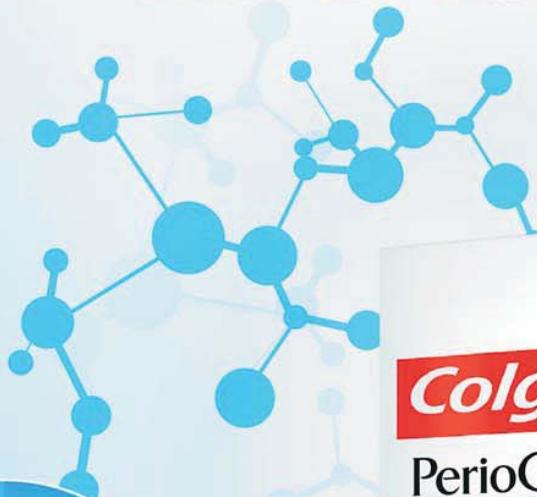

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Methodological considerations for a model of endodontic treatment in Wistar rats

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ABSTRACT

The use of correctly designed animal models is a fundamental step prior to clinical trials in humans. Although rats are easy to house and handle, and have molars that resemble those of humans, very few researchers use them as a model for root canal treatment, probably due to their small size and the lack of relevant data necessary to reproduce the model. Our aims were to describe the anatomic and histologic characteristics of the mandibular first molar of the Wistar rat and present a standardised model for its experimental endodontic treatment. Twenty female rats were used. The characteristics of the mesial and distal roots were described histologically and the quality of the results achieved following the treatment protocol presented herein was assessed by means of digital radiographs, micro-

CT and histological sections. The age of 55 days was found to be the most adequate for performing this technique, but we consider the interval of 50 to 60 days to be suitable. Both canals are oval, although in opposite planes, and the furcating-facing walls present the minimum dentine thickness. It was essential to become familiar with these aspects in order to decide upon the most appropriate instrumentation and obturation techniques that would enable replication of this model in basic science research.

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Keywords: endodontics - laboratory animal models - rats - anatomy and histology - root canal.

Consideraciones metodológicas para el modelo de tratamiento endodóntico en ratas Wistar

RESUMEN

El uso de modelos animales correctamente diseñados es un paso fundamental previo al desarrollo de ensayos clínicos en humanos. A pesar de resultar fáciles de criar y manipular y de poseer molares que se asemejan a los humanos, muy pocos grupos utilizan a la rata como modelo experimental para el tratamiento endodóntico probablemente debido a su pequeño tamaño y a la escasa información disponible para poder aplicar los modelos existentes. Nuestros objetivos fueron describir las características anatómicas e histológicas del primer molar inferior de la rata Wistar y presentar un modelo estandarizado para el tratamiento endodóntico experimental de esta pieza. Se utilizaron 20 ratas hembra. Las características de las raíces mesial y distal fueron descritas histológicamente y los resultados obtenidos fueron evaluados mediante radiografías digitales,

microCT y cortes histológicos. La edad de 55 días demostró ser la más adecuada para ejecutar la técnica, pero consideramos que el intervalo de 50 a 60 días puede resultar apropiado. Se observó que ambos canales presentan una morfología oval, aunque en direcciones opuestas, y que las paredes furcales resultaron ser las que presentan el menor espesor de dentina. Familiarizarse con estos aspectos de la anatomía e histología del molar de la rata resultó fundamental para decidir sobre las técnicas de preparación y obturación más apropiadas que permitieran la replicación de este modelo en el campo de las ciencias básicas.

Palabras clave: endodoncia - modelo experimental - ratas - anatomía e histología - conducto radicular.

INTRODUCTION

Animal models are fundamental in dental research, especially in the field of Endodontics, as many aspects of periapical tissues and their response and

healing impose an ethical barrier for their assessment in humans, and they are better explored in animal models that allow histological evaluation. These models include different species, from rodents¹⁻⁴ to

animals higher up in the phylogenetic scale such as dogs⁵⁻⁷ and primates⁸.

Wistar rats were selected in the present study because they can be easily housed and handled, and enable histological sections to be obtained relatively fast, at a lower acquisition and maintenance cost, with fewer breeding, management and ethical concerns. Furthermore, the reaction of their pulpal³ and periapical tissues⁹ is similar to that of humans, which makes them a suitable option for experimental models in Endodontics.

Albino rats lack primary dentition –they are monophyodont– and present four permanently-growing incisors with a wide-open apex separated from the three molars in each quadrant by a large diastema. Interestingly, rat molars present developmental, anatomical, histological and physiological features similar to those of human teeth¹⁰. The mandibular first molar of the rat is the largest and has four roots—one mesial, one distal and two smaller central ones on the buccal and lingual side, respectively¹⁰. In 1967, the research group led by Erausquin at the School of Dentistry of the University of Buenos Aires described a protocol for performing the root canal treatment of the mesial root of this molar, in accordance with the existing endodontic procedures, in order to study the reaction of the periapical tissues in response to different root canal sealers⁴. However, to date, few research groups have used the rodent model of experimental endodontics *in vivo* in their lines of research, which can range from periapical repair^{1,2} to post-endodontic pain¹¹. In fact, when it comes to evaluating endodontic sealers, subcutaneous implantation models are preferred^{12,13}. It is likely that the scant research using this model is largely due to the difficulty of the technique and the lack of studies describing a standardised protocol according to age and size of the animals, as well as to the lack of information on the morphology of the root canal system of the rat molar. Such information is vital to predictable endodontic treatment¹⁴, since what is not known cannot be adequately treated.

Given the need to present relevant data regarding the reproducibility of the model, the aim of the present study was to describe the anatomic and histologic characteristics of the mandibular first molar of the Wistar rat and to develop a standardised model for experimental root canal treatment of this tooth.

MATERIALS AND METHODS

The experimental protocol and use of animals in the present study was authorised by the Institutional Committee for the Care and use of Laboratory Animals of the School of Dentistry, University of Buenos Aires (CD No. 012/2016 CICUAL–ODON/FOUBA) and all procedures were performed in accordance with the 2011 8th edition of the National Research Council Guide for the Care and Use of Laboratory Animals. Animals were housed in cages in groups of two or three per cage at the animal facility in the Department of Histology and Embryology of the School of Dentistry of University of Buenos Aires under 12:12 h light/dark cycles, and allowed *ad libitum* access to food (standard chow, Cooperación, Buenos Aires, Argentina) and water.

Characterisation of the mandibular first molar in control animals

For the first stage of this study, 15 healthy female Wistar rats were divided into three groups (n=5) according to age –30, 55 and 75 days– for histologic analysis of the length of the mesial and distal roots of the mandibular first molars. Thereafter, the characteristics of the root canals and walls were further assessed in animals from the age group in which root length was found to be optimal for endodontic procedures, using mesio-distally and bucco-lingually oriented sections (n=5 right and n=5 left molars).

Root canal treatment procedure in experimental animals

For the second stage of this study, aimed at standardising the endodontic procedure, 50- to 60-day-old healthy female Wistar rats (n=5) weighing about 180 g were used. Animals were anaesthetised with a combination of 50 mg/kg of ketamine (Ketamid; Holliday-Scott S.A., Beccar, Buenos Aires, Argentina) and 10 mg/kg of xylazine (Kensol; König S.A., Avellaneda, Buenos Aires, Argentina) administered i.p. in order to attain deep levels of anaesthesia. Procedures were carried out using a stretcher that incorporated original modifications to the one described by Erausquin in 1967⁴: once anaesthetised, the animal is laid in dorsal position and its head immobilised by the upper incisors, which are placed through a metal wire attached to the headrest. Mouth opening, tongue separation and mandibular first molar isolation

are achieved by an *ad hoc* aluminium clamp with projections that grip the first molar and an orifice for securing the lower incisors. This makes the mandibular first molar readily accessible (Fig. 1) and enables its isolation, enhanced by the placement of light-cured resin barrier. All procedures can thus be performed without difficulty. Prior to endodontic access, the surface of the isolated molar was cleaned using 0.5% sodium hypochlorite. The mesial pulp horn was then exposed by drilling a small hole through the roof of the pulp chamber using a size 1/4 round dental bur mounted on an electric engine-driven micromotor and placed on the mesial pit with a disto-mesial inclination. Using the same bur, the distal pulp horn was immediately exposed and a size 1/2 round dental bur was used to refine the access until all four root canal orifices were visible. While performing these procedures, it is important to bear in mind that the cusp tips are separated from the pulp chamber floor by only 1.5–2 mm, so care is required to avoid perforation (Fig. 2).

Initial catheterisation was performed using a #08 K-file (Dentsply Sirona, Ballaigues, Switzerland) and a working length of 4 mm (Fig. 2), and confirmed using an electronic apex locator (Root ZX mini; J Morita corp., Tokyo, Japan). Root canal enlargement was performed using K-files #10 to #25 with in and out and clockwise and counter-clockwise rotation motions. At each change of instrument, canals were irrigated with 0.3 ml of freshly-prepared 2.5% sodium hypochlorite delivered by means of



Fig. 1: A metal wire holds the upper incisors, immobilising the head, while a specially designed clamp integrated into the stretcher fixes the position of the lower jaw, keeping the mouth open and displacing the tongue and cheek, thus making the intact mandibular first molar clearly visible and accessible for access and further preparation and obturation.

a carpule dental syringe and an extra-short 30G needle (Misawa Medical Industry, Kasama City, Ibaraki Prefecture, Japan) while aspirating it with a micro-cannula (Roeko SurgiTip-Endo; Coltène/Whaledent, Altstätten, Switzerland). In central canals –buccal and lingual– only #08 and #10 K-files were used in order to achieve pulp removal without further enlargement, given the thinness of their walls.

Canals were then dried using sterile paper points #25 and filled with a #25 gutta-percha master cone, accessory FF cones (Meta Biomed; Cheongju, North Chungcheong, South Korea) –both previously decontaminated in 2.5% sodium hypochlorite– and a ZOE-based sealer (Grossman's Root Canal Sealer; Farmadental, Buenos Aires, Argentina) using cold lateral condensation technique and paying special attention to the distinctive morphology of each canal. After sectioning the filling material to the cervical level with an adapted heated #40 digital plugger (VDW, München, Germany) and vertically compacting it, the pulp chamber was filled with light-cured glass-ionomer cement (SDI Riva, Victoria, Australia).

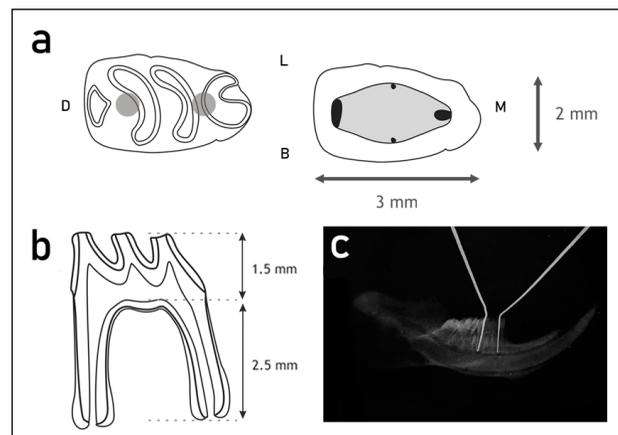


Fig. 2: a) Diagram of the occlusal and pulp chamber topography of the mandibular first molar and the size of the crown. Adequate refinement of the endodontic access enables visualisation of the mesial, distal, and the smaller buccal and lingual root canal orifices. M: mesial, L: lingual. Grey dots mark the starting location for penetration of the pulp chamber roof. b) Diagram of the internal anatomy of the mandibular first molar. The distance between the molar cusp tips and the pulp chamber floor is 1.5 mm and from this point to the root apex, 2.5 mm. Note the scarce dentine thickness separating the pulp chamber from the furcal area, resulting in high risk of perforation during the access phase. Buccal and lingual roots are not depicted. c) Radiograph of a 55-day-old rat hemimandible (*ex vivo*) showing files placed in the mesial and distal canals at a working length of 4 mm.

