.12
P. gingivalis (n=1)	0.12	8

Values ≤ 8ug/ml for metronidazole and ≤ 4ug/ml for tetracycline are considered sensitive.

DISCUSSION

Periodontitis is considered to be a mixed infectious bacterial disease caused mainly by Gram negative anaerobes[9] which interact with host tissues and cells causing the release of a wide range of cytokines, chemokines and inflammatory mediators, leading to the destruction of periodontal structures[31].

P. gingivalis is a rod-shaped, Gram negative, immobile, asaccharolytic, strict anaerobe[9]. Because of its ability to produce a large quantity of virulence factors, it is considered to be a major pathogen and very important microbiological indicator in the onset and development of periodontal disease[31].

This study reports the frequency and antimicrobial susceptibility of *P. gingivalis* isolated from patients with chronic periodontitis. *P. gingivalis* was found with a frequency of 34.5% (30/87) and all 30 isolates were highly sensitive to metronidazole (100%-30/30) and tetracycline (90%-27/30). This frequency was lower than those reported since 2007 in patients with chronic periodontitis in Iran (41.7%)[20], Spain (77.8%)[2], Japan (78.5%)[32] and Chile (83.8%)[2]. Previous studies in Colombia report frequencies of *P. gingivales* in patients with chronic periodontitis of 60.7%, 65.9%, 67.1%, 68.2 and 76.47% [1-4,33]. It would seem that these variations in frequency are a result of differences in sample taking, transportation and processing, isolation by bacteriological culture and the use of molecular techniques, and particular socio-cultural, demographic and living conditions [1,3-5,17,20,32,33]. The study by Sanai et al.[17] clearly shows that the transportation of samples could have caused loss of viability in *P. gingivalis, P. intermedia* and *P. nigrescens.* The differences in sensitivity obtained by culture may also be due to situations generating changes in the subgingival microflora, including deficient hygiene habits and attitudes, chronic baseline diseases, smoking, alcohol use and previous antimicrobial therapies [2-4,11,23].

Thioglycollate broth is an enrichment medium designed to facilitate rapid growth of a wide variety of fastidious, aerobic, microaerophilic microorganisms, and in particular obligate anaerobes[24-27]. This study used thioglycollate enriched with hemin and menadione, containing a base of casein, L-cystine, dextrose, yeast extract, sodium chloride, sodium thioglycollate, resazurin and a low proportion of agar to provide a soft consistency[24-27]. L-cystine and sodium thioglycollate are reducing agents that maintain a low oxidation-reduction potential which allows survival and adequate metabolism of obligate anaerobes. Hemin and menadione stimulate the multiplication of bacteria that produce black-brown pigment, among which *P. gingivalis* is included[26,27].

According to scientific and technical principles (DNA amplification), molecular techniques (in particular PCR –polymerase chain reaction) are assumed to be more sensitive and specific than the culture method [34,35]. Studies conducted using cultures on patients with chronic periodontitis have found some sensitivities which are low, while others are very close to or even higher than (41.7, 60.7,

67.1, 65.9, 76.47, 77.8 and 83.8%)[1,2,4,20,33] those reported in studies using the PCR technique (68.2 and 78.5%)[3,32]. It should be noted that these sensitivities were found in different populations and social-demographic situations, in addition to which they were conducted on different sample sizes. There is currently only one paper which attempts to resolve the inconsistencies in sensitivity between the two methods[35]. The study by Urban et al.[35] detects periodontal pathogenic bacteria using the traditional anaerobic culture method and commercial PCR. The PCR test detected almost the same number of positive samples for *P. gingivalis* as the culture method[35], with 94% concordance and only two discrepant results. From these results it can be deduced that commercial PCR can be recommended for use in an oral microbiological diagnosis laboratory for its speed (2-3 hours), sensitivity and specificity. However, even though the culture method is tedious, slow and requires expertise, it allows antimicrobial susceptibility to be evaluated and enables other studies that require the live bacteria to be used in typing or studies of virulence and pathogenicity. Upon selecting a method, laboratories should assess their needs, the impact the method may have on diagnosis and its limitations. The results of culture and PCR seem to indicate that joint use of both methods may be required due to the individual contributions of each[5, 35,36].

