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GENETIC-RELATEDNESS OF PERI-IMPLANTS AND BUCCAL *CANDIDA ALBICANS* ISOLATES DETERMINED BY RAPD-PCR

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ABSTRACT

Molecular techniques have been used in recent studies to identify a wide range of potential bacterial pathogens in peri-implant pockets of the oral cavity. However, the prevalence and molecular epidemiology of yeasts and species distribution related to peri-implantitis are as yet unknown. The aim of this study was to determine the prevalence and distribution of yeasts in peri-implant biofilm and to study genetic relatedness of Candida albicans.

Yeasts recovered from peri-implant biofilm samples (n=89) and buccal samples (n=120) were studied in 40 immunocompetent non-smoking patients who visited the dental clinic of the Asociación Implantodontológica Argentina, Buenos Aires, Argentina, and had received oral rehabilitation with implants for more than five years. Yeasts recovered from samples were studied by typing assays using RAPD-PCR. The prevalence of yeasts in the peri-implant sulcus was 73% (n=29). C. albicans was the most prevalent species identified in this study population. The RAPD analysis showed identical genotypes in most C. albicans spp. from the two different sampling sites: buccal and peri-implant. These findings suggest that peri-implant biofilm is an ecological niche that favors the growth of yeast species. Most C. albicans found in peri-implant biofilm originate from the endogenous infection caused by commensal strains.

Key words: *Implants; biofilm; Candida albicans; RAPD-PCR; peri-implantitis.*

RELACIÓN GENÉTICA DE AISLAMIENTOS DE *CANDIDA ALBICANS* POR RAPD-PCR EN SURCOS PERI-IMPLANTARIOS DE CAVIDAD BUCAL

RESUMEN

Las técnicas moleculares se han utilizado en estudios recientes para identificar una gran diversidad de patógenos bacterianos de surcos periimplantarios de cavidad bucal. Sin embargo, la prevalencia y epidemiología molecular de especies de levaduras en relación con la periimplantitis son aún desconocidas. El objetivo de este estudio fue determinar la prevalencia y distribución de las levaduras en la biopelícula periimplantaria y estudiar la relación genética de Candida albicans. Se estudiaron 40 pacientes inmunocompetentes no fumadores que se asistieron en la clínica dental de la Asociación Implantodontológica Argentina, Buenos Aires, Argentina, y que habían recibido rehabilitación oral con implantes durante más de cinco años. Las levaduras aisladas de las muestras de biopelícula periimplantaria (n = 89) y bucales (n = 120), fueron identificadas por métodos micológicos tradicionales y moleculares. Se obtuvo el ADN de C. albicans y se realizaron estudios moleculares por RAPD -PCR. La prevalencia de levaduras en el surco alrededor del implante fue de 73 % (n = 29). C. albicans fue la especie más frecuente identificada en esta población de estudio. El análisis RAPD permitió identificar idénticos genotipos de C. albicans en ambos nichos ecológicos estudiados, periimplantar y bucal.

Según los resultados obtenidos, el surco periiplantario es un nicho ecológico que favorece el crecimiento de especies de levaduras del género Candida. La mayoría de los aislamientos de C. albicans periimplantarios se originan a partir de la infección endógena causada por cepas comensales.

Palabras clave: *Implantes; biopelícula; Candida albicans; RAPD-PCR; periimplantitis.*

INTRODUCTION

The use of osseointegrated implants, as well as their complications and problems, have increased in recent decades. Successfully osseointegrated titanium implants usually harbor low quantities of plaque and present little marginal inflammation. Supra- and sub-gingival microbiota at well maintained implant sites seem to resemble the microbiota associated with healthy gingiva. An increased proportion of putative periodontal pathogens has been documented at implant sites, suggesting that the periodontal pocket may serve as

a reservoir for colonization of titanium implants. Peri-implantitis is a chronic progressive marginal infection, defined as an inflammatory reaction that affects the tissue surrounding osseointegrated dental implants, resulting in the loss of the supporting bone. Microbiota resembling that of adult peiodontitis has been found in peri-implantitis [1-4].

Extensive antibiotic treatment and irrigation with chlorhexidine may cause etiological changes. Microorganisms not primarily associated with periodontitis, such as *Staphylococcus spp*., enterics and *Candida spp*., have also been isolated [2-5]. Molecular techniques have been used in recent studies to identify a wide range of potential bacterial pathogens in peri-implant pockets [6,7]. However, the prevalence of yeasts and species distribution related to peri-implantitis are as yet unknown.

The same has been found to be true for dental biofilm [2,8] . Dahlen *et al.* [9], and Reynaud *et al.*[10] claim that there was colonization by the genus *Candida spp*. in periodontal pockets, refractory periodontitis[3,10,11], and implant failure. Other studies report presence of *Candida albicans* in the subgingival plaque microbiota of human immunodeficiency virus (HIV) positive individuals [12].

In recent years, several molecular typing methods have been used to characterize *Candida spp*. isolates and to delineate strain relatedness, the most widely used being polymerase chain reaction (PCR) based methods. Among these, the random amplified polymorphic DNA (RAPD) method of DNA fingerprinting has become quite popular for all infectious fungi and has been successfully applied to assess the genetic relatedness of *Candida spp.* [13-18]. These methods have greatly enhanced knowledge on the epidemiology of oral and subgingival *Candida spp*., and can provide valuable information through their ability to distinguish distinct isolates of the same species. Some studies have demonstrated that commensal yeasts dominate in oral candidiasis, whereas controversial evidence shows that genetically homogeneous, hypervirulent strains of *C. albicans* are involved in the disease[19].

Since there is no available data on the epidemiology of yeasts and genetic characterization of peri-implant *C. albicans*, the aim of this study was to characterize peri-implant biofilm and mucosal *C. albicans* isolates recovered from immunocompetent subjects with more than 5 years of implant treatment, and to assay the genetic similarity of *C. albicans* isolates from the two niches in the same patient by RAPD.

MATERIAL AND METHODS

Study population

This study was approved by the Ethics Committee of the School of Pharmacy and Biochemistry, University of Buenos Aires (Res. 41, File 727.071/10). Yeasts recovered from peri-implant plaque (n=89) and buccal samples (n=120) were studied in 40 immunocompetent non-smoking patients with more than five years of implant treatment on oral prosthesis who attended the dental clinic of the Asociación Implantodontológica Argentina, Buenos Aires, Argentina.

Evaluations included clinical examination and radiographs with clinical measurements: pocket depth (PD), considered regular up to 3 mm around implants, plaque index, gingival index [11,20] and bleeding on probing. Measurements were taken at four sites per tooth (mesial, buccal, distal and lingual positions) on 15 teeth, excluding third molars.

Bone resorption was assessed by comparing the radiographic examination in the patients' medical records taken at the time of implant placement to those taken at the appointment for this study. In order to analyze bone resorption, implants were classified into two groups according to time of implant placement: "immediately loaded implants" if they were placed during the same session as tooth extraction or "delayed loaded implants" if they were placed on healed bone, months or years after extraction.

Participation in our survey was voluntary and all patients provided written informed consent.

The volunteers were requested to rinse their mouths thoroughly with sterile distilled water, after which sterile swabs were used to take samples from tongue, palate and cheek.

The dental professional then isolated the area using cotton rolls and a high-speed suction device. Following removal of the supragingival plaque using a Teflon curette to avoid salivary contamination, peri-implant biofilm was collected from the interdental plate by inserting 3-4 sterile paper points number 30-35-40 for 15-30 minutes in the four sites: mesial, buccal, distal and lingual positions. Samples were cultured in a differential chromogenic medium

(CHROMagar Candida, Paris, France). Yeast isolates were identified using conventional mycological methods: colony color on the chromogenic medium, micromorphology in agar milk with 1% Tween-80 [21], carbohydrate assimilation tests using a commercially available kit API ID 32D (BioMérieux, Lyon, France), and specific PCR[22].

Random amplified polymorphic DNA (RAPD) analysis

Yeast DNA was isolated using a technique described previously [22-24]. Five different primers were included in the typing assays. Primer sequences were as follows:

OPA 02 (TGCCGAGCTG), OPA 09 (GGGTAACGCC), M13F (CGACGTTGTAAAACGACGCCCAGT), M13R (CAGGAAACAGCTATGAC), and OCP 5 (GATGACCGCC). They were all used in RAPD-PCR, following the method developed by Williams *et al*.[23]. Arbitrary amplification was performed in a total volume of 50 µl containing: 1_buffer 2.5 mM MgCl2, 0.2 mM each of the dNTP, 0.5 mM of the primer, 1.25 U Taq DNA polymerase (Invitrogen), and 75 ng of template DNA. The cycling program consisted of 4 min at 94ºC, 35 1-minute cycles at 94ºC, 1 min at 25ºC, 2 min at 72ºC followed by a final extension of 5 min at 72ºC.

These steps were carried out in a Minicycler DNA thermal cycler (TM MJ Research Inc., NY, USA). Products were separated by electrophoresis in 1.4% agarose gel and stained with ethidium bromide. They were visualized under UV light and digitalized by image analyzer software (EPI-Chemi Darkroom. UVP Laboratory Products, California, USA). Band profiles were analyzed and compared visually. Each band was scored as positive or negative for all isolates; and the presence or absence of each band was recorded for each isolate. The resulting matrix was interpreted using the Treecon program, where isolates were grouped according to the resemblance of their patterns. Based on matrix of similarity coefficients (SC), a dendrogram was generated by the unweighted pair group method using arithmetic averages (UPGMA). The criterion used for genotyping was as follows: arbitrary threshold at an SC of 90% for closely related isolates.

Statistical analysis

Statistical analysis was performed using Statistix 7.0 and the SPSS 11.0 software. Confidence interval was 95% (CI 95%). Fisher and ANOVA were calculated at 95% using the Epi-Info 6.04 program (Atlanta University, GA).

RESULTS

Clinical features

The 40 subjects included in the study ranged in age from 33 to 76 years (mean age 56 years), 50% were female (20/40). None of them had received antibacterial or antifungal agents before this treatment. Of the total population, 68% were non-smokers. This population had an average of 12.80 teeth and 2.58 implants; 1.85 loaded implants and 0.38 non-loaded implants.

Of the total number of original implants (n=103) in the study population, we found that only 89 were present. The percentage of bone resorption in immediately loaded implants (n=13), was significantly higher ($p<0.001$) than in delayed loaded implants (n=76) (Fig.1).

Comparison of bone resorption in relation to the kind of prosthesis placed on the implants (n=89) showed significantly higher resorption rates ($p<0.001$) in the group with removable prostheses (36/48) than in the group with fixed prostheses (6/26) and without load (7/15) (Table 1).

Pocket depth (PD) was more than 3mm in 18/40 patients and less than 3 mm in 22/40 patients (Table 2).

Carriage of *C. albicans* and other yeast species

The prevalence of yeasts in the peri-implant sulcus was 73% (n=29, CI 95%:55.9- 84.9). In buccal mucosa, the distribution of yeasts was: 73% in palate and cheek (n=29), CI 95%; 0.559 0.859), and

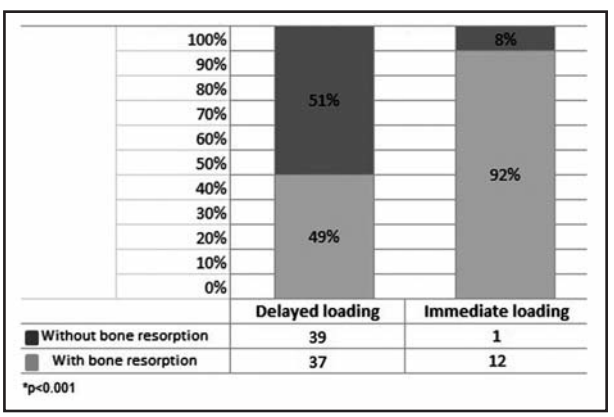

Fig. 1: Percentage of bone resorption in immediately-loaded and delayed-loaded implants. (N=89).

Table 1: Study of bone resorption in 89 implants.

Prosthetic load		With bone resorption	Without bone resorption
Totals	**89**	**49**	**40**
Fixed prosthesis	26	6	20
Removable prosthesis	48	36	12
Without prosthesis	15	7	8

Table 2: Pocket depth greater and smaller than 3mm.

Cultures	PD>3mm.		PD≤3mm.		Total
Positive	15	83%	13	59%	28
Negative	3	17%	9	41%	12
Total patients	18	100%	22	100%	40

Table 3: Prevalence of yeasts in the peri-implant sulcus and mucosa.

Cultures	Cheek	IC95%	Tongue*	IC95%	Palate	IC95%	Sulcus	IC95%
Positive	29 (73%)	55.9 84.9	34 (85%)	70.2 94.3	29 (73%)	55.9 84.9	29 (73%)	55.9 84.9
Negative	11(27%)	15.1 44.1	6 (15%)	05.7 29.8	11 (27%)	15.1 44.1	11 (27%)	15.1 44.1

*p<0.001

85% in lingual mucosa (n=34, CI 95%; 70.2- 94.3), representing a high statistically significant prevalence (*p< 0.001*) (Table 3).

Table 4 summarizes species distribution of yeast isolates in peri-implant biofilm and buccal mucosa. Of the 140 yeasts recovered, *C. albicans* was the species most frequently found in all niches, peri-implant and mucosa.

The prevalence of *C. albicans* was 55% (n=22) in peri-implant biofilm. Other non-*C. albicans spp*. and other yeasts were found: *C. dubliniensis* (n=11), *C. parapsilosis* (n=5), *Saccharomyces cereviciae* (n=5), *C. krusei* (n=2), *C. tropicalis* (n=1), *C. lusitaniae* (n=1) and *Rhodotorula spp*. (n=1).

Table 4: Prevalence of Candida albicans in peri-implant sulcus.

Yeast Species	Sulcus	%	IC95%
C. albicans	22	**55.0**	38.7 70.4
C.dubliniensis	11	**27.5**	15.1 44.1
C. parapsilosis	5	12.5	4.2 26.8
C.tropicalis	1	2.5	0.1 13.2
C. guilliermondii	0		
C. krusei	2	5.0	0.6 16.9
Saccharomyces cerevisiae	5	12.5	4.2 26.8
C. glabrata	0		
C. lusitaniae	1	2.5	0.1 13.2
Rhodotorula spp.	1	2.5	0.1 13.2
Total	**48**		

The occurrence of two or three co-isolated species was observed in 22/120 buccal mucosa samples. *C. albicans* and *C. krusei* (n=6) followed by *Saccharomyces cereviciae* and *C. dubliniensis* (n=4) were the associations most frequently observed.

The combinations in peri-implant sulcus was 16.7% (n=8). Of the associations of the species found, the most predominant were *C. dubliniensis* with *C. krusei,* and *C. albicans* with *C. glabrata* (2% each) (Table 5).

In relation to pocket depth and presence of yeasts, patients with peri-implant sulcus >3 mm exhibited an increase in positive cultures (83%, 15/18) compared to negative cultures (17%, 3/18), whereas patients with peri-implant sulcus ≤3 mm, positive cultures (59%, 13/22) and negative cultures (41%, 9/22) exhibited much lower discrepancy. This difference was not statistically significant (Table 6).

Of the 89 implants studied, 43 showed no colonization by *Candida*, of which 23 had bone resorption (53 %) and 20 did not (47%). Of the 46 implants where there was colonization by *Candida*, 26 had resorption (47%) while the other 20 did not (43%). In all four cases, the percentages were similar. According to these results, peri-implant *Candida* colonization would not be the determining cause of bone resorption around implants. (Fig. 2)

Table 5: Distribution of yeasts in mucosa.

Colonization of yeasts in mucosa	CHEEK	TONGUE	PALATE	TOTAL CULTURES	Percentage of total
Negative	11	6	11	28	
C. albicans	14	11	12	**37**	40%
C. dubliniensis	5	3	4	**12**	13%
C. parapsilosis	2	5	2	9	10%
Saccharomyces cerevisiae	3	2	1	6	7%
C. tropicalis	0	1	1	2	2%
C. glabrata	0	1	0	1	1%
C. krusei	0	1	0	1	1%
C. guillermondii	1	0	0	1	1%
C. lusitaniae	1	0	0	1	1%
C. krusei and C. albicans	1	3	2	**6**	7%
Saccharomyces cerevisiae and C. dubliniensis	1	2	1	**4**	5%
C. parapsilosis and C. tropicalis	0	1	2	3	3%
C. parapsilosis and C. albicans	0	2	0	2	2%
C. parapsilosis and dubliniensis	0	0	2	2	2%
C. guillermondii and C. tropicalis	0	1	1	2	2%
Saccharomyces cerevisiae and C. glabrata	0	1	0	1	1%
C. glabrata and C. dubliniensis	1	0	0	1	1%
C. krusei and C. dubliniensis	0	0	1	1	1%
Total Positive	29	34	29	92	100%

Implants with removable prostheses exhibited significantly higher ($p<0.001$) rates of *Candida spp.* colonization (19/22) than those with fixed prostheses (9/18) (Table 7).

RAPD-PCR ASSAY

We selected five RAPD primers, based on their reproducibility, after the pre-screening process in order to analyze 68 *C. albicans* isolates. The number of bands ranged from two to three splitters (M13r) to 12 (M13f). Three of five primers were the most informative (M13f, OPA 9 and OPC5) and generated the highest number of band patterns (10 to 12).

The dendrogram generated by the UPGMA clustering method, using the RAPD-PCR technique

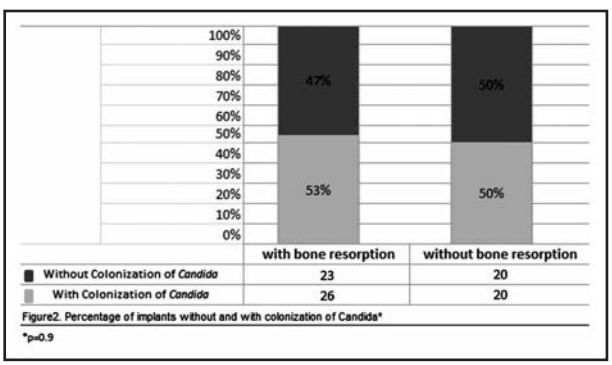

Fig. 2: Percentage of implants with and without Candida colonization.

Table 6: Presence of yeasts in relation to pocket depth.

Cultures	PD>3mm.		PD≤3mm.		Total
Positive	**15**	**83%**	**13**	**59%**	28
Negative	*3*	*17%*	*9*	*41%*	12
Total patients	**18**	**100%**	**22**	**100%**	40

Table 7: Colonization of *Candida spp.* in implants with removable and fixed prosthesis.

Culture	Fixed prosthesis		Removable prosthesis		Total
Positive	**9**	**50%**	**19**	**86%**	28
Negative	**9**	**50%**	**3**	**14%**	12
Total	**18**	**100%**	**22**	**100%**	40

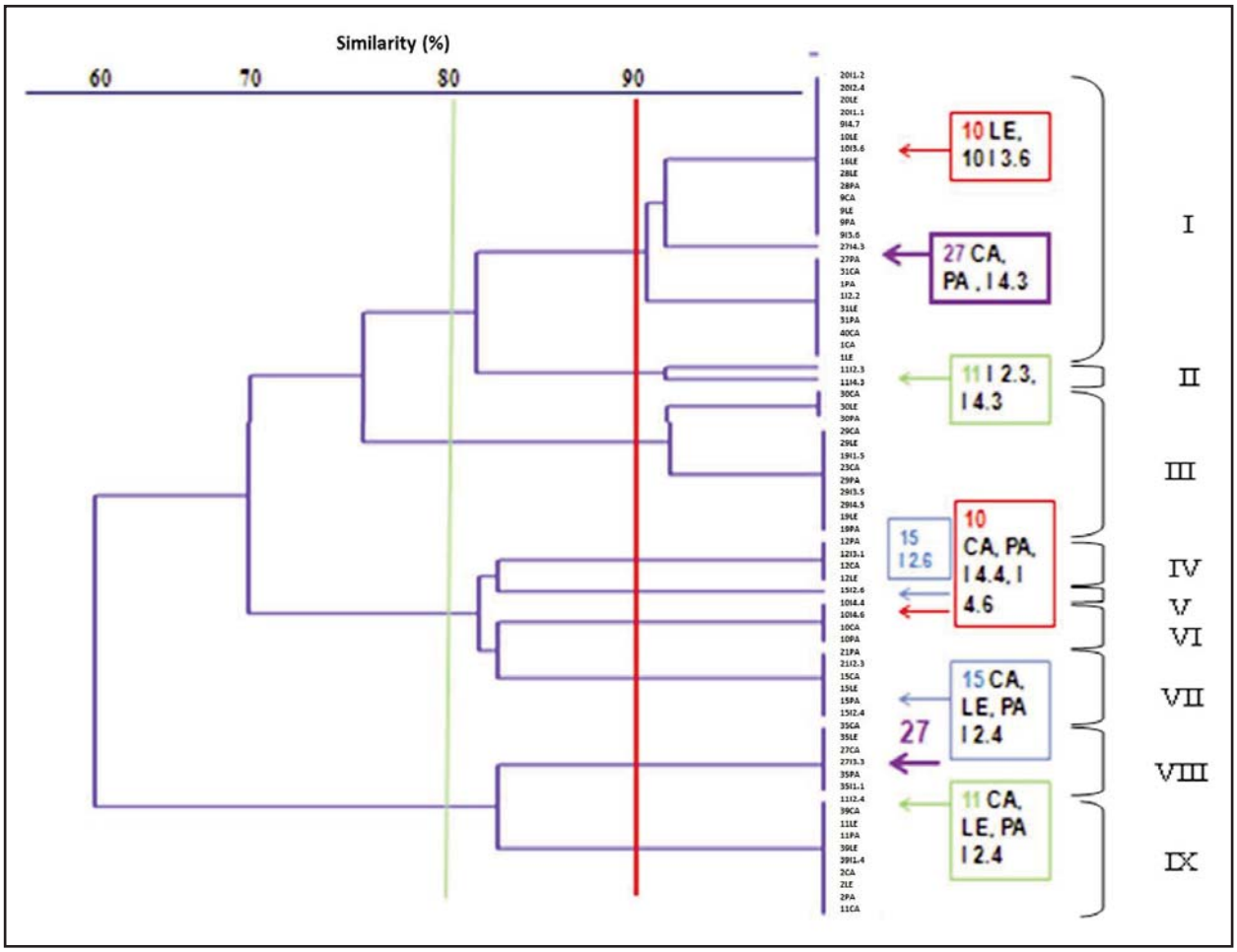

Fig. 3: The dendrogram generated by the UPGMA clustering method, using the coefficient of similarity between RAPD-PCR of C. albicans in oral cavity, tongue (LE), palate (PA), cheek (CA), and peri-implant sulcus (I) shows that the similarity coefficient (SC) ranged from 60 to 100%. Thirteen genetic clusters and nine main genotypes were obtained at a similarity coefficient (SC) of 90%, genotypes I, II, III, IV and V.

for *C. albicans* in oral cavity, tongue (LE), palate (PA), cheek (CA), and peri-implant sulcus (I) shows similarity coefficient (SC) ranging from 60% to 100%. Thirteen genetic clusters and nine main genotypes were obtained at a similarity coefficient (SC) of 90%, genotypes I, II, III, IV, and V. (Fig 3)

DISCUSSION

In this study, 40 immunocompetent adult patients with more than 5 years' treatment were recruited and grouped according to their health status and pocket depth into peri-implantitis or healthy. As expected, patients with peri-implantitis presented more infectious sites, including higher rates of percentage similarity (PS) (Anova Test $p< 0.001$).

Eighty-nine peri-implant sulcus samples and 120 swabs from buccal mucosa were cultured directly in CHROMagar Candida medium to enable the presumptive identification of *C. albicans* or *C. dubliniensis, C. tropicalis* and *C. krusei*. This also enabled identification of the presence of infections caused by more than one species simultaneously. Similar findings have been reported by other authors who analyzed other populations[22,25,26-28].

The prevalence of yeasts in sulcus was 73% (n=29), showing that the surrounding ecological niche and peri-implant sulcus enabled yeast growth. Other studies have reported the presence of *Candida spp*. in peri-implant lesions[29,30], and found *Candida spp*. in 55% of peri-implant sites.

The comparison of yeast distribution in relation to clinical markers of peri-implantitis revealed no significant difference in the prevalence of yeasts at sites with PD >3 mm or at sites with bone resorption.

These findings revealed the presence of yeast species in peri-implant sulcus as well at sites with or without peri-implantitis.

Of the 120 buccal mucosa samples studied here, the tongue was the site with highest prevalence of *Candida spp*. (85, CI95%, 0.702 0.943), in contrast to cheek and palate, with a statistically significant difference ($p< 0.001$).

Candida spp. prevalence was higher in our study than in previously reported series[31-34] in which it ranged from 25% to 65%, suggesting that the presence of implants in our study population increases prevalence.

In relation to the type of implant rehabilitation −fixed or removable− the latter yielded significantly higher ($p< 0.001$) prevalence of yeasts. It is worth noting that these findings suggest that peri-implant plaque is an ecological niche that favors the growth of yeast species; especially in implants with removable rehabilitation, even though they can be removed for cleaning. Moreover, these implants are made of acrylic, which favors adhesion of *Candida spp.* These are the first data results reported in Argentina.

The use of buccal devices induces alterations within the oral cavity. Hägg *et al.*[35] observed that the presence of prosthesis or other buccal devices increases the number of *Candida spp*., not only at the site but throughout the mucosa. Dental prostheses are made of acrylic resins in which surface defects favor the development of plaque and prevent its removal[36]. The surface of the prosthesis is very porous and thus susceptible to being colonized by large numbers of microorganisms, which may give rise to different pathologies in the oral cavity.

Comparison of the two study samples showed "high" concordance, with colonization or infection by the same yeast in both ecological niches in 95% of the patients ($Kappa= 0.8$).

In relation to the distribution of yeast species, *C. albicans spp*. was the most prevalent (55%, n=22), but it is important to highlight that non-*C. albicans spp.* were also found in peri-implant sulcus: *C. dubliniensis* 27.5% (n=11), *C. parapsilosis* 12.5% (n=5), *Saccaromyces cereviciae* 12.5% (n=5), *C. tropicalis*, *C. lusitaniae* and *Rhodotorula spp.* 2.5% (n=1), and *C. krusei* 5% (n=2), (Table 1). Many of these less prevalent species are emerging and characterized by the presence of diminished sensitivity to antifungals[37]. No data is available in the literature reviewed.