After recovery from anaesthesia, animals were monitored for signs of discomfort or pain, including alteration of feeding and hydration patterns and behaviour, to detect any unexpected post-treatment reaction. If any of the animals showed signs of severe pain according to the score suggested by Wolfensohn and Lloyd¹⁵, or any signs of infection, they were to be removed from the experimental group and administered antibiotic and/or analgesic medication. However, this was not necessary, as all operated animals returned to normal behaviour, including feeding and social interaction, after the first day post-treatment. After two weeks, animals were euthanised by i.p. injection of a lethal dose of pentobarbital sodium (Euthanyle; Brouwer S.A., Buenos Aires, Argentina) at approximately the same time of the day in order to minimise the effect of changes owed to circadian rhythm. Right and left hemimandibles were dissected and fixed in buffered formalin (pH 7.3-7.5) for 48 h.

Radiographic analysis

Radiographic analysis of the endodontically treated molars was performed by taking periapical radiographs of hemimandibles using a digital sensor (MyRay; Imola, Bologna, Italy) and a dental X-ray unit operated at 70 kV and 8 mA, with a 15 cm source-to-object distance and 0.15-second exposure time.

Micro-tomographic analysis

Micro x-ray computed tomography of endodontically treated molars was carried out using a custom attachment and a micro-CT system (SkyScan 1272; Bruker, Kontich, Belgium). Scans were obtained using a tube voltage of 90 kV and current of 110 mA through 180 rotation around the vertical axis with a rotation step of 0.2. Image pixel resolution was 15 µm and a 0.5 mm aluminium filter was used to reduce beam hardening. Projection images were reconstructed using NRecon v.1.7.3.1 software (Bruker, Kontich, Belgium) and Data Viewer software (Bruker, Kontich, Belgium) was used to obtain representative images.

Histologic and histomorphometric analysis

Following decalcification in EDTA 10% pH 7 for 5 weeks, hemimandibles were dehydrated and embedded in paraffin, and serial sections of the mandibular first molars of approximately 7 µm were obtained. Mesio-distally (MD) and bucco-lingually

(BL) oriented sections were obtained from control animals to study the characteristics of this molar while mesio-distally (MD) oriented sections were obtained from experimental animals to evaluate the quality of the treatment; both were stained with haematoxylin-eosin (HE). Only sections in which continuity between coronal and radicular pulp and between the latter and the apical periodontal ligament was evident were selected, in order to ensure adequate orientation of the axis of each root. Digitalised microphotographs were then taken with a light microscope (Nikon Eclipse Ni, Nikon Instruments Inc, Melville, NY, USA) and analysed using ImagePro Plus 4.5.0.29 software to determine:

- The length of the mesial and distal roots (MR, DR) in MD sections of the initial three animal groups (i.e. 30, 55 and 75 days old). In order to do this, one tangent line was drawn to the pulp chamber floor and another one to the root apex, and the distance between the two measured perpendicularly to them and running through the centre of the corresponding root canal.
- The width of the root canal and the thickness of the furcal (F), external (E), buccal (B) and lingual (L) walls in the cervical (C), middle (M) and apical (A) thirds in MD and BL sections of the 55-day-old animals. For each wall, total and dentine thickness were measured along nine straight parallel equidistant lines each. Three determinations per third were averaged to obtain a single value for each parameter and third. The thickness corresponding to cementum was calculated as the difference between the aforementioned measurements.

Statistical analysis

InfoStat v2019 was employed. Normality of data distribution was verified through Shapiro-Wilk test. All the results were expressed as mean and standard deviation (SD), and analysed statistically using ANOVA followed by Tukey's post-hoc test, setting the significance level at 5%.

RESULTS

Histomorphometric description of the mandibular first molar

The histologic characteristics of the roots of the mandibular first molar of 30, 55 and 75 day old rats were analysed. In 30 day old animals, root

development was still incomplete, with thin root canal walls, a wide root canal, a wide-open apex and the presence of Hertwig's Epithelial Root Sheath (HERS) (Fig. 3a). Animals aged 55 days had a completely developed apex with no evidence of HERS (confirming that the radicular dentine had reached its maximum length), cementum lining the external surface and increased root canal wall thickness (Fig. 3b). In older animals (75 days old) further cellular cementum deposition occurred at the apex, sometimes invading the apical third of the canal, thus increasing radicular length and altering apical morphology (Fig. 3c). The length of the mesial and distal roots did not differ significantly. However, mean root length increased progressively and significantly from 30 days ($1.9\pm 0.24\text{mm}$), to 55 days ($2.5\pm 0.18\text{mm}$) and up to 75 days of age ($3.1\pm 0.11\text{mm}$) (Fig. 4).

These histological observations led us to rule out the 30- and 75-day-old groups from further endodontic protocol experiments and to select the 55-day-old group for the rest of the radicular analysis and experimental root canal treatment. In these animals, in addition to root length, root canal width and wall thickness of both mesial and distal root canals were measured in both orientations (MD and BL) and all three radicular thirds (cervical, middle and apical).

The mean width (μm) of the mesial root canal was significantly greater in the MD direction (MD: 293 ± 120 vs BL: 165 ± 54 , $p < 0.0001$), while the distal root canal was found to be wider in the BL direction (MD: 192 ± 69 vs BL: 398 ± 147 , $p < 0.0001$) (Fig. 5).

The analysis of all four root canal walls (furcal, external, buccal and lingual) showed that the relationship between dentine and cementum is inversely proportional in each of the three thirds as the greatest thickness corresponds to dentine in the cervical third and cementum in the apical third. Considering these parameters, we can state that the total wall thickness (μm) remains constant (without significant differences between thirds) showing a progressive decrease in dentine and increase in cementum thickness from the cervical to the apical third ($p < 0.0001$ between thirds of each root canal wall) (Fig. 5 and Table 1).

Since preserving dentine is an important aspect of endodontic shaping procedures, dentine thickness (μm) was analysed within each root (MR and DR) in each of the three thirds (C, M and A) and considering all four walls (F, E, B and L). The furcal wall was found to be the thinnest in both roots, and this was significantly more noticeable in the cervical third of the mesial root (MRC: F: 154 ± 27 , E: 256 ± 33 , L: 233 ± 32 , B: 237 ± 19 , $p < 0.0001$) and the middle third

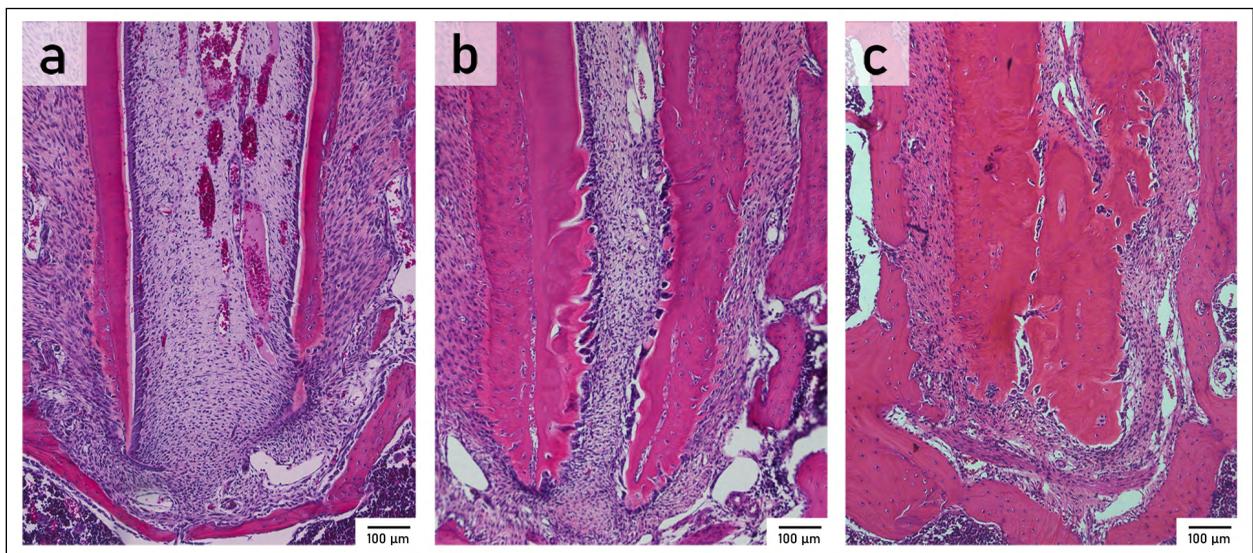


Fig. 3: Microphotographs of HE stained histological sections of the distal root of the mandibular first molar depicting different stages of root development. In 30-day-old animals a) root development is still incomplete and Hertwig's Epithelial Root Sheath (HERS) can still be observed. By 55 days of age b), radicular dentine has been completely laid down, with cementum laid parallel and externally to it and no evidence of HERS. In 75-day-old animals c) greater amounts of cellular cementum can be seen at the root apex, increasing radicular length and often invading the apical third of the root canal, thereby altering canal morphology. Bars represent 100 μm .

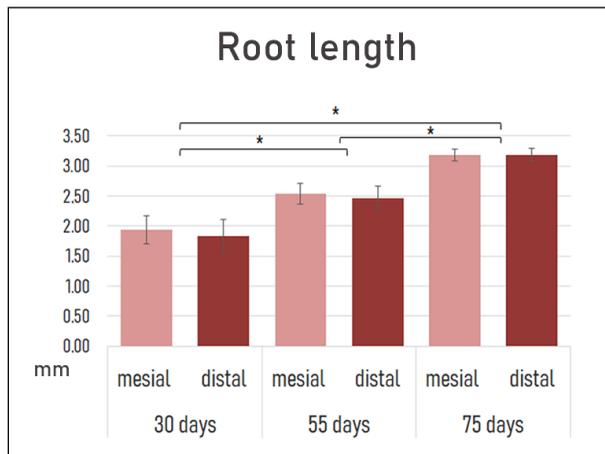


Fig. 4: The graph shows the mean length and SD (in mm) of the mesial and distal roots of the mandibular first molar. No differences were found between roots within each age group but root length was found to increase significantly with age ($p < 0.0001$).

of the distal root: F: 98 ± 10 , E: 176 ± 20 , L: 237 ± 43 , B: 184 ± 42 , $p < 0.0001$) (Fig. 5 and Table 1).

Post-endodontic observations

The average time needed to perform each root canal treatment was 40 minutes, from the time of drilling for endodontic access to placing the coronal seal. When the animals were euthanised (2 weeks post-treatment), all coronal seals were intact and in place. Mesial and distal root canal fillings showed

satisfactory radiographic or micro-tomographic three-dimensionality and adequate length, and there was no evidence of surrounding alveolar bone loss (Fig. 6). Histological observations were consistent with the aforementioned. Furthermore, no pulpal remnants were found in treated animals and the apical limit was confirmed to be correct (Fig. 7), demonstrating satisfactory achievement of the physical and chemical goals of chemomechanical preparation. The presence of remaining dentine surrounding the filling material proved that the calibre of the preparation was in accordance with the internal anatomy.

A moderate amount of lymphocytic infiltrate was observed in the periapical tissues, as well as the presence of small fragments of eosinophilic material with a tubular pattern resembling dentine, possibly consisting of debris or, in absence of this pattern, root canal sealer extruded during the shaping and obturation phases, respectively (Fig. 7). No areas of necrosis were observed within the adjacent periodontal ligament or cellular cementum.