Metronidazole is a synthetic, primarily bactericidal chemotherapeutic agent. Its antibacterial action is limited to a wide range of anaerobic bacteria[14]. It is often used in the treatment of severe periodontitis, and is frequently the medication of choice and used empirically in combination with one or more other antimicrobial agents[14]. Tetracyclines are a family of large structures, natural or semi-synthetic antibiotics, and basically bacteriostatic agents which act by stopping protein synthesis[14].

The M.I.C.Evaluator system used in this study has been perfectly proven and provides fast, reliable results for determining MICs, as it does not require dilutions of the antimicrobial agent and it avoids the excessive use of culture mediums[37]. All 30 *P. gingivalis* isolates were sensitive to metronidazole with MIC values ranging from 0.015 to 4 ug/ml. For tetracycline, 27 isolates (90%) were sensitive, with MIC values ranging from <0.015 to 4 ug/ml, with the remaining 3 isolates (10%) being resistant to tetracycline with MIC values of 8 ug/ml. Similarly, the study by Andrés et al.[16] in 1998 reports that 100% of 31 *P. gingivalis* were susceptible to metronidazole and tetracycline, with MIC <0.125-2 ug/ml for metronidazole and <0.125.0.5 ug/ml for tetracycline. Kulik et al.[21] evaluated the antimicrobial susceptibility of 152 *P. gingivalis* strains to metronidazole and tetracycline, among other antimicrobial agents. All isolates were 100% susceptible to both these antimicrobial agents, with MIC <0.016-0.016 ug/ml for metronidazole and <0.016-2 ug/ml for tetracycline. Japoni et al.[20] reported 100 and 94% susceptibility, respectively, to doxycycline and metronidazole of 50 *P. gingivalis* strains isolated from patients with chronic periodontitis in Iran. Van Winkelhoff et al.[38] report susceptibilities of 100% to metronidazole and tetracycline in clinical isolates of *P. gingivalis* from Holland and Spain. In contrast to these high susceptibilities, Ardila et al.[19] report 21.56 % (11/51) resistance of *P. gingivalis* to metronidazole with MIC values of 0.08-16 ug/ml. With the exception of this high resistance to metronidazole, all other studies report high sensitivity to it. High resistance to metronidazole and other antimicrobial agents may be due to the excessive and inadequate use of antimicrobial agents, which foster the development of highly resistant strains[19,]. Situations leading to bacterial resistance should be avoided in day to day practice[14,15].

In our study, the 10% resistance to tetracycline is noteworthy. The most common resistance mechanism to tetracycline is by protein synthesis of the efflux pumps, which in Gram negative microorganisms are encoded by the *tet* genes[14]. Sanai et al.[17] (2002) determined the presence of the gene providing resistance to tetracycline (tet-Q) in 3 out of 5 (60%) *P. gingivalis* isolates from children, and these isolates seem to belong to the same original clone. It is important to consider that bacteria which are resistant to antimicrobial agents and live in the oral cavity may be an important source of transmission of genes providing antimicrobial resistance to other pathogenic bacteria. In the near future, the search for these resistant genes should probably look at the 3 tetracycline-resistant *P. gingivalis* strains reported herein.

The aim of this study was to determine whether gender and age were related in any way to the presence or absence of *P. gingivalis* in patients with chronic periodontitis. No relationship was found. These

results agree with those reported in other studies conducted in Colombia[1,4].

Different studies show that there is a very high association between *P. gingivalis* and signs of periodontal disease: inflammation, increase in probing depth, poor oral hygiene, loss of alveolar bone, loss of clinical attachment, bleeding on probing and severity of periodontitis[22,31]. In addition, the presence of *P. gingivalis* seems to play a very important part in the progression of chronic periodontitis[22,24]. Nevertheless, this study found no relationship between pocket depth, bleeding on probing, attachment level or severity of periodontitis and presence or absence of *P. gingivalis,* in agreement with the results reported by Lafaurie et al.,[3] and in contrast to other studies which found that *P. gingivalis* is strongly related to pocket depths > 5mm[3,9,39].

The results presented in this study are a contribution to the microbiological study of chronic periodontitis in the population of Colombia. They should contribute to devising preventive measures and developing adequate policies for managing antibiotics, to delay the appearance of antimicrobial resistance.