Epidemiological surveillance is very important for identifying the prevalence of yeast species in the biofilm of peri-implant sulcus since they create reservoirs for opportunistic microorganisms which, in certain clinical situations such as patients with immune deficiencies, play a significant role in diseases such as buccal candidiasis and disseminated diseases [34, 38].

In this study, *C. albicans* isolates from the buccal cavity and peri-implant sulcus of the same patient were considered to be closely related in 90% of the cases (16/20) according to RAPD-PCR. Similarity among isolates from both ecological niches suggests that the source of *C. albicans* colonization in peri-implant biofilm is the patient's buccal cavity. Thus, it can be assumed that most *C. albicans spp*. found in peri-implant biofilm originate from endogenous infection by commensal strains.

Coincidentally, other authors have found identical genetic patterns in yeasts from different anatomical sites in the same patient. However, the results obtained highlight the fact that the same patient carries different species[39]. It is important to consider that *C. albicans* colonization in peri-implant sulcus could also occur due to the presence strains adaptable to the peri-implant environment, which is likely as a result of genetic variations such as gene conversion and/or chromosomal translocations [15, 19].

To date, scientific literature has not provided any information on the genetic characterization of *C. albicans* isolates in peri-implant sulcus. Hence, yeast isolates were analyzed by RAPD-PCR, which has proved to be a rapid, simple, cost-effective technique and discriminatory for the molecular typing of *C. albicans* isolates. Other authors have used the same techniques to assay several yeasts species [13, 15-17, 22].

This is the first study conducted in Argentina on the molecular characterization of clinical *C. albicans* isolates in peri-implant sulcus by RAPD-PCR.

We confirm that the peri-implant plaque is an ecological niche that favors the growth of yeast species; especially in implants with removable rehabilitation.

C. albicans spp. were the most prevalent in peri-implant samples, but it is important to highlight that non-*C. albicans spp*. were also found in peri-implant sulcus, e.g. *C. dubliniensis*, *C. parapsilosis*, *Saccaromyces cereviciae*, *C. tropicalis*, *C. lusitaniae* and *C. krusei*.

The findings suggest that most peri-implant *C. albicans* originate from endogenous infection by commensal strains.

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ASSESSMENT OF KNOWLEDGE ON TEMPOROMANDIBULAR DISORDERS AMONG MEXICAN DENTAL EDUCATORS

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ABSTRACT

Temporomandibular disorders (TMDs) is an umbrella term that embraces a group of musculoskeletal and neuromuscular conditions that involve the temporomandibular joints, muscles and all associated tissues. Because of the relatively high number of patients with TMDs in the population, instruction in this area of health care should be included on all dental curricula. Although levels of knowledge among dentists have been evaluated in several countries, they have not been in Mexico. This study evaluates the dental faculty's range of knowledge about TMD at five dental schools in Puebla, Mexico.

Using an observational design, a survey was administered to 161 educators in order to assess their knowledge of TMD. Four domains were assessed, including: a) pathophysiology; b) psychophysiology; c) psychiatric disorders; and d) chronic pain. Overall knowledge of TMD was measured using a consensus of TMD experts' answers as a reference standard[1] The results show that educators' overall knowledge had 55% agreement with the reference standard. Individually, the psychophysiological domain was correctly recognized by 77.7% of the educators; correct responses on the other domains ranged from 38% to 56%. This study demonstrates the need to incorporate standardized TMDs instruction into the dental curricula at Mexican Universities, without which graduating dentists will lack the necessary knowledge or experience to diagnose and manage their TMD patients.

Key words: *Temporomandibular joint disorders; dental education; teaching.*

EVAUACIÓN DEL CONOCIMIENTO SOBRE TRASTORNOS TEMPOROMANDIBULARES EN DOCENTES DE ODONTOLOGÍA EN MÉXICO

RESUMEN

Los Trastornos Témporomandibulares (TTM) incluyen un grupo de condiciones musculoesqueléticas y neuromusculares que afectan a la Articulación Temporomandibular (ATM), los músculos masticadores y otros tejidos asociados.

Debido al número relativamente alto de pacientes con TTM en la población, la educación en esta área de la salud debe ser incluida en las currículas de las escuelas de odontología.

A pesar de que el nivel de conocimiento sobre TTM ha sido evaluado en diversos países, esto no ha sido realizado en México, por lo que el objetivo del presente estudio fue evaluar el nivel de conocimiento sobre los TTM de los profesores de odontología en cinco universidades de Puebla, México.

Bajo un diseño observacional, se administró una encuesta a 161 docentes de odontología para evaluar el nivel de conocimiento sobre los TTM. La encuesta incluyó cuatro dominios: a) patofisiología; b) psicofisiología; c) trastornos psiquiátricos y d) dolor crónico. Se usaron las respuestas otorgadas con un consenso de expertos como estándar de referencia[1] para evaluar el nivel global de conocimiento sobre los TTM. Los resultados mostraron que los docentes tuvieron un nivel global de conocimiento del 55% de acuerdo al estándar de referencia. El dominio psicofisiológico individualmente fue el mejor reconocido con el 77% de acuerdo con los expertos; las respuestas correctas en los otros dominios oscilaron entre el 38% y el 56%. El presente estudio demostró la necesidad de incorporar educación sobre los TTM estandarizada en la currícula de las escuelas o facultades de odontología en las universidades mexicanas. Hasta que esto suceda, las generaciones de odontólogos no tienen el conocimiento ni la experiencia necesarios para diagnosticar y manejar a los pacientes con Trastornos Temporomandibulares.

Palabras clave: *Trastornos Temporomandibulares; educación dental; enseñanza.*

INTRODUCTION

Temporomandibular disorders are recognized by the American Association of Dental Research (AADR) as a collective term that embraces a group of musculoskeletal and neuromuscular conditions that involve the temporomandibular joints, the muscles and all associated tissues [2]. TMDs have been identified as a major cause of non-dental pain in the orofacial region and are considered to be a sub-classification of musculoskeletal disorders [3]. It

has been speculated that the onset of TMD is complex and multifactorial, and such factors have been classified as predisposing, precipitating and perpetuating [4].

The reported prevalence of TMD, according to population-based studies, ranges from 6.3% to 15% in women and 2.8% to 10% in men. TMD conditions have been found to have an age-specific pattern, peaking at 35 to 45 years of age [5-10]. Studies have shown that the prevalence of signs or symptoms associated to TMD can be observed in up to 50% of the general population, of which only 3% to 7% seek professional help, depending on the severity of their symptoms [5]. Additionally, it has been demonstrated that patients with more than one TMD diagnosis have a greater chronicity as well as greater psychosocial involvement [10-13].

In the United States, there have been several attempts to improve education in this field. Since 1990, the First Educational Conference to Develop the Curriculum in Temporomandibular Disorders and Orofacial Pain proposed several curriculum models specifically for predoctoral, postdoctoral, and continuing education [14-22]. A second educational conference was held in 1992, at which the educational methodologies for the implementation of formal curriculum guidelines in dental education, problem-based learning, decision analysis, and computer technology were discussed [23]. Finally, in 2000, the Third Educational Conference was held, sponsored by the American Academy of Orofacial Pain, the Association of University TMD and Orofacial Pain Programs, the American Academy of Oral Medicine, the Canadian Academy of Oral and Maxillofacial Pathology and Oral Medicine, and the Association of Canadian Faculties of Dentistry. Over 130 educators participated with the goal of improving the teaching of TMD and OFP at predoctoral level [23,24].

Today, TMDs are being studied and treated with a medical perspective that involves orthopedic principles combined with a biopsychosocial understanding of how chronic pain disorders affect those who suffer them [25,26]. Despite this progress, there are still controversies among those in the field of dental and advanced dental education. LeResche *et al*. [1] evaluated the extent of knowledge of TMD in a random sample of general dentists and TMD specialists. They reported that practicing dentists tended to agree with the opinion of experts on psycho-physiological aspects, but they generally disagreed on issues related to the domain of pathophysiology. The study concluded that there is a high degree of consensus in knowledge among specialists and general dentists on some items; however, there is a need to reach a more consistent consensus among all domains.

Based on the information presented above, there is no doubt that teaching TMD should be a fundamental component of the dental curriculum, not only at the didactic level, but also incorporated into the student's clinical experiences, which influence knowledge and skills for treating TMD patients [13,15,26,27-32]. As far as we know, there is no published study evaluating the knowledge of dental educators or clinicians in the area of TMD in Mexico. Therefore, the aim of this study was to evaluate knowledge of TMD among dental educators at five dental schools in Puebla, Mexico.

MATERIALS AND METHODS

An observational, cross-sectional study was conducted on 161 dental educators from five, out of a total of twenty-one, dental schools in the city of Puebla, Mexico. All twenty-one universities were invited to participate, but only 5 accepted to participate voluntarily and obtained approval from the relevant institutions. A published survey conducted in Seattle was used as reference[1] in which thirteen researchers who publish extensively in the refereed TMD literature formed the TMD expert group. These experts belonged to the International Association for Dental Research (IADR) and/or the International Association for the Study of Pain (IASP), and all had extensive clinical and/or research experience with TMD patients. The Seattle study was translated and adapted by an expert panel into Spanish. This survey consisted of 35 items divided into four domains: a) pathophysiology: assessing knowledge of biomedical or biomechanical aspects of TMD etiology, diagnosis and treatment, b) psychophysiology: assessing knowledge of the interaction of physical and psychological factors in TMD etiology, diagnosis and treatment, c) psychiatric disorders: assessing knowledge about anxiety, depression and somatization disorders associated with TMD, and d) chronic pain: assessing knowledge about the causes, diagnosis and appropriate treatment of chronic pain conditions as applied to TMD, according with survey proposal by LeResche et al [1].

In the original Seattle study, the statements were evaluated by panels of experts. The expert responses used in the Seattle study were also used for the present study. The statements were said to generate expert consensus if more than 75% of the experts in the designated group endorsed an "agree" response (scored 7 to 10) or a "disagree" response (scored 0 to 3).

The answers were considered "correct" if the response matched the reference standard or response provided by the consensus of TMD experts. Otherwise, the responses were considered "incorrect", even those in which the participants answered "I don't know".

All the participants answered the survey at their respective institutions in the presence of the researcher.

Statistical Analysis

Descriptive statistics including mean, median, standard deviation (SD), and percentages are presented. In addition, the median percentage of correct responses for each domain and total instrument scores were calculated. Comparisons by gender and by year of graduation from dental school were performed using the Mann-Whitney test. Comparison by academic level was performed using the Kruskal Wallis test. A significance α level of 0.05 was used. SPSS version 17 was used for the statistical analysis. The dependent variable was TMD knowledge in dental educators. The independent variables were: gender, academic level, and year of graduation.

RESULTS

A total 161 dental educators participated in this study. Mean age was 40 years with a standard deviation of 10 years. Gender distribution was 55% female and 45% male. Regarding academic level, 24% were general dentists, 37% had a clinical specialty (not specifically TMD), and 39% had either an MS or PhD degree.

In the first domain (pathophysiology), the median percentage of correct responses by dental educators was 38% (Table 1). Within this domain, the item with the lowest rate of correct responses was "Occlusal equilibration is a useful early treatment for TMD", for which only 2,5% of educators had

Table 1: Pathophysiology Domain.

Items	Expert Response	Right answer according Expert
1. Balancing interferences are commonly related to TMD.	*Disagree* 85%	14.3%
2. Occlusal equilibration is a useful early treatment for TMD.	*Disagree* 85%	2.5%
3. Orthodontic treatment can prevent the onset of TMD.	*Disagree* 77%	21.1%
4. Arthroscopic surgery is almost completely effective in repositioning the disc in patients with internal derangements.	*Disagree* 100%	38.5%
5. Orthodontic therapy is the best treatment to resolve TMD in a patient with a skeletal malocclusion.	*Disagree* 92%	65.8%
6. TMD caused by trauma is much more difficult to treat and has far worse prognosis than other types of TMD.	*Disagree* 83%	54.7%
7. Transcranial films are the most accurate method for viewing the TM Joint.	*Disagree* 77%	50.9%
8. The presence of arthritic changes on tomograms, along with crepitus in the joint indicates the need for treatment.	*Disagree* 77%	18.0%
9. The position of the condyle in the fossa as seen in tomograms is a very accurate indication of internal derangement.	*Disagree* 92%	40.4%
10. Mandibular repositions splints are more effective than maxillary splints.	*Disagree* 100%	45.3%
11. Nocturnal bruxism is caused by occlusal interferences.	*Disagree* 85%	67.7%
12. Ice packs and/or heat packs and passive muscle stretching are good early treatments for TMD.	*Agree* 100%	58.4%
13. All individuals with clicking TMJs require treatment.	*Disagree* 100%	36.0%

Median percentage of right answers according to experts = 38.4

adequate knowledge. The item with the highest number of correct responses in the same domain was "Nocturnal bruxism is caused by occlusal interferences", for which 68% of educators had adequate knowledge. This particular domain presented a wide range of variability.

In the second domain (psychophysiology), dental educators had better knowledge of the subject, and the median percentage of total correct answers was 78% (Table 2).Within this domain, the item with the lowest rate of correct answers was "Stress is a major factor in the development of TMD", with only 47% of the educators demonstrating adequate knowledge. The item with the highest percentage of correct answers was "Stress management is indicated for many TMD patients", with 88% of the educators having adequate knowledge.

In the third domain (psychiatric disorders), the median percentage of total correct answers by dental educators was 50% (Table 3). The item with the lowest rate of correct answers was "Clinical depression is rare in chronic TMD patients", with only 47% of educators having adequate knowledge. The item with the highest percentage of correct answers in this domain was "Depression can be an important etiologic factor in chronic pain", with 62% of educators demonstrating adequate knowledge.

Finally, in the fourth domain (chronic pain), the median percentage of correct answers was 56% (Table 4). Within this domain, the item with the lowest rate of correct answers was "Prescription of narcotics, as needed for pain as treatment of choice when TMD pain is severe", where only 26% of the participants had adequate knowledge. The item with the highest rate of correct answers in this domain was "Behavior modification treatments are appropriate for patients with chronic TMD pain", where 63% of dental educators agreed with experts on TMD.

Table 2: Psychophysiologic Domain.

Items	Expert Response	Right answer according Expert
1. The mechanisms of acute and chronic pain are the same.	*Disagree* 100%	79.5%
2. Biofeedback can be useful for treating TMD.	*Agree* 100%	65.2%
3. Oral parafunctional habits are often significant in the development of TMD.	*Agree* 85%	72.7%
4. Patients with TMD who clench/brux do so either during the day or at night, but not both.	*Agree* 85%	72.7%
5. Stress management is indicated for many TMD patients.	*Agree* 77%	88.2%
6. Stress is a major factor in the development of TMD.	*Agree* 100%	46.6%
7. Tension and stress increase jaw muscle EMG levels in susceptible patients.	*Disagree* 82%	76.4%
8. Progressive muscle relaxation is not an effective treatment for TMD.	*Agree* 92%	54.0%
9. Information on the daily pattern of TMD symptoms can be helpful for identifying contributing factors.	*Disagree* 92%	79.5%

Median percentage of right answers according to experts = 77.7

Table 3: Psychiatric Disorders Domain.

Items	Expert Response	Right answer according Expert
1. Clinical depression is rare in chronic TMD patients.	*Disagree* 100%	47.2%
2. Depressed mood is fair common in chronic TMD patients.	*Agree* 86%	52.8%
3. Anxiety disorders are more common in TMD patients than in the population at large.	*Agree 79%*	59.0%
4. Depression can be an important etiologic factor in chronic pain.	*Agree* 79%	62.1%

Median percentage of right answers according to experts = 50.0

Table 4: Chronic Pain Domain.

Items	Expert Response	Right answer according Expert
1. Chronic TMD patients should be advised to rest and limit their work and social activities when they are experiencing pain.	*Disagree* 85%	46.6%
2. PRN narcotics (i.e., "as needed " for pain) are a treatment of choice when TMD pain is severe.	*Disagree* 93%	25.5%
3. Antidepressants are never indicated in the management of TMD.	*Disagree* 88%	49.1%
4. An extensive history of previous treatment failures in a TMD patient is usually an indication for surgery.	*Disagree* 100%	55.3%
5. Chronic pain is a behavioral as well as a physical problem.	*Agree* 96%	36.0%
6. Although some TMD patients have psychological problems, these problems are usually unrelated to their pain.	*Disagree* 85%	37.9%
7. Difficulty with sleep is a common finding in chronic pain.	*Agree* 96%	58.4%
8. Some patients use pain as an excuse to avoid unpleasant chores.	*Agree* 89%	60.9%
9. Behavior modification treatments are appropriate for patients with chronic TMD pain.	*Agree* 88%	63.4%

Median percentage of right answers according to experts = 55.5

Table 5: Comparison by gender.

Domain	Male (n=72) Median Correct Percentage	Female (n=89) Median Correct Percentage	p*
Pathophysiology	40.2	38.7	0.564
Psychophysiologic	70.0	70.7	0.837
Chronic Pain	47.7	48.3	0.768
Psychiatric disorders	53.4	56.2	0.576
Across all domains	**52.8**	**53.1**	**0.816**

*U de Mann-Whitney

Comparison by gender (Table 5), year of graduation (Table 6), and academic level (Table 7) showed no statistically significant difference among groups ($p>0.05$).

DISCUSSION

This research shows that participating dental educators' knowledge ofTMD differs greatly from the knowledge of experts in TMD reported in the literature[1,33.] Several countrieshave made efforts to assess knowledge of TMD among dentists[1,25,31-36,38,39.] Researchers have shown that even among professionals with advanced education in TMD, there is no homogeneity of concepts on the pathophysiology of these conditions [1,34,36-38.] In Mexico there is no specialty in TMD, and patients with this condition are treated by specialists in different areas of stomatology and general dentists. This study represents the first evaluation conducted in Mexico, and clearly indicated the inconsistency of knowledge and understanding of these disorders, and consequently, the low priority that has being assigned to the field of TMD in dental education. We believe that this study highlights the need for dental educators to be prepared and teach the most updated knowledge in the field to their dental students.

Our results are consistent with data previously reported by several researchers. No difference was found by gender, academic level and year of graduation[1,35.] This is also consistent with Glaros et al [33] who claims that general dentists and specialists in areas other than the TMD do not differ in knowledge about these disorders. However, other

Table 6: Comparison by time that the educators finished the last academic level.

Domain	Under 15 years (n=63) Median Correct Percentage	15 and over years (n=98) Median Correct Percentage	p*
	Median	Median	
Pathophysiology	38.4	38.4	0.932
Psychophysiologic	66.6	77.7	0.084
Chronic Pain	55.5	49.9	0.926
Psychiatric disorders	50.0	50.0	0.856
Across all domains	**52.6**	**55.0**	**0.531**

*U de Mann-Whitney

Table 7: Comparison by academic level.

Domain	General Dentists	Dental Specialists	Dentists with MD/PHD	p*
		Median Correct Percentage		
Pathophysiology	39.4	41.4	37.5	0.369
Psychophysiologic	68.3	71.4	70.8	0.676
Chronic Pain	50.2	46.0	48.6	0.526
Psychiatric disorders	62.5	52.5	52.7	0.275
Across all domains	54.6	52.5	52.3	0.635

*Kruskal Wallis

authors have found controversial results, with specialists obtaining better scores [34].

Our data are also consistent with previously reported results on the pathophysiological domain, representing the lowest rate of 38% [1,33,35,38]. The results illustrate a poor understanding of the etiology, diagnosis, and treatment of TMD. Our research showed the greatest weakness (only 2.5% of correct answers according to the experts) is in the belief that occlusal balance is a useful option in early treatment of temporomandibular disorders. Occlusal equilibrations are still being used in Mexico for the early management of patients with TMD, despite the vast worldwide evidence against such treatment. This particular finding contrasts with values from other previously reported studies in which the percentage of agreement of general dentists and other specialists was about 30% and 26%[1,33]. On the other hand, the correct percentage, according to the experts in this research, about the statement "orthodontic treatment can prevent TMD" (21%), was slightly lower in studies by Glaros et al [33] (19%) and Le Resche [1](14%), although all results are low.

Conversely, it is noteworthy that the domain of psychophysiology (mechanisms of acute and chronic pain, biofeedback, oral parafunctional habits, stress, etc.) in the etiology of temporomandibular disorders was well recognized by the participants (78%). This highlights the understanding of most educators of the role of psychophysiological factors in the field of TMD. Previous studies [1,33] have shown correct knowledge of this domain in 50% to 90% of general dentists and other specialists, consistent with the results of our study (46% to 88%).With respect to the domain of psychiatric disorders, our study has found that depression and anxiety are recognized as determining factors in patients with TMD, with 52% to 62% of participants answering those items correctly. Studies by Le Resche[1] and Glaros[33] found success rates higher than those reported in our study. Finally, domain analysis of chronic pain denotes that participants have acceptable knowledge of said domain (55%). However, issues such as "PRN narcotics (i.e., "as needed" for pain) are a treatment of choice when TMD pain is severe" and "Chronic pain is a behavioral as well as a

physical problem", remain poorly understood by participants.

Despite the high prevalence of TMD reported in the literature, knowledge of TMD among dental educators needs improvement, as previous studies have reported [1,33,34,37,38]. The results denote a high level of variability in the domain of the pathophysiology diagnosis and treatment as well as a need to improve in the other domains. Knowledge among educators is not influenced by gender, academic level, or year of graduation. These results support the conclusion that there is an important need for improvement in the knowledge of TMD in the dental educational system in Puebla, México.

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PERCEPTION OF DISCOMFORT DURING INJECTION AND THE NEED FOR SUPPLEMENTAL ANESTHESIA IN THE INTRAOSSEOUS TECHNIQUE USING 4% ARTICAINE

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ABSTRACT

The authors conducted an experimental study to determine patient perception of discomfort during injection and the need for supplemental anesthesia using the intraosseous technique with 4% articaine with 1:100,000 epinephrine in patients with symptomatic pulpitis in mandibular molars. At different clinical sessions, researchers used 4% articaine with 1:100,000 epinephrine to apply intraosseous injection (Group 1) or inferior alveolar nerve block (Group 2). Each technique was applied in 35 patients. In each group, the need for additional anesthesia was determined and patient discomfort during injection was assessed with a Visual Analogue Scale (VAS) test. In the intraosseous group, no supplemental technique was needed in 22 patients (62.85 %), and results were similar for the inferior alveolar technique (n: 23 - 65.71%). The intraosseous technique proved to be more comfortable than the mandibular technique (18 patients – 25.7%). This study found that the use of intraosseous technique with 4% articaine shows promising results regarding patient comfort and reducing the need for additional anesthesia.

Key words: *Articaine; lidocaine; local anesthesia dental anesthesia.*

PERCEPCIÓN DE INCOMODIDAD DURANTE LA INYECCIÓN Y NECESIDAD DE ANESTESIA SUPLEMENTARIA EN ANESTESIA INTRAÓSEA USANDO ARTICAÍNA AL 4%

RESUMEN

Los autores condujeron un estudio experimental para determinar la eficacia de la técnica anestésica intraósea usando articaína al 4% con epinefrina 1:100.000, en pacientes con pulpitis aguda en molares mandibulares. En diferentes sesiones clínicas, los miembros del equipo de investigadores usaron articaína al 4% con epinefrina 1:100.000 para inducir anestesia mandibular con la técnica intraósea (Grupo 1) o con el bloqueo del nervio alveolar inferior (Grupo 2), se aplicó cada técnica en 35 pacientes con diagnóstico de pulpitis aguda en molares inferiores. En cada grupo, se determinó la necesidad de hacer anestesia complementaria y la comodidad del paciente con un test Escala Visual Analoga. Un total de 70 pacientes fueron enrolados en este estudio (35 sujetos por grupo). En el grupo de intraósea no fue necesaria la aplicación de técnicas complementarias en 22 pacientes (31.4%), resultados similares en la técnica alveolar inferior (n: 23 - 32.8%). La técnica intraósea demostró ser más cómoda al compararla con técnica mandibular (18 pacientes - 25.7%). Este estudio demostró que el uso de la técnica intraósea conarticaína al 4%, arrojó resultados prometedores en lo que a comodidad y reducción en la anestesia complementaria hace referencia.

Palabras clave: *Articaína; anestesia local; anestesia dental.*

INTRODUCTION

Pain control in dentistry is based on the use of different anesthetics. When conventional techniques do not provide adequate anesthesia, alternatives are needed. The success rate of anesthesia is variable, with 10% to 20% failure reported when inferior alveolar nerve block is used[1]. It is therefore necessary to include new techniques[1,2] as well as active principles such as 4% articaine, which supplements good anesthesia technique using an active principle which was introduced in the USA in the year 2000[3]. Articaine appears to have greater residual diffusion capacity, providing an intermediate period of anesthesia. Although it is an amide-type anesthesia, it has an additional ester group and is thus less toxic, since 90% is metabolized by plasma estearates. Its higher liposolubility enables greater diffusion than other amides in soft tissues and bone, so it has been proposed for use in infiltrations in the mandibular

molar region[3, 4].The onset of the anesthetic effect occurs within 1-6 minutes, and it lasts for about an hour, with maximum effect at 25 minutes.[5]

The use of intraosseous anesthesia dates back to 1910, when *Masselink BH* published a technique for placing the solution inside the medullar bone through a perforation in the cortical bone made with a round carbide drill.[6] Intraosseous anesthesia is indicated for removing deep caries, pulpotomy, carving abutments in living teeth, endodontic treatment of teeth with pulpitis and exodontic treatment of permanent teeth. Its advantages include providing deep pulp anesthesia which is highly effective for endodontic treatment of teeth with pulpitis. Its use is somewhat limited by the fact that it requires a special kit for application, and should therefore be planned before beginning a procedure so that it may be used immediately as a supplement during the operation.[7-10]Potential complications reported are perforation of the roots of the tooth under treatment or neighboring teeth, laceration of the inferior alveolar nerve and perforation of the maxillary sinus.[11]Different authors have recommended infiltration of the mandible with 4% articaine 4%, demonstrating that anesthesia efficacy improves when using this anesthetic solution.[12-14]

This paper reports the results of a clinical study to determine the efficacy of intraosseous anesthetic technique using 4% articaine with 1:100,000 epinephrine compared to conventional mandibular block technique in patients diagnosed with acute pulpitis in mandibular molars.