DISCUSSION

Correctly designed animal models using rodents have been extensively used in dental research^{1-4,9,16,17} because these animals can be easily housed and handled and enable histological sections to be

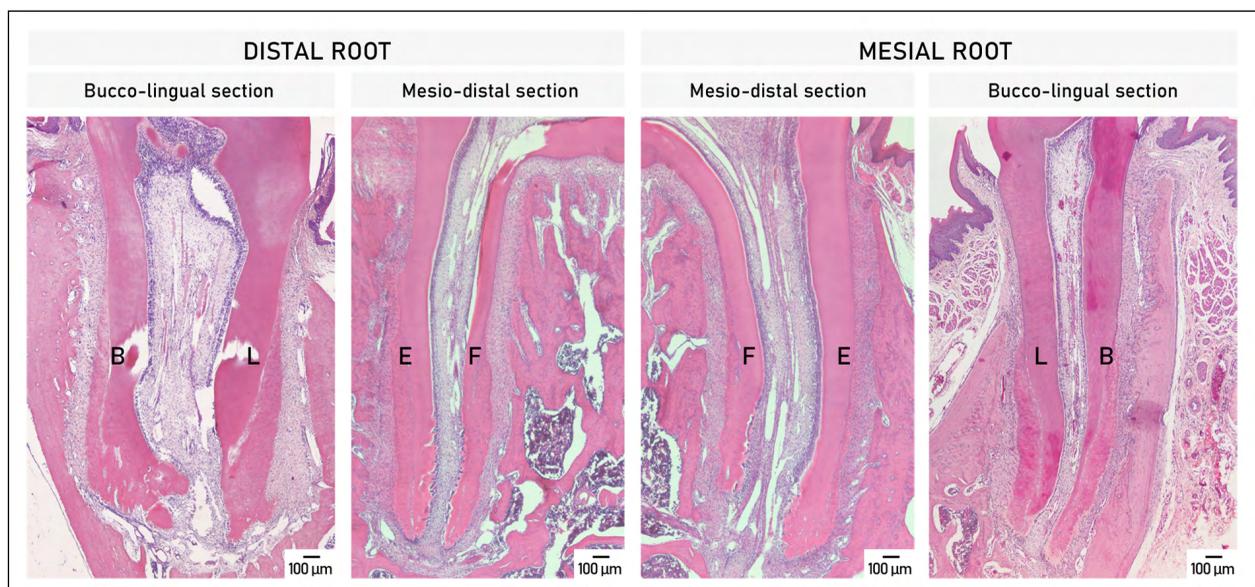


Fig. 5: Microphotographs of HE stained histological sections of the mesial and distal roots of the mandibular first molar. Bucco-lingual sections of both roots show an even thickness of buccal and lingual walls while mesio-distal sections reveal that furcation-facing walls are notably thinner: B: buccal, L: lingual, E: external, F: furcal. Original magnification 40X. Bars represent 100 μ m.

Table 1. Root canal wall thickness

Third	Mesial root (55 days). Mean thickness (μm)				Distal root (55 days). Mean thickness (μm)				
	Buccal	Lingual	External	Furcal	Buccal	Lingual	External	Furcal	
Cervical	T	242 \pm 19 ^{Aa}	238 \pm 32 ^{ABa}	259 \pm 33 ^{Aa}	171 \pm 25 ^{Ba}	209 \pm 48 ^{Ab}	311 \pm 50 ^{Ba}	250 \pm 23 ^{ABa}	162 \pm 29 ^{Aa}
	D	237 \pm 19 ^{Aa}	233 \pm 31 ^{Aa}	256 \pm 32 ^{Aa}	154 \pm 27 ^{Ba}	198 \pm 48 ^{Ab}	300 \pm 50 ^{Ca}	245 \pm 21 ^{BCa}	155 \pm 31 ^{Aa}
	C	4 \pm 1 ^{Aa}	5 \pm 2 ^{Aa}	3 \pm 1 ^{Aa}	17 \pm 8 ^{Aa}	10 \pm 6 ^{Aa}	11 \pm 3 ^{Aa}	5 \pm 3 ^{Aa}	7 \pm 2 ^{Aa}
Middle	T	269 \pm 54 ^{Aa}	269 \pm 54 ^{Aa}	269 \pm 54 ^{Aa}	212 \pm 17 ^{Aa}	253 \pm 40 ^{Aab}	311 \pm 56 ^{Aa}	236 \pm 29 ^{Aa}	125 \pm 9 ^{Ba}
	D	169 \pm 10 ^{ABb}	190 \pm 26 ^{Aa}	209 \pm 16 ^{Ab}	144 \pm 19 ^{Ba}	194 \pm 42 ^{Aa}	237 \pm 43 ^{Aa}	176 \pm 20 ^{Aa}	98 \pm 10 ^{Bab}
	C	58 \pm 35 ^{Ab}	54 \pm 20 ^{Aa}	60 \pm 41 ^{Ab}	69 \pm 13 ^{Ab}	69 \pm 24 ^{Aa}	75 \pm 23 ^{Aa}	60 \pm 25 ^{Aab}	27 \pm 14 ^{Aab}
Apical	T	187 \pm 20 ^{Aa}	220 \pm 33 ^{Aa}	227 \pm 24 ^{Aa}	225 \pm 36 ^{Aa}	312 \pm 91 ^{Ab}	287 \pm 73 ^{Aa}	236 \pm 28 ^{ABa}	151 \pm 21 ^{Ba}
	D	52 \pm 9 ^{Bc}	100 \pm 22 ^{Ab}	114 \pm 10 ^{Ac}	73 \pm 10 ^{ABc}	100 \pm 38 ^{Ab}	89 \pm 23 ^{Ab}	104 \pm 8 ^{Ab}	48 \pm 6 ^{Ab}
	C	135 \pm 14 ^{Ac}	120 \pm 26 ^{Ab}	113 \pm 19 ^{Ac}	152 \pm 42 ^{Ac}	212 \pm 90 ^{Ab}	198 \pm 72 ^{Ab}	131 \pm 25 ^{Bb}	103 \pm 19 ^{ABb}

The table shows mean and SD (in μm) of the total, dentine and cementum thickness of the root canal walls of the mesial and distal roots of the mandibular first molar in the bucco-lingual (buccal and lingual walls) and mesio-distal (external and furcal walls) direction. T: total, D: dentine, C: cementum. For comparisons within each root, different lowercase letters indicate statistically significant differences between rows and different uppercase letters indicate differences between columns ($p < 0.0001$).

obtained relatively fast. In the field of Endodontics, larger animals such as dogs, whose teeth have a single canal and are more comparable in size to human teeth, have been preferred for studying root canal treatment procedures⁵⁻⁷. However, in addition to dogs being more expensive and often drawing opposition from public opinion¹⁸, it has been reported that their apical anatomy is complex and invariably presents an apical delta^{19,20}, making them a less than ideal model for understanding the repercussions of root canal chemomechanical debridement and obturation on periapical tissues. Although scarce, some studies using the rat as a model for endodontic treatment are available in literature, the vast majority using the mandibular first molar: some have focused on treating either the mesial^{1,4} or distal^{21,22} root and even fewer have addressed both^{2,23}. Sadly, the careful dissection of some of the endodontic treatments reported reveals suboptimal results regarding working length or excessive tapers which turn out to be inadequate for the root canal anatomy present in the rat. These problems are probably owed to the lack of studies describing a standardised protocol for root canal treatment in accordance with animal age, size and root canal morphology, as well as the adequate age for treatment. Moreover, to our knowledge, there is no study addressing the anatomic and histologic characteristics of the mandibular first molar of the Wistar rat.

According to early published data, in albino rats, molar crown formation is completed between postnatal days 11-21, and root development in first,

second and third mandibular molar is completed by days 25, 28 and 40, respectively¹⁰. Nevertheless, the data presented in our study revealed that by day 25, root development of the mandibular first molar is not yet complete and Hertwig's Epithelial Root Sheath (HERS) can still be observed. In our study, root development of the mandibular first molar was found to be complete at around day 55, which is in agreement with recent data². From this age onwards, root length increases as a result of the continuous deposition of cellular cementum at the root apex. Interestingly, in adult rats, up to one third of the total root length can be composed of cementum alone. This mechanism, termed hypercementosis, is considered to be physiological, continuous²⁴ and necessary to compensate for the attrition resulting from mastication and diet characteristics¹⁰, and potentially complicates the achievement of patency and satisfactory endodontic debridement and obturation. Taking all this into account, we decided to use Wistar rats aged 55 days which, according to histological observations, have a completely developed root (no HERS is observed) and cementum –including the cellular variety– lining the external surface of radicular dentine, sometimes extending slightly apically to it, and maintaining a relatively stable root length of 2.5 mm and a radicular canal that is accessible for instrumentation and the establishment of an apical stop.

Our findings regarding the distinctive anatomy of the mesial and distal canals revealed that both are oval, as within each sample the diameter in

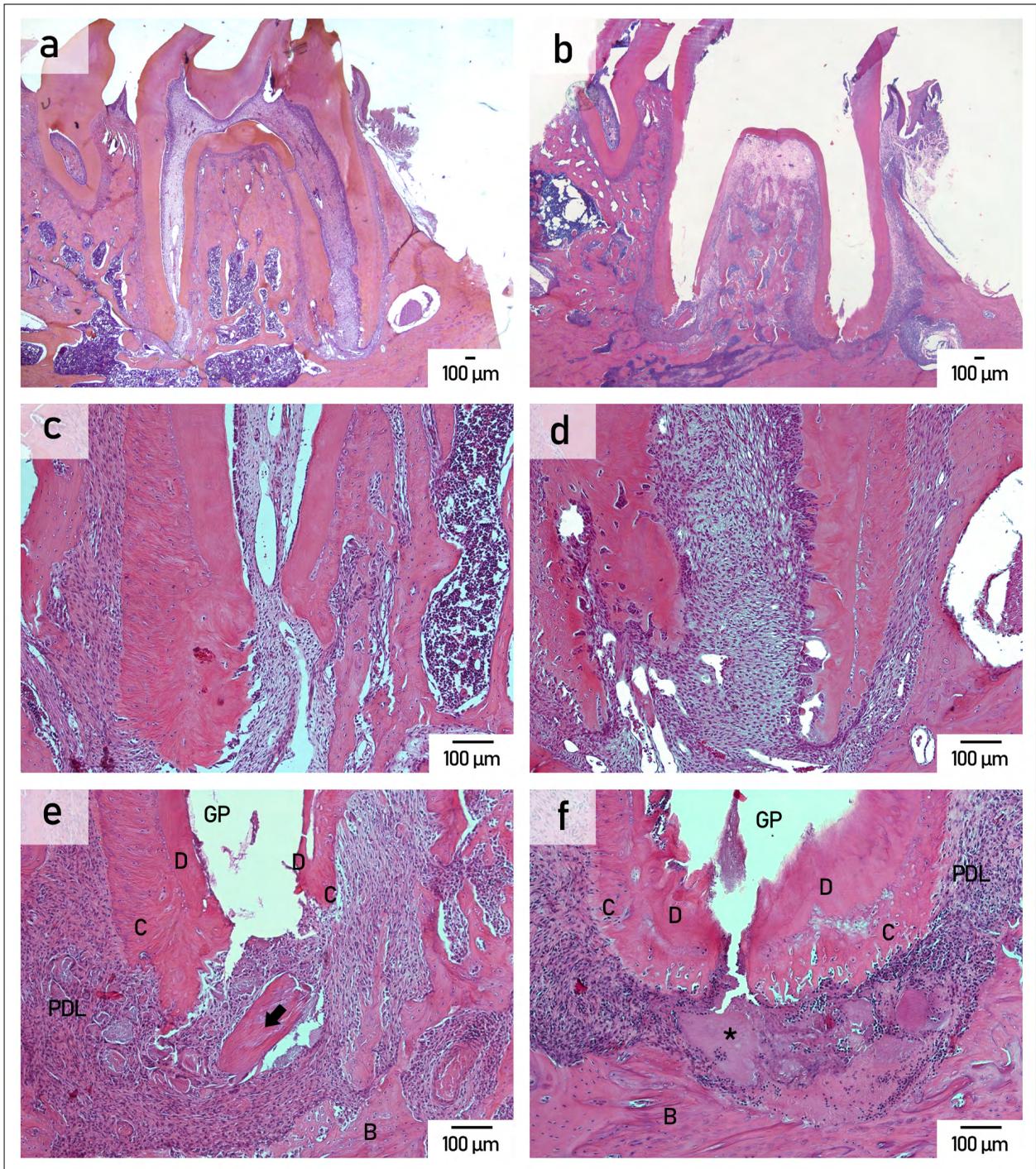


Fig. 6: Periapical radiograph of an endodontically treated a) molar depicts achievement of adequate working length and proper filling of both distal and mesial root canals. This was confirmed by micro-tomographic slices that also enabled more detailed assessment of the condition of the surrounding bone. Sagittal slices b) confirmed that no stripping of the canal walls had occurred, which is consistent with an intact interradicular bone, while coronal slices of the distal c) and mesial d) roots showed correct adaptation of the obturation mass to the root canal walls and only small pores within it, even in the distal root c) which is fairly wide in the bucco-lingual plane. In some cases, a minor extrusion of sealer was seen through the foramen of both distal and mesial roots, but it seems not to have caused loss of apical bone.

one of the directions –either MD or BL– was two times greater than the diameter in the other direction²⁵. Furthermore, while the mesial canal was found to be wider in the mesio-distal plane, the opposite occurred with the distal canal. These characteristics should be taken into account when pondering the question of which type of preparation and obturation techniques to use. In terms of preparation, attention should be paid to cleaning and shaping the canal entirely in order to eliminate inflamed or necrotic pulp. Most rotary instruments produce a round preparation^{26,27}, leaving unprepared recesses in oval-shaped canals²⁸. Even if this problem can be partially surmounted by the use of laterally directed brushing working strokes²⁹, unfortunately this would not be feasible in the rat, given that the restricted angle for instrumentation and the short length of the roots limit the amplitude

and control of movements. We therefore consider manual circumferential instrumentation to be more appropriate, as previously suggested for this type of anatomy in human teeth²⁵.