To conclude, this study reports a *P. gingivalis* frequency of 34.5% in patients with chronic periodontitis, and the clinical isolates identified had high sensitivity to metronidazole and tetracycline.

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IN VITRO EVALUATION OF THE FILM THICKNESS OF SELF-ETCHING RESIN CEMENTS

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ABSTRACT

The aim of this study was to evaluate the film thickness of self-etching resin cement. The following materials were used: Group1, Relyx U100 (3M /ESPE); Group 2, BisCem (Bisco); Group 3, Max Cem (Kerr); Group 4, Set (SDI) and Group 5, Relyx ARC (3M/ESPE) as control. Two 5.4 x 76.2 x 1 mm glass slides were marked in the center to identify the area where the material would be placed. A volume of 0.05 ml was used for each specimen material. After 1, 3 and 6 minutes, a 50 N load was applied for one minute. The thickness of each specimen was then measured using a digital micrometer to the nearest 1 µm; (Digimatic, Mitutoyo Corporation, Japan.). Data were analyzed using ANOVA and Bonferroni's multiple comparison tests. No significant difference was found between the materials tested (p = 0.0921) or material/time interaction (p = 0.0864), but there were differences in the time factor (p = 0.0001). At one minute, the thinnest film was Relyx ARC (control) (14 µm), followed by Relyx U100 (17 µm), and Maxcem and SeT (19 µm). At 3 minutes, Group 5 (control) was also the thinnest film (19 µm), followed by Group 1 (21 µm), Group 3 (25 µm), Group 2 (29 µm), and Group 4 (31 µm). At 6 minutes, Group 4 was the thinnest (34 µm), followed by Group 1 (38 µm), Group 5 (40 µm), Group 2 (41 µm) and Group 3 (42 µm). The film thickness of resin cements was influenced by time and polymerization reaction. The film thickness of self-etching cements was low.

Key words: *resin cements, film thickness.*

EVALUACIÓN IN VITRO DEL ESPESOR DE PELÍCULA DE CEMENTOS RESINOSOS DE AUTOGRABADO

RESUMEN

El objetivo fue evaluar el espesor de película de cementos resinosos de autograbado. Se utilizaron los siguientes materiales Grupo 1: Relyx U100 (3M /ESPE), Grupo 2 BisCem (Bisco), Grupo 3: Max Cem (Kerr), Grupo 4: Set (SDI) y Grupo 5: Relyx ARC (3M/ESPE) como control. Se emplearon dos superficies de vidrio de 25.4 x 76.2 x 1 mm., señaladas en su parte media con una marca, para ubicar el material sobre la misma área. Se utilizó un volumen de 0,05 ml. de material para cada probeta. Se esperó 1, 3 o 6 minutos para aplicar una carga de 50 N durante 1 minuto. Trascurrido dicho lapso cada probeta se sometió a lectura de espesor de película utilizando un micrómetro digital, con una precisión de 1um; (Digimatic, Mitutoyo Corporation, Japón.). Los datos fueron analizados mediante ANOVA y test de comparaciones múltiples de Bonferroni, no hubo diferencias significativas entre los materiales evaluados (p = 0,0921), ni en la interacción material / tiempo (p = 0,0864), pero si existieron diferencias en relación al factor tiempo (p = 0,0001). Al minuto el menor espesor de película correspondió a Relyx ARC (control) con un valor de 14 µm, seguido por Relyx U100 (17 µm), BisCem, Maxcem y SeT presentaron un valor de 19 µm. A los 3 minutos el grupo 5 (control) presentó también el menor espesor (19 µm), seguido por el grupo 1 (21 µm), grupo 3 (25 µm), grupo 2 (29 µm), y grupo 4 (31 um). A los 6 minutos el grupo 4 mostró el menor valor con 34 µm, seguido por grupo 1 (38 µm), grupo 5 (40 µm), grupo 2 (41 µm) y grupo 3 (42 µm). El espesor de película de los cementos resinosos se vió influenciado por el tiempo y reacción de polimerización. Los cementos de autograbado presentaron un reducido espesor de película.