MATERIALS AND METHODS

A double-blind randomized uncontrolled study was performed to determine patients' perception of discomfort during use of the intraosseous technique, and the need for supplemental injection with 4% articaine with 1:100,000 epinephrine in cases of symptomatic pulpitis in mandibular molars. Sample size (N = 70) was obtained using *Epi Info*. As there was no quantifiable target population, non-probabilistic sampling by criterion was used and a technique assigned randomly.

Patients underwent clinical and radiographic examination and sensitivity tests to confirm the diagnosis of acute symptomatic pulpitis. Exclusion criteria were periapical lesions, temporary dentition, allergy to anesthetic solution and radiologic evaluation determining existence of anatomical structures preventing the application of the intraosseous technique. Seventy patients were divided randomly into two groups (n = 35): Group 1, intraosseous technique and Group 2, inferior alveolar nerve block. Anesthetics were applied by a standardized researcher. The need for supplemental anesthesia during root canal treatment was determined and patient perception of discomfort after the use of either the intraosseous technique or inferior alveolar never block was assessed using a Visual Analogue Scale (VAS).

Data were recorded in standardized collection instruments and tabulated on an Excel spreadsheet. Statistical analyses included descriptive statistics, frequency tables and comparison of variables. The Chi^2 test with Yates correction was applied assuming $p < 0.05$, using STATA 9® statistic software. Ethical considerations were followed as set forth in Colombia's Ministry of Health Resolution 008430 of 1993. All participants signed informed consent.

RESULTS

Demographics: 70 patients were treated: 35 in the intraosseous injection group and 35 in the inferior alveolar nerve block group. There was no statistical difference between groups for age and sex (Table 1).

Table 1: Demographic Data.

	INTRAOSSEOUS GROUP	IANB GROUP		P Value
GENDER	n (%)	n (%)	n (%)	
MALE	19 (45.71)	16 (54.29)	35 (100)	0.47
FEMALE	16 (45.71)	19 (54.29)	35 (100)	
AGE (YEARS)				
MEAN ± STD DEV	42.14±13.95	41.82±18.83	70 (100)	0.93

*STD DEV: Standard Deviation,
*IANB: Inferior Alveolar Nerve Block

Table 2: Supplementary Anesthesia.

	INTRAOSSEOUS GROUP n (%)	IANB GROUP n (%)	Total n (%)	P Value
No	22 (62,85)	23 (65,71)	64,28%	0.80
YES	13 (37,15)	12 (34,28)	35,71%	

*IANB: Inferior Alveolar Nerve Block

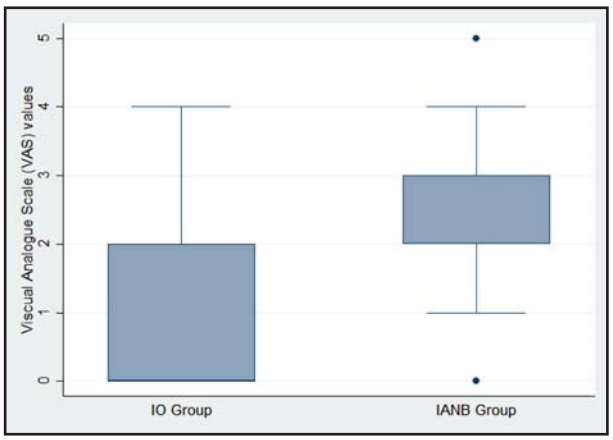

Fig. 1: Perception of injection and perforation discomfort during intraosseous injection and inferior alveolar nerve block.

Supplemental Injection: 35.71% (95% CI: 25.18 – 47.83) (n= 25) of the sample required supplemental injection (Table 2). 37.15% of the patients in the mandibular intraosseous injection group required supplemental injection compared to 34.28% in the inferior alveolar nerve block group (Table 2) (p=0.80).

Patient perception of injection and discomfort: the mean values on the visual analogue scale (VAS) were 0.94 (SD: 1.21) for Group 1 (mandibular intraosseous injection) and 2.6 (SD: 1.24) for Group 2 (alveolar nerve block group), differing statistically (p=0.00) (Fig. 1).

DISCUSSION

Pain control in mandibular molars with pulpitis is one of the main challenges during pulp removal for canal preparation, so alternatives to conventional nerve blockage are needed in order to achieve deep anesthesia in the mandibular molar zone. One of the best options is to combine an active principle with high bone diffusion, e.g. 4% articaine, using the intraosseous anesthetic technique. In this study, intraosseous technique was proposed as the primary anesthesia with 4% articaine including 1:100,000 epinephrine for mandibular molars with asymptomatic pulpitis. The intraosseous technique was found to be similar to the conventional technique with regard to the need for supplemental anesthesia: 13 (18.57%) and 12 patients (17.14 %), respectively. With regard to patient discomfort during the injection of anesthesia, as reported on the Visual Analog Scale (VAS), 18 patients (25.79%) reported no pain during the intraosseous technique, in contrast to the mandibular technique group, in which only 2 (2.85%) reported no pain. This shows that the intraosseous technique with the X Tip Kit is more comfortable than puncture using the conventional technique at the level of the inferior dental orifice. The results of this research differ from those reported by Pereira LA et al.Faltancitas 10 a la 14 inclusive[15] in a double blind study in 60 patients comparing anesthetic effectiveness and cardiovascular changes after the application of 0.9 ml of 4% articaine with 1:100,000 and 1:200,000 epinephrine using the intraosseous technique, in mandibular molars with acute pulpitis, which showed that both solutions had a highly anesthetic effect (96.8% and 93.1% respectively) with minimal cardiovascular effects. Their results are comparable with our study if we consider that for the intraosseous technique, 18.57% required supplemental anesthesia, reflecting 81.43% effectiveness. Mohammad *et al*.[16] conducted a double blind clinical trial in 100 patients diagnosed with irreversible pulpitis in maxillary teeth, who received infiltrative anesthesia with 2 ml of 4% articaine with 1:100,000 epinephrine or with 2% lidocaine with 1:80,000 epinephrine. The response was evaluated with an electronic pulp measuring device and found no significant statistical difference compared to 4% articaine with 1:100,000 epinephrine and lidocaine with 1:80,000 epinephrine, in anesthesia of maxillary teeth infiltrated in the buccal zone. They additionally

determined that only 67% of the patients expressed numbness with the mandibular technique and 33.3% required a supplemental technique. Our study found that only 13 patients required supplemental anesthesia (18.57%). Inferior alveolar nerve blockage is the technique most often used to numb the mandibular posterior teeth in root canal treatments. Jung IY *et al.*[17] report that the local anesthesia used to block the inferior alveolar nerve can provide a success rate of 70% in non-inflamed pulps, but the success rate decreases dramatically to 30% in patients with irreversible pulpitis, who are 8 times more likely than normal patients to undergo failure. These are the cases for which we highly recommend the use of intraosseous injection with 4% articaine as a primary technique, improving the success ratio during the preparation of root canals.

Nussteine*t al*[18] recommend the intraosseous technique due to a 98% success rate with lidocaine and 1:100,000 epinephrine with rapid-onset pulpal anesthesia. Our study suggests that intraosseous anesthesia can be used as a primary technique in root canal treatments in patients with symptomatic pulpitis because it provides an adequate level of anesthesia and a high degree of comfort.

The use of intraosseous technique with 4% articaine shows promising results regarding comfort and reduction of the need for additional anesthesia. The intraosseous technique is probably the best alternative when deep pulpar anesthesia is required in mandibular molars

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TEMPOROMANDIBULAR JOINT INVOLVEMENT IN RHEUMATOID ARTHRITIS PATIENTS: ASSOCIATION BETWEEN CLINICAL AND TOMOGRAPHIC DATA

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ABSTRACT

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation and synovial hyperplasia, which usually affects multiple joints. The temporomandibular joint (TMJ) becomes susceptible to the development of changes resulting from RA. The aim of this study was to evaluate the presence of TMD and degenerative bone changes in TMJ in patients diagnosed with RA (rheumatoid arthritis). The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) questionnaire was used for clinical evaluation of the TMJ and for TMD classification of 49 patients of both sexes and all ages. Individuals who had already undergone prior treatment for TMD and/or with a history of craniofacial trauma were excluded. The participants underwent cone beam computed tomography (CBCT) exams to assess possible degenerative changes in the mandibular condyle and the articular eminence. The frequencies of the changes found are presented and the possible associations between clinical and CT findings analyzed using the chi-square test. It was found that 75% of the patients had complaints of pain in the orofacial region, including arthralgia, myalgia or both. As for the diagnoses, 100% of the sample was diagnosed as RDC/TMD Group III (arthralgia, osteoarthritis or osteoarthrosis). The presence of degenerative bone changes was found in 90% of the subjects, the most prevalent being flattening (78.7%) and osteophytes (39.3%). The association test suggested a greater tendency to develop degenerative changes in asymptomatic individuals (p = 0.01). The asymptomatic nature of the involvement of the TMJ in RA can hide structural damage seen in imaging. Thus, the importance of early diagnosis and treatment to reduce structural and functional damage is emphasized.

Key words: *Rheumatoid arthritis; temporomandibular joint; cone beam computed tomography, temporomandibular disorder.*

ENVOLVIMENTO DA ARTICULAÇÃO TEMPOROMANDIBULAR EM PACIENTES COM ARTRITE REUMATOIDE – ASSOCIAÇÃO ENTRE DADOS CLÍNICOS E TOMOGRÁFICOS

RESUMO

A artrite reumatoide (AR) é uma doença sistêmica, autoimune, caracterizada por inflamação crônica e hiperplasia sinovial, que usualmente afeta múltiplas articulações. Dentre estas, a articulação temporomandibular (ATM), torna-se susceptível ao desenvolvimento de alterações. O estudo objetiva avaliar a presença de desordem temporomandibular (DTM) e alterações ósseas degenerativas da ATM (articulação temporomandibular) de pacientes diagnosticados com AR (artrite reumatóide). Como metodologia, aplicou-se o questionário Research Diagnostic Criteria for Temporomandibular Disorder (RDC/TDM) em para avaliação clínica da ATM e classificação da desordem temporomandibular em 49 pacientes de ambos os sexos e idade variável. Foram excluídos os indivíduos que já haviam realizado tratamento prévio para DTM e/ou com histórico de traumatismo crânio-facial. Posteriormente os participantes foram submetidos a exames de tomografia computadorizada de feixe cônico (TCFC) para avaliação de possíveis alterações degenerativas no côndilo mandibular e na eminência articular. Foram apresentadas as frequências das alterações encontradas e verificou-se a associação entre os achados clínico-tomográficos por meio do teste do Qui-quadrado. Após a avaliação clínica verificou-se que 75% dos pacientes possuíam queixas de dor na região orofacial, variando entre a presença de artralgia, mialgia ou ambas. Quanto aos diagnósticos, 100% da amostra apresentou diagnóstico do Grupo III do RDC/TMD (artralgia, osteoartrite ou osteoartrose). A presença de alterações ósseas degenerativas foi encontrada em 90% dos indivíduos avaliados, sendo que as mais prevalentes foram aplainamento (78,7%) e osteófito (39,3%). O teste de associação sugeriu uma maior tendência de desenvolvimento de alterações degenerativas nos indivíduos assintomáticos (p = 0.01). O caráter assintomático do envolvimento da ATM na AR pode ocultar danos estruturais vistos em imagem. Assim, ressalta-se a importância do diagnóstico e tratamento precoces para redução de danos estruturais e funcionais.

Palavras-chave: *Artrite reumatoide; articulação temporomandibular; tomografia computadorizada de feixe cônico.*

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune systemic disease characterized by chronic inflammation and synovial hyperplasia which usually affects multiple joints. Currently, it is considered the main inflammatory joint disorder, with a prevalence of 0.5-1% in the general population[1]. The incidence is higher in women, with varying proportions documented in the literature: 4:1[2], 7:1[3], and 9:1[4].

While its autoimmune pattern is emphasized, various factors contribute to development of the disease, such as genetic predisposition, micro-biological, hormonal and environmental factors[5-8]. Extensive infiltration of immune cells occurs, with high levels of inflammatory mediators in synovial tissue of peripheral joints[9,10], and the abnormalities in the structure and function of such joints appear as the primary and characteristic manifestations in these patients.

The temporomandibular joint (TMJ) becomes susceptible to the development of changes resulting from RA. However, it is rarely the first joint to be affected[5,6,11,12]. It is estimated that more than half of the patients with RA present clinical evidence of involvement of the TMJ[5,7,9,10], the most frequent being bilateral involvement.

Clinically, the involvement of the TMJ follows the same destructive path as the other joints, correlating directly with the severity and duration of the RA that has taken hold[7,13]. In the initial phase, there may be synovial hyperemia, lymphocyte infiltration, fibrinoid degeneration and pannus formation (granulation tissue). The cartilage is destroyed and the granulation tissue can be seen in the joint cavity. From that point on, there may be fibrosis and scarring, generating fibrous adhesion. Fibrous ankylosis is the rarest and final stage that the TMJ affected by RA can attain[10,14].

The most common clinical signs and symptoms in the TMJ are arthralgia, swelling, stiffness during mouth opening and upon waking, weakness of the masticatory muscles with decreased bite force, joint noises, and limited joint function[2,5-7,10,15].

Studies using cone beam computed tomography (CBCT), considered the gold standard for the analysis of degenerative bone changes of the TMJ, demonstrated that it is possible to find, in the TMJ of patients with RA, high prevalence of reduced joint space and serious erosion of cortical or subcortical bone, which can lead to destruction of the mandibular condyle[11,16]. Bone sclerosis, osteophytes and subcortical cysts may also occur[6,7]. However, the presence of changes in the TMJ of RA patients is often ignored by rheumatologists and even by patients, especially when treatment is focused on other joints of the upper extremities and/or weight-supporting extremities. These changes in the TMJ may lead to complications and disability, making its treatment and monitoring extremely important.

The aim of this study was to evaluate the presence of TMD and degenerative bone changes in TMJ in patients diagnosed with RA by performing a clinical examination and analysis of CBCT images.

MATERIAL AND METHODS

This is a cross-sectional, observational, descriptive study, approved by the Research Ethics Committee of the Federal University of Juiz de Fora (UFJF), under code number 771.869.

Being an observational study, the sample was consisted of patients recruited randomly in one year, totalizing 49 volunteers.

The study assessed patients previously diagnosed with RA treated at the Rheumatology Clinic of the University Hospital, UFJF. The diagnosis of RA was based on criteria established by the American College of Rheumatology, 1987, and ACR/EULAR 2010[17].

The patients diagnosed with RA were informed about the study and we included those who agreed to participate. All patients signed informed consent prior to participating in the research. Participation in the study was open to patients of both sexes and all ages. Exclusion criteria were pregnant women, individuals who had already undergone prior treatment for TMD and/or with a history of cranio-facial trauma.

The assessment was divided into two stages: assessment of clinical signs and symptoms in the TMJ and analysis of CBCT images. To assess the clinical signs and symptoms, we used Axis I of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD), the gold standard for clinical diagnosis of TMD[18], which enabled patients to be classified into distinct groups according to the type of disorder: Group I) muscle disorders; Group II) disk displacement, and Group III) arthralgia, osteoarthritis and osteoarthrosis. The questionnaire

was administered to all patients by a single trained and calibrated examiner according standards established by RDC/TMD[18].

CBCT images were obtained using thei-Cat Next Generation system (Imaging Sciences International, Hatfield, Pennsylvania, USA) operating at 120 kV and 3-8 mA, with the following acquisition protocol: 26.9 s exposure time, 8 cm FOV and 0.25 mm voxel. All images were obtained in the closed mouth position (maximum habitual intercuspation - MHI). For the examination of each TMJ, a representative coronal section (most central region) of the condyle was selected, obtained via the TMJ window of the ICatVision software (Imaging Sciences International, Hatfield, Pennsylvania, USA). From this coronal section, sequential parasagittal sections were generated, where the articular bone changes were evaluated.

The bone surfaces of the mandibular condyle and the articular eminence of each TMJ were classified as: healthy (no change); flattening (loss of rounded contour of surfaces); with erosion (loss of continuity in cortical bone); with osteophytes (exophytic formation growing from surfaces); and with sclerosis (any increase in cortical thickness in load-bearing areas). Each possible change could appear alone or in combination. To identify the presence of bone changes, the condition had to be observed in at least two sequential parasagittal sections[19]. This evaluation was performed by a radiologist with experience in TMJ CBCT images, without knowledge of the patient's clinical data. The evaluation was based on established criteria for tomographic analysis of the TMJ[19].

The absolute and relative frequencies of the changes found were presented and the association between clinical and CT findings were verified using the chi-square test. Calculations were performed using version 14.0 of the SPSS for Windows software, with a significance level of 5% ($p \leq 0.05$) and a 95% confidence interval.

RESULTS

Initially, 49 individuals who were undergoing clinical examination (RDC/TMD) for diagnosis of TMD were included in the study. Sixteen of them did not return to the institution for the CBCT exam, and were therefore excluded, with 33 subjects remaining in the study. All participants were female, with mean age 59.4 (± 10.3) years.

Mean RA duration was 12.04 (± 8.57) years, being 13.36 (± 8.48) years in participants with presence of degenerative bone changes, and 3.66 (± 1.24) in individuals with no changes observed in CT.

Through the combination of the RDC/TMD and the CBCT images, it was possible to observe that all subjects were diagnosed with TMD, presenting at least one diagnosis of Group III (arthralgia, osteoarthritis or osteoarthrosis).

Of the 33 participants, 8 (24.2%) presented osteoarthritis, 21 (63.6%) had osteoarthritis, and 4 (12.1%) presented arthralgia. In association with the diagnosis of Group III changes, 13 (39.3%) individuals were also diagnosed with muscular disorder (Group I), and only 1 (3.0%) participant was diagnosed with Group II TMD (disc displacement).

In relation to the degenerative bone changes in the TMJ diagnosed in CBCT examinations, there was greater involvement of the condyle than the articular eminence, particularly from the flattening of the bone surfaces. It is also worth noting that a greater number of individuals with associated conditions of degenerative bone changes was observed. Considering the combined assessment of condyle and articular eminence, only 3 (9%) of the patients presented healthy structures, 11 (33.3%) had only one isolated bone change, and most (19 / 57.5%) presented more than one bone change simultaneously. Table 1 shows the prevalence of the degenerative bone changes of the TMJ, classified into flattening, osteophytes, erosion, and sclerosis.

A total of 12 (36.36%) participants were considered as having arthralgia: four diagnosed with arthralgia alone and eight who also had osteoarthritis. Among the 30 (90.9%) individuals who had bone changes, nine (27.27%) also had arthralgia and 21 (60%) were asymptomatic. The three (9.09%) individuals without degenerative bone changes had joint pain, and no participant was free of the two conditions simultaneously. From the chi-square test, there was a significant association between the groups (p=0.01), suggesting a greater tendency to develop degenerative bone changes in asymptomatic individuals, compared to those who felt pain (Table 2).

Similarly, the association was tested between the presence of degenerative changes and the cases of myalgia (Group I). Among the patients whose images showed condylar and/or articular eminence changes, 40% had muscle pain while 60% were

Table 1: Prevalence of degenerative bone changes of the TMJ by individual.

	Healthy N (%)	Flattening N (%)	Osteophytes N (%)	Erosion N (%)	Sclerosis N (%)
Condyle	4 (12.1)	26 (78)	13 (39.3)	8 (24.2)	8 (24.2)
Eminence	17 (51.5)	12 (36.3)	0 (0)	1 (0.3)	5 (15.1)

Table 2: Association between the presence of degenerative change and arthralgia.

	Arthralgia			
	Presence	Absence	*p*-value	OR
Presence of degenerative change	9 (30%)	21 (70%)	0.01	0 (0-1.20)
Absence of degenerative change	3 (100%)	0(0)		

Table 3: Association between the presence of degenerative change and myalgia.

	Myalgia			
	Presence	Absence	*p*-value	OR
Presence of degenerative change	12 (40%)	18 (60%)	0.82	1.33 (0.08-41.99)
Absence of degenerative change	1 (33.3%)	2 (66.6%)		

asymptomatic. Among the patients with healthy bone structures, 66.6% presented no pain symptoms. As shown in Table 3, there was no significant association between the groups (p=0.82).

DISCUSSION

Rheumatoid arthritis acts as a systemic etiological factor with major impact on the development of temporomandibular disorders. However, since its clinical manifestations are often silent, the involvement of the TMJ in patients with RA has been ignored[5,20]. However, it was observed that 75% of the patients had complaints of pain in the orofacial region including arthralgia, myalgia, or both. In addition, 100% of the sample was diagnosed as RDC/TMD Group III, with impairment of the TMJ.

By means of the association between the clinical (RDC/TMD) and the CT exams −considered the gold standard for the diagnosis of degenerative bone changes associated with Group III[21]− the presence of changes was found in 90% of the participants. Flattening of the condylar surface, the bone change with the highest prevalence in the study, can be understood as a sign of adaptation and remodeling due to the person's age and/or degree of disk displacement; however the degree of flattening observed may be related to condylar destruction in these individuals[19]. The presence of osteophytes is directly related to the progression of osteoarthritis[21]. Erosion, observed in eight participants, seen as a cortical bone discontinuity, is characteristic of inflammatory degenerative processes such as in RA. While subcortical sclerosis of the condylar surface or fossa is considered a variation, especially when it comes to aging, remodeling, or association with mandibular hyperfunction as an attempt at protection. However, manifestation of generalized sclerosis of the subchondral bone is associated with joint degradation and may be a result of the presence of RA[19].

The duration of RA, especially when it is more than five years, is regarded as an aggravating factor for the involvement of the TMJ[5,22]. Degenerative bone changes such as the presence of erosion and flattening in the mandibular condyle, are usually noticed in CT scans 5 to 10 years after the onset of symptoms[4]. Corroborating this statement, in the

present study the patients with degenerative changes had an average RA duration of 13.36 (± 8.48) years, while patients with bilaterally healthy bone surfaces had an average RA duration of 3.66 (± 1.24) years. Therefore, the study suggests that the involvement of the articular bone structures occurs in more advanced stages of the disease.

Nevertheless, it is worth noting that among patients with degenerative changes, the presence of joint pain in the TMJ was not proportional to the presence of bone changes. Of the 30 participants who had imagological changes, only nine complained of pain in the joint region, and 12 reported myalgia. Results show that the presence of arthralgia in the TMJ is associated with the early stages of the disease, acting as a signal of impairment in this joint in RA patients, as patients with advanced joint damage reported no pain. Similarly, one study[7] showed images of the TMJ which revealed erosions in RA patients who had only mild discomfort or appeared free from symptoms in the TMJ, characterizing the silent manifestation in this joint. It should be considered, however, that the TMJ is structurally different from all the other joints, since it has retrodiscal tissue, rich in blood vessels, which can act as a highly efficient drainage system for common exudate. Thus, joint effusion and pain can be minimized or even extinguished in this joint[5,6]. However, the absence of pain in the RA patient does not mean the absence of TMJ impairment.

Temporomandibular disorders generally have prevalence characteristics similar to those affecting patients with RA, higher prevalence in females and the most common age group is 30 to 65 years, and thus may cause dentists to disregard the presence of RA. Similarly, due to the silent manifestation of TMJ, rheumatologists dismiss recommendations and treatments for this joint[5,20]. Thus, the quality of life and the pain levels in these patients may become compromised, making their treatment even more complex.

A multidisciplinary team should work on this complex type of patient in an attempt to reduce the physical and psychological consequences of RA[23]. In addition, case-control studies and longitudinal studies on larger samples would help understand the involvement of the TMJ and its evolution in patients with RA, with the aim of helping them to achieve better quality of life.

CONCLUSION

According to this study, arthralgia of the TMJ in patients with RA acts as a signal of the onset of an active inflammatory process, in an attempt at protection, since individuals with advanced lesions did not complain of pain. The asymptomatic nature of the involvement of the TMJ in RA can hide structural damage seen in imaging. Therefore, the importance is emphasized of early diagnosis and treatment to reduce structural and functional damage.

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FUNGAL CONTAMINATION AND DISINFECTION OF DENTAL CHAIRS, TERESINA, PIAUI, BRAZIL

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ABSTRACT

The aim of this study was to analyze fungal contamination on dental chairs at the clinic of a Higher Education Institution in Teresina-PI, Brazil, and to evaluate the effectiveness of different disinfectants: 70% alcohol and 1% sodium hypochlorite. We selected the five sites with most contact between patient and chair: headrest, backrest, armrests, seat and foot rest. Samples were collected from these sites on 14 chairs and inoculated in agar Sabouraud culture medium containing chloramphenicol. Pathogenic fungi were isolated from all sampling sites on the chairs. Highest frequencies were found on footrest, followed in decreasing order by seat, backrest, armrests and headrest. Fourteen species of filamentous fungi were identified, belonging to the genera Alternaria, Aspergillus, Cladosporium, Curvularia, Drechslera, Fusarium, Penicillium and Paecillomyces. After sampling, seven chairs were disinfected with 70% alcohol and seven with 1% sodium hypochlorite, and samples were taken again using the same procedure. No fungal growth was detected following disinfection with sodium hypochlorite, which was clearly more effective than alcohol, after which there was still fungal growth. This study highlights the need for the biosafety protocol to include cleaning and disinfection of dental chairs with 1% sodium hypochlorite after each attendance in order to prevent cross-infection.

Key words: *Dental equipment; fungi; cross infection; disinfection.*

CONTAMINAÇÃO DE FUNGOS E DESINFECÇÃO EM CADEIRAS ODONTOLOGICAS, TERESINA, PIAUÍ, BRASIL

RESUMO

O objetivo deste estudo foi analisar contaminação fúngica em cadeiras odontológicas na clínica de uma Instituição de Educação Superior em Teresina-PI, Brasil, e avaliar a efetividade de diferentes desinfetantes: álcool 70% e hipoclorito de sódio 1%. Nós selecionamos os cinco locais com maior contato entre o paciente e a cadeira: encosto da cabeça, das costas, dos braços, assento e encosto dos pés. As amostras foram coletadas destes locais das 14 cadeiras e inoculadas em meio de cultura agarSabouraud contendo cloranfenicol. Fungos patogênicos foram isolados de todos os locais de amostragem das cadeiras. As frequências mais altas foram encontradas no encosto dos pés, seguido em ordem decrescente pelo assento, encosto das costas, dos braços e encosto da cabeça. Quatorze espécies de fungos filamentoso foram identificados, pertencente aos gêneros Alternaria, Aspergillus, Cladosporium, Curvularia, Drechslera, Fusarium, Penicillium e Paecillomyces. Após a coleta, sete cadeiras foram desinfetadas com álcool 70% e sete com hipoclorito de sódio 1%, e as amostras foram colhidas novamente usando o mesmo procedimento. Não foi detectado crescimento fúngico após desinfecção com hipoclorito de sódio, que foi claramente mais efetivo que o álcool, do qual ainda houve crescimento fúngico.Este estudo destaca a necessidade da inclusão no protocolo de biossegurança a limpeza e desinfecção das cadeiras odontológicas com o hipoclorito 1% após cada atendimento, a fim de prevenir infecções cruzadas.