In this regard, it is important to consider dentine thickness in each radicular third in order to preserve as much as microbiologically admissible, since excessive loss of dentine may contribute to increased susceptibility to fracture³⁰. Even though micro-CT technology could prove to be a powerful non-destructive method for understanding the anatomy of the rat molar, segmentation of dentine and cementum based on pixel grey level values would be very difficult –if not practically impossible when considering acellular cementum– and require very high resolutions, resulting in time-consuming scanning protocols (estimated resolution of 2 μm or less, and >10 h). These difficulties are caused by the small size of the sample and the fact that dentine and cementum have similar mineral densities³¹. We therefore decided to use HE stained histological sections, which enabled us to identify and measure the thickness of each tissue³². We thus found furcation-facing walls to be thinner than their external counterparts, especially in the coronal third. This is analogous to what is observed in human molars, in which the limited amount of dentine associated with the concavity and root curvature makes them *danger zones*³³ where overzealous filing must be avoided in order to prevent strip perforations. An analysis of the diameters of different NiTi instruments with equal tip size (i.e. 0.25 mm) over the first 3 mm of their active portion –including one used in a recent study proposing a model for root canal treatment in the rat²– based on the premise that they produce centred preparations³⁴ reveals that all of them would cause excessive thinning of dentine. In the direction in which each canal attains its minimum diameter –BL and MD for the mesial and distal canals respectively– this could take up from 33% to 97% of the dentine wall in the cervical and middle thirds of the root, depending on the system, while the use of K files of equal diameter would cause a decrease in thickness of no more than 39%. This shows that more conservative tapers (i.e. 2%) should be preferred to larger tapers (i.e. 4%, 6%, 7%, 8%). As for the obturation phase, a single cone technique would not be likely to satisfactorily fill the entire canal space, which is why we consider



*Fig. 7: Microphotographs of HE stained histological sections of a control a, c, d) and an endodontically treated molar b, e, f). Comparison shows that the preparation technique achieved satisfactory elimination of dental pulp tissue at an adequate working length and an apical size in accordance with the anatomy of each root. Original magnification 20X (a-b) and 100X (c-f). GP: gutta-percha, PDL: periodontal ligament, D: dentine, C: cellular cementum, B: bone, →: dentine debris, *: extruded root canal sealer. Bars represent 100 µm.*

cold lateral condensation with accessory gutta-percha points to be a better choice.

Another important aspect is final restoration of the access cavity, which has been shown to have impact on the rate of success of endodontic therapy³⁵ and is important in preserving the treatment throughout experiment. Materials such as amalgam, as proposed by other groups²², tend to result in lack of retentiveness due to the shallowness of the access cavity (1.5 mm in depth) or cracks/fracture of the walls upon compaction. The use of composites, on the other hand, demands a strict adhesive technique and, once cured, can result in premature contacts that would lead to a state of inflammation of the periodontal ligament, in addition to muscle fatigue and/or temporomandibular joint disorders³⁶. Furthermore, their high elastic modulus could complicate the obtention of histological sections using a microtome once the hemimandible has been decalcified. We therefore suggest the use of type II restorative glass-ionomer cement, given its compressive and tensile strength, as well as its excellent adhesion to dentine and enamel and hardness that permits surface wear when in occlusion with the antagonist tooth³⁷. Other groups have also used this material^{1,21,23}. In our experience, a thorough removal of excess gutta-percha from the pulp chamber is key to guaranteeing sufficient contact of the material with the walls and to ensure greater longevity of the restorations.

In view of achieving operator training and carrying out a successful treatment, we have considered the methodological steps followed and prepared the following protocol and recommendations for experimental root canal treatment in Wistar rats:

- Select appropriately aged animals in order to avoid intraoperative complications. We found 55-day-old rats to be optimal models, but consider the interval of 50 to 60 days of age to be adequate.
- Attain deep levels of general anaesthesia for up to 40-60 minutes.
- Use a stretcher that provides proper positioning of the animal's head and enough mouth opening in order to ensure clear visualization view of the molar and operator's access to perform shaping,

cleaning and filling procedures.

- Use a specially designed clamp and an aspiration micro-cannula to enable soft-tissue separation and provide a thoroughly dry field while maintaining oropharynx permeability and avoiding ingestion and/or obstruction of the respiratory airway.
- Position the dental bur on the mesial pit with a disto-mesial inclination so as to enter the mesial pulp horn and avoid perforation of the pulp chamber floor.
- Perform catheterisation of the canals using #08 K-files and confirm working length (estimated in 4 mm) using an electronic apex locator.
- Shape and enlarge mesial and distal canals using manual circumferential instrumentation because of their oval shape and using the minimum taper available (i.e. 2%) because of the thinness of their walls.
- Fill canals using a cold lateral condensation technique instead of a single cone technique, given the oval shape of the canals.
- Remove excess gutta-percha from the pulp chamber and seal it with type II restorative glass-ionomer cement.
- Throughout the procedure, hydrate the animal's eye and monitor its breathing and heartbeat, in order to provide immediate care should any emergency or complication arise.
- Monitor animals until after anaesthesia has worn off. The postoperative recovery will depend upon basic sanitary care, place (cage) in which animals are being kept, proper ventilation and hygiene.
- Consider rodents to be living beings whose biological responses are similar to humans', but faster, hence, whenever this model is used, results and their extrapolation to humans should be interpreted carefully.

To conclude, the experimental protocol presented herein enables the study of biological responses that would otherwise be ethically inaccessible in humans and could not be replicated effectively by using extracted teeth. We consider that this model for experimental root canal treatment could contribute to future research, unravelling a variety of aspects related to endodontic treatment.

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Brazilian dentists' perceptions of using bone grafts: an inland survey

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ABSTRACT

Pre-clinical assessments of bone substitute materials are frequent in the literature, but research papers about the clinical situation of bone graft use and consumer market acceptance are rare. The aim of this study was to evaluate the dental use of bone grafts according to the perception of dentists in the city of Sobral, Brazil. We interviewed 183 professionals and analyzed their professional data, knowledge of the subject, specific use, and opinions on cost-effectiveness and biosafety. Most of the respondents had 10 years' or less experience in the profession, and lacked specialization though they had been familiar with the subject since they graduated. The most frequently mentioned compositions were ceramics, followed by composites. Only a quarter of the respondents had performed bone grafts, generally with up to 50 cases, with the most frequent applications being using simultaneously with a dental implant, fresh dental sockets and maxillary sinus lifting. Autogenous and xenogeneic grafts were more frequent than alloplastic and allogeneic; ceramics were the most frequently used composition, and the association

of bone graft with membrane was more frequent than bone graft alone or associated with autologous fibrin. Professional and patient satisfaction was high, cost was considered moderate, and differences were found regarding patient participation in the choice and country of origin of the product. Bone grafts were used more often in the private than public service. Dentists stated that they follow the instructions. Inflammatory or infectious complications were found to be related to the frequency of the procedure performed and safety regarding the origin. Opinions were favorable regarding the use of grafts derived from animals and unfavorable to grafts derived from cadavers. Very few respondents were registered in human bone tissue banks. The good level of acceptance for dental use of bone grafts in a Brazilian inland city provides a promising scenario for the development of the sector.

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Keywords: bone transplantation - biocompatible materials - bone regeneration.

Uso de enxertos ósseos na percepção de cirurgiões-dentistas do interior do Brasil

RESUMO

Avaliações pré-clínicas sobre materiais osseosubstitutos são frequentes na literatura, mas são raras pesquisas sobre o panorama clínico de uso de enxertos ósseos e aceitação do mercado consumidor. O objetivo deste estudo foi avaliar o uso odontológico de enxertos ósseos na percepção de dentistas da cidade de Sobral, Brasil. Foram entrevistados 183 profissionais e analisados dados profissionais, conhecimento temático, uso específico, relação custo-efetividade e biossegurança. A maioria dos cirurgiões-dentistas exibiu até 10 anos na profissão, ausência de especialização, porém familiaridade com a temática desde a graduação. As cerâmicas, seguidas de compósitos, compuseram as composições mais citadas. Apenas um quarto dos cirurgiões-dentistas já realizaram enxertos ósseos, em geral com realização de até 50 casos, com maior tendência de aplicação em alvéolo dentário fresco, simultânea a implante dentário e para levantamento de seio maxilar. As origens autógena e xenógena tiveram maior destaque do que aloplástica e alógena, as cerâmicas foram a composição mais usada e a associação do

enxerto ósseo com membrana foi mais frequente do que enxerto ósseo sozinho ou associado à fibrina autóloga. A satisfação do profissional e paciente foi alta, o custo considerado moderado, havendo diferenças quanto a participação do paciente na escolha e no país de origem do produto, com maior uso no serviço privado do que no público. Os dentistas afirmaram seguirem a bula, que as complicações inflamatórias ou infecciosas estão relacionadas com a frequência de procedimento realizado e segurança quanto à origem. Houve opinião favorável ao uso de enxertos derivados de animais e desfavorável ao de cadáveres, havendo raros profissionais cadastrados em bancos de tecidos ósseos humanos. A boa aceitação para uso odontológico de enxertos ósseos em cidade do interior do Brasil configura um cenário promissor para o desenvolvimento do setor.

Palavras-chave: transplante ósseo - materiais biocompatíveis - regeneração óssea.

INTRODUCTION

Bone regeneration in the human body has been studied over the past century with a wide variety of purposes, approaches and surgical materials¹. Bone grafts are indicated in medical and dental practice for restoring compromised function and aesthetics due to bone defects². In oral surgery, bone grafts contribute to the treatment of small to moderate bone defects resulting from periodontal or endodontic disease, dental socket filling after extraction, maxillary sinus lifting or rehabilitation of bone atrophy prior to implants, and even large bone defects caused by traumas, congenital deformities, pathologies and infections involving the maxillofacial complex³.

The choice of a bone substitute should be guided by its physicochemical, mechanical and biological characteristics, including architecture, porosity, resistance, biocompatibility, biodegradability, osteoconduction, osteoinduction and/or osteogenesis, in order to mimic the bone tissue to be repaired⁴. Biomaterials can be classified according to their origin (autogenous, allogeneic, xenogeneic or alloplastic), composition (metals and alloys, ceramics and bio-glass, polymers or composites) or interaction with the biological environment (bioinert, bioabsorbable or bioactive)⁵.