Palabras clave: *cementos resinosos, espesor de película.*

INTRODUCTION

Luting agents are used to fix rigid restorations to teeth and prevent them from becoming detached, and to achieve an adequate marginal seal that will ensure that the restorations last inside the mouth[1,2]. It is important to select and use luting agents correctly because many of their advantages are lost if the wrong system is used[2].

Composite or resin cements have interesting physical and mechanical properties compared to other dental cements such as zinc phosphate[3,4]. These advantages include low solubility, high flexural resistance, low marginal leakage and better retention[5-8]. Another important factor to consider is film thickness in order to achieve a rigid restoration without alterations in the seating, to prevent mar-

ginal misfit and alterations in its location in the occlusal direction. Adequate cement thickness (40-50 µm)[9,10] and an appropriate volume of material reduce the need to create escape routes, and optimize the seating of the restoration, improve marginal fit, provides less exposure of the cement to mouth fluids and minimize contraction stress during polymerization by reducing the interface.[11-13] Film thickness may directly affect the long-term clinical success of cemented restorations. Various factors affecting thickness have been analyzed, including physical surface phenomena, chemical interactions between materials and tooth, pressure applied upon seating the restoration, how the different materials are handled, dead spaces within the restoration, time between mixing and seating the rigid restoration[14-16].

When attaching a restoration, during the luting technique it is necessary to consider both the intensity and the duration of the seating force of the structure to be attached. It is advisable to apply high pressure, which should be maintained until the material hardens, and to manipulate the material quickly and carefully in order to improve its physical, chemical and mechanical properties[17].

Resin cement composition is based on a Bis-GMA, UDMA, TEGDMA type resin matrix with an inorganic reinforcing filler of glass, zirconium, silica or silicates, usually 50%-70% by weight[6].

Self-etching resin adhesive cements have recently been introduced on the market. They do not require treatment of the tooth structure, which incorporates monomers with phosphoric acid groups (phosphorylated methacrylates) whose acidity enables demineralization of the tooth tissue and adhesion[18]. This simplifies the technique and saves operational time for the adhesive procedure[19,20].

Progress in the field of research, technological development and the production of a wide range of self-etching adhesive cements led us to conduct this study in order to determine whether these cements have one of the desirable properties of cementing agents: thin film.

MATERIALS AND METHODS

The testing method was adapted from ISO standard 4049:2000 for polymer-based restorative and luting dental materials[10].

The tests were performed in a laboratory under controlled temperature (21°C ± 2°C) and relative moisture ambient relative humidity (60% ± 10°C).

Table 1 shows the experimental materials used in Groups 1 to 4 – self-conditioning resin cements, and Group 5 (control) – a conventional resin cement.

Two 25.4 mm x 76.2mm x 1 mm glass slides were used. Their centers were marked so that the material would always be placed in the same area. An additional mark was made at one end of the slides to ensure the same orientation for all samples (Fig.1). The thickness of paired glass slides was measured with a digital micrometer (Digimatic, Mitutoyo Corporation, Japan) to the nearest 1 µm.

Materials were handled following the instructions of the respective manufacturers.

A tuberculin syringe with modified tip (Figs. 2 and 3) was used to measure 0.05 ml of each material. The mixture was placed on a glass slide and covered with another. After 1, 3 and 6 minutes, constant pressure of 50 N was applied for 1 minute using a dynamometer (Fig. 4), after which the film thickness of each sample was measured using a digital micrometer (Fig. 5).

Thickness was measured at three points for each specimen and time variable, and the arithmetic mean calculated. Sample size was three specimens for each material and time unit (n=9).

The values were subject to analysis of factorial variance and Bonferroni's multiple comparisons test.

Table 1: Experimental materials.

Group	Experimental Material	Manufacturer	Lot
1	RELYX U100	3M ESPE. DENTAL PRODUCTS. USA.	424360
2	BISCEM	BISCO INC. USA.	1100002087
3	MAXCEM	KERR CORPORATION. U.S.A.	3498965
4	SET PP	SDI. AUSTRALIA	S0905891
5	RELYX ARC	3M ESPE DENTAL PRODUCTS. USA.	N166655

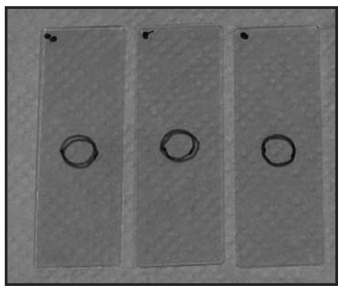

Fig. 1: Marks on glass slides – in the center for placing the cement and at the top end for positioning.