Palavras chave: *Equipamentos odontológicos; fungos; infecção cruzada; desinfecção.*

INTRODUCTION

Dental offices harbor various forms of contamination by microorganisms, including fungi, exposing dentists to the risk of infections transmitted in various ways, including direct contact with infectious lesions and secretions; indirect contact by means of microorganisms on instruments, equipment and rigid surfaces; aerosols, and interpersonal contact [1,2].

High-speed handpieces can spread fungi by creating aerosols that settle on surfaces and equipment such as chair, spotlight, dental equipment and

instruments. This is particularly risky when air-conditioning further aggravates the occurrence of fungi and the area is not cleaned regularly [3,4].

Biosafety in dentistry includes a set of actions to protect staff and patients in a clinical setting, including ergonomic practices, control of physical and chemical hazards, use of specific protocols, and appropriate handling of products and equipment, in addition to the sterilization process, disinfection, antisepsis, use of barriers and individual protective equipment (IPE).[5]

Controlling disease transmission is a challenge to dentists, since the oral cavity contains microorganisms, many of which can cause diseases ranging from a mild cold to pneumonia and from tuberculosis to herpes. These infections may be transmitted by contaminated saliva, blood, fluid from the gingival sulcus, and even the patient's respiratory secretions [2,6]. This study analyzed fungal contamination on dental chairs at the clinic in a Higher Education Institution in Teresina-PI, and evaluated the effectiveness of two disinfecting agents: 70% alcohol and 1% sodium hypochlorite.

MATERIAL AND METHODS

This was a quantitative and descriptive study performed at the Research Laboratory of the University Center UNINOVAFAPI in Teresina-PI from January to November 2015, after authorization from the management.

Samples were collected from 14 (93.3%) of the dental chairs by rubbing sterile swabs soaked in

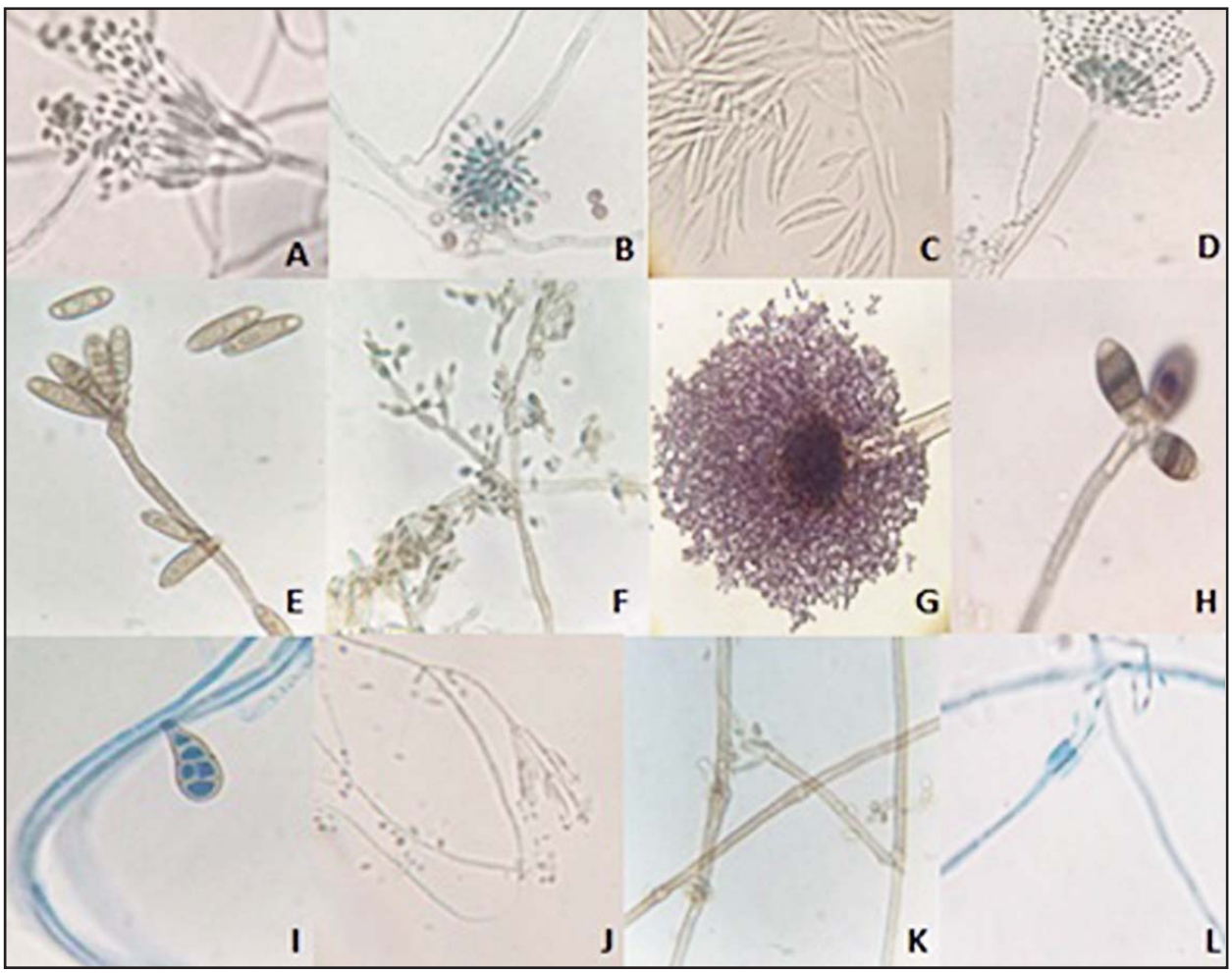

Fig. 1: Micromorphology of filamentous fungi isolated in dental chairs of a Higher Education Institution (HEI):
A- *Penicillium oxalicum;* B- *Aspergillus flavus;* C- *Fusarium aff. incarnatum;* D- *Aspergillus carnus;* E- *Drechslera biseptata;*
F- *Cladosporium cladosporioides;* G- *Aspergillus niger;* H- *Curvularia clavata;* I- *Curvularia brachyspora;*
J- *Penicillium piceum;* K- *Cladosporium oxysporum;* L- *Penicillium decumbens.*

Table 1: Presence of fungal species per dental chair site at a clinic at a Higher Education Institution (HEI).

Fungal Species	Regions				
	Headrest	Backrest	Seat	Armrest	Footrest
Fusarium aff. *Incarnatum*		•			
Paecilomyces variotii		•			
Curvularia lunata		•		•	•
Alternaria infectoria	•	•			•
Aspergillus niger	•	•	•	•	•
Aspergillus flavus			•		•
Cladosporium oxysporum			•		
Cladosporium cladosporioides	•		•		
Drechslera biseptata			•		
Penicillium decumbens			•	•	
Penicillium oxalicum				•	
Aspergillus carneus				•	
Penicillium piceum					
Curvularia brachyspora					

Source: Research Laboratory UNINOVAFAPI.

saline on the five sites selected: headrest, backrest, armrests, seat and footrest.

From each sample, 100µL were inoculated in Petri dishes containing Sabouraud Dextrose agar (Difco™) culture medium plus chloramphenicol (0.05g/L) and incubated at room temperature to enable the growth of the fungal colonies.

After growth of the colonies, microcultures were mounted for recognition of species using identification keys previously described[7,8].

The same procedure was used to test the disinfectant efficacy of 70% ethanol and 1% sodium hypochlorite. For statistical analysis, the chi-square test for goodness of fit was applied with a significance level (α) of 5%.

RESULTS

We identified 14 species belonging to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Penicillium* and *Paecillomyces* (Fig.1).

Aspergillus niger was the most frequent species, being found at all sites of the dental chair. *Curvularia clavata* and *Alternaria infectoria* were identified from three site seach (Table 1).

The region with the highest level of contamination was footrest, with 50.0%, followed by seat (42.9%), backrest and armrest (35.7% each), and finally headrest (21.4%) (Table 2).

Following disinfection with 70% alcohol, fungal growth occurred in the samples from all sites, whereas following disinfection with 1% hypochlorite, no fungal growth was observed.

DISCUSSION

Dental chairs can become contaminated by fungi in several ways: via air conditioning, failure to apply biosafety standards, lack of internal protocols and/or failure to comply with them, and invasive procedures performed during treatment.

Table 2: Frequency of fungi on the dental chair, Teresina – PI, 2015.

Dental chair sites	Number of fungal species	%*
Headrest	3	21.4
Backrest	5	35.7
Seat	6	42.9
Armrest	5	35.7
Footrest	7	50.0
Total found	14	100.0

(*) amounts to more than 100% because more than one type of fungus may be found per location.

Table 3: Disease occurrence caused by fungi isolated from dental chairs from a HEI in Teresina - PI.

Fungal Species	Diseases[7]
Penicillium piceum Raper&Fennell	Fungaemia
Penicillium oxalicum Currie & Thom	Eye infection
Aspergillus flavus Link: Fr.	Allergic bronchial aspergillosis, lung infections, ear infections, sinus infections and endocarditis.
Fusarium aff. *incarnatum* (Rob.) Sacc.	Endocarditis
Paecilomyces variotii Bain.	Pneumonia, sinusitis, endophthalmitis
Drechslera biseptata (Sac. & Roum.) Richardson & Fraser	Sinusitis
Cladosporium cladosporioides (Fres.) (de Vries)	Lung and cutaneous infection
Cladosporium oxysporum Berk. & Curt.	Keratitis and cutaneous infection
Aspergillus carneus (v. Tiegh.) Blochwitz	Lung infections
Curvularia clavata Jain	Sinusitis, cerebritis
Alternaria infectoria Simmons	Phaeohyphomycosis
Penicillium decumbens Thom	Fungaemia
Aspergillus niger v. Tiegh.	Pulmonary aspergillosis, endophthalmitis, endocarditis, peritonitis, onychomycosis, cutaneous infections
Curvularia brachyspora Boedijn	Keratitis and cutaneous infection

Source: De Hoog et al. 2000.

All the species found in this study are pathogenic and may cause infections ranging from cutaneous to systemic infections. *Aspergillus niger*, the most common species in this study, causes pulmonary aspergillosis, endophthalmitis, endocarditis, peritonitis and cutaneous infections[7]. The table 3 shows the main diseases caused by the species found in this study.

There is high risk of cross-infection in the dental office as a result of the invasive procedures performed and environmental contamination by biological agents or bioaerosols which may come from internal or external air conditioning, furniture and carpets [2, 3, 9].

Mobin and Salmito found the following pathogenic fungal genera in air conditioners of an Intensive Care Unit (ICU): *Acremonium, Aspergillus, Paecillomyces, Penicillium, Trichoderma, Cladosporium, Curvularia* and *Nigrospora.* Several other studies show that air conditioners provide a favorable environment for fungal growth, thus actively contributing to worsening health status of patients, whether in ICUs or dental offices [10].

Sousa and Fortuna studied air conditioned dental offices in Bahia, and in all cases found the highest frequency of microorganisms such as *Aspergillus* and *Fusarium* near the spittoon [3]. This might be due to high-speed drills spreading microorganisms through aerosols, which may become attached to equipment and accessories as well as remaining airborne.

A study comparing microbial load between a dental clinic and a non-dental public area found that the risk in the dental clinic may be greater than in the public area due to the diversity of microorganisms, susceptibility of the host and exposure time. In addition to bacteria, they found fungi, as *Aspergillus niger* and *Aspergillus flavus* [11].

Another study reports high number of aerosol and bioaerosol particles during dental procedures and a variety of microorganisms present in a dental office, including the fungal genera *Penicillium, Cladosporium* and *Alternaria* [9].

Internal air conditioning also contributes to proliferation and spread of fungi in dental offices. Re-circulating air and airing the room after each patient is recommended to prevent accumulation of fungal spores [10].

After disinfecting the chairs with 70% alcohol, there was still fungal growth from the samples, showing the method to be less effective than use of 1% sodium hypochlorite. Although alcohol is a

commonly used disinfectant, the results of this and of other studies suggest that it is ineffective against fungi [12].

Regarding the action of alcohol on bacteria, several studies claim that its effect is more bacteriostatic than bactericidal. Moreover, there are studies that claim that the use of 70% alcohol is inappropriate for removing saliva layers on instruments, and it has been demonstrated that even water is more appropriate than alcohol for removing blood and organic matter [13].

The biosafety protocol should therefore include cleaning and disinfecting chairs with 1% hypochlorite after each patient in order to prevent cross-infection.

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MUCOEPIDERMOID CARCINOMA OF THE SALIVARY GLANDS. A RETROSPECTIVE STUDY OF 51 CASES AND REVIEW OF THE LITERATURE

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ABSTRACT

The aim of this study is to present the casuistic of mucoepidermoid carcinoma of salivary glands in patients diagnosed at "Dr. Eduardo Cáceres Graziani" National Institute for Neoplastic Diseases, Lima, Perú.

From January 2002 to December 2012, 51 cases were diagnosed as mucoepidermoid carcinoma. The number of female patients was higher, with 28 cases (54.9%), and regarding age distribution, 33.3% of the patients were under 30 years old. Pain was one of the main symptoms, and 74.5% of the mucoepidermoid carcinomas were located in the parotid gland.

It is concluded that epidemiology regarding age and gender of the 51 cases analyzed was in the same range as other studies, and that most cases were located in major salivary glands, in agreement with reports on other populations. Other characteristics showed a homogeneous distribution.

Key words: *Salivary gland neoplasms; mucoepidermoid tumor, epidemiology, neoplasms.*

CARCINOMA MUCOEPIDERMOIDE DE GLÁNDULAS SALIVALES. ESTUDIO RETROSPECTIVO DE 51 CASOS Y REVISIÓN DE LA LITERATURA

RESUMEN

El propósito de este estudio es presentar la casuística del carcinoma mucoepidermoide de glándulas salivales de pacientes diagnosticados en el Instituto Nacional de Enfermedades Neoplásicas "Dr. Eduardo Cáceres Graziani" Lima, Perú, desde el 2002 hasta el 2012.

Realizamos un estudio retrospectivo en el cual fueron incluidos sujetos con diagnóstico primario de carcinoma mucoepidermoide en glándulas salivales. Entre enero de 2002 y diciembre de 2012, se registraron 51 casos. El número de pacientes de sexo femenino fue mayor, con 28 casos (54,9%) y con respecto a la distribución por edades, el 33,3% de los pacientes eran menores de 30 años de edad. El dolor fue uno de los síntomas principales. El 74,5% de los carcinomas mucoepidermoides se localizaron en la glándula parótida.

De los hallazgos obtenidos se concluye principalmente que en lo que respecta a la distribución epidemiológica de edad y género de los 51 casos analizados estas variaron en el mismo rango de otros estudios. También se distingue que el mayor número de casos estuvieron localizados en glándulas salivales mayores, dato en concordancia con otras poblaciones reportadas. Las demás características presentaron una distribución homogénea.

Palabras clave: *Neoplasias de las glándulas salivales; carcinoma mucoepidermoide; epidemiología; neoplasias.*

INTRODUCTION

Neoplasms of the major and minor salivary glands are a challenge for clinicians and histopathologists because they are infrequent and have a wide range of histological, clinical, epidemiological and developmental characteristics[1].

In salivary glands, the most frequent benign tumor is pleomorphic adenoma and the most frequent malignant tumor is mucoepidermoid carcinoma (MC), according to the Armed Forces Institute of Pathology (AFIP) in Washington[2].

MC is defined as a malignant epithelial neoplasm of the salivary glands caused by proliferation of secretory cells, formed by a variable proportion of mucous, epidermoid, intermediate, columnar and clear cells, often with a cystic component. Its

biological behavior is related to the histologic tumor grade (low, intermediate or high)[2-4].

MC was studied, described and reported for the first time by Stewart, Foote and Becker in 1945 with regard to tumors with double metaplasia or double constitution, epidermoid cells and mucous producing cells[5].

Etiology

Little is known regarding the etiological agents of salivary gland neoplasms. Apparently, low-dose radiotherapy used in benign disorders such as acne or obstructive lesions of the lymphoid tissue in the oral cavity or nasopharynx is the main factor involved in the genesis of these tumors. They have not been associated to radiotherapy for the treatment of malignant neoplasms, suggesting that irradiation at high doses is a lower risk factor than irradiation at low doses. Local trauma has also been implicated in the genesis of MC in minor salivary glands[6].

Epidemiology

Neoplasms of the salivary glands are rare, accounting for less than 2%[7] of all human neoplasms and about 3% of head and neck tumors[7,8]. Malignant tumors of the salivary glands are infrequent and account for about 3% of all malignant neoplasms of head and neck[8].

Malignant neoplasms originating in minor salivary glands are less than 25% of all salivary neoplasms[9,10], and most of the tumors arising in minor salivary glands are malignant[9,11].

MC is the most common malignant neoplasm in major and minor salivary glands[12], accounting fornearly 30% of all malignant neoplasms of the salivary glands. Approximately half the MCs occur in the major salivary glands, with 80% in the parotid gland, 8 to 13% in the submaxillary gland and 2 to 4% in the sublingual gland[13]. Central mucoepidermoid tumors located in the jaws are a recognized entity. Browand and Waldron reported 9 cases and examined the 41 previously published cases[14].

Some authors report that MC is evenly distributed between sexes[15], but most authors report that glandular MC is more frequent in females, with a female:male ratio of 2:1[6,16] or 3:2[17,18].

The onset occurs between the 2nd and 8th decades of life, and it is the most frequent malignant tumor in persons under 20 year of age, in whom there is a predilection for the hard palate. There is also clear predilection for white race[6,16].

Clinical manifestation

Between 70% and 80% of neoplasms in general of the salivary glands are located in the parotid, while the palate is the most common site for neoplasms of minor salivary glands[2]. Similarly, just over 70% of MCs are located in major glands, with the parotid being the most frequent site[19], (with almost half the cases) (Fig. 1 and 2), followed by the submandibular gland and sublingual gland[3]. MCs represent 23% of

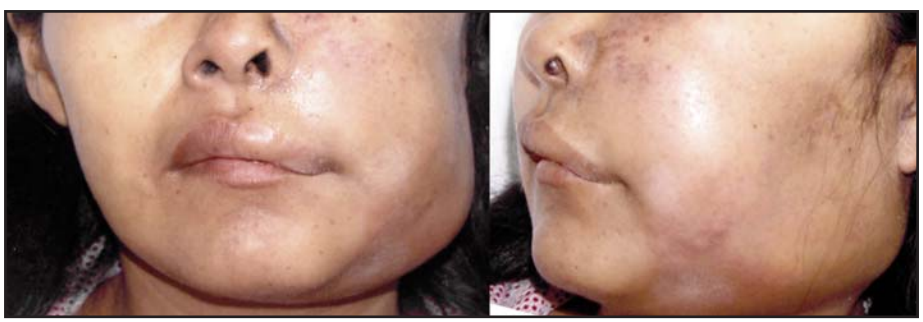

Fig. 1: Female patient, 30 years old. Tumorous lesion in left parotid gland. Patient complained of pain and paresthesia. One year of illness with slow, progressive growth. Diagnosed with high-grade MC and died six months later, after radiation treatment.

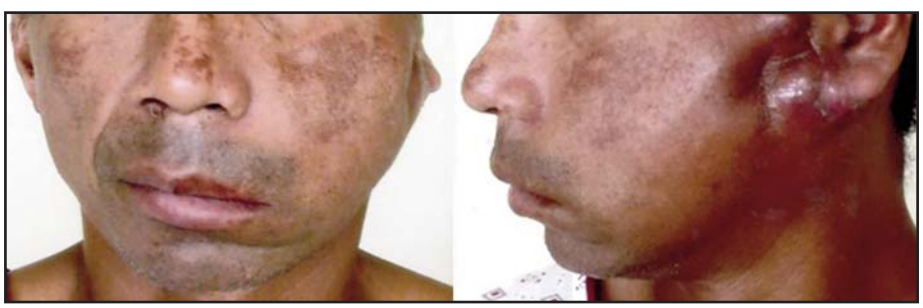

Fig. 2: Male patient, 48 years old. Tumorous lesion in left parotid gland. Patient complained of paresthesia. Diagnosed with high grade MC. Seventeen months after surgery and radiotherapy, presented pulmonary metastasis and died 5 months later.

all tumors in the minor salivary glands[20], with the palate being the most commonly affected site (with almost half the cases in hard palate, the second most frequent location after parotid gland), and they are found less frequently in other minor salivary glands such as those of the tongue, floor of the mouth, gum, lips and cheek mucosa, the ectopic salivary tissue being another location, though exceptional.

Clinically, in major glands, MCs appear as solitary, asymptomatic enlargements of the parotid body or pole, or of the submaxillary region[8]. In minor glands they appear as blue or purplish-red fluctuating masses with a smooth surface, and are often clinically mistaken for mucoceles[21].

The average latency period is one year, but may vary widely. MCs sometimes grow rapidly after a period of quiescence. In high-grade lesions there is onset of pain, facial paralysis and fixation in neighboring structures[8].

Discovery in minor glands is sometimes accidental during a routine mouth exploration. Rapidly growing tumors are very unusual. The surface is usually smooth, but if it is ulcerated, it is usually associated to more aggressive forms. If it is located at the base of the tongue it may cause dysphagia, while if it affects the bone it may cause insensitivity in teeth[6].

Histology

According to the bicellular theory, salivary gland tumors are formed by[22]:

a) Duct luminal cells and/or acinar cells plus myoepithelial cells,
b) Duct luminal cells or acinar cells, or
c) Myoepithelial cells only.

Histopathology thus identifies mucosecretory, epidermoid, intermediate[8,17,18,21], columnar or clear cells, proliferating alone or in different combinations, in a cystic or solid pattern[8,21].

In addition to this cell pattern, there is extracellular matrix produced by the neoplastic myoepithelial cells, collagen, elastic fibers, glycoproteins, glycosaminoglycans and proteoglycans[23].

The anatomical pathology shows they are partially encapsulated, with full encapsulation being very rare[8,21].

Most authors consider three grades of differentiation[8,21] depending on intracystic component, neural invasion, necrosis, mitotic activity and pleomorphism[24]. The histological grade in this study was classified following AFIP[25], published in the World Health Organization Classification of tumours[26] and shown in Table 1 and Figs. 3, 4 and 5.

Diagnosis

The differential diagnoses considered are: necrotizing sialometaplasia (of the palate), mucocele, inverted papilloma or cystadenoma, cystadenocarcinoma, primary or metastatic epidermoid carcinoma, and low-grade polymorphic adenocarcinoma[17].

The most useful and popular techniques for evaluating neoplasms of the salivary glands are currently Computerized Axial Tomography (CAT) and biopsy by needle aspiration. The latter, however, should be taken with precaution because its diagnostic precision is 60-80%, sometimes requiring a repetition of the aspiration or surgical biopsy. The histological information from the biopsy and delimitation of the lesion provided by CAT enable appropriate medical and surgical management[27].

Treatment

The treatment depends on the location, clinical aspect and histopathological grade. Low-grade MC is generally treated with surgery only, while high-grade tumors also require radiation and dissection of neck lymph nodes[28]. The management of intermediate-grade tumors is controversial, perhaps reflecting the controversy in tumor classification[29].

For tumors in accessory salivary glands, surgical excision is recommended, leaving 1-2 cm safety margins around the tumor[8].

For treatment of tumors in major glands, gland exeresis is recommended (superficial or deep parotidectomy, submaxilectomy)[8].

Cervical lymph notes are removed when lymph nodes are clinically affected (higher incidence of lymph node metastasis in submaxillary location)[8].

Radiotherapy after surgical treatment with tumor-free margins does not increase local control, as it does in advanced cases, cases with infiltrated resection margins and cases located at the base of the tongue[30]. Response to chemotherapy is low (best results have been achieved with methotrexate y cisplatin)[31].

Standard treatment for the main types of salivary gland cancer is surgical resection with adjuvant radiation to reduce failure rates[32-34] and even though

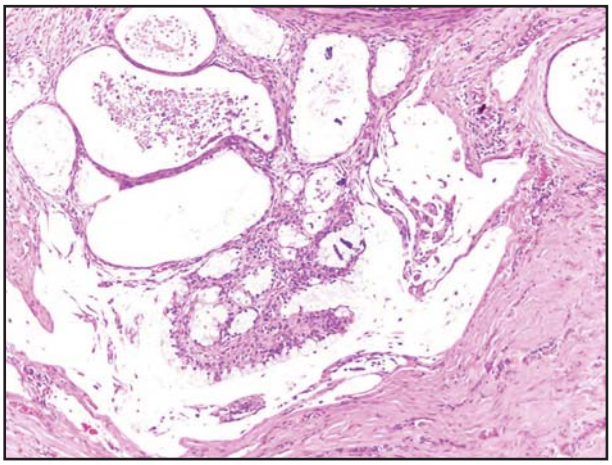

Fig. 3: MC, low grade. Histological image showing cystic structures lined with mucinous, squamous and intermediate cells with slightly atypical nuclei and low mitotic activity. Adjacent connective tissue without perineural invasion (H&E Orig. Mag. 100x).

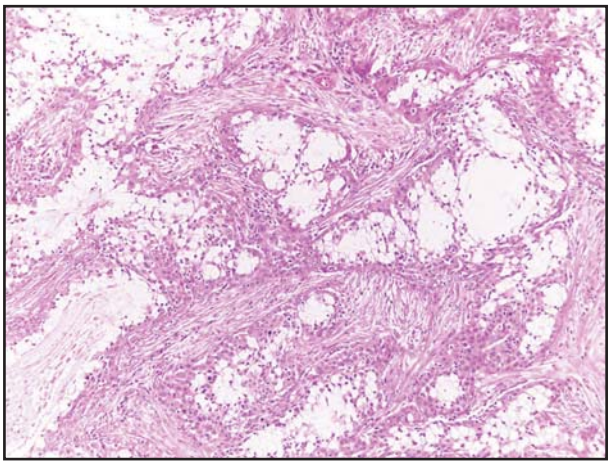

Fig. 4: MC, intermediate grade. Histological image showing tumor with small cystic structures, predominance of intermediate cells, with mucosecretory and epidermoid cells (H&E Orig. Mag. 100x).