There are advantages and limitations in the use of bone grafts. Higher success rates have been achieved with autogenous bone, despite its limited supply and higher morbidity^{5,6}. Donated allografts are more readily available, having been reported over the last sixty years and used increasingly in Europe. However, difficulties in processing the biomaterial and risk of an immunogenic inflammatory response may reflect delay or decrease in osteogenesis⁶. Xenografts have been popular since the 1960s, especially bovine bone, despite the controversies with relation to the risk of transmission of zoonoses, which, in fact, have never been documented in dental practice⁷. Currently, animal byproducts could subsidize the generation of natural materials and contribute to the consolidation of a sustainable industry⁸. Alloplastic materials are satisfactory alternatives to natural materials, but face the challenge of controlling the great variability of results depending on their composition, associated with resorption and inflammatory or repair responses⁹. In view of this evidence, there is still no ultimate or single biomaterial for bone reconstruction, which motivates the biotechnological development of the sector¹⁰.

The growth of the industry and the consumer market for bone substitute materials for dental use converges with the higher life expectancy of the population and the increase in purchasing power in developing countries¹¹. This market is markedly significant in North America, followed by Europe, Asia, Latin America, Oceania and Africa¹². A worldwide increase of 4.4%¹³ to 9.5%¹² is estimated for the period from 2018 to 2026 in guided bone regeneration procedures involving bone grafts and membranes, which may be associated with greater clinical demand from both professionals and patients seeking better results¹³. In the United States and Europe, more than half a million people annually receive treatment for bone defect repair, and the international bone graft market moved USD 493 million in 2018 and expects to generate USD 931 million by 2025¹⁴. In Latin America, this market was valued at USD 116.8 million in 2018¹⁵. In Brazil, the political and economic crisis in the country and the bureaucracy involved in opening new companies are expected to delay the growth of this sector¹³. In a price-sensitive market, the costs and benefits of bone graft procedures should be considered for clinical choice¹⁶.

In this broad market scenario, there is a lack of evidence in the literature about the permeability to bone grafts in dental practice in medium and small cities. The city of Sobral, located in the interior of the State of Ceará, northeastern Brazil (3°41'42.0"S 40°20'28.3"W) is located 232 km from the state capital, Fortaleza. It has an estimated population of 208,935, being the fifth-largest city in this Brazilian state, with a rate of 24.6% of professional occupation, average of 2 minimum monthly wages, gross domestic product per capita of R\$ 20,258.09 or USD 4,771.08, municipal human development index of 0.714, 88.35% of urban population, 67.83% in the 15- to 64-year age group and Gini index 0.56¹⁷. There are 39 outpatient units with dental care, 1 emergency facility with maxillofacial surgery¹⁷, 44 oral health teams in primary care, 2 dental specialty centers (1 municipal and 1 regional) in secondary care¹⁸ and 2 schools of dentistry (1 public and 1 private) with dental care for the population. In the Sobral Campus of the Federal University of Ceará, it was estimated that in 2019, about 4,000 procedures involving oral surgery, periodontics or implantology were performed at the dental clinic.

Thus, the aim of this study was to assess the general

profile of use of bone grafts in the perception of dentists in the Brazilian inland.

MATERIALS AND METHODS

Ethical and legal aspects

This research adopted the ethical principles of respect for people, non-maleficence, beneficence and justice described in the Belmont Report (1978) and the Brazilian guidelines of the Resolution of the National Health Council No. 466 (2012), which establishes the basic requirements for research involving human beings in the country. This research was assessed and approved by the local Ethics Committee of the Universidade Estadual Vale do Acaraú via Plataforma Brasil (register CAAE #04644918.4.0000.5053 and approval protocol #3.145.268), before data collection started.

Participants' consent was registered through a Free and Informed Consent Form (FICF), which provided information in appropriate language about the purpose of the research, as well as ensuring the confidentiality of identities.

Type of study

This was an exploratory, descriptive, cross-sectional study by intensive direct observation with a quantitative, structured approach. The target population consisted of dentists working in the city of Sobral.

Sample

In a previous consultation with the Federal Council of Dentistry, which is a professional-class entity responsible for managing all enrollments and the legality of dental practice in Brazil, it was informed that there are 233 registered practitioners in the city of Sobral. Considering a population of 233 dentists in the city of Sobral and calculating a representative sample with 99% confidence interval and 5% margin of error, we idealized a sample of 173 dentists.

To achieve significant, homogeneous sampling, a random field study was conducted from February to September 2019, restricted to the Sobral municipality, using the snowball technique to access these professionals.

Inclusion criteria were dentists working in the city of Sobral, in private practice or public assistance in primary, secondary or tertiary care of the Brazilian Unified Health System, duly registered with the

Federal Council of Dentistry, of legal age (over 18 years old), of either sex, with no limitation regarding year of graduation, and who accepted to participate voluntarily in this study. Professionals who did not sign the FICF or cases of error in filling out forms were excluded.

Data collection

For the standardization of data collection and analysis, an anonymous, self-reported, face-to-face form was used, applied in individual voluntary interviews in a dental office or health unit.

The variables considered in this study were based on the methodology developed by Castro-Silva and Coutinho (2012)¹⁹. The form consisted of closed objective or semi-open questions, grouped into five basic categories (professional data, subject knowledge, specific use, cost-effectiveness and biosafety).

Data analysis

After the generation of an electronic bank with data collected in the Excel for Windows software (Office 2010™, Microsoft Corporation, USA), the quantitative variables were described in terms of absolute and relative values, being presented in the form of a table. Intergroup inferential statistical analysis of frequencies was performed using the Statistical Package for the Social Sciences software version 20 (IBM™, USA) applying the chi-square test and Fisher's exact post-test for nonparametric data, considering significant differences if $P < 0.05$.

RESULTS

Among the 233 dentists in the city of Sobral, a total 183 volunteers comprised the sample, equivalent to 78.54% of the city's professionals. Remarkably, the sample used in this study was greater than the size determined using statistical parameters as mentioned above. The representative sample of dentists in this study increases the level of confidence in the results found for the city of Sobral.

The prevalence of females (117 participants) was almost double that of males (66 participants) (Fig. 1A). Mean age \pm standard deviation was 35.37 ± 10.21 years and there was prevalence of three quarters of the sample in the age group up to 40 years (Fig. 1B). The vast majority of the sample did not have any specialization or expertise in Orthodontics (Fig. 1C). Table 1 shows respondents' general knowledge about

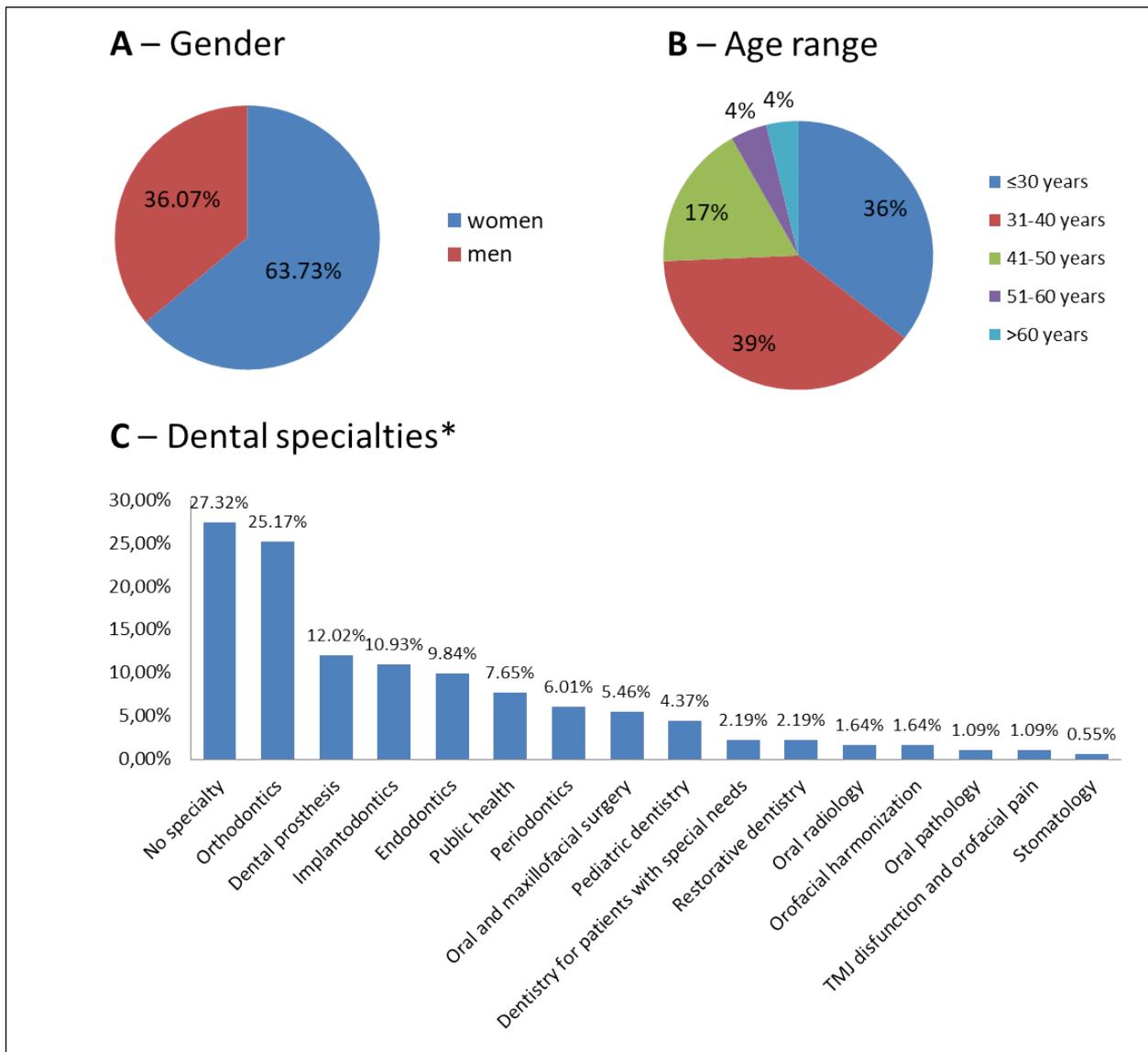


Fig. 1: Profile of dentists in the city of Sobral, Brazil (n=183)

bone grafts. Most respondents sample had graduated up to 10 years previously and demonstrated significant familiarity with the subject. They had acquired such knowledge mainly at undergraduate level, in comparison to postgraduate studies and dental conferences. Among the natural or synthetic compositions mentioned, ceramics were the most prevalent, followed by composites, while there was little mention of polymers and glasses. Bovine mineral bone was the material most frequently cited by all participants.

Table 2 shows that only a quarter of the respondents (45 or 24.59%) had performed bone grafts. Regardless of the intensity of use (from ≤ 10 to >100

cases), the most frequent bone graft applications were simultaneously with a dental implant, fresh dental sockets and maxillary sinus lifting, while the least frequent were periodontal use, paraendodontic use and extensive bone reconstruction. Autogenous and xenogeneic grafts were more often used than alloplastic and allogeneic grafts. Ceramics were the most frequently used composition of bone grafts and their association with membrane was more frequent than bone graft alone or associated with autologous fibrin.

Table 3 shows the high satisfaction of professionals and patients with bone grafts, with material or technical costs being considered moderate.

Table 1. Knowledge about the subject of bone grafts among dentists in Sobral, Brazil (n=183).

Variable	Categories	Frequency of time since graduation (years)					P**
		≤ 10	11-20	21-30	31-40	≥ 40	
Knowledge about bone grafts or bone substitute biomaterials	None	1(0.55%)	0	1(0.55%)	0	0	<0.01
	Yes, in undergraduate level	88(48.09%)	34(18.58%)	7(3.83%)	0	0	
	Yes, in postgraduate studies	41(22.40%)	35(19.13%)	11(6.01%)	4(2.19%)	3(1.64%)	
	Yes, at dental conferences	15(8.20%)	16(8.74%)	4(2.19%)	5(2.73%)	3(1.64%)	
Citation of bone grafts (by composition)*	Ceramics	59(32.24%)	57(31.15%)	12(6.55%)	0	3(1.64%)	0.22
	Glasses	1(0.54%)	1(0.54%)	0	0	0	
	Polymers	2(1.09%)	4(2.18%)	2(1.09%)	0	1(0.54%)	
	Composites	30(16.39%)	15(8.20%)	9(4.92%)	2(1.09%)	1(0.54%)	

*Possibility of more than one option **Statistical chi-square test

Table 2. Specific use of bone grafts by dentists in Sobral, Brazil (n=45).