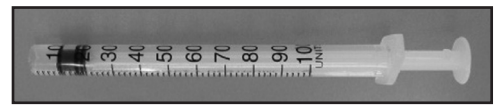

Fig. 2: Tuberculin type syringe with modified tip for standardization of the amount of luting agent to be used.

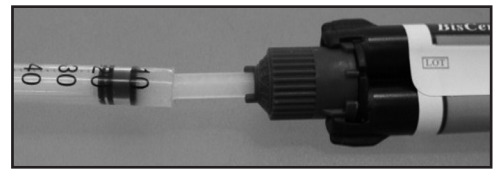

Fig. 3: Mixture loaded in the syringe.

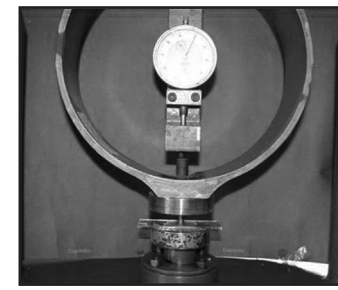

Fig. 4: 50 N constant load applied by dynamometer.

Fig. 5: Measuring luting agent film thickness with a digital micrometer (Mitutoyo, Japan).

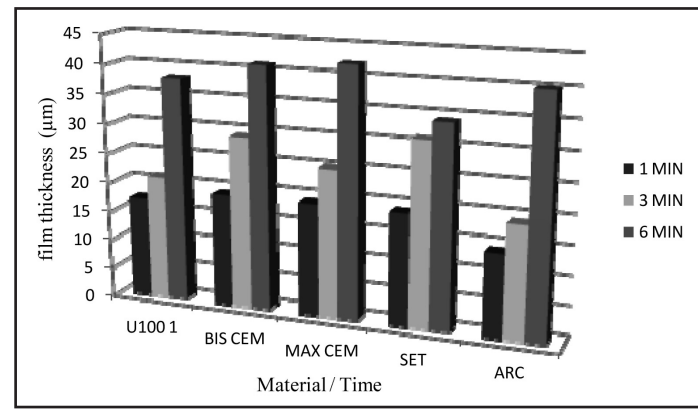

Fig. 6: Graphic representation of film thickness results in µm (mean values), expressed in Table 2.

RESULTS

Table 2 shows the mean values for film thickness, standard deviations for each experimental material and study times. The thinnest film was Relyx ARC (control) (14 µm), followed by Relyx U100 (17 µm), and BisCem, Maxcem and SeT (19 µm).

At 3 minutes, Group 5 (control) was also the thinnest (19 µm), followed in increasing order by Group 1 (21 µm), Group 3 (25 µm), Group 2 (29 µm), and Group 4 (31 um). At 6 minutes, Group 4 was thinnest (34 µm), followed by Group 1 (38 µm), Group 5 (40 µm), Group 2 (41 µm) and finally Group 3 (42 µm). These values are shown in Fig. 6.

Analysis of variance (Table 3) showed no statistically significant difference between materials ($p = 0.0921$) or material/time interaction ($p = 0.0864$), but there were significant differences with regard to time ($p = 0.0001$).

Bonferroni's multiple comparison test shows significant differences in time for the different experimental materials (Table 4).

Table 2: Arithmetic means and standard deviation.

Material	Time in minutes	Mean (µm)	S.D.
Relyx U100	1	17	2
Group 1	3	21	1.2
	6	38	2.4
BisCem	1	19	3
Group 2	3	29	3.6
	6	41	2.1
MaxCem	1	19	2.6
Group 3	3	25	7.5
	6	42	1.7
SeT	1	19	4.2
Group 4	3	31	7.8
	6	34	9.8
Relyx ARC	1	14	1.7
Group 5	3	19	1
	6	40	3.2

Table 3: Analysis of variance (SS type III).