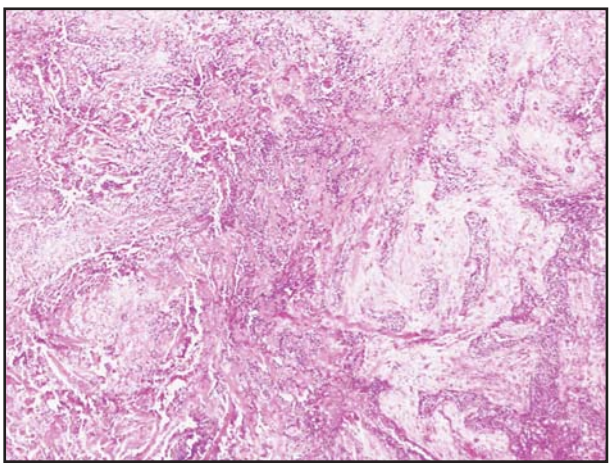

Fig. 5: MC, high grade. Histological image showing proliferations of solid islands of epidermoid and intermediate cells with few mucinous cells, with atypical nuclei and greater mitotic activity (H&E Orig. Mag. 100x).

Table 1: Histopathologic features used in grading MC. World Health Organization[26].

Histopathologic feature	Point value
Intracystic component <20%	2
Neural invasion	2
Mitosis (≥4/10 HPFs*)	3
Necrosis	3
Anaplasia	4
Tumor grade	**Point score**
Low grade	0 - 4
Intermediate grade	5 - 6
High grade	≥7

*HPF, High-Power Fields

the role of adjuvant chemotherapy has not been proven, it has been used to treat distant metastasis and non-excisable disease, and to reduce the effects of local/regional recurrence[35].

Adjuvant radiotherapy has been shown to have an advantage in survival of patients with tumors larger than 4 cm, but little benefit for patients with small tumors, suggesting that, together with the margins involved, tumors larger than 4 cm are an absolute indication for post-surgical radiotherapy[32,36-38].

Further progress in therapy is needed to improve the outcomes of histologic high-grade disease[39].

Prognosis

Prognosis depends on clinical stage[40], (related to anatomical location[41,42]), histologic grade (patients with low or intermediate-grade tumors have local control and favorable survival rates[39]) and treatment[40].

Variable prognostic factors have also been reported, such as neural invasion, vascular invasion, and local or distant metastasis, which are associated to clinical outcome for patients[25].

Other authors mention the same factors that influence survival, such as histologic grade, clinical stage, paralysis of facial nerve and lymph node

metastasis, in addition to location (worse prognosis in submaxillary), age and sex (better prognosis in young people and females). Mucoepidermoid tumor is one of the few tumors of salivary glands in which flow cytometry has high prognostic value, with greater survival in patients with diploid DNA patterns[6].

Regarding histologic grade, it has been reported that at the same histopathological grade, tumors in the parotid gland have better prognosis than tumors in the submandibular gland[43]. Prognosis is better for low-grade lesions and for high-grade lesions when they arein stage I or II[32,36,37,44]. MC prognosis appears to depend largely on tumor grade, with reports of 5-year MC survival rates of 92%-100% for low grade, 62-92% for intermediate grade and 0-43% for high grade tumors[25]. MCs with high grade malignancy have a 50% probability of presenting metastasis[17].

Some molecular factors of malignant cells also influence survival rate, e.g. p27, which is a highly favorable prognostic factor for MC, whereas Ki67 is not identified as a survival indicator[45].

Even with complete resection, there is still a substantial risk of local recurrence (16%-27%) and distant metastasis (13%-26%)[46,47].

The aim of this study is to present the epidemiology of salivary gland MC through thecasuistic in patients diagnosed at the "Dr. Eduardo Cáceres Graziani" National Institute for Neoplastic Diseases from 2002 to 2012.

MATERIALS AND METHODS

This is an observational, descriptive, cross-sectional, retrospective study which included subjects with primary diagnosis of mucoepidermoid carcinoma of the salivary glands diagnosed at "Dr. Eduardo Caceres Graziani" National Institute for Neoplastic Diseases, Lima, Peru, from January 2002 to December 2012. Clinical records with incomplete data were excluded.

The study was approved by the institutional review board (protocol: INEN 13:27) and conducted in accordance with the World Medical Association Declaration of Helsinki on medical research protocols and ethics.

The collected data were transferred to a Microsoft Excel program, on which the table was prepared.

The analysis was performed using Windows XP® Operative System (Washington, USA), with the assistance of the statistical program SPSS (Statistical Package for Social Sciences) version 20.0 for Windows (SPSS, Chicago, IL, USA).

All values found in the different statistical tests were considered with a significance level of 0.05 ($p<0.05$).

RESULTS

Between January 2002 to December 2012, 349 new cases were found of malignant tumors in salivary glands, of which 4.61% (51)cases were mucoepidermoid carcinoma.

All cases were diagnosed by pathological anatomy studies.

Table 2 shows the distribution of the different characteristics evaluated according to sex. There are more female patients (28 cases; 54.9%; female:male ratio 1.2:1). Age range was 12 to 88 years, with 64.71% of patients aged 60 years or less. In most cases (62.75%), tumor size was smaller or equal to 4 cm at the time of the first visit, and the most frequent location was parotid gland (74.51%). No significant difference was found for these characteristics. Among the signs and symptoms evaluated, the presence of paresthesia was statistically significant ($p=0.038$). Considering the TNM (T: primary tumor, N: cervical nodules, and M: distant metastasis) staging system for salivary gland carcinoma, cases were most frequently stage T2 (43.14%), which are tumors measuring 2-4 cm with no macroscopic extracapsular extension, N0 (86.27%), which are lesions without metastasis in regional nodules and M0 (68.63%), which is when there are tumors without distant metastasis. With regard to clinical stage, the largest number of cases was in clinical stage IV C (31.37%), followed by clinical stage II (29.41%). Regarding histological grade, cases were distributed evenly between low and high grade, with 39.22% each, and regarding treatment, surgery combined with radiotherapy was the treatment of choice, with 42.18% of the cases.

DISCUSSION

Most epidemiological studies of MC have found females to be affected more frequently than males, e.g Goode *et al*. studied 234 MC of the major salivary glands, finding that females accounted for 51.3%[43]. In our study, females accounted for 54.9%, a value similar to those reported by Villavicencio *et al*.[48] and Schwarz *et al*.[49]. Some authors report a 3:2 female:male ratio[16,18], and McHugh *et al*. report a

Table 2: Clinical pathological features of subjects with MC of the salivary glands.

	Clinical characteristics		Female		Male		*p* Value
			n	%	n	%	
Age (months)	Average		43.107		49.04		
	Minimum		18		12		
	Maximum		79		88		
	Up to 60 years		20	60.6	13	39.4	0.268
	Over 60 years		8	44.4	10	55.6	
Size (cm)	Average		4.25	3.56	5.17	2.96	
	Minimum		1.5		1		
	Maximum		20		12		
	Up to 4 cm		20	62.5	12	37.5	0.157
	Over 4 cm		8	42.1	11	57.9	
Location	Parotid gland		18	47.4	20	52.6	0.165
	Submaxillary gland		5	71.4	2	28.6	
	Minor glands		5	83.3	1	16.7	
Signs	Ulcer	No	24	57.1	18	42.9	0.487
		Yes	4	44.4	5	55.6	
	Pain	No	17	54.8	14	45.2	0.991
		Yes	11	55.0	9	45.0	
	Paresthesia	No	25	62.5	15	37.5	0.038*
		Yes	3	27.3	8	72.7	
	Dysphagia	No	27	56.3	21	43.7	0.439
		Yes	1	33.3	2	66.7	
	Trismus	No	27	54.0	23	46.0	0.360
		Yes	1	100	0	00.0	
TNM (T)	T1		7	70.0	3	30.0	0.235
	T2		13	59.1	9	40.9	
	T3		6	50.0	6	50.0	
	T4A		1	16.7	5	83.3	
	T4B		1	100	0	00.0	
TNM (N)	N0		25	56.8	19	43.2	0.224
	N1		3	75.0	1	25.0	
	N2A		0	00.0	2	100	
	N2B		0	00.0	1	100	
TNM (M)	M0		20	57.1	15	42.9	0.634
	M1		8	50.0	8	50.0	
Clinical stage	I		4	66.7	2	33.3	0.051
	II		9	60.0	6	40.0	
	III		4	57.1	3	42.9	
	IV A		1	50.0	1	50.0	
	IV B		2	40.0	3	60.0	
	IV C		8	50.0	8	50.0	
Histological grade	Low grade		14	70.0	6	30.0	0.068
	Intermediate grade		7	63.3	4	36.4	
	High grade		7	35.0	13	65.0	
Treatment	Sg		10	66.7	5	33.3	0.504
	Sg + Rt		10	47.6	11	52.4	
	Sg + Rt + Cht		0	0.0	1	100	
	Rt + Cht		1	100	0	0.0	
	Rt		7	53.8	6	46.2	

* Statistically significant value
TNM: Classification of Malignant Salivary Gland Tumor Stages.
Cht Chemotherapy; Rt Radiotherapy; Sg Surgery.

1:1.2 male:female ratio[39]. Another recent study is consistent with the above, finding 67% of the cases in females[29].

MC is the most common malignant neoplasm of the salivary glands in persons over 40 years of age[13,50]. Chomette *et al*. report that the onset of MC according to age is variable and may occur between the 2nd and 8th decades of life[6], in agreement with our study, in which on set ranged from the 2nd to 9th decades of life. Villavicencio *et al*. report onset from the 4th to 6th decades of life[48], and Rapidis *et al*. report 3rd to 9th decades of life[17]. Average age in all the aforementioned studies was the 6th decade of life, thus, average age of on set is reported as 52.2 years by Chomette *et al*.[6]; 54.3 years by Villavicencio *et al*.[48], and 56.7 years by Rapidis *et al*.[17]. These values are similar to the average age found in our study, which was 45.78 years. It is worth noting that this study found 8 cases of patients aged 19 years or under over the 10-year study period (0.8 cases per year), similar to findings reported by Techavichit *et al*. of 14 cases over a period of 15 years (0.9 cases per year) in patients aged 19 years or under[51].

Villavicencio *et al*. report that the clinical size of the primary tumor varies according to the anatomical site affected; with median size 8 cm (4 to 10 cm) in submaxillary gland and 3 cm (1 to 5 cm) in minor salivary glands[48]. Rapidis *et al*. found no statistically significant difference between tumor size according to site[17]. Katabi *et al*. report that tumor size was 0.5 to 9 cm, with an average value of 1.77 cm[29]. In our study, the average size was 4.67 cm.

MC is a neoplasm that usually affects major salivary glands, although 10% can arise in minor or accessory salivary glands in the head and neck area[24], including maxillary sinus, nasopharynx, nasal cavity, oropharynx, larynx and trachea. Our study was consistent with this observation, finding 74.51% of the cases in parotid gland, 13.73% in submaxillary gland and 11.76% in minor glands. Ellis *et al*. report that 74.5% of neoplasms of the salivary glands in general are found in the parotid gland[2], with a percentage identical to the one found in our study. The location of MC in our study was also consistent with Villavicencio *et al*. who reported 74.5% in major salivary glands (32 cases in parotid and 3 cases in submaxillary gland)[48]; Védrine *et al*., who reported 18 cases, of which 77.78% (14 cases) were in parotid gland, 11.11% (2 cases) in submaxillary gland and 11.11% (2 cases) in minor salivary glands[7]; Schwarz *et al*. who reported 60% (24 cases) in parotid gland, 10% (4 cases) in submandibular gland and 30% (12 cases) in minor glands[49], and Rapidis *et al*. who reported 18 cases, of which 66.6% (12 cases) were in major salivary glands (10 in parotid and 2 in submaxillary)[17]. Regarding location in minor salivary glands, Triantafillidou *et al*. report 56.25% in palate[40], while in our study, 100% of the minor salivary gland cases were located in the palate.

Signs and symptoms are varied, with none appearing as characteristic or pathognomonic, but the most outstanding in our study was pain, in agreement with Villavicencio *et al*., who report pain in 55.3% of their 47 cases[48]. It is worth noting that it is statistically significant (p=0.038) that most patients do not report paresthesia as a symptom at the time of the initial clinical examination.

Regarding the clinical stage of MC, Schwarz *et al*. report declining percentages for increasing stages, with 40% (16 cases) Type I, 30% (12 cases) Type II, 15% (6 cases) Type III and 15% (6 cases) type IV[49]. In our study, the highest number of cases was found for the last stage, IV (16 cases), followed by stage II (15 cases).

In most epidemiological studies presenting data on the histological grade of the disease, the distribution of the number of MC cases generally declines as the level of histologic grade rises, i.e. there is an inverse relationship. Rapidi *et al*. report a decline according to histological grade, with 9 tumors classified as low grade, 5 as intermediate grade and 4 as high grade[17]; Katabi *et al*. report 90.38% (47 cases) low grade, 3.85% (2 cases) intermediate grade and 5.77% (3 cases) high grade[29]; Védrine *et al*. report 68.75% (11 cases) low grade, 18.75% (3 cases) intermediate grade and 12.5% (2 cases) high grade[7], and Schwarz *et al*. report 67.5% (27 cases) low grade, 7.5% (3 cases) intermediate grade and 25% (10 cases) high grade[49]. Our study found equal numbers of cases for low grade and high grade with 39.22% (20 cases) each, and 21.56% (11 cases) intermediate grade. This higher percentage of cases with high histological grades may be due to the health culture in the Peruvian population, since people tend to consult a professional only when the disease is at an advanced stage.

Treatment is fairly well established, with most papers reporting surgical treatment and adjuvant radiation therapy, similarly to our casuistic, which shows that 41.18% was treated thus, which depends on the histologic grade. This is also reflected by follow-up case research such as Chen *et al*., which follows a series of 61 cases, all of which were treated with surgery and postoperative radiation, for over 10 years[52], and other papers which report casuistic of surgical treatment only, such as Rapidis *et al*., who treated 9 cases of parotid gland tumors with superficial or total parotidectomy, all with radical dissection of neck or suprahyoid dissection, with one parotid gland case being treated with hemimandibulectomy and suprahyoid dissection[17].

Based on the abovementioned findings, other studies were conducted which, in addition to the epidemiological contribution regarding this salivary gland disease, present the respective patient follow-up analyses for survival.

It is concluded that the epidemiology regarding age and gender of the 51 cases analyzed was in the same range as other studies, and that most cases were located in major salivary glands, in agreement with reports on other populations. Other characteristics showed a homogeneous distribution.

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MICROBIOLOGICAL CONTAMINATION IN DIGITAL RADIOGRAPHY: EVALUATION AT THE RADIOLOGY CLINIC OF AN EDUCATIONAL INSTITUTION

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ABSTRACT
The aim of this study was to evaluate the contamination rate of intra- and extraoral digital X-ray equipment in a dental radiology clinic at a public educational institution. Samples were collected on three different days, at two times in the day: in the morning, before attending patients, and at the end of the day, after appointment hours and before cleaning and disinfection procedures. Samples were collected from the periapical X-ray machine (tube head, positioning device, control panel and activator button), the panoramic X-ray machine (temporal support, bite block, control panel and activator button), the intraoral digital system (sensor), and the digital system computers (keyboard and mouse). The samples were seeded in different culture media, incubated, and colony-forming units (CFU/mL) counted. Biochemical tests were performed for suspected colonies of Staphylococcus, Streptococcus and Gram-negative bacilli (GNB). Fungi were visually differentiated into filamentous fungi and yeasts. The results indicated the growth of fungi and Staphylococcus from all sampling locations. GNB growth was observed from all sites sampled from the intraoral X-ray equipment. On the panoramic unit, GNB growth was observed in samples from activator button, keyboard and mouse. In general, a higher number of CFU/mL was present before use. It can be concluded that more stringent protocols are needed to control infection and prevent X-ray exams from acting as vehicle for cross contamination.

Key words: *Radiology; digital radiography; infection control; microorganisms; bacteria; fungi.*

CONTAMINAÇÃO MICROBIOLÓGICA EM RADIOGRAFIAS DIGITAIS: AVALIAÇÃO DA CLÍNICA DE RADIOLOGIA DE UMA INSTITUIÇÃO DE ENSINO

RESUMO
O objetivo neste estudo foi avaliar o índice de contaminação dos equipamentos de radiografias digitais intra e extrabucais da clínica de radiologia odontológica de uma instituição pública de ensino. As amostras foram coletadas em três dias distintos, em dois momentos: pela manhã, antes dos atendimentos clínicos, e ao final do dia, após os atendimentos e antes dos procedimentos de limpeza e desinfecção. As amostras foram coletadas do aparelho de raios X periapical (cabeçote, braço articular, painel de controle e botão disparador); do aparelho de raios X panorâmico (apoio temporal, bloco de mordida, painel de controle e botão disparador); do sistema digital intrabucal (sensor); dos computadores dos sistemas digitais (teclado e-mouse). As amostras foram semeadas em diferentes meios de cultura e, após incubação, foram contadas as unidades formadoras de colônia (UFC/mL). Testes bioquímicos foram realizados para colônias suspeitas de Staphylococcus, Streptococcus e bastonetes Gram negativos(BGN). Os fungos foram diferenciados visualmente em fungos filamentosos e leveduras. Os resultados indicaram crescimento de fungos e Staphylococcus em todos os locais amostrados. Em relação aos BGN, houve crescimento em todos os locais coletados do equipamento radiográfico intrabucal. No aparelho panorâmico, houve crescimento de BGN apenas no botão disparador, teclado e mouse. De maneira geral, houve maior número de UFC/mL antes do uso. Pode se concluir que é necessário implantar protocolos mais rigorosos de controle de infecção na prática radiológica, evitando que a obtenção de exames radiográficos seja um veículo para contaminação cruzada na FO/UFJF.

Palavras-chave: *Radiologia; Radiografia Digital; Controle de Infecção; Microorganismos; Bactérias; Fungos.*

INTRODUCTION

Radiographic exams area complementary tool for diagnosing major diseases of the oral cavity. Their increasing popularity has encouraged private colleges and clinics to adopt them[1]. The advantages of digital radiography include reduced radiation exposure, rapid image acquisition, easy digital storage, and electronic image transmission, as well

as elimination of darkroom requirements and the possibility of image quality enhancement, such as changes in contrast and density[2-4].

In digital radiography, image receptors can be of two types: 1) solid electronic sensors such as charge-coupled devices (CCD) and complementary metal oxide semiconductor (CMOS) sensors, which produce images directly; and 2) photostimulable storage phosphor (PSP) plates that must be scanned to convert latent images into visible images[3,5-8].

Digital radiography has many advantages over conventional techniques that use radiographic film as the image receiver. However, since the receptor is reused a number of times, as opposed to the single use of radiographic film, digital systems clearly increase problems associated with infection control[1]. The sensors or phosphor plates cannot be sterilized; thus, it is important to use effective physical barriers to protect them[7,9-11]. At dental schools, the sensors are handled by a large number of operators and used ona great many patients, further challenging the effectiveness of infection control[3]. In addition to the sensors, care should be taken with the other equipment involved with digital systems, such as the computer, particularly the keyboard and the mouse, and the actual X-ray machine, whether intraoral or extraoral.

Given the need to evaluate the infection control protocol followed at the Radiology Clinic (School of Dentistry), the aim of this study was to evaluate microbial contamination on the surfaces of the intraoral and extraoral digital radiology equipment used at the Radiology Clinic to identify the different groups of microorganisms present on them, and to compare contamination rates before and after clinic office hours.

MATERIALS AND METHODS

Samples were collected at the Radiology Clinic over three non-consecutive random days at two different times: in the morning, before attending patients, and at the end of the day, after appointment hours and before cleaning and disinfection procedures.

Samples were collected from the following surfaces of the radiological equipment and accessories: 1) digital intraoral system: periapical X-ray machine (tube head, positioning device, control panel and activator button), digital system (sensor), and computer (mouse and keyboard); 2) digital extraoral system: panoramic X-ray machine (temporal support, bite block, control panel and activator button) and computer (mouse and keyboard).

Material was collected using a sterile swab soaked in a test tube containing 10 mL sterile saline solution (0.85% NaCl). After using the swab, it was stored in the same tube until samples were processed. Sterilized 5x5 cm^2 templates were used to standardize the sampling area. The total area sampled per device was 125 cm^2. For the activator button, sensor and bite block, where the surface is extremely small, the entire area was used for collection. Throughout the collection process, personal protective equipment was used to avoid cross contamination.

The collected samples were subjected to serial dilution in which 1 mL aliquots were transferred to tubes containing 9 mL sterile saline solution (0.85% NaCl), and so on, until 10^{-2} dilution. After homogenization, 100 µL aliquots were dispensed using a pipette and seeded with a Drigalski loop on the surfaces of plates, in duplicate. The following culture media were used: mannitol agar (HiMedia, Mumbai, India), which is selective for the isolation of staphylococci; MacConkey agar (HiMedia, Mumbai, India), which is selective for the isolation of enterobacteria and other Gram-negative non-fermenting bacilli; Sabouraud dextrose agar (HiMedia, Mumbai, India), which is used for the isolation of molds and yeasts; and sheep blood agar (HiMedia, Mumbai, India), which is used for the recovery of streptococci/enterococci, staphylococci and Gram-negative bacilli.

The seeded plates with mannitol agar and MacConkey agar culture media were incubated at 35 ± 2°C in a bacteriological incubator for 24 to 48 hours. The plates containing the blood agar medium were subjected to microaerophilic conditions with a candle in an augmented atmosphere of 5% CO_2 at 35 ± 2°C for 24 to 72 hours. The Sabouraud agar plates were incubated at 27 ± 2°C for up to seven days. After the incubation period, the colonies were counted. The dilution formula was applied to calculate colony-forming units per milliliter (CFU/mL) of each sampling site and averaged, considering the duplication.

Colonies suggestive of staphylococci that were identified in mannitol agar and blood agar were subcultured in tryptone soya agar (TSA; HiMedia, Mumbai, India) to remove selective agent interference for Gram stain test confirmation and for biochemical analyses. In the Gram stain test, the

observation of grouped Gram-positive cocci is confirmatory of the group. The catalase test was used to differentiate staphylococci from streptococci/ enterococci because staphylococci behave as catalase-positive bacteria. The coagulase and DNase tests served to differentiate *Staphylococcus aureus* from other species. At all stages, reference cultures were used as positive controls (*Staphylococcus aureus* ATCC 33591 and *Staphylococcus epidermidis* ATCC 12228). Novobiocin antimicrobial susceptibility testing was used to differentiate between *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*; the positive control for sensitivity in this test was the presence of a halo equal to or greater than 15 mm. To avoid overestimating the results, the count of staphylococcal colonies on blood agar was disregarded for the locations that presented growth in both mannitol agar and blood agar in the same collection.

Colonies suggestive of streptococci/enterococci in the blood agar medium were subcultured in TSA for confirmation by the Gram stain test, in which Gram-positive cocci are observed in long chains, while for colonies suggestive of enterococci, Gram-positive cocci are observed in short chains or in pairs. To differentiate staphylococci from streptococci/ enterococci, the colonies subcultured in TSA were subjected to the catalase test, where catalase-negative colonies were selected. These colonies were further subcultured in blood agar and incubated under microaerophilic conditions for 24 hours to observe the hemolysis pattern.

Colonies suggestive of Gram-negative bacilli (GNB) in MacConkey agar and blood agar were subcultured in TSA and submitted to the Gram stain test. To differentiate between glucose-fermenting and non-fermenting bacilli, the fermentation test was conducted in a glucose broth. Oxidase test strips were used to differentiate between oxidase-positive and oxidase-negative colonies. Tests were also run using the Bactray system (Laborclin, Pinhais, Paraná, Brazil) for the biochemical identification of GNB via pH changes, substrate hydrolysis, and metabolic production. To avoid overestimating the results, the count of GNB colonies in blood agar was disregarded for the locations that presented growth in both MacConkey agar and blood agar in the same collection.

Fungi growing in Sabouraud agar were visually differentiated into filamentous fungi and yeasts. No biochemical test was performed.

The Kolmogorov-Smirnov test was used to verify that the values were normally distributed. The Wilcoxon test allowed a comparison of the CFU counts before and after the clinic's activities. We used SPSS version 13.0 (SPSS Inc, Chicago, USA). The significance level was 5% ($p \leq 0.05$).

RESULTS

Altogether, 78 samples were collected from the Radiology Clinic, with half the samples (three per site) collected before the clinical procedures and the other half (three per site) collected after clinic office hours and before cleaning and disinfection procedures.

Tables 1 and 2 present the data describing the quantitative distribution of the different microorganisms from the collection sites for intraoral and extraoral digital systems, respectively. Fungi were present at all collection sites, both before and after clinic office hours. Staphylococci were also highly prevalent, being absent only in a few specific collection periods. In contrast, GNB were found less frequently, although they were present at all the collection sites before the use of the intraoral equipment.

In assessing the growth of *Staphylococcus*/ *Streptococcus* in the intraoral digital X-ray system, all the colonies tested positive for catalase, i.e., no growth of *Streptococcus*/ *enterobacteria* was observed. After coagulase and DNase testing, *Staphylococcus aureus* was not isolated. Regarding the novobiocin susceptibility profile, 23.81% were resistant and were identified as *Staphylococcus saprophyticus*, whereas 76.19% were sensitive and characterized as *Staphylococcus epidermidis*. In the extraoral digital X-ray system, only one sample, which was collected after the use of the control panel, had a negative result for catalase and was visible as Gram-positive cocci in long chains, which are representative of *Streptococcus*. This colony was subcultured in blood agar and incubated under microaerophilic conditions for 24 hours, at which point a partial hemolysis pattern was observed that was alpha-hemolytic. The rest of the colonies, which were catalase-positive bacteria, were subjected to coagulase and DNase testing. Only one colony, which was obtained from the activator button before use, showed positive results for both tests and was identified as *S. aureus*. Regarding the novobiocin susceptibility profile, 31.34% were

Table 1: Descriptive data (mean, median, minimum, and maximum) of the quantitative distribution of the various microorganisms in the respective collection locations in the intraoral digital system. Data are presented in CFU/mL.