Variable	Categories	Frequency of bone grafts (cases)				P**
		≤ 10	11-50	51-100	> 100	
Application*	Fresh dental socket	14(31.11%)	8(17.78%)	2(4.44%)	7(15.56%)	0.85
	Simultaneously with a dental implant	17(37.78%)	10(22.22%)	3(6.67%)	6(13.33%)	
	After periodontal treatment	7(15.56%)	5(11.11%)	1(2.22%)	0(0%)	
	Maxillary sinus lifting	13(28.89%)	7(15.56%)	3(6.67%)	7(15.56%)	
	Paraendodontic surgery	2(4.44%)	3(6.67%)	1(2.22%)	2(4.44%)	
	Extensive bone reconstruction	5(11.11%)	2(4.44%)	3(6.67%)	4(8.89%)	
Origin*	Autogenous	10(22.22%)	9(20%)	3(6.67%)	6(13.33%)	0.86
	Allogeneic	4(8.89%)	1(2.22%)	1(2.22%)	0	
	Xenogeneic	13(28.89%)	10(22.22%)	2(4.44%)	6(13.33%)	
	Alloplastic	5(11.11%)	2(4.44%)	2(4.44%)	2(4.44%)	
Composition*	Ceramics	24(53.33%)	16(35.55%)	9(20%)	16(32.55%)	0.80
	Glasses	0	1(2.22%)	0	0	
	Polymer	2(4.44%)	0	1(2.22%)	1(2.22%)	
	Composites	5(11.11%)	4(8.89%)	1(2.22%)	2(4.44%)	
Association*	Bone graft alone	9(20%)	5(11.11%)	2(4.44%)	5(11.11%)	0.53
	Bone graft+Cement	0	0	0	1(2.22%)	
	Bone graft+Membrane	20(44.44%)	9(20%)	3(6.67%)	6(13.33%)	
	Bone graft+Autologous fibrin	3(6.67%)	3(6.67%)	2(4.44%)	5(11.11%)	

*Possibility of more than one option **Statistical chi-square test

However, the fact that professionals never or rarely include the patient in the choice of this form of treatment is remarkable. The reported country of origin of the bone graft used differed significantly according to how many bone grafts the respondent had performed: most of those who had performed up to 10 cases did not know country of origin or had used imported material, while respondents who had performed over 50 cases reported using imported or imported+national materials. Use of bone grafts was higher in the private service than in the public for most of the analyzed ranges.

Table 4 shows that professionals always follow datasheets and recommendations for the use of bone grafts. Respondents who had performed up to 50 cases did not overall report having patients with inflammatory or infectious complications after bone grafts, while the profile is significantly reversed for

respondents who had performed more than 50 cases, although they reported feeling safe regarding the origin of the materials. In general, both the opinion of the professionals and their perception of the belief of their patients were more favorable to the use of bone grafts from animals than from humans. Within all three dental specialties, the low number of professionals registered in the Brazilian Transplant System for use of bone tissue banks confirms the low level of acceptance among professionals of allografts, with only 2 registered dentists in the specialties of Oral and Maxillofacial surgery or Implantodontics.

DISCUSSION

The local register of 233 dentists for a population of 208,935 inhabitants in Sobral¹⁷ shows the ratio of 1 professional to 896 individuals, which is a lower rate

Table 3. Cost-effectiveness of bone grafts for dentists in Sobral, Brazil (n=45).

Variable	Categories	Frequency of bone grafts (cases)				P*
		≤ 10	11-50	51-100	≥ 100	
Professional satisfaction	Yes	24(53.33%)	11(24.44%)	3(6.67%)	7(15.55%)	1.00
	No	0	0	0	0	
	Don't know	0	0	0	0	
Patient satisfaction	Yes	24(53.33%)	11(24.44%)	3(6.67%)	7(15.55%)	1.00
	No	0	0	0	0	
	Don't know	0	0	0	0	
Material or technical costs	Low	1(2.22%)	1(2.22%)	0	0	0.40
	Moderate	11(24.44%)	5(11.11%)	3(6.67%)	6(13.33%)	
	High	1(2.22%)	5(11.11%)	0	1(2.22%)	
	Don't know	1(2.22%)	0	0	0	
Patient participates in the choice	Always	4(8.89%)	3(6.67%)	1(2.22%)	1(2.22%)	0.82
	Often	5(11.11%)	3(6.67%)	0	2(4.44%)	
	Rarely	3(6.67%)	3(6.67%)	1(2.22%)	0	
	Never	11(24.44%)	2(4.44%)	1(2.22%)	4(4.44%)	
	Don't know	1(2.22%)	0	0	0	
Country of origin	National	3(6.67%)	3(6.67%)	0	0	0.02
	Imported	12(26.67%)	2(4.44%)	2(4.44%)	5(11.11%)	
	National+Imported	6(13.33%)	6(13.33%)	1(2.22%)	2(4.44%)	
	Don't know	13(28.89%)	0	0	0	
Service*	Public	11(24.44%)	3(6.67%)	2(4.44%)	2(4.44%)	0.67
	Private	19(42.22%)	11(24.44%)	3(6.67%)	7(15.55%)	

*Statistical chi-square test

Table 4. Biosafety of bone grafts for dentists in Sobral, Brazil (n=45).

Variable	Categories	Frequency of bone grafts (cases)				P***
		≤ 10	11-50	51-100	≥ 100	
Professionals always follow datasheets and recommendations	Never	0	0	0	0	0.86
	Always	21(46.67%)	11(24.44%)	3(6.67%)	6(13.33%)	
	Sometimes	2(4.44%)	0	0	1(2.22%)	
	Don't know	1(2.22%)	0	0	0	
Patients with inflammatory or infectious complications after bone grafts	No	19(42.22%)	6(13.33%)	1(2.22%)	1(2.22%)	0.04
	Yes	4(8.89%)	5(11.11%)	2(4.44%)	6(13.33%)	
	Don't know	1(2.22%)	0	0	0	
Professional feels safe regarding the origin	No	1(2.22%)	1(2.22%)	0	0	0.83
	Yes	21(46.67%)	10(22.22%)	3(6.67%)	7(15.55%)	
	Don't know	2(4.44%)	0	0	0	
Professional favorable to use of bone graft from animals	No	3(6.67%)	0	0	0	0.55
	Yes	19(42.22%)	11(24.44%)	3(6.67%)	7(15.55%)	
	Don't know	2(4.44%)	0	0	0	
Patient favorable to use of bone graft from animals	No	4(8.89%)	1(2.22%)	0	1(2.22%)	0.44
	Yes	9(20.00%)	8(17.78%)	2(4.44%)	5(11.11%)	
	Don't know	11(24.44%)	2(4.44%)	1(2.22%)	1(2.22%)	
Professional favorable to use of bone graft from human beings	No	9(20.00%)	5(11.11%)	1(2.22%)	3(6.67%)	0.95
	Yes	7(15.55%)	4(8.89%)	1(2.22%)	3(6.67%)	
	Don't know	8(17.78%)	2(4.44%)	1(2.22%)	1(2.22%)	
Registration in the Brazilian Transplant System*	OMS**	1(2.22%)	0	0	1(2.22%)	0.39
	Implantodontics	0	0	0	1(2.22%)	
	Periodontics	0	0	0	0	
Patient favorable to use of bone graft from human beings	No	9(20.00%)	2(4.44%)	1(2.22%)	3(6.67%)	0.65
	Yes	1(2.22%)	2(4.44%)	0	0	
	Don't know	14(31.11%)	7(15.55%)	2(4.44%)	4(8.89%)	

*Possibility of more than one option **OMS: Oral and maxillofacial surgery ***Statistical chi-square test

than the Brazilian average of 1 dental professional to 668 inhabitants²⁰. Even in the face of favorable demand, the low number of experts would explain the generalist profile of dentists in the city and may explain the low use of bone grafts in the sample.

Inorganic bioceramics were bone substitute materials most frequently cited and used by respondents, in agreement with most studies on the subject^{9,21,22}. This preference is supported by osteoconductive action, ability to carry osteogenic cells^{9,21} or osteoinductive growth factors²³, versatility of concentrations, formats and particle sizes²² as well as the possible association of apatites with collagen or other polymers with the aim of achieving biological synergism^{15,21,24}. Thus, the basic understanding of the properties of biomaterials for bone regeneration and their appropriate applications is very important in making a good clinical choice¹⁹.

The most frequent clinical applications were simultaneous use with a dental implant, fresh dental sockets and maxillary sinus lifting, in agreement with the two most prevalent surgical beds in the dental literature^{19,24,25}. The present study found a higher frequency of bone graft in association with membranes or autologous fibrin than in isolated form, disagreeing with another Brazilian study¹⁹ and international reports, which state that dentists make less use of membranes next to the bone graft because it represents an additional cost to the patient^{16,26}. The adjunct use of autologous fibrin has been promising, as it has a high concentration of angiogenic and osteogenic growth factors, evidencing the evolution of bone regeneration techniques²⁷.

The prevalence of use of autogenous and xenogeneic grafts compared to alloplastic and allogeneic, associated with a more favorable perception of the use of bone grafts from animals than from humans, agrees with other studies on the topic^{19,28}. Autografts are a gold standard for the treatment of bone defects²⁸, but the preference of professionals for xenografts and alloplastic grafts has increased gradually in Latin America¹⁵ and Brazil²⁶ for medical and dental applications^{3,22}. The advantages of xenografts and alloplastic grafts are biomimicry of human bone tissue²⁴ and absence of patient's donor bed, which explains their wide range of clinical uses^{9,19,22}. Stigmatization of the cadaverous source, lack of disclosure²⁹ and ethical, moral or religious values of users could explain low choice of allografts^{30,31}.

Satisfaction with bone grafts in this study was high,

confirming an Indian study with 59% of utilization³¹. Refusal rates to bone grafts has been more often associated to the geopotential context than to age, gender, education and religion, ranging from 2% for alloplastic grafts to 20% for allografts in Chilean study³⁰ or from 11% for autografts to 67% for xenografts in Saudi Arabia²⁹.

The present study confirmed the existing knowledge on use of materials of imported^{12,13} or national origin²⁴. Multinational companies, predominantly US, currently dominate the global market for dental bone grafts^{12,13}. Brazilian industry gains representation among companies already consolidated in the market, with 3 out of 5 institutions responsible for 65% of dental bone grafts in the country²⁶.

Costs were considered moderate by the respondents, predominantly from private practice in Sobral, a finding similar to those from a large city in southeastern Brazil¹⁹. However, the country suffers from chronic underfunding in public health³², where the costs of procedures can be substantial limitations^{13,33} and only a small percentage of its population has undergone bone reconstruction procedures^{16,34}. In private practice, costs are associated to the loyalty of dentists to the use of bone grafts³³.

Dentists may select therapeutic options that involve high cost, based on their perception of the patient's profile and oral condition or on the patient's feedback regarding motivation or being able to afford the cost of the treatment³⁵. The current study highlighted the low level of patient participation in the choice of material to be grafted, in contrast to most international studies^{29-31,35}. This emphasizes the need to improve professional interaction with patients in order to make successful joint decisions regarding the use of bone graft.

High occurrence of inflammation or infection in grafted patients was proportional to high density of surgical care performed by the respondents. The rigid biosafety involved in processing, sterility and standardized validation tests of grafting^{12,36} reduces fears regarding the transmission of animal or human diseases^{30,31} and increases the safety perceived by dentists regarding their use³⁷. Nevertheless, grafting procedure failure can range from 5 to 10% as a result of infections or inappropriate graft stabilization¹⁵. Factors such as filling large bone defects with bone block, mixed grafts (autogenous + synthetic) and diabetes mellitus may also be associated with

infections³⁸. To reduce potential out-of-office complications with bone grafting, it can be helpful to apply antibiotic therapy used sparingly³⁹ and intensification of care with oral hygiene habits³⁴. Although there is still little dental use of bone grafts in Sobral, their cost-effectiveness and biosafety are

generally satisfactory and in accordance with the literature. This study confirms good acceptance by dentists and their knowledge of the subject of bone grafts in Sobral, a medium-sized city in the Brazilian inland, which constitutes a promising scenario for the development of the sector.