S.V.	SS	d.f.	MS	F	p-value
Model	0.004	14	2.90E-04	14.586	<0.0001
Material	1.70E-04	4	4.40E-05	2.207	0.0921
Time	0.004	2	0.002	89.828	<0.0001
Material* Time	3.10E-04	8	3.90E-05	1.965	0.0864
Error	0.001	30	2.00E-05		
Total	0.005	44			

Table 4: Bonferroni's multiple comparison test.

Test:Bonferroni Alfa=0,05 DMS=0,00412
Error: 0,0000 gl: 30

Time in minutes	Means	n	E.E.			
1	0,018	15	0,001	A		
3	0,025	15	0,001		B	
6	0,039	15	0,001			C

Means with a letter in common are not significantly different (p<= 0,05)

DISCUSSION

Resin cements are used to achieve attachment and sealing when a rigid structure is attached to tooth tissues.

These materials need to have adequate consistency – fluid enough according to the restoration to be cemented – achieving appropriate film thickness[8,21-23]. Cement spreading is a property that depends on the time factor when there is a reaction that determines its setting or change in state during that time[7].

ISO standard 4049/2000 for polymer-based dental materials[10] establishes that polymer-based dental cement films should be less than 50 µm thick. Accordingly, this study used three waiting times: 1,3 and 6 minutes after preparing the mixture, followed by the application of a 50 N load. Our results showed significant differences for the time variable (p = 0.0001) for 6 minutes, compared to 1 and 3 minutes after preparing the mixture, for all experimental materials. For all the self-etching cements evaluated and the conventional resin cement, viscosity was found to increase with time from the mixture. Thus, it should be highlighted that the operator should work quickly and effectively to achieve adequate film thickness. Manufacturers' instructions provide working times for their products, specifying mixing time, working time, photopolmerization time and finally, self-polymerization time. The sum of all these times was not less than 7 minutes for any of the groups evaluated. This may explain why the thickness was less than 50 µm at 6 minutes for all groups. Still, film thickness increases significantly with time, so that timing should never be exceeded, since the viscosity of the material will increase, with a consequent increase in film thickness, reducing marginal fit and inducing greater polymerization tension leading to subsequent adhesive failure of the restoration[16,24-26].

Resin cement film thickness is also influenced by the load applied during restoration seating. A load of sufficient strength and duration should be used to achieve a thin layer of material between the parts to be joined until the material hardens completely[17,12,16,26,27]. This study obtained film thicknesses of 14 µm to 19 µm, at 1 minute, 19 µm - 31 µm at 3 minutes and 34 µm to 42 µm at 6 minutes by applying a 50 N load for one minute, from which it may be inferred that the self-etching luting agents studied meet one of the desirable properties for resinous cements, which is thin films, with no significant difference among the different experimental materials (p = 0.0921). These results agree with those of Kious AR, Roberts HW, and Brackett WW [25]. Moreover, Moraes RR, Boscato N, Jardim PS,

Schneider LFJ [26] report that self-adhesive resin cements polymerize more slowly and with a lower degree of final conversion, providing more time during seating, which might explain the low thicknesses recorded, even at 6 minutes, when they were not greater than 50 µm.

The viscosity of self-etching cements is different from that of traditional cements, which is related to the percentage of inorganic reinforcement filler and varies according to the material selected[28-30]. According to Han L, Okamoto A, Fukushima M and Okiji T [2], a lower percentage of filler particles may provide a thinner film thickness, according to the results reported in their paper.

Of the luting agents evaluated in this study, the thinnest film was obtained with Relyx ARC (14 µm at 1 minute), which contains 67.5% inorganic filler by weight and particle size 1.5 µm. The others ranged from 17 µm to 19 µm at 1 minute, with the following filler percentages: Relyx U 100 (70%), Bis Cem (60%), Max Cem (66%) and SeT (65%). This may confirm the above.

CONCLUSIONS

Resinous luting agent film thickness was affected by the time from mixture and finally by the polymerization reaction.

Self-etching luting agents produced thin films.

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INDEXES TO VOLUME 27 (2014)

SUBJECT INDEX

INDEXES TO VOLUME 27 (2014)

AUTHOR INDEX

INDEXES TO VOLUME 26 (2013)

AUTHOR INDEX