	BEFORE USE											
	Cocci*				GNB				Fungi			
	Mean	Med	Min	Max	Mean	Med	Min	Max	Mean	Med	Min	Max
Tube head	0.03	0	0	0.10	0.88	0.40	0	2.25	8.46	0.35	0.05	25
Positioning device	0.16	0.05	0.05	0.40	15.66	0	0	47	0.51	0.05	0	1.50
Control panel	0.01	0	0	0.05	0.16	0.10	0.05	0.35	2.16	0.90	0.05	5.55
Activator button	0	0	0	0	0.15	0	0	0.45	0.06	0.10	0	0.10
Sensor	0.01	0	0	0.05	0.05	0	0	0.15	0.11	0.10	0	0.25
Mouse	0.05	0.05	0.05	0.05	0.01	0	0	0.05	5.90	0.15	0.05	17.50
Keyboard	0.10	0.05	0	0.25	0.01	0	0	0.05	0.78	0.20	0.15	2
	AFTER USE											
	Cocci*				GNB				Fungi			
	Mean	Med	Min	Max	Mean	Med	Min	Max	Mean	Med	Min	Max
Tube head	0.01	0	0	0.05	0	0	0	0	0.06	0.05	0	0.15
Positioning device	0.10	0.10	0	0.20	0	0	0	0	0.03	0	0	0.10
Control panel	0.01	0	0	0.05	0	0	0	0	0.01	0	0	0.05
Activator button	0.01	0	0	0.05	0	0	0	0	0.03	0	0	0.10
Sensor	0.08	0.10	0.05	0.10	0.06	0	0	0.20	0.03	0	0	0.10
Mouse	0	0	0	0	0	0	0	0	0.05	0.05	0	0.10
Keyboard	0.01	0	0	0.05	0.46	0	0	1.40	0.13	0.10	0	0.30

*Cocci: *Staphylococcus*

resistant and identified as *S. saprophyticus*, whereas 64.93% were sensitive and characterized as *S. epidermidis* (Fig. 1).

In the evaluation of GNB growth on the intraoral digital X-ray system, all samples were negative for the glucose fermentation test. Approximately 30% of the samples were oxidase-positive GNB, and 70% were oxidase-negative GNB. Tests were conducted using the Bactray system, which identified *Proteus mirabilis* on the positioning device, control panel, tube head and keyboard before use and on the sensor and keyboard after use in 52.94% of the samples, with a 100% probability. *Pseudomonas pseudoalcaligenes* was identified on the tube head, positioning device, control panel, button and mouse before use in 29.41% of the samples, with a probability of 83.18%. *Acinetobacterbaumannii/ calcoaceticus* was identified on the sensor, positioning device and tube head before use in 17.65% of the samples, with a probability of 65.5%. Regarding the glucose fermentation test for the colonies identified on the extraoral digital X-ray system, 23.08% of the samples were fermentation-positive bacteria, and 76.92% were fermentation-negative bacteria. Only one oxidase-positive sample was identified. The Bactray system identified *Acinetobacter baumannii/ calcoaceticus* on the keyboard before use and on the keyboard and activator button after use in 53.85% of the samples, with a probability of 65.5%. *Serratiaplymuthica* was identified on the keyboard and mouse before use in 23.08% of the samples, with a probability of 62.84%. *Klebsiellarhinoscleromatis* was identified on the keyboard after use in 15.38% of the samples, with a probability of 30%. *Burkholderiapseudomallei* was identified on the keyboard after use in 7.69% of the samples, with a probability of 48.78% (Fig. 1).

In the evaluation of fungal growth fromthe intraoral digital X-ray system, 3.64% filamentous fungi and

Table 2: Descriptive data (mean, median, minimum, and maximum) of the quantitative distribution of the various microorganisms in the respective collection locations in the extraoral digital system. Data are presented in CFU/mL.

	BEFORE USE											
	Cocci*				GNB				Fungi			
	Mean	Med	Min	Max	Mean	Med	Min	Max	Mean	Med	Min	Max
Temporal support	0.08	0	0	0.25	0	0	0	0	0.06	0	0	0.20
Biteblock	0	0	0	0	0	0	0	0	0.16	0.20	0.10	0.20
Control panel	0	0	0	0	0	0	0	0	0.85	0	0	2.55
Activator button	16.56	0	0	49.70	0	0	0	0	0.23	0.30	0	0.40
Mouse	0.03	0	0	0.10	0.01	0	0	0.05	0.18	0.25	0	0.30
Keyboard	0.46	0.40	0.15	0.85	0.20	0.15	0	0.45	0.36	0.40	0.20	0.50
	AFTER USE											
	Cocci*				GNB				Fungi			
	Mean	Med	Min	Max	Mean	Med	Min	Max	Mean	Med	Min	Max
Temporal support	0.10	0.15	0	0.15	0	0	0	0	1.83	0.05	0	5.45
Biteblock	0.06	0.05	0	0.15	0	0	0	0	0.96	0.30	0	2.60
Control panel	4.11	0.05	0	12.30	0	0	0	0	0.06	0.05	0	0.15
Activator button	0.15	0.15	0	0.30	2.41	0	0	7.25	0.45	0.30	0.05	1
Mouse	0.10	0.10	0.05	0.15	0	0	0	0	0.01	0	0	0.05
Keyboard	0.20	0.05	0.05	0.50	0.36	0.30	0	0.80	0.16	0.15	0.05	0.30

*Cocci: *Staphylococcus e Streptococcus*

Table 3: Comparison of CFU/mL counts (median) before and after the clinical procedures, for the intraoral digital X-ray system.

	Cocci*			GNB			Fungi		
	Before	After	p	Before	After	p	Before	After	p
Tube head	0	0	0.17	0.40	0	0.06	0.35	0.05	0.02*
Positioning device	0.05	0.10	0.46	0	0	0.17	0.05	0	0.46
Control panel	0	0	1.00	0.10	0	0.10	0.90	0	0.02*
Activator button	0	0	0.17	0	0	0.17	0.10	0	0.17
Sensor	0	0.10	0.06	0	0	0.46	0.10	0	0.17
Mouse	0.05	0	1.00	0	0	0.17	0.15	0.05	0.02*
Keyboard	0.05	0	0.06	0	0	0.17	0.20	0.10	0.02*

*Cocci: *Staphylococcus*
p: significance level
$p \leq 0.05$: statistically significant difference by Wilcoxon test.

96.36% yeasts were distinguished visually. For the extraoral system, 68.74% filamentous fungi and 31.26% yeasts were distinguished (Fig. 1).

The results of the comparisons between CFU/mL counts before and after the clinical procedures are shown in Tables 3 and 4. For the intraoral digital system, the Wilcoxon test indicated significant differences only for the fungi collected from the tube head, control panel, mouse and keyboard. For the extraoral digital system, the CFU/mL counts

Table 4: Comparison of CFU/mL counts (median) before and after the clinical procedures, for the extraoral digital X-ray system.

	Cocci*			GNB			Fungi		
	Before	After	p	Before	After	p	Before	After	p
Temporal support	0	0.15	0.91	0	0	1.00	0	0.05	0.06
Biteblock	0	0.05	0.06	0	0	1.00	0.2	0.3	0.46
Control panel	0	0.05	0.06	0	0	1.00	0	0.05	0.46
Activator button	0	0.15	0.91	0	0	0.17	0.3	0.3	0.06
Mouse	0	0.1	0.06	0	0	0.17	0.25	0	0.06
Keyboard	0.4	0.05	0.11	0.15	0.3	0.46	0.4	0.15	0.02*

*Cocci: Staphylococcus e Streptococcus
p: significance level
p≤0.05: statistically significant difference by Wilcoxon test.

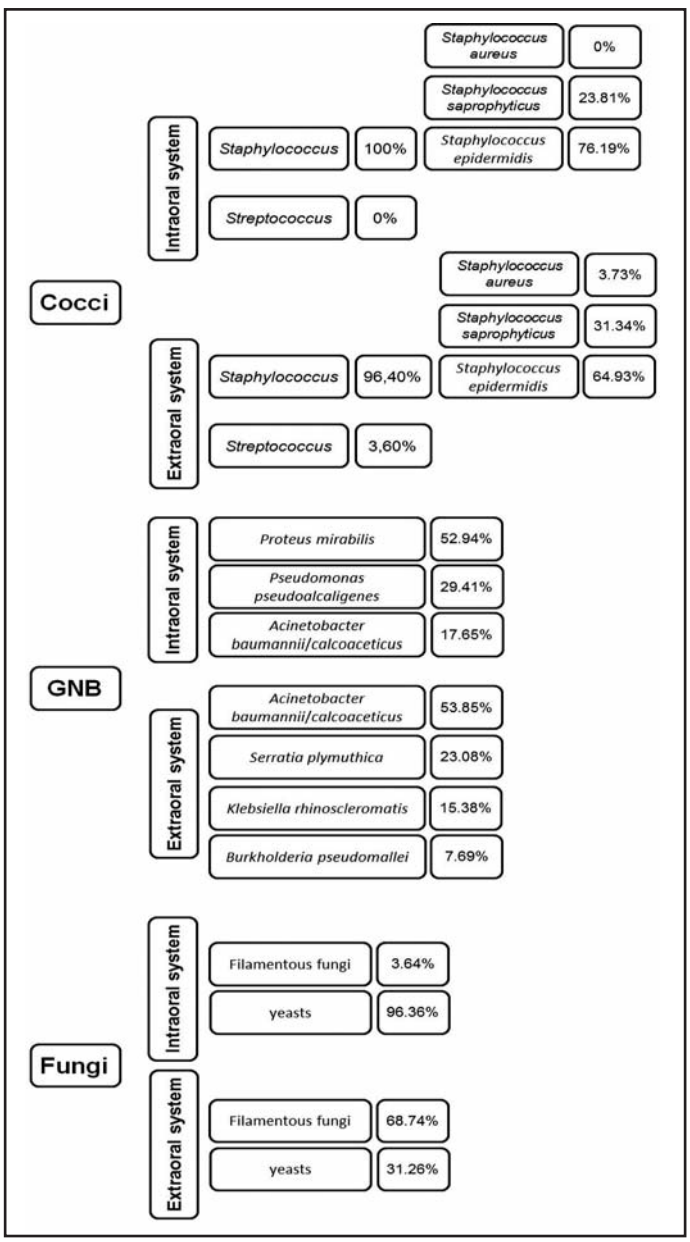

Fig. 1: Isolation percentage of the different microorganisms detected.

before and after clinical procedures showed a significant difference only for the fungi collected from the keyboard. Notably, the number of colonies was always higher before clinical procedures.

DISCUSSION

Digital X-ray is a major advance in radiographic imaging and is increasingly being adopted by dental professionals. It is already used even in undergraduate courses in dentistry and has great potential to aid diagnosis and treatment procedures and to facilitate image storage, transfer and retrieval[4,6,8,12]. However, the substitution of films by sensors or phosphor plates does not free the digital equipment from cross-contamination; on the contrary, the reuse of the image receptors increases the importance of infection control.

Bacterial, viral, and fungal infections pose a significant hazard in dental practice, and biosafety principles must be followed to prevent contamination of equipment, operators and patients. Dental radiography, which is normally overlooked because it is not routinely associated with needles, sharp instruments and waste blood, has recently become a concern because infectious

diseases may be transmitted by contamination of the materials and equipment used to obtain intraoral and extraoral radiographs.

The protocols suggested as universal precautions for infection control emphasize the use of barriers or the chemical disinfection of surfaces. However, the barriers used for radiographic equipment should allow visual access to the control panel and should not interfere with the configuration of the machine. Moreover, disinfecting surfaces with chemicals is not common practice with electronic equipment[13]. Digital X-ray sensors, which cannot be sterilized by heat, should be covered with protective barriers[2,14] However, some studies have shown that receptors for digital images are potential sources of contamination even when surrounded by a plastic barrier[10,11,15,16].

In dental schools, problems with infection control are more critical because of the large number of patients and radiographic equipment operators involved. In addition, the inexperience of most operators (undergraduate students) can further complicate the installation of strict infection control protocols. Some authors have suggested gas sterilization with ethylene oxide for digital sensors at dental schools[3]. Additionally, for installations that do not have access to gas sterilization systems, a disinfectant with adequate cleaning effectiveness and short contact time, such as propanol-ethanol or a chlorine-based disinfectant[3] can be considered.

Although the literature describes the actions that should be taken to control infection before, during and after radiographic film exposure and emphasizes that aseptic practices applied in dental radiography are relatively simple and inexpensive, a great amount of negligence by operators still occurs[17,18]. Qudeimat et al reported that changing gloves between patients was rare among dentists and dental assistants in a teaching center in Jordan[19]. McCarthy and MacDonald, who compared infection control practices among general dentists and specialist groups, indicated that better compliance with infection control practices is needed in both groups[20]. These situations indicate that cross-infection control issues do not arouse interest among dental surgeons or that there is a deficiency in continuing education regarding how to avoid cross-infection in dental practice. Biosafety in radiology should be more clearly required by the authoritative bodies, not only for students in dental programs but also for professionals in public and private practices[21].

Many microorganisms are related to dental practice. Even though some of them belong the normal microbiota, they should be considered opportunistic pathogens which can cause human infections constituting a disease when introduced into unprotected sites or in situations of immune system deficiency, depending on their virulence factors. The upper respiratory tract and oral cavity are colonized by numerous microorganisms such as *Staphylococcus*, *Streptococcus*, *Porphyromonas*, *Prevotella*, *Haemophilus*, *Eubacterium*, *Enterobacteriaceae*, *Actinomyces*, *Acinetobacter* and *Candida*[22,23].

The genus *Staphylococcus* is composed of diverse species that can be found in human clinical samples. These microorganisms are important pathogens and, in general, involved in various diseases mediated by toxins, such as skin diseases, bacteremia, endocarditis, pneumonia, osteomyelitis, septic arthritis, urinary tract infections and opportunistic infections. The species most commonly associated with human diseases are *Staphylococcus aureus* – the most virulent and best-known member of the genus– and *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis* and *Staphylococcus saprophyticus*[22]. The results of the present study indicated growth of *Staphylococcus* from all sampling sites, and *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* were also identified. The rate of contamination with *Staphylococcus* is of concern because, although these bacteria are members of the normal microbiota of the skin and mucous membranes of humans, they also cause suppuration, abscess formation, various pyogenic infections, and even fatal septicemia[22].

Streptococci and enterococci are Gram-positive, catalase-negative, and oxidase-negative bacteria that usually grow into pairs and in chains. Among the important streptococci is *Streptococcus pyogenes*, which is responsible for suppurative afflictions such as pharyngitis, soft tissue infections, and streptococcal toxic shock syndrome and for non-suppurative disorders such as rheumatic fever and glomerulonephritis. *Streptococcus agalactiae* is responsible for diseases in newborns and infections in pregnant women. Several β-hemolytic streptococci

are important. *Streptococcus viridans* is responsible for abscess formation in deep tissue; *Streptococcus anginus*, for septicemia in neutropenic patients; *Streptococcus mitis* and *Streptococcus salivarius*, for subacute endocarditis; *Streptococcus mutans*, for tooth decay; *Streptococcus bovis*, for cancer of the gastrointestinal tract; *Streptococcus pneumoniae*, for pneumonia, meningitis, and bacteremia[22]. According to Jorge[23], only four groups are considered oral streptococci, i.e., the *mutans*, *anginosus*, *mitis*, and *salivarius* groups. In this study, streptococcal contamination of the extraoral X-ray device control panel proved the existence of oral cavity microorganisms on the radiographic equipment; these microorganisms are often present in oral mucosa and in saliva[22].

The bacilli or Gram-negative rods belonging to the *Enterobacteriaceae* family are widely distributed in nature; found in soil, water, vegetables, and the intestinal tracts of humans and animals[24]. They cause a variety of diseases in humans, including 30% to 35% of all bacteremia, over 70% of urinary tract infections, and a large number of intestinal infections. The *Salmonella* serotype *Typhi*, and *Shigella* and *Yersinia pestis* species are always associated with human disease, while other species such as *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus mirabilis* are commensal members of the normal microbiota that can cause opportunistic infections[22]. *Escherichia coli*, *Klebsiellaspp*and *Yersinia* have been detected in the human oral cavity and subgingival samples[23]. Gram-negative non-fermenting bacilli are a non-spore-forming aerobic group that do not use carbohydrates as an energy source or degrade them by fermentative pathways, and have special requirements for growth[24]. They constitute a group of opportunistic pathogens of plants, animals and human beings, and their taxonomic classification has undergone changes in recent years. However, most of the clinically significant isolates are members of five genera: *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, *Actinobacter* and *Moraxella*[22]. In the present study, GNB growth was observed from all locations sampled from the intraoral radiographic equipment. On the panoramic unit, GNB growth was observed only from the activator button, keyboard and mouse. The following species were identified: *Proteus mirabilis*, *Pseudomonas pseudoalcaligenes*, *Acinetobacterbaumannii*/ *calcoaceticus*, *Serratiaplymuthica*, *Klebsiellarhinoscleromatis* and *Burkholderiapseudomallei*.

Approximately 80,000 identified species of fungi exist; however, less than 400 are medically important, and fewer than 50 species cause approximately 90% of fungal infections. Most pathogenic fungi are exogenous, and their natural habitats are water, soil and organic waste. Candidiasis and dermatophytosis are the fungal infections (mycoses) of highest incidence, caused by fungi of the resident microbiota which are highly adapted to survival in the human host[25]. In the present study, although fungi were the most prevalent microorganisms, with growth in all samples and from all sampling sites, they were only identified visually as filamentous fungi or yeasts.

Although no study in the literature has determined the maximum amount of microorganisms allowed in a clinical dental setting, the goal is to reduce this amount as much as possible to promote health and prevent disease. Importantly, we can never be certain whether we are dealing with an immunocompromised patient during a radiographic examination. For these patients, a low number of microorganisms can cause disease, or normal microbiota can cause opportunistic infections.

The results of this study also showed a higher CFU/mL count before the use of the radiographic equipment, possibly due to the timing of the sampling, because the swabbing actions during collection before the use of the equipment may have cleaned the collection sites. A second hypothesis is that the equipment and surfaces are poorly sanitized.

Because potentially infectious individuals are not always identified through information from their medical history or through physical, clinical, and laboratory exams, protective measures should be adopted to prevent or reduce the transmission of pathogenic microorganisms that can cause various types of infectious or contagious diseases. Thus, the dentist is primarily responsible for cross-infection control in the clinical workplace, and must maintain asepsis while conducting X-ray exams and verify that the necessary measures for safe and effective infection control are being followed by all team members. Methods for sterilization, disinfection, mechanical barriers and personal protective equipment should be used in all dental specialty

work, including radiology, to ensure a favorable environment for maintaining the health of staff and patients[26].

Based on the results of this study, acquisition of intraoral and extraoral digital radiographs increases the possibility of cross-infection, creating the need for more stringent protocols for infection control in radiological practice, in order to prevent X-ray exams from being vehicles for cross contamination, particularly at educational institutions.

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EVALUATION OF FRACTURE TORQUE RESISTANCE OF ORTHODONTIC MINI-IMPLANTS

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ABSTRACT

This study sought to assess the fracture torque resistance of mini-implants used for orthodontic anchorage. Five commercially available brands of mini-implants were used (SIN®, CONEXÃO®, NEODENT®, MORELLI®, and FORESTADENT®). Ten mini-implants of each diameter of each brand were tested, for a total 100 specimens. The mini-implants were subject to a static torsion test as described in ASTM standard F543. Analysis of variance (ANOVA) with the Tukey multiple comparisons procedure was used to assess results. Overall, mean fracture strength ranged from 15.7 to 70.4 N·cm. Mini-implants with larger diameter exhibited higher peak torque values at fracture and higher yield strength, regardless of brand. In addition, significant differences across brands were observed when implants were stratified by diameter. In conclusion, larger mini-implant diameter is associated with increased fracture torque resistance. Additional information on peak torque values at fracture of different commercial brands of mini-implants may increase the success rate of this orthodontic anchorage modality.

Key words: *Dental implants, orthodontics, torque.*

RESISTÊNCIA DE FRATURA AO TORQUE DE MINI-IMPLANTES ORTODÔNTICOS

RESUMO

O objetivo do presente estudo foi avaliar a resistência de fratura ao torque de mini-implantes ortodônticos. Foram utilizadas cinco marcas comerciais (SIN®, CONEXÃO®, NEODENT®, MORELLI® e FORESTADENT®). Para cada diâmetro, de cada marca comercial, foram testados 10 mini-implantes, totalizando 100 amostras. Os mini-implantes foram submetidos a um Ensaio Estático de Torção, conforme a norma técnica ASTM F543. Os resultados foram submetidos à Análise de Variância (ANOVA) complementado pelo teste de comparações múltiplas de Tukey. Os valores médios de resistência de fratura ao torque variaram de 15,7 a 70,4 N·cm e mini-implantes de maior diâmetro apresentaram maiores valores de torque máximo de fratura e de limite de escoamento, independente da marca comercial. Além disso, foram observadas diferenças significativas entre as marcas comerciais quando agrupadas de acordo com o diâmetro. Conclui-se que mini-implantes de maior diâmetro apresentaram maiores valores de resistência de fratura ao torque. Informações sobre o torque máximo de fratura das diferentes marcas comerciais podem aumentar o índice de sucesso deste método de ancoragem ortodôntica.

Palavras-chave: *Implantes dentários; ortodontia; torque.*

INTRODUCTION

The control of loads placed on teeth and their bony foundations is one of the principles of orthodontics[1]. For every action force there is a reaction force of equal size and opposite direction, which causes movement of the anchorage unit[2]. Therefore, management of orthodontic anchorage, which may be defined as the resistance offered by a group of teeth or extraoral supports when a force is applied, thus preventing or limiting unwanted movement, is essential to the success of orthodontic treatment [3, 4]. In recent years, alternative orthodontic anchorage methods have become the focus of substantial research and mini-implants have been introduced into the market, broadening the range of options available [5, 6].

The success of mini-implants is related to their minimally invasive nature, ease of insertion and removal, low cost, immediate loading, versatility, and little discomfort to the patient[6-8]. Overall, their success rate is over 80%.[9] However, failure in the placement of these devices has been reported[10, 11].

Research into factors that interfere with the stability of these devices and their resistance to fracture at insertion and removal has therefore been encouraged[5].

Fracture torques of 5 N·cm to 50 N·cm during implant placement have been reported in the literature[8, 10, 11], although few manufacturers report such reference values. Studies have also suggested that factors associated with mini-implant design, thread profile, and material may also influence outcomes[12-14]. In addition, mini-implants with larger diameter have been found to have superior fracture strength[15].

In view of the foregoing, the present study sought to assess the fracture torque resistance of orthodontic mini-implants from different manufacturers.

MATERIALS AND METHODS

This was a laboratory-based *in vitro* study and may be described as a static torsion test of bone screws. The study was conducted at Laboratório de Ensaios Mecânicos – Soluções em Ensaios de Materiais e Produtos (LEM-SCITEC, Palhoça, SC, Brazil), a facility accredited by the Brazilian National Institute of Metrology, Quality and Technology – Inmetro (CRL 0495).

Five brands of orthodontic mini-implants commercially available on the Brazilian market, with fully threaded, cylindrical, solid shafts, were used. The material from which mini-implants are made is defined by ASTM standard specification F136 (Ti 6Al-4V). It is a titanium alloy containing 6% aluminum and 4% vanadium used for manufacturing medical and dental implants. Ten mini-implants of each diameter of each brand were tested, for a total 100 specimens. Diameters ranged from 1.3 to 2 mm. All implants had a transmucosal profile of 1 mm (Table 1). All specimens had fully threaded cylindrical shafts 8 to 9 mm in length. The following characteristics were assessed in each specimen: mode and site of failure, angle of rupture, resistance to fracture at insertion, and yield torque.

Static torsion testing was performed as described in ASTM standard F543 - *Standard Specification and Test Methods for Metallic Medical Bone Screws* [16]. Each screw was secured in locking pliers to prevent rotation during testing, keeping five threads exposed above the transmucosal profile.

Tests were conducted at a constant speed of 1 rpm, under dry conditions, at a temperature of 20 ± 5º C. A torque (N·m)-angle (°) curve was plotted for each tested specimen, and the test was terminated at the time of screw failure. The equipment used in torsion testing is described in Table 2.

Ten specimens were used as a comparator group for the present study. Taking into consideration a mean fracture torque value of 39.2 N·cm (SD=4), reported in a previous study conducted with 1.7-mm mini-implants[17], the present study has 90% statistical power and a 95% confidence level to detect a 15% difference between groups. The collected data were assessed by analysis of variance (ANOVA) with the Tukey multiple comparisons procedure.

Table 1: Identification of mini-implants evaluated in the study.

Brand	Diameter/length (mm)	Reference	Lot
Neodent® (Curitiba, PR, Brazil)	1.3 x 9 1.6 x 9	109.488 109.497	800076729 800073022
SIN® (São Paulo, SP, Brazil)	1.4 x 8 1.6 x 8 1.8 x 8	POT 1418 POT 1618 POT 1818	M040069950 M070077671 M010063381
Conexão® (Arujá, SP, Brazil)	1.5 x 8 1.8 x 8 2.0 x 8	98758199 98788199 98708199	140379 135536 131059
Morelli® (Sorocaba, SP, Brazil)	1.5 x 8	37.10.202	1732607
ForestaDent® (Pforzhein, Germany)	1.7 x 8	1101A2308	11301454

RESULTS

Rupture was the characteristic mode of failure for the tested mini-implants. Fractures occurred along the free end formed by the five exposed screw threads, with the fracture angle ranging from 89° to 406.8°. On ANOVA with Tukey multiple comparisons, neither failure site nor rupture angle were significantly associated with mini-implant brand or diameter at the 5% significance level.

Table 3 shows the fracture torque resistance and yield torque values of the tested mini-implants. Mean fracture strength at insertion and yield limit ranged from 15.7 to 70.4 N·cm and 9.2 to 53.1 N·cm, respectively. Minimum and peak torque curves obtained during mechanical testing are shown in Figs. 1 and 2.

Significant differences were observed between brands. In addition, mini-implants with larger diameter exhibited superior fracture strength and yield limits, regardless of brand. The worst performance was observed for the specimen with the narrowest diameter (NEODENT® 1.3) and the best performance for the specimen with the largest diameter (CONEXÃO® 2.0).