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Efficacy of two soft-bristle toothbrushes in plaque removal. A randomized controlled trial

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ABSTRACT

The aim of the present examiner-blind randomized controlled clinical study was to compare the efficacy two soft-bristle toothbrushes in terms of plaque removal. Seventy volunteers were randomly allocated to Group A (tapered-tip toothbrush) or Group B (end-rounded toothbrush). At baseline appointment (Day 0), volunteers underwent plaque examination using the Improved Plaque Identification Index. Under supervision, they brushed their teeth for 1 minute with their assigned toothbrushes and the plaque examination was repeated. Volunteers continued the oral hygiene regimen (assigned toothbrush and a regular dentifrice provided by the researchers) for 7 days. The experimental procedures of Day 0 were then repeated. Separate statistical analyses were performed for mean percent reduction of plaque in the whole-mouth, interproximal and gumline scores at both times, using Mann-Whitney test, $p < 0.05$. After a single

toothbrushing, on Day 0, mean percent plaque was significantly reduced in both groups ($p < 0.05$), with statistically greater reductions of whole-mouth (21.39 ± 12.44 vs. 11.40 ± 11.17), gumline (6.32 ± 7.37 vs. 2.89 ± 4.57) and interproximal (10.82 ± 10.49 vs. 5.21 ± 7.68) for Group A as compared to Group B. However, on day 7, no significant difference was observed between groups for whole-mouth (29.94 ± 20.91 vs. 26.58 ± 18.64), gumline (14.04 ± 18.82 vs. 13.78 ± 17.63) and interproximal surfaces (26.41 ± 22.77 vs. 23.12 ± 20.98) ($p > 0.05$). In conclusion, on Day 0, Group A presented higher efficacy in supragingival plaque removal than Group B, as reflected by whole-mouth, gumline and interproximal plaque scores.

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Keywords: toothbrushing - biofilms - oral hygiene

Eficácia de duas escovas de cerdas macias na remoção de Placa. Um ensaio clínico randomizado

RESUMO

O objetivo desse ensaio clínico, examinador-cego, randomizado e controle foi de comparadas a eficácia de duas escovas de cerdas macias em relação ao controle de placa. Setenta voluntários foram randomicamente alocados para Grupo A (escova com ponta cônica) ou Grupo B (escova com ponta arredondada). Na consulta inicial (dia 0), voluntários receberam exame de placa utilizando o Índice de identificação de placa melhorado. Sob supervisão, eles escovaram seus dentes por 1 minuto com as escovas designadas e o exame de placa foi repetido. Voluntários continuaram seu regime de higiene oral (escova dental alocada e dentífrico comum fornecidos pelos pesquisadores) durante 7 dias. Os procedimentos da consulta inicial foram novamente repetidos. Análises estatísticas distintas foram realizadas para percentual de redução média de placa para os escores de boca toda, interproximal e linha gengival em ambos os momentos, utilizando teste de Mann-Whitney,

$p < 0,05$. Após único uso da escova, no dia 0, o percentual de redução média de placa foi significativamente reduzido em ambos os grupos ($p < 0,05$), com reduções significativas para boca toda ($21,39 \pm 12,44$ vs. $11,40 \pm 11,17$), linha gengival ($6,32 \pm 7,37$ vs. $2,89 \pm 4,57$) e interproximal ($10,82 \pm 10,49$ vs. $5,21 \pm 7,68$) no Grupo A quando comparada com o Grupo B. Entretanto, no dia 7, nenhuma diferença significativa foi observada entre os grupos para boca toda ($29,94 \pm 20,91$ vs. $26,58 \pm 18,64$), linha gengival ($14,04 \pm 18,82$ vs. $13,78 \pm 17,63$) e interproximal ($26,41 \pm 22,77$ vs. $23,12 \pm 20,98$) ($p > 0,05$). Em conclusão, no dia 0, a escova do Grupo A apresentou eficácia superior na remoção de placa supragengival quando comparada com a escova do Grupo B, como demonstrado nos escores de placa de boca toda, linha gengival e interproximal.

Palavras-chave: escovação dentária - biofilmes - higiene bucal

INTRODUCTION

Supragingival plaque removal is undeniably the best way to ensure better oral health in the population. It has been proven to prevent caries, periodontal diseases and tooth loss and, more recently, to improve the outcome of dental implants¹⁻³.

Oral hygiene routine demands time, dexterity and motivation. Therefore, the limits of the clinical effectiveness of self-performed oral hygiene are evident. Some studies have demonstrated the presence of remaining biofilm despite efforts to remove dental plaque^{2,4}. The new classification of periodontal diseases and conditions established 10% as maximum number of bleeding sites for patients to be classified as healthy, which is achieved with good oral hygiene habits^{3,5}.

Some studies have investigated the use of oral hygiene products, showing that there has been an increase worldwide. For example, while in Brazil in the 1990s, less than one toothbrush per capita was used per year^{4,6}, this use had virtually doubled by 2010^{5,7}. However, the estimation of use of oral hygiene products does not necessarily imply better clinical results in effectiveness of plaque control.

Toothbrushes are the gold standard product used to remove plaque from teeth. They differ in texture, tip, hardness, and even in their material, which may be natural or synthetic. The literature contains few studies comparing the efficacy of available toothbrushes. The use of a soft-bristle toothbrush has been recommended to improve plaque reduction while minimizing gingival tissue lesions^{8,9}. Studies comparing the efficacy of available products are therefore important to better support the indication of any given toothbrush.

The aim of the present examiner-blind randomized controlled clinical study was to compare the efficacy two soft-bristle toothbrushes in terms of plaque removal with regular fluoride toothpaste in controlling established dental plaque over a 7-day period.

MATERIAL AND METHODS

Study Design

This study was designed as a phase III randomized, single-center, two-cell, examiner-blind, parallel-group clinical study.

Ethical Considerations

The protocol was approved by the Institutional Review Board of the Federal University of Rio

Grande do Sul, Brazil and all the volunteers signed an informed consent form. The study was conducted according to good clinical practice standards¹⁰.

Sample Size Estimate

Sample size was estimated after a pilot study. Based on the standard deviation for the response measures of 0.58, a significance level of $\alpha=0.05$ and 80% level of power, the present study was powered to detect a minimal statistically significant difference between study group means of 15%, and 32 individuals per group were considered necessary. A 10% possible attrition rate was added. Thus, 35 individuals per group were considered for participation.

Participants

Seventy healthy male and female individuals, aged 21-70 years, were enrolled. Recruitment was performed at the Federal University of Rio Grande do Sul, Brazil. The following inclusion criteria were used: good oral health; initial mean plaque index of at least 0.6, determined by the Improved Plaque Identification Index^{6,11}; and ≥ 20 natural uncrowned teeth, excluding third molars. Individuals were not included in the study if they had orthodontic bands, removable partial dentures, tumor or significant pathology in the soft or hard tissues of the oral cavity, moderate or advanced periodontal disease (purulent exudate, tooth mobility, and/or extensive loss of periodontal attachment or alveolar bone), antibiotic use in the month prior to entering the study, participation in any other clinical study, pregnant or breast-feeding status, dental prophylaxis in the 2 weeks prior to the baseline examination, history of allergy to oral/personal care consumer products or their ingredients, use of any prescription medicine that might interfere with the study outcome, medical condition preventing abstinence from eating/drinking/chewing gum for 4 hours prior to the scheduled visit, and history of alcohol or drug abuse.

Experimental Procedures

Participants were instructed to refrain from any oral hygiene measure for 12 hours before reporting to the clinical site, and from eating, drinking or smoking for 4 hours. The baseline examination comprised evaluation of the oral cavity soft tissue and perioral region followed by plaque disclosure with 10ml of 0.04% basic fuchsin solution (Replasil; Iodontosul,

Porto Alegre – RS, Brazil). On Day 0, plaque examination was performed using the Improved Plaque Identification Index before toothbrushing¹¹. Supragingival plaque was assessed on the facial and lingual surfaces of each tooth. Scores of 0 to 4 were assigned to all disclosed surfaces of the maxillary and mandibular teeth using a dental light and dental mirror. Teeth were divided into nine units (Fig. 1, A-I). From these site-wise scores, a whole-mouth plaque score was determined for each participant by calculating the proportion of sites in the mouth at which plaque was present. Three areas are near the gingival margin, two are interproximal, and four are on the body of the tooth. This index enables stratification of area(s) that are of concern during the analysis.

Simple randomization was performed by a computer-generated list by an external researcher responsible for allocation concealment. Toothbrushes were kept inside numbered opaque plastic bags. The experimental groups were:

Group A. A soft-bristle, tapered-tip toothbrush

Group B. A soft-bristle, end-rounded toothbrush.

All participants were instructed to brush their teeth for one minute under supervision with the assigned toothbrush and a commercially available standard fluoride toothpaste (Colgate Cavity Protection; Colgate, São Paulo, Brazil), after which plaque was evaluated (Day 0 after toothbrushing). They were instructed to use the product at home twice daily (morning and evening) for the next 7 days and to refrain from any interproximal cleaning. On day 7, all participants returned to the clinical facility and the procedures performed on Day 0 were repeated. The same examiner (CKR), who was unaware of group allocation, performed all plaque examinations.

Statistical Analysis

The main study outcome is plaque score reduction, determined by the Improved Plaque Identification Index. Plaque Index was dichotomized into absence of dental plaque (scores 0 or 1) and presence of dental plaque (scores 2, 3 or 4). The percentage of sites with presence of dental plaque was calculated for each individual for both experimental periods (Day 0 and Day 7). The percentage reductions of plaque before and after toothbrushing were calculated for each experimental period as follows: percent of dental plaque after toothbrushing x 100 / percent of dental plaque before toothbrushing.

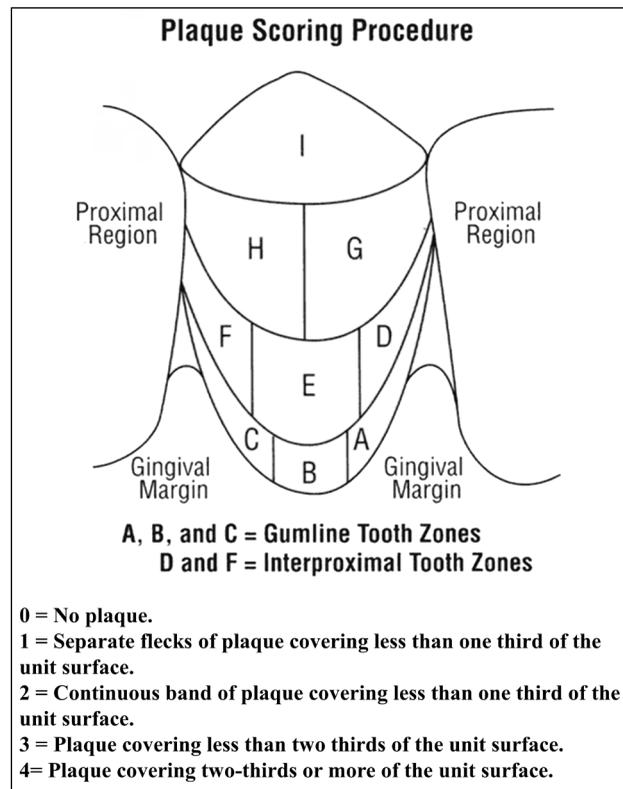


Fig. 1: Improved plaque identification index (according to Ayad et al., 2016).