Table 4 shows the results for mini-implants stratified into three groups by diameter: small (1.3 mm/1.4 mm/1.5 mm), medium (1.6 mm/1.7 mm) or large (1.8 mm/1.9 mm/2.0 mm). Mean fracture strength for small, medium, and large specimens was 25.9 N·cm, 33.9 N·cm, and 54.2 N·cm, respectively. In the small-diameter group, MORELLI® brand mini-implants had the best performance. In the medium-diameter group, the SIN® and NEODENT® brands stood out, whereas in the large-diameter group, CONEXÃO® brand mini-implants exhibited superior resistance to fracture at insertion.

Table 2: Identification of mini-implants evaluated in the study.

Internal reference	Description	Manufacturer/model	Certificate of calibration
IM 0143	Single-axis torsion testing machine – Servo Mecânica 9	OF (OFCME 30 Nm)	—
IM 0144	Motion control motor	OF	INMETRO DIMCI 0789/2014 23/04/2015
IM 0145	Torque measurement system – 2Nm-Maq 9	OF (OFTCN 20 KC) S371806/2013	K&L 20/08/2014
IM 0036	Digital caliper, 300 mm	INSIZE (IS11137-300)	CERTI 0244/14 05/02/2015

Table 3: Mean (standard deviation) fracture torque resistance and yield torque of orthodontic mini-implants.

Brand – diameter (mm)	Fracture torque resistance (N·cm)	Yield torque (N·cm)
SIN® – 1.4	26.1^{EF} (0.6)	17.3^{E} (0.6)
SIN® – 1.6	36.1^{D} (2.7)	24.5^{C} (1.9)
SIN® – 1.8	50.2^{B} (1.5)	33.6^{B} (1.0)
FORESTADENT® – 1.7	28.1^{E} (0.5)	20.1^{D} (0.8)
MORELLI® – 1.5	37.7^{D} (2.9)	25.2^{C} (2.8)
CONEXÃO® – 1.5	24.2^{F} (1.2)	16.3^{E} (1.6)
CONEXÃO® – 1.8	45.9^{C} (0.8)	32.7^{B} (1.5)
CONEXÃO® – 2.0	70.4^{A} (3.1)	53.1^{A} (3.7)
NEODENT® – 1.3	15.7^{G} (0.7)	9.2^{F} (0.4)
NEODENT® – 1.6	37.5^{D} (0.8)	23.1^{C} (0.9)

Means followed by different capital letters indicate significant differences according to ANOVA followed by the Tukey multiple comparisons test, at a significance level of 5%.

Table 4: Mean (standard deviation) fracture torque resistance of orthodontic mini-implants, stratified into three groups by diameter.

	Fracture torque resistance (N·cm)					
Brand	**Small diameter 1.3–1.5 mm**		**Medium diameter 1.6–1.7 mm**		**Large diameter 1.8–2.0 mm**	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
SIN®	26.1^{B}	0.7	36.1^{A}	2.7	50.2^{B}	1.4
ForestaDent®	-	-	28.2^{B}	0.5	-	-
Morelli®	37.7^{A}	2.9	-	-	-	-
Conexão®	24.3^{B}	1.2	-	-	58.2^{A}	12.8
Neodent®	15.7^{C}	0.7	37.5^{A}	0.8	-	-

In each diameter group, means followed by different capital letters indicate significant differences according to ANOVA followed by the Tukey multiple comparisons test, at a significance level of 5%.

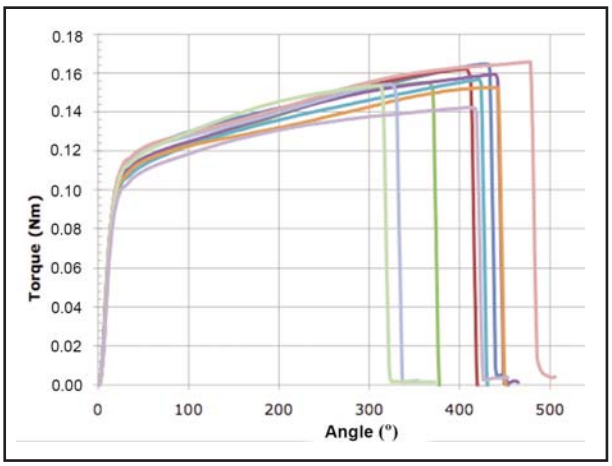

Fig. 1: Torque curve obtained for the 10 specimens with the lowest fracture resistance (Neodent® 1.3x9 mm).

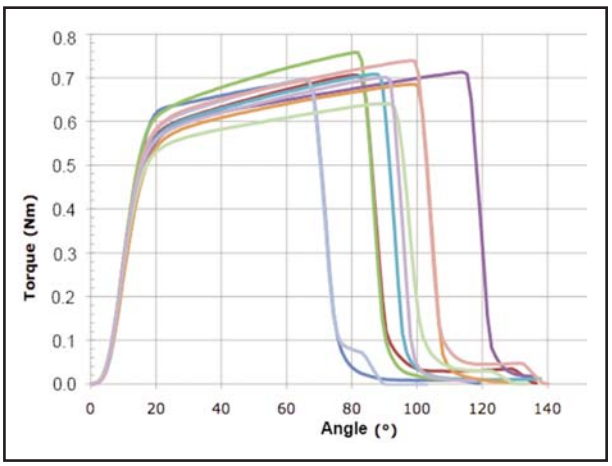

Fig. 2: Torque curve obtained for the 10 specimens with the highest fracture resistance (Conexão® 2.0x8 mm).

FORESTADENT® brand mini-implants, despite having a larger diameter than MORELLI® brand specimens, were less resistant to fracture at insertion. Fig. 3 illustrates the relationship between fracture torque resistance and yield torque values and the different diameters of the tested mini-implants. Both variables increased with increasing implant diameter, in similar distribution patterns. The results show that the yield torque is immediately below the fracture limit.

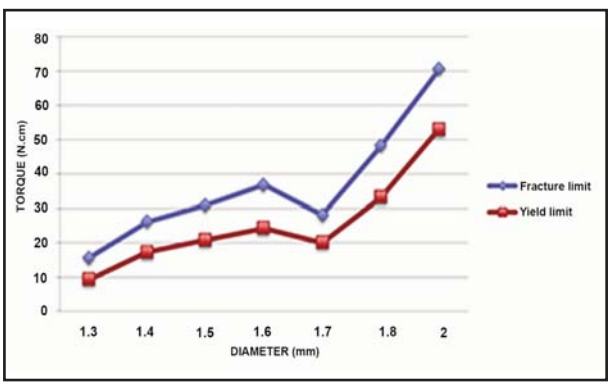

Fig. 3: Mean torque values for fracture and yield limit and their association with mini-implant diameter.

DISCUSSION

Conventional orthodontic anchorage systems have biomechanical limitations and are dependent on patient compliance[18]. The ease of insertion and removal and the high success rate of mini-implants have encouraged their adoption as an efficient method for skeletal anchorage[5,19-21]. However, differences in torsional strength and peak fracture torque between different commercial brands have prompted additional research, with a view to enhancing clinical safety and reducing failure rates [22-24].

The usual length of orthodontic mini-implants ranges from 5 to 12 mm, while diameter and transmucosal profile usually range from 1.2 to 2 mm and from 0 to 3 mm, respectively[10,11,25]. Studies have shown that a progressive increase in implant diameter provides improved primary stability due to increased bone contact area[4,26-30], but also increases the risk of damage to surrounding structures, particularly to the roots of adjacent teeth[31-33].

Mini-implants with smaller diameter and length, however, have increased risk of fracture due to lower mechanical resistance. Despite its importance to peri-implant health, the transmucosal profile has no influence on resistance to fracture at implant insertion [34,35].

In the present study, static torsion testing was performed in accordance with ASTM standard F543. This method ensures replicability of the study and prioritizes mechanical analysis of the specimen, regardless of substrate. Mechanical data are obtained from the specimen alone, without external interference, as the implant is isolated and secured in a clamp. Conversely, studies performed in acrylic, porcine bone, and artificial bone are subject to interference from other variables [32,33, 36, 37].

In addition, a previous study evaluated titanium alloy quality and microstructure of the mini-implant brands tested herein[14]. According to the authors, these devices were free from internal structural defects and compliant with current standards[14].

In the present study, the characteristic mode of failure was mini-implant rupture. Site of failure along the exposed threads and angle of rupture were not associated with brand or diameter of the devices evaluated. Technically, fracture sites may occur randomly, as all screw threads are subject to the same strain condition and intensity. The rupture angle should preferably be high, as this would allow the practitioner to detect during insertion that the implant is undergoing elastic deformation and not driving into bone, halt the procedure, and alter the technique accordingly before fracture occurs.

Mean fracture torque resistance ranged from 15.7 to 70.5 N·cm. These values are consistent with those reported in studies that employed similar methods. Lima *et al*.[15] observed values ranging from 30 to 36 N·cm in 1.6-mm NEODENT® implants, whereas Wilmes *et al.*[11], in a study of 41 commercially available brands 1.3 to 2 mm in diameter, reported values ranging from 10.9 to 64.1 N·cm.

Our results also showed that fracture strength is directly related to mini-implant diameter. Mini-implants with larger diameter exhibited higher fracture torque resistance, regardless of manufacturer. In the present study, the CONEXÃO® 2.0-mm mini-implants performed best overall. According to Barros *et al*.[13], a 0.1-mm increase in mini-implant diameter significantly reduces the risk of fracture. Toyoshima and Wakabayashi[38] also observed that increasing diameter improves fracture torque resistance.

Yield torque represents the time point at which alloy deformation shifts from elastic (reversible) to plastic (irreversible). Optimally, in clinical practice, dentists should always work within the elastic limit of the alloy, thus preventing permanent deformation of the device. The higher the yield limit of a device, the greater its ability to resist plastic deformation. According to the results obtained, the yield limit behaves similarly to and is immediately below the fracture limit of these devices. Therefore, manufacturers should adopt this limit as a reference value, as it represents the point at which fatigue and deformation occur; devices torqued beyond this limit may be at increased risk of fracture during removal. Further research into this mechanical parameter is warranted before this paradigm can be adopted and thus increase operator safety.

Stratification of the mini-implants into groups by diameter revealed differences across the tested brands. MORELLI® and CONEXÃO® brand devices exhibited the best fracture torque resistance in the small-diameter and large-diameter groups, respectively.

The use of mini-implants for orthodontic anchorage is effective and widespread in clinical practice. However, the success of this method depends largely on primary stability. Fracture torque thus plays a critical role in clinical protocols involving placement of these devices. Precise information on the peak fracture torque and yield limit of mini-implants should be made available by manufacturers. In addition, torque-sensing instruments should always be coupled to mini-implant drivers in order to ensure measurement of the forces applied.

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GENERALIZED AGGRESSIVE PERIODONTITIS: MICROBIOLOGICAL COMPOSITION AND CLINICAL PARAMETERS IN NON-SURGICAL THERAPY

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ABSTRACT

The aim of this study was to determine the variations in periodontal parameters and microbiological composition in periodontal pockets at the baseline and 3 and 6 months post-treatment in patients with Generalized Aggressive Periodontitis (GAP) undergoing non-surgical periodontal treatment combined with chlorhexidine and systemic antibiotics. Medical and dental history was taken from 10 subjects, average age 30.6±2.7 years, diagnosed with GAP. A non-surgical periodontal treatment combined with 0.12% chlorhexidine, 875 mg amoxicillin and 500 mg metronidazole every 12 hours for ten days was conducted. At each visit, the following measurements were recorded: bacterial plaque (BP), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), hypermobility, and furcation lesions, and a sample of subgingival plaque was taken from the site of the deepest probing depth of each sextant to identify Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia and Aggregatibacter actinomycetemcomitans using molecular biology techniques. After 6 months, the Wilcoxon test showed an increase of 0.97 mm in CAL (p=0.0047) and 2.54 mm in PD (p=0.009). A healthy site was defined as having a PD <5 mm, negative BOP and no pathogenic bacteria detected at 6 months, indicating significant improvement (p=0.008), with OR (95% CI) =4.7 (1.1022-20.11). With the treatment protocol used in this study, 6 months after treatment, patients had an approximately 4-fold higher possibility of presenting PD <5 mm and periodontal pockets without periodontal pathogenic bacteria.

Key words: *Aggressive periodontitis, periodontal treatment.*

PERIODONTITIS AGRESIVA GENERALIZADA: COMPOSICIÓN MICROBIOLÓGICA Y PARÁMETROS CLÍNICOS EN LA TERAPIA NO QUIRÚRGICA

RESUMEN

En este trabajo, nos propusimos determinar las variaciones de los parámetros periodontales y la composición microbiológica de las bolsas periodontales al inicio, a los 3 y 6 meses después del tratamiento en pacientes con periodontitis agresiva generalizada (GAP), sometidos a tratamiento periodontal no quirúrgico combinado con clorhexidina y antibióticos sistémicos. Se elaboró historia médica y dental en 10 sujetos, con una edad media de 30,6 ± 2,7 años, con diagnóstico de GAP. Se les practicó tratamiento periodontal no quirúrgico combinado con clorhexidina al 0,12%, 875 mg de amoxicilina y 500 mg de metronidazol. Los antibióticos se prescribieron cada 12 horas durante diez días. Se registraron: la placa bacteriana (BP), sangrado al sondaje (BOP), la profundidad de sondaje (PD), el nivel de inserción clínica (NIC), hipermovilidad y lesiones de furcación. En cada visita, se tomaron las mediciones, y se tomó una muestra de la placa subgingival en sitio de la mayor profundidad al sondaje en cada sextante para identificar mediante técnica de biología molecular: Porphyromonas gingivalis, Treponema denticola, forsythia Tannerella, Prevotella intermedia, y Aggregatibacter actinomycetemcomitans. Después de 6 meses, el análisis de la prueba de Wilcoxon mostró un aumento de 0,97 mm de CAL (p = 0,0047) y 2,54 mm en la PB (p = 0,009). Se definió sitio sano, cuando se determinó un PD <5 mm, BOP negativo, y no se detectaron bacterias patógenas a los 6 meses, lo que indicó una mejora significativa (p = 0,008), con (IC 95%) = 4,7 (1,1022 a 20,11). Con el protocolo de tratamiento presentado, es posible especular que a los 6 meses después del tratamiento, un paciente puede tener aproximadamente 4 veces más posibilidades de presentar una PD<5 mm y bolsillos periodontales sin bacterias patógenas.

Palabras clave: *periodontitis agresiva, tratamiento periodontal.*

INTRODUCTION

Generalized Aggressive Periodontitis (GAP) comprises a group of periodontal pathologies characterised by rapid evolution, severe bone loss, few clinical manifestations, low-grade inflammation and an amount of microorganisms which does not appear to be proportional to the severity of destruction [1]. The condition is diagnosed when

more than 30% of the teeth are affected, including at least three permanent teeth other than incisors and first molars, and is mainly diagnosed in systemically healthy persons at an early age [1]. It is also speculated that there is a hereditary component in susceptibility [2].

A highly virulent microbial flora has been described, with *Aggregatibacter actinomycetemcomitans* considered to be a risk factor because its persistence is associated with recurrence[3,4]. Other authors suggest that *Porphyromonas gingivalis, Tannerella denticola* and *Prevotella intermedia*, together with *A. actinomycetemcomitans*, are responsible for the low response to treatment in GAP patients because of a relationship between the rapid loss of periodontal insertion in patients undergoing maintenance and the persistence of these species [5,6].

The primary objective of treating this kind of periodontal disease is to modify the pathogenic subgingival microbiota to non-pathogenic and to prevent the reestablishment of virulent bacteria in the subgingival biofilm. These changes should improve clinical parameters such as bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) [7,8]. The identification of the bacterial species that colonise the pockets might be a tool for predicting the outcome or success of treatment, especially with the use of additional antiseptic and antibiotic treatment [9, 10].

Several studies have reported the effectiveness and clinical safety of non-surgical periodontal therapy combined with systemic antibiotics for the treatment of GAP [11- 13]. However, there is no clear consensus on the choice of antibiotics or the protocol for the use and duration of antibiotic treatment. Moreover, several important issues related to the therapy have yet to be clarified. There is no previous report in Argentina about the effectiveness of non-surgical therapy combined with antibiotics such as amoxicillin and metronidazole for the management of periodontal flora in GAP. The aim of this study was to analyze the periodontal clinical parameters and the microbiological composition of periodontal pockets in patients diagnosed with GAP, and their response to non-surgical periodontal treatment combined with 12% chlorhexidine and 875 Amoxicillin plus Metronidazole 500 mg by comparing baseline to the situation at 3 and 6 months.

MATERIALS AND METHODS

Study Population

Ten unrelated patients of both sexes, aged 15 to 35 years, were recruited from the Department of Periodontics of the Independencia Foundation in the city of Cordoba, Argentina. Detailed systemic and oral anamnesis procedures were performed to assess the inclusion criteria, and GAP was diagnosed according to the definition of the American Academy of Periodontology [14]. The inclusion criteria for the patients were: 1) having at least 20 teeth, presenting interproximal bone loss in at least 3 teeth other than incisors and first molars and 2) being systemically healthy, having no previous history of periodontal treatment or history of prescribed antibiotic medication within the previous 6 months. Patients who were pregnant, breastfeeding, diabetic, smokers, immunosuppressed or drug addicts were excluded. Teeth with lesions with furcation were excluded. The Ethics and Discipline Committee of the Independence Foundation approved the protocol. All patients received a precise explanation of the research protocol, signed an informed consent to take part in the study, and were treated following the principles of the Helsinki Declaration.

Clinical Records and Treatment Protocol

A complete medical and clinical dental history, including serial radiographies and periodontal charting (Go- Probe* R), was performed on each patient. Researchers MMU and JM recorded all clinical parameters at the beginning of the protocol and at 3 and 6 months after treatment. The following clinical parameters were recorded: 1) presence/absence of supragingival bacterial plaque (BP), presence/absence of bleeding on probing (BOP), dental hypermobility, PD in mm, and loss of clinical attachment level (CAL) in mm. The measurements were taken from 6 different sites per tooth, excluding the third molar, using a Hu Friedy periodontal probe (PCP-UNC-15, Hu-Friedy, Chicago, IL). The inter-rater correlation coefficient for the PD measurements was 0.85, indicating a high level of reliability between MMU and JM.

Periodontal treatment consisted of detailed oral hygiene technique instructions, followed by root scaling and planing, and rinsing with a solution of 0.12% chlorhexidine digluconate (GlaxoSmithKline). Following their oral hygiene routine, patients were

instructed to rinse with 15 ml clorhexidine for 30 seconds twice a day for 60 days, beginning after the first session of mechanical debridement. Mechanical debridement consisted of progressive sessions of root scaling and planing once a week divided into approximately 5 to 7 sessions, depending on the case. The treatment was performed under local anaesthesia, by quadrant and with specific Gracey curettes (Hu-Friedy, Chicago, IL) for each sector and face of the teeth. The treatment was considered complete when BP was less 10%, there was no bleeding or any visible sign of inflammation, and no calculus was detected by the calculus detection device (Hu-Friedy, Chicago, IL). Patients received a prescription of 875 mg amoxicillin and 500 mg metronidazole every 12 hours for 10 days following the first scaling session. The dosage and duration of the antibiotic prescription are in relation to the pharmacological presentation. After completing the basic treatment, the clinical parameters were re-evaluated after 3 and 6 months with reinforcement of the hygiene instructions.

Sample Collection for the Microbiological Identification of Periodontal Pathogens

The samples for microbiological analysis were taken at the beginning of the non-surgical periodontal treatment and at 3 and 6 months. The material for the analysis was collected on the site presenting the deepest PD on each subgingival sector of each sextant, on the proximal faces: mesial-vestibular, mesiopalatal, distovestibular and distopalatal.

Prior to taking samples, the supragingival plaque was removed from the interproximal surfaces using a sterile curette. The surfaces were isolated with sterile gauze and five consecutive #35 paper points were inserted with catheterisation movements to the depth of the periodontal pocket for 60 seconds each. The cones were placed into Eppendorf tubes and stored at 4°C until processing.

Sample Processing

1- DNA extraction:

Two hundred microliters of sterile water were added to the Eppendorf tubes containing the paper points impregnated with the material extracted from the periodontal pockets. Each tube was incubated at 37ºC for 10 minutes and centrifuged at 14,000 g for 5 minutes. DNA was extracted using conventional techniques [15]. To verify the presence of DNA in the supernatant, electrophoresis was performed on 0.8% agarose gel stained with ethidium bromide and visualised using ultraviolet (UV) light.

2- Identification by conventional PCR:

First, we amplified a highly preserved specific sequence (960 bp) of the 16S rRNA gene to identify gram-negative bacteria in the sample. Once Gram-negative bacteria were confirmed in the sample, PCRs with specific oligonucleotides for *A. actinomycetemcomitans, T. forsythia, P. gingivalis, P. intermedia*, and *T. denticola* were performed following the protocol described by Ashimoto *et al* [16]. The assay was performed twice for each sample, including a negative control by adding to each reaction one tube without DNA and one tube with DNA from organisms isolated in a culture and donated by the Bacteriology Laboratory at the Reina Fabiola Hospital (Catholic University of Cordoba). The PCR products were analysed by electrophoresis on 1.6% agarose gels. The gels were stained with ethidium bromide and photographed under UV light. A molecular marker indicated the size of the amplification product in the gel (Perkin Elmer 100-bp Marker). For doubtful results, the samples were sequenced to corroborate the specificity of the amplified fragment (ABI PRISM 310, Applied Biosystems, USA).

Statistical analysis

Statistical analysis was performed using SPSS (Ver. 9; SPSS, Inc.) and Infostat vs2007e data analysis software. Significance was defined as p values under 0.05 ($p<0.05$). A sample size of 10 subjects was needed to provide an 80% power for detecting an average 0.75% reduction in probing depth between baseline and 6 months. Once patients had been selected for treatment, it was not possible to include additional patients to compensate for any potential dropouts. The intra-class correlation coefficients for mean PD and CAL were 0.92 and 0.91 respectively. The examiner's reproducibility of measurements taken at baseline and at 6 months was good (0.93 and 0.92 respectively).

The quantitative variables (PD, CAL) are expressed as means and standard deviations, and the qualitative variables BP, BOP, S and presence of bacteria in the pockets are expressed as absolute and percent frequencies. The statistical significance

between the mean values for the same periods of assessment was evaluated using the paired Student T test, and the differences between 2 periods of treatment were assessed using the Wilcoxon Signed Rank test. The presence of bacteria and the CAL and PD values at the end of the treatment were correlated with 2x2 contingency tables, using Pearson's chi-square test or Fisher's exact test when few samples were analysed. Subsequently, raw odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to calculate the relative risk of disease. Status was considered healthy when after six months, pathogenic bacteria were not detected, BOP was negative and PD<5 mm was recorded in the treated pockets.

RESULTS

This study analysed 60 periodontal sites in 4 males and 6 females, average age 30.6±2.7 years (range 21-35 years) who completed all stages of the project. Patients had an average 27 tooth, although one patient lost two teeth and another patient lost one tooth during the treatment. The complete oral examination revealed an average 42±7.9 pockets over 5 mm and 121.5±6.9 pockets under 5 mm. BOP was present in the upper maxilla in 47.6% of the cases, and in the mandible in 47.9%. Table 1 summarises the changes in periodontal parameters at the different time points of the assessment. Mean PD and CAL values in mm decreased significantly throughout the treatment in all patients (p<0.035). After six months post-treatment, according to the Wilcoxon Signed Rank test, there was a 0.97 mm increase in CAL (p=0.0047) and a 2.54-mm recovery in PD (p=0.009). Positive BOP decreased by 40.47% in the upper maxilla and by 37.2% in the lower maxilla after treatment. At six months, the number of periodontal pockets deeper than 5 mm decreased by 36.6%, whereas the frequency of pockets less than 5 mm deep increased significantly. Regarding the distribution throughout treatment of the five pathogens identified in the periodontal pockets at all sites, *P. gingivalis, T. denticola*, and *T. forsythia* were identified with greater frequency at the beginning of the treatment. *P. gingivalis* was not detected at the 3-month follow-up and only recognised at one site six months post-treatment, whereas *T. denticola and T. forsythia* persisted at all sites after completion of the 6-month period.

P. intermedia was identified in association with *A. actinomycetemcomitans* only at the beginning of the study. These bacteria were not isolated again at any site throughout the study. The absence of *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans* after treatment concurred with the increase in non-virulent Gram-negative bacteria during the 3- and 6-month periodontal maintenance period (Table 2).

To analyse whether the reduction in PD observed at the 6-month follow-up was associated with

Table 1: Periodontal Clinical Parameters during the treatment.

	Baseline (n=60)	3m (n=60)	6m (n=60)	p
PD(mm)	7.0±2.6*	4.63±3.8	4.46±3.7	0.002
CAL(mm)	7.6±6.5*	6.8±4.5	6.63±5.4	0.035
Upper BOP (%)	47.6	42.9	4.047	0.007*
Lower BOP (%)	47.9	39.08	37.19	0.039*
Pockets>5mm	42*±0.9	25±0.2	22±0.3	0.037
Pockets<5mm	121*±6.4	137±0.1	140±04	0.045

The scores represent means ± standard error; in millimeters of probing depth (PD) and clinical attachment level (CAL), and the number of pockets of more than or less than 5 mm at all sites measured at each stage of treatment at 3m (3 months) and 6 m (6 months). BOP bleeding on probing; the numbers represents the percentage of the average score. *Significant p value between baseline and 6m.

Table 2: Distribution of Periodontal Bacteria at baseline and 3- and 6- months post-treatment.

	Pg Sites (%)	*Td* Sites (%)	*Tf* Sites (%)	*Pi* Sites (%)	*Aa* Sites (%)	*Others* Sites (%)
Baseline	13 (43.3)	15 (47)	13 (40)	12 (37)	4 (10)	2 (6)
3 months	0	6 (20)	7 (23)	0	0	18 (60)
6 months	1 (3)	5 (17)	8 (27)	0	0	16 (53)

The numbers represent the number of sites where each bacterium was identified; percentages between brackets. *Porphiromonas gingivalis (Pg), Treponema denticola (Td), Tannerella forsythia (Tf) Prevotella intermedia (Pi)* and *Agreggatibacter actinomycemcomitans (Aa)*, Others: Gram-negative bacteria.

the presence/absence of periodontal bacteria, a univariate analysis was performed to correlate the presence of 1 or more pathogenic bacteria with PD values compatible with clinical improvement. For this purpose, healthy status was considered to be PD<5 mm plus absence of pathogenic bacteria in the pocket and absence of BOP. The chi-square test revealed a statistically significant association of X^2=5.6, p=0.008, a statistically significant improvement between baseline and the 6-month measurement, with OR (95% CI) = 4.7 (1.1022-20.11). This finding indicated that the chance of pockets improving to PD<5 mm at 6 months post-treatment was 4-fold higher when periodontal pathogenic bacteria were absent from the periodontal pocket.

DISCUSSION

In our study, the analysis of clincal parameters before and after treatment revealed positive results for mechanical periodontal therapy combined with chlorhexidine and antibiotics in patients with GAP, showing significant improvements in clinical periodontal variables such as decreased PD, increased CAL, and decreased percentage of plaque and sites with BOP, which were maintained at the 6-month follow-up. Notably, the average increase in CAL was 0.97 mm at six months after the beginning of the treatment, and the PD decreased by 2.54 mm during the same period. This clinical success included a significant reduction in periodontal pathogens at the treated sites. These results are in agreement with other studies reporting that non-surgical treatment including the use of amoxicillin and metronidazole leads to clinical benefits and significant reduction in periodontal pathogens.[10- 13].

Hughes et al.[17] conducted a study on patients with GAP following treatment with superficial root debridement and oral hygiene instructions. In the re-assessment, they report a mean decrease of 2.11 mm in PD and an increase of 1.77 mm in CAL. They report that 32% of the patients with a negative response to treatment were smokers and suggest that smoking is the most important factor associated with treatment failure [17]. The fact that our study excluded smokers may explain our higher PD values after 6 months.

At the 3- and 6-month follow-ups we found significant reduction in the presence of *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans* in the periodontal pockets treated, indicating the efficacy of mechanical therapy combined with 875 mg amoxicillin and 500 mg metronidazole every 12 hours for 10 days and Clorhexidine 0.12% rinses to eliminate pathogens and thereby restore periodontal tissue. It is also worth noting that the *T. denticola* and *T. forsythia* were identified at all stages of the treatment, and although they were detected in fewer pockets, the association between *T. denticola* and *T. forsythia* may be resistant to the combined treatment. Other authors have described the bacterial relationship between *P. gingivalis, A. actinomycetemcomitans* and *T. forsythia* in GAP [18]. We note that different studies use different doses and duration of the antibiotic treatment [11]. The amoxicillin dose used in our study has not been reported in other studies. For example, Gomes Baeta et al. [19] prescribed 500 mg amoxicillin and 250 mg metronidazole 3 times per day for 10 days, and detected *A. actinomycetemcomitans* in all samples up to the 9-month post-treatment follow-up session. Haffajee et al. [20] showed that systemic administration of these antibiotics may suppress periodontal bacteria efficiently and thereby improve the therapeutic response [18]. We believe that one weakness of the current study is that the high doses of amoxicillin used did not prevent the persistence of *T. denticola* or *T. forsythia* either at 3 or at 6 months. Further studies are needed to analyse whether a combination of amoxicillin/metronidazole might induce persistence or antibiotic resistance in the periodontal microflora. The risk of side effects or adverse events also remains to be addressed [12].

Mechanical instrumentation significantly changed the composition of the subgingival microflora by decreasing virulent microorganisms and increasing beneficial cocci and bacilli, with this bacterial pattern leading to a healthy bacteriological profile [20]. Some authors suggest a limited effect of root scaling and planning on certain pathogenic species because their complete elimination might be difficult as a result of re-infection of successfully treated sites. Johnson et al.[21] suggest that after 6 months, these bacteria recolonise the plaque when they remain in the epithelial cells of the oral mucosa and are unaltered by treatment.[21] These data support the hypothesis that extracrevicular bacterial deposits contribute to the development of recurrent diseases

in some patients [19,20]. We observed recolonization by *P. gingivalis* in 1 patient at the 6-month follow-up. According to our data**,** *P. gingivalis* in a periodontal pocket might be a risk factor for unsuccessful treatment or relapse. It is important to note that the reinforcement of oral hygiene and re-instrumentation at follow-up appointments contributed to the continuous reduction of certain pathogenic species. Our observations, as well as studies by other authors, have noted a significant decrease in the bacterial population in the treated pockets at three months, and repopulation at six months [22]. This repopulation may be caused by the use of systemic antibiotic therapeutic regimen. While adjunctive therapy is effective, few studies have reported whether periodontal species have developed resistance to antibiotics prescribed [18].

Because GAP is rarely diagnosed in our location, the sample size in our study is limited. Furthermore, the 6-month follow-up period might be too brief for longitudinal assessment. An increase in the number of patients and a longer follow-up period would enhance the study's strength and contribute to establishing appropriate intervals for maintaining periodontal health. Notably, although the clinical outcome was positive after 6 months, the elimination of certain bacteria was shown to be transitory. This persistence may contribute to the recurrence of the disease in some patients. To achieve long-term stability in clinical outcomes, it is essential to implement strict maintenance programmes. We believe that controlling the microbiological composition of the pocket may help reduce the use of antibacterial agents and the risk of producing bacterial resistance.

The rationale for adding amoxicillin to the prescription of this combined drug regimen is based on a synergistic effect of amoxicillin on metronidazole and its hydroxymetabolite against *A. actinomycetemcomitans* [23]. In our study, only 10% of the periodontal pockets were found to contain *A. actinomycetemcomitans* at baseline. Bazzano et al. report similar frequencies in patients with severe chronic periodontitis and treated only with root scaling and planning [24]. A study that evaluated metronidazole, alone or combined with amoxicillin, as an adjunct to nonsurgical therapy in chronic periodontitis subjects, showed that the clinical and microbiological benefits of both treatment options were very similar [25]. Further studies are needed to discuss the balance of the risk/benefits ratio of the prescription adjunctive antimicrobial regimen, in particular when high doses of amoxicillin are used (825mg/10 days/ 2 times a day/) and *A. actinomycetemcomitans* is identified in low frequency.

Our study verified that in patients diagnosed clinically, radiologically and microbiologically with GAP, virulent bacterial species may be eliminated by mechanical treatment combined with specific systemic antibiotics (875 mg amoxicillin and 500 mg metronidazole), antiseptics (such as 0.12% chlorhexidine digluconate) and a 6-month maintenance period. The above protocol leads to a significant recovery in periodontal parameters such as PD and BOP, achieving values that are compatible with periodontal health.

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EFECTIVENESS OF THE WAVEONE AND PROTAPER D SYSTEMS FOR REMOVING GUTTA-PERCHA WITH OR WITHOUT A SOLVENT

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ABSTRACT

Endodontic retreatment requires complete removal of the filling material and access to the apical foramen. The purpose of this study was to evaluate the effectiveness of the WaveOne reciprocating system and compare it to the ProTaper D rotary system, with or without the use of a solvent, in removing filling material from root canals. The time required for each filling removal technique employed was also determined and compared. Forty extracted human mandibular premolars with a single, straight, flattened canal were prepared and filled. They were divided into four groups (n = 10): Group 1: ProTaper D NiTi rotary instruments; Group 2: ProTaper D NiTi rotary instruments, with a solvent; Group 3: WaveOne primary instrument; and Group 4: WaveOne primary instrument, with a solvent. The teeth were then split along their long axis and photographed using an operating microscope with 5X magnification. The amount of remaining filling material was assessed with Image Tool software. The results were compared using the Kruskal-Wallis test ($p < 0.05$). There was no significant difference between groups regarding the amount of residual filling material ($p > 0.05$). Operative time was significantly longer in Group 3 than in groups 1, 2 and 4 ($p < 0.05$). The WaveOne system and the ProTaper D system were equally effective, with or without a solvent. The time required to remove the filling material from the canals was significantly longer in Group 3 than in the other groups.

Key words: *Gutta-Percha; root canal; reciprocating.*

EFICIÊNCIA DOS SISTEMAS WAVEONE E PROTAPER D NA REMOÇÃO DE GUTA-PERCHA COM OU SEM USO SOLVENTE

RESUMO

No retratamento endodôntico, a completa remoção do material obturador e o acesso ao forame apical são necessários para permitir a limpeza do sistema de canais. O propósito deste estudo foi avaliar a eficácia do sistema reciprocante WaveOne e compará-la ao sistema rotatório ProTaper Universal, com ou sem o uso de solvente, na remoção do material obturador.O tempo necessário a cada técnica empregada foi determinado e comparado. Quarenta pré-molares inferiores humanos extraídos com canal único, reto e achatado foram preparados e obturados. Foram então divididos em quatro grupos (n = 10) de acordo com o sistema utilizado, como segue. Grupo 1: ProTaper D Niti; Grupo 2: Sistema ProTaper D com solvente; Grupo 3: Sistema WaveOne instrumento Primary; e Grupo 4: Sistema WaveOne instrumento Primary com solvente, sendo o tempo registrado. Os dentes foram clivados longitudinalmente e fotografados utilizando microscópio operatório com aumento de 5 vezes.A quantidade de material remanescente foi avaliada com o uso do software Image Tool 3.0. Os resultados foram comparados utilizando o teste de Kruskal-Wallis($p < 0.05$). Em relação aos resultados, não houve diferença significativa entre os grupos quanto à quantidade de material obturador residual ($p > 0.05$). O tempo operatório no Grupo 3 foi significativamente maior do que nos grupos 1, 2 e 4 ($p < 0.05$).O sistema WaveOne foi tão efetivo quanto o ProTaper D, com ou sem solvente.tempo necessário à desobturação dos canais no Grupo 3 (WaveOne sem solvente) foi significativamente maior do que nos demais grupos.

Palavras-chave: *Guta-percha; canal radicular; movimento reciprocante.*

INTRODUCTION

Although endodontic treatments have a high success rate, they sometimes require a second intervention[1,2]. Endodontic retreatment requires the complete removal of the filling material and access to the apical foramen for adequate cleansing and refilling of the root canal system[3].

Conventional techniques using Gates-Glidden and manual files have been losing their popularity due to the need for solvents. More current techniques

using motors facilitate endodontic retreatment and reduce working time.

Reciprocating systems were introduced[4] with the aim of improving resistance to fracture, since the reciprocating motion reduces the cyclic and torsional fatigue to which the instrument is submitted[5,6] .Furthermore, the instruments in these systems are made from an alloy subjected to a thermal treatment, known as M-Wire and considered more resistant than the conventional NiTi alloy[7]. The WaveOne system (Dentsply Maillefer, Ballaigues, Switzerland) was designed specifically for use in reciprocating motion. It comprises three instruments: small (21.06), primary (25.08 in three first milimeters) and large (40.08 in three first millimeters)[8].

Reciprocating systems were not originally designed for use in retreatment procedures; nevertheless, Rios et al.[9] compared the efficacy of two reciprocating systems and one rotary system in removing filling material from straight canals. They observed that the reciprocating systems and the rotary system tested were equally effective in removing filling material. Others studies have also compared reciprocating files in retreatment [26,27].

Zuolo et al.[10] compared the effectiveness of a reciprocating system and a rotary system to that of a manual instrumentation technique in removing filling material from root canals. Chloroform was used as a solvent in all groups. The authors concluded that the operative time involved in using the reciprocating system with a solvent was significantly shorter than that required by the other systems tested. In spite of these results, no consensus has been reached to date on the effectiveness of reciprocating systems for endodontic retreatment procedures. The use of a solvent as an aid in removing the filling mass is also somewhat controversial. Some authors have suggested that its use could increase working time due to the formation of a residual slurry which adheres to the canal walls[11], whereas others do not confirm these results[12,13]. Some authors point out that a solvent may be responsible for improved cleanliness of the root canal system [14], whereas others do not observe this correlation[13,15].

Tagger's hybrid filling technique was chosen to plasticize gutta-percha and produces good adaptation of the filling material to the canal walls[16].

The purpose of this study was to evaluate the effectiveness of the WaveOne reciprocating system, with or without the use of a solvent, and compare it to that of the ProTaper D rotary system in removing filling material from straight canals. The time required by each retreatment technique was also determined and compared.

MATERIALS AND METHOD

Specimen preparation

All the specimens used in this study were obtained from the tooth bank at the Dental Research Center, São LeopoldoMandic University, Campinas, SP, Brazil. The study protocol was reviewed and approved by the Research Ethics Committee of the same institution (protocol no. 2012/0420).

Forty extracted human mandibular premolars were used for this study. All teeth had a single, straight, flattened canal, a completely formed root and no calcification or internal resorption. The teeth were selected based on an evaluation of apical radiographs taken in orthoradial and mesiodistal direction. The teeth were preserved in a 0.1% thymol solution (Fórmula e Ação, São Paulo, SP, Brazil).

The crowns were removed with a diamond disc (KG Sorensen, Cotia, SP, Brazil), and root length was standardized at 16 mm. A #10 K-type file (Dentsply Maillefer) was then inserted into the canal until it could be seen at the apical foramen with an operating microscope (D. F. Vasconcelos, São Paulo, SP, Brazil) at 5X magnification. The working length was determined 1 mm short of this measurement.

Initial endodontic treatment

A single operator instrumented the canals using the rotary technique. Pre-flaring was performed with Gates Glidden burs (Dentsply Maillefer) and the SX and S1 files of the ProTaper Universal rotary system. Apical preparation was performed with ProTaper S1, S2, F1 and F2 files. The canal was irrigated with 2 mL 2.5% sodium hypochlorite (Fórmula e Ação) after each instrument change. Once the instrumentation was completed, the canal was irrigated with 17% EDTA (Fórmula e Ação) for 3 minutes followed by 5 mL 2.5% sodium hypochlorite.

The canals were dried with absorbent paper points and filled with ProTaper F2 gutta-percha cones (Dentsply Maillefer), accessory cones (Dentsply Maillefer), and AH plus sealer (Dentsply Maillefer) using Tagger's hybrid technique[16], 1 mm short of the apical foramen. Filling quality was confirmed with periapical radiographs taken in the orthoradial and mesiodistal direction.

The coronal access was sealed with Cavit G temporary filling material (3M Espe, Seefeld, Germany), and the teeth were stored under 100% humidity for 30 days for subsequent removal of the root canal filling.

Endodontic retreatment

The 40 teeth were randomly divided into 4 groups with 10 specimens assigned to each group using a computerized algorithm.

The crowns were removed and the roots were standardized at 16 mm to avoid any possible interferences related to canal access and crown anatomy[11,14,21]. After filling the teeth, they were stored at 100% humidity for 30 days to ensure complete setting of the endodontic cement[22,23]. After removing the temporary seal, the filling material was removed using one of the following techniques:

Group 1, filling removal with ProTaper D – The filling material was removed using ProTaper D1 and D2 retreatment files, in that order, up to the established working length. The files were driven by an X-Smart electrical motor (Dentsply Maillefer) in continuous rotation at a constant speed of 500 rpm for instrument D1, and 400 rpm for instrument D2, with a torque of 4 Ncm. After applying the last rotary instrument, canal patency was confirmed by introducing a #10 manual file up to the foramen.

Group 2, filling removal with ProTaper D + chloroform – The filling material was removed using a technique similar to that employed in Group 1; however, after using the D1 file, 0.1 mL chloroform (Fórmula e Ação) was placed in the canal with a micropipette (Digipet, Curitiba, PR, Brazil). The D2 instrument was then applied up to the working length.

Group 3, filling removal with WaveOne – Filling material removal was performed with the WaveOne primary instrument driven by the X-Smart Plus electrical motor in WaveOne system mode, according to the manufacturer's instructions, with the reciprocating handpiece used with pecking motion. This instrument was inserted in 3 mm steps, always accompanied by irrigation, until the working length was reached. Canal patency was confirmed by inserting a #10 manual file up to the foramen.

Group 4, filling removal with WaveOne + chloroform – In this group, 0.1 mL chloroform was placed at the canal orifice, and the WaveOne primary instrument was then applied as in Group 3. Each file of the ProTaper system was discarded after use in 5 canals[11,17,18] , and each file of the WaveOne system was discarded after use in 4 canals[19].

After each use, the instruments were thoroughly cleaned by removing any filling material residue. The irrigating solution used during retreatment was 2.5% sodium hypochlorite, with a total 20 mL per specimen. All teeth were retreated by a single operator.

Filling material removal was considered completed when no filling material residue was observed on the endodontic instruments or detected inside the root canal with the operating microscope under 12.5X magnification.

The actual time spent on the removal procedure was measured and recorded for each specimen with a digital stopwatch. The stopwatch was started at the beginning of the filling removal procedure and stopped when it ended.

Filling removal evaluation

After completing the filling removal procedures, grooves were made along the long axis of the tooth in bucolingual direction, and the grooves were deepened with a diamond disc up to the vicinity of the canal wall. The specimens were then cleaved into two halves, mesial and distal, and both halves were assessed[10].

All of the specimens thus obtained were coded and photographed with a Canon T3I camera (Canon, Inc., Taichung City, Taiwan) and an F1.4 50 mm lens (Nagasaki Canon Inc., Nagasaki, Japan) coupled to an operating microscope set at a 5X magnification.

Image Tool 3.00 software (University of Texas Health Science Center, San Antonio CA, USA) was used to measure both the canal area and the filling material remainder area, given in square pixels (Fig. 1). The data were converted into percentages to allow a comparison among techniques employed and among specimens with different areas.

Statistical analysis

The Kruskal-Wallis test was used to compare the amount of remaining filling material and the time required for the filling removal procedure in the different study groups ($p < 0.05$).

RESULTS

All teeth examined showed some amount of residual filling material inside the canals. The amount of filling remainder in each group is shown in Table 1. There was no significant difference between groups regarding the amount of residual filling material ($p > 0.05$). Table 2 shows that the operative time required in Group 3 was significantly longer than that required in groups 1, 2 and 4 ($p < 0.05$). There was no significant difference between groups 1, 2 and 4 as regards operative time.

DISCUSSION

The complete removal of filling material from the root canal system is one of the main objectives of nonsurgical endodontic retreatment. Only by attaining this goal can the apical foramen be accessed and the action of endodontic instruments and irrigating solutions used during instrumentation be effective, preventing the necrotic tissue and microorganisms from remaining inside the canal[3].

We chose to work with mandibular premolars because they are flattened mesiodistally and have a greater buccolingual dimension, making it harder for endodontic instruments to touch all of the dentinal walls[20]. Thus, adequate removal of the filling material in these canals is also rendered more difficult[21], a fact that cannot be ignored when comparing the effectiveness of filling removal techniques.

The use of a solvent as an aid in removing the filling mass is somewhat controversial. A solvent can facilitate penetration of the instrument into the filled canal[24,25], but its use may result in the formation of a thin and hard-to-remove layer of filling material adhered to the dentinal walls[11,12]. Chloroform is the most widely used solvent, and it is ability to dissolve gutta-percha is superior to that of most other solvents[25]. Furthermore, it can be safely used in endodontic procedures owing to its limited toxicity.

None of the techniques employed in this study was effective in completely removing filling material, confirming the results obtained in previous studies[9,10,21,23]. However, the working time required in Group 3 was significantly longer than that required in groups 1, 2 and 4.

The first instrument (D1) in Group 1 (ProTaper D without solvent) has a working tip which makes it easier for it to penetrate the filling mass. The following instrument (D2) penetrates the filling material and causes it to be removed en bloc. This may explain the shorter working time recorded for this system compared to Group 3 (WaveOne without solvent). However, it is important to note that, owing to its working tip, instrument D1 may only be used safely in straight canals; its use in curved canals may cause deviations from the canal's original path.

Fig. 1: Evaluation of filling material remainder.

Table 1: Means and standard deviations of residual filling material (expressed as percentage area) on canal walls after application of the filling material removal methods.

Groups	Proportion (%) of residual material in relation to canal total area
1	3.08 (2.11)
2	2.08 (1.25)
3	1.45 (0.86)
4	2.52 (3.28)
p value	0.2431

Table 2: Means and standard deviations of the time (in seconds) required for the filling removal procedure in the different study groups ($p < 0.01$).

Group 1	Group 2	Group 3	Group 4
285.40 (64.33)[a]	327.50 (49.14)[a]	676.00 (218.36)[b]	372.70 (141.95)[a]

Different letters indicate significant statistical difference ($p < 0.01$).

Even though a solvent softens gutta-percha, no statistically significant difference was observed between Group 1 (ProTaper D without solvent) and Group 2 (ProTaper D with solvent) as regards operative time, confirming the results of previous studies[12,13]. This may be explained by the effectiveness of the ProTaper D system in removing the filling mass, rendering it unnecessary to use a solvent to soften the gutta-percha[12]. These results differ from those of the study conducted by Takahashi et al.[11], in which using a solvent increased the working time required by the ProTaper D system to remove the filling material.

The longer working time was recorded for Group 3 (WaveOne without solvent). This may be explained by the difficulty, perceived by the operator, in introducing the WaveOne primary instrument into the filling mass. The use of a solvent with this system (Group 4) softened the gutta-percha, rendering instrument penetration easier, thus significantly reducing working time.

Even though the solvent used here proved beneficial in reducing operative time with the WaveOne system, the use of solvents in general should be undertaken in a controlled manner owing to the risk of extrusion into the periapical region. Furthermore, studies have shown that single-file systems working in reciprocating motion produce significantly more debris then systems working in continuous rotation during the canal instrumentation phase. It could therefore be hypothesized that combining the use of solvents with reciprocating systems might aggravate the risk of extrusion.

Comparing the effectiveness of a manual instrumentation technique to that of the Mtwo rotary system (VDW, Munich, Germany) and of the Reciproc (VDW) reciprocating system in removing filling material with the use of chloroform, Zuolo et al.[10] observed that operative time was significantly shorter in the reciprocating system than in the other techniques. In contrast, our study found no significant difference in operative time between the ProTaper D and WaveOne systems (with solvent). This discrepancy is probably related to the fact that Zuolo et al.[10] used a sequence of 4 instruments of the Mtwo system, in addition to Mtwo R instruments (15.05 and 25.05), to reinstrument the canals, whereas we used a sequence of only 2 instruments of the ProTaper D system in the present study.

Considering that reciprocating systems are specifically designed to reduce the taper-lock effect, hence imparting greater safety to the instrumentation procedure[6,7]; and, further, that three clockwise and counterclockwise motions are required to complete a whole turn of the instrument inside the canal, it could be assumed that reciprocating systems would be in disadvantage compared to rotary systems in terms of filling removal operative time. This alleged disadvantage seemed to have been reflected in our results, since the working time for Group 3 (WaveOne without solvent) was the longest. On the other hand, reciprocating systems are more resistant to fracture, and thus safer, particularly in retreatment of curved canals. We conclude that the WaveOne system and the ProTaper D system were equally effective, with or without a solvent, in removing filling material. Removing the filling material from the canals took significantly longer in Group 3 (WaveOne without solvent) than in the other groups.

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SAIO
SOCIEDAD ARGENTINA
DE INVESTIGACION ODONTOLOGICA
División Argentina de la International Association for Dental Research

On November 10, 11 and 12, 2016, the 49th Annual Meeting of the Argentine Society for Dental Research (SAIO), the Argentine Division of the IADR, was held in Mar del Plata, Buenos Aires Province.

The XLIX Annual Meeting was held
in loving memory of Dr. María Beatriz Guglielmotti.

The meeting was honored by the presence of IADR Vice-President Dr. Rena D'Souza, and SUIO Past-President Dr. Marcelo Kreiner, attending as special guests.

The following conferences were given at the meeting:
1- "A NEW DECADE OF OPORTUNITY FOR DENTAL AND CRANIOFACIAL RESEARCH", by Dr. Renna D´ Souza
2- "A NOVEL CURE FOR CLEFT PALATAL", by Dr. Renna D´ Souza
3- " THE 3Rs IN ANIMAL RESEARCH AND TESTING", by Astauskas Julia
4- " SINUS RESPONSE TO GRAFT PLACEMENT", by Prof. Hector Alvarez Cantoni

A highlight of the meeting was the appointment of Drs. Ricardo Macchi, María Elina Itoiz, Ángela Ubios, Noemí Bordoni and Omar Gani as Honorary Members, and Dr. Alcira Rosa de Nastri as Life Member.

The meeting was attended by 340 participants, and 200 research works were presented.

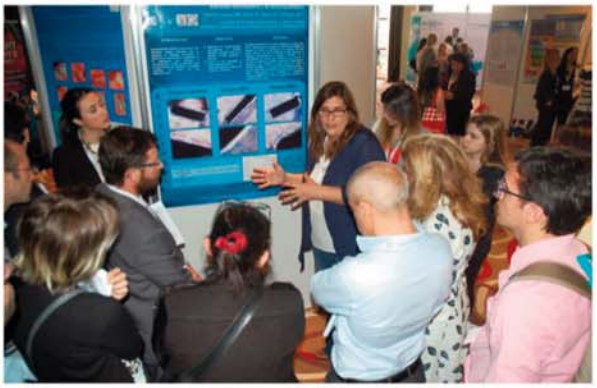
The following prizes and fellowships were awarded:
Unilever Division Travel Award, Colgate - Palmolive Award for students, Colgate - Palmolive Award for Clinical Science Professionals, Colgate - Palmolive Award for Basic Science Professionals, "María Ines Egozcue" Award, Dental Education Award, National Academy of Dentistry Award, Oral Health Award, Rins de David Award, Prof. Dr. H. Maddalena Award, Omar Tumilasci Award, "Rodolfo Erausquin" Award, Orthodontics Group Award, and Scozzarro Award

The Editorial Committee of the Acta Odontológica Latinoamericana met with SAIO members during the event.

Dr Renna D'Souza's Conference

Dr. Rena D'Souza with the Members of de SAIO Board
From left to right: Drs. Débora Tasat, Carlos Rozas, Tammy Steimetz, Mariana Picca, Daniel Olmedo, Rena D'Souza, Susana Molgatini, Luciana Sánchez, Gabriel Sánchez, Analía Garrofé and Andrea Kaplan

From left to right: Drs. Daniel Olmedo, Noemí Bordoni, Patricia Mandalunis, Luciana Sánchez, Sandra Renou, María Elena Itoiz, Ricardo Macchi, and Rómulo Cabrini



A Poster Session

Honorary Members: Drs. Ángela Ubios, Ricardo Macchi, María Elina Itoiz, and Noemí Bordoni

From left to right: Drs. Rena D'Souza (IADR Vice-President), Daniel Olmedo (Argentine Division President) and Sebastián Puia (XLIX Annual Meeting President)

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‡ Resultados de un estudio de 6 meses para evaluar las mejoras en caries del esmalte usando el método QLFTM (Fluorescencia Cuantitativa Inducida por Luz) contra una crema dental regular sólo con fluoruro, ambas con 1450 ppm de fluoruro.

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