Separate statistical analyses were performed for whole-mouth, interproximal, and gumline plaque scores. A per-protocol analytical approach was used. The statistician was blinded to participants' product allocation. Percentage reductions between before toothbrushing and after toothbrushing were calculated for both Day 0 and Day 7 for whole-mouth, interproximal and gumline regions. Comparisons between groups were performed using the Mann-Whitney test, as a non-parametrical data distribution was observed (Kolmogorov-Smirnov test showed a p-value <0.05).

Non-parametrical distribution was also identified for the number of present teeth, so the Mann-Whitney test was used to compare the number of teeth between groups. Age was compared between groups by t-test for independent samples. Categorical data (sex and smoking exposure) were compared between groups by chi-square test. A significance level of $\alpha=0.05$ was established for all analyses. Data analysis was performed using the statistical package SPSS 21.0 (SPSS Inc., Chicago, USA).

RESULTS

One individual of the 71 initially screened for the study was not included (Fig.2). Out of the 70 included participants, 67 completed the 7-day clinical trial. Three participants – 2 in Group A and 1 in Group B did not complete the study. Reasons for dropouts are mentioned in Fig. 2. Groups did not differ in gender or mean age (Table 1).

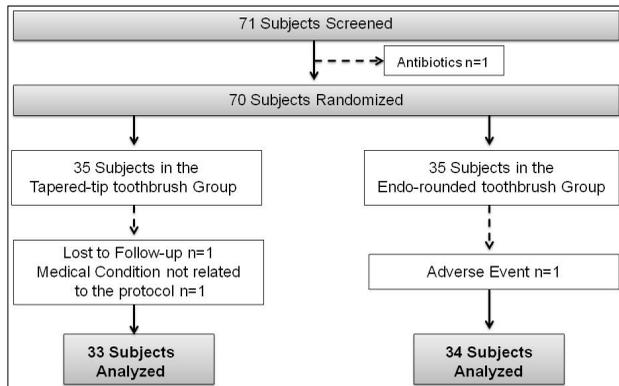


Fig. 2: Study Flowchart.

With relation to scores on Day 0 before toothbrushing, a statistically significant difference between groups was detected ($P < 0.001$) (Table 1), and therefore the present study analyzes percent reductions rather than means. Fig. 3 shows the mean percent reduction (from before toothbrushing to after toothbrushing) on Day 0, considering the whole-mouth, interproximal and gumline and surfaces. Group A showed significantly greater reduction in mean whole-mouth ($21.39 \pm 12.44\%$), interproximal ($10.82 \pm 10.49\%$) and gumline ($6.32 \pm 7.37\%$) plaque scores compared to Group B, in which reductions were $11.4 \pm 11.17\%$, $5.21 \pm 7.68\%$ and $2.89 \pm 4.57\%$, respectively (Fig. 3).

In the Day 7 follow-up, a statistically significant difference was observed in the scores before

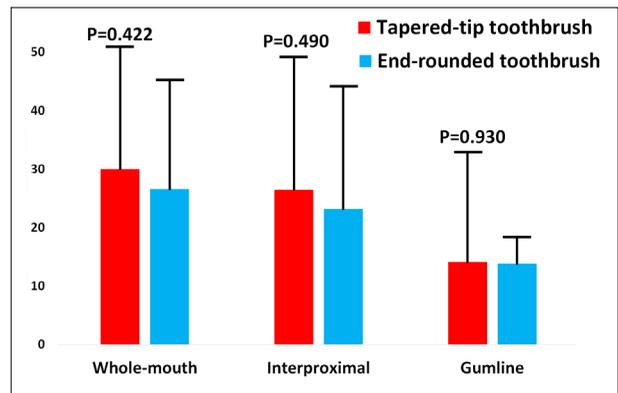


Fig. 3: Mean percent reduction of plaque for Group A (tapered-tip toothbrush) and Group B (end-rounded toothbrush) on Day 0.

toothbrushing ($P < 0.007$) (Table 1) in whole-mouth surfaces. Participants in Group A showed no significant difference in the whole-mouth ($29.94 \pm 20.91\%$), interproximal ($26.41 \pm 22.77\%$) or gumline (14.04 ± 18.82) percent reduction of plaque scores when compared to Group B, which had $26.58 \pm 18.64\%$, $23.12 \pm 20.98\%$ and 13.78 ± 17.63 , respectively. However, no statistically significant difference was observed in mean percent reduction of plaque (Fig. 4).

Three adverse events possibly related to the study were reported during the 7-day study: traumatic brushing, mouth ulcers and severe pain in teeth irradiating to ear and jaw as reported by the subjects. All three (3) adverse events were reported in Group B and one individual preferred not to continue in the study due to the discomfort. All participants who reported adverse events were carefully followed until the issue was resolved. As toothbrushing technique may explain these findings, all individuals received individualized oral hygiene instruction after all experimental periods were completed.

Table 1. Demographics and baseline characteristics of the study participants.

Sex	Male – n (%) Female – n (%)	17 (48.6) 18 (51.4)	17 (48.6) 18 (51.4)	1.000*
Mean age \pm SD		35.89 \pm 10.80	32.29 \pm 9.86	0.150 #
Mean number of teeth \pm SD	(median – min.; max.)	27.37 \pm 1.29 (28 – 23;28)	27.20 \pm 1.41 (28 – 23;28)	0.548 μ
Mean percent of dental plaque (Whole-Mouth) on Day 0 before toothbrushing	Mean \pm SD	98.34 \pm 12.78	97.26 \pm 16.34	<0.001 μ
Smoking exposure	Non-smokers – n (%)	29 (82.86)	35 (100.0)	0.025 β
	Smokers – n (%)	6 (17.14)	0 (0.0)	

Legend: *Chi-square test; # t-test for independent samples; μ Mann Whitney test, β Fisher's exact test.

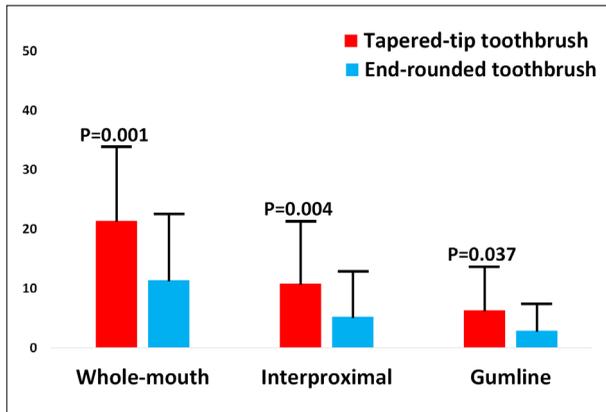


Fig. 4: Mean percent reduction of plaque for Group A (tapered-tip toothbrush) and Group B (end-rounded toothbrush) on day-7.

DISCUSSION

The present study was designed as a phase III randomized, single-center, two-cell, examiner-blind, parallel-group clinical study, with the aim of comparing the plaque removal efficacy of two soft-bristle toothbrushes in 70 healthy volunteers. Plaque reduction was greater in Group A (tapered-tip toothbrush) than in Group B (end-rounded toothbrush) in the first usage. This result was statistically significant in the percent reduction on Day 0 but was not significant on Day 7.

Toothbrushing is known to be the most frequently used method of oral hygiene. The contemporary paradigm of oral hygiene is based on the highest efficacy with the least harm. In order to increase effectiveness and reduce the effects of improper brushing techniques, manufacturers set the goal of evolving their toothbrushes in terms of design and bristle characteristics. It is well known that soft-bristle toothbrushes are efficacious and have less harmful potential^{8,9}. Different toothbrushes have been developed with the aim of achieving better cleaning effect in the whole mouth, regardless of the type of movements used by the user^{12,13}. A variety of studies can be found in the literature regarding different toothbrush designs and morphologies. It is not easy to compare the different studies, particularly due to high variety in design, follow-up, brands used, etc. The findings of such studies are important as different types of toothbrushes are launched on the market and there is constant need of support from sound studies in terms of efficacy and safety.

The present study was designed to compare the effect of a novel soft-bristle toothbrush (Group A)

with a toothbrush that is already well established on the market (Group B). It should be emphasized that even though both brushes are considered soft, the brush being tested in the study (Group A) has softer bristles. Although it is well known that softer bristles can be less efficacious¹⁴, there is no doubt about the benefit of their being less harmful⁸. This is not a double-blind study, since the participants received different toothbrushes. However, all additional procedures were identical for both groups. The study was designed as an efficacy study, which demonstrates the potential of a novel device. Even though initial plaque scores were numerically similar, they differed significantly between groups, so in order to normalize data, the present study analyzes percent reduction in plaque scores. The results showed that Group A presented higher efficacy in reducing whole-mouth and interproximal plaque on Day 0 (which was the first contact with the brushes), even though the bristles were softer. The results after 7 days did not present a statistically significant difference. The separate analyses performed in his study are important because they consider the ability of the different types of toothbrushes to act on specific parts of the teeth. Their ability to remove plaque from interproximal areas, where other devices (such as dental floss) present limitations, is of special interest in terms of occurrence of dental caries, gingivitis and periodontitis.

Plaque was measured using the Improved Plaque Identification Index¹¹, which was designed to quantify the presence of dental plaque in each of the 9 regions identified on teeth by the Navy Plaque Index. The Improved Plaque Identification Index could be more sensitive and provide more accurate demonstration of differences in plaque levels. However, this kind of quantification tends to generate regression to the mean, so we dichotomized the index to facilitate clinical interpretation. The results showed higher efficacy in Group A upon the first usage. The leveling of the results on Day 7 might be explained in part by the Hawthorne effect, since participant behavior may be expected to change in response to awareness of being observed¹⁵. In addition, the effect of a learning curve should not be disregarded.

Tapered bristle toothbrushes have been tested and shown to be more effective in reaching not only interproximal areas of the teeth along the gingival margin and under gumline, where plaque

accumulates the most, but also to reach fissures, when compared to end-rounded bristles. This result was also positive in an *in vitro* study with artificial plaque in which tapered-tip bristles have demonstrated significantly better results in reducing plaque scores in the interproximal area¹⁶. The use of tapered-tip bristle toothbrushes could benefit patients who do not include flossing in their daily hygiene routine, as it was shown in an updated review¹⁷ that flossing did not necessarily reduce dental caries and periodontal disease.

As in the study by Rösing et al. 2016¹⁸, a 12-hour period established to refrain from any type of oral hygiene before both evaluations showed that plaque accumulation was sufficient to test the efficacy of the toothbrushes, although it was significantly different before toothbrushing Day 0 in Group A. It should be noted that no brushing technique was imposed on the participants, in order to highlight the outcome of their real daily brushing techniques.

Three adverse events were reported in the present study, none of which was related to the study protocol. Most of the reported adverse events could be explained by causes related to patients' brushing habits. One of the participants was excluded. It is also important to highlight that soft and extra-soft bristle toothbrushes have been reported in a systematic review to be safer than other toothbrush stiffness when oral injuries are evaluated, with tapered or rounded bristle end-shape⁸ presenting no clinical relevance concerning soft oral tissue damage.

Participants were asked to brush their teeth for one (1) minute. Many studies have used the same brushing time, the main reason being to avoid the

patient needing to brush excessively¹⁸⁻²⁰. Moreover, it has been demonstrated that patients do not spend longer than one minute on toothbrushing in general. This study has strengths and limitations that should be noted. Among the strengths are the study design, with randomization of the participants, use of a standardized brushing time, examiner reproducibility, examiner and statistician blinding, and very high compliance rate. The limitation of the study is the efficacy design, in which a short time (7 days) was used. Neither effectiveness nor effect on gingival inflammation can be ascertained from our results.

Despite the abovementioned limitations, it should be noted that there were better interproximal results in Group A, which may be relevant, considering that toothbrushes were not meant to reach this area. Other studies have demonstrated similar results with tapered-tip toothbrushes¹⁸⁻²⁰. It is important to remember that a systematic review published by the Cochrane collaboration and revisited in 2019 showed concerning results regarding the levels of periodontal disease and caries in dental floss users, concluding that dental floss users do not necessarily have less diseases¹⁷. Thus, it is always important to study newly designed toothbrushes, especially as a possibility of compensating for the limited effects of flossing, according to how they are used. Longer term randomized controlled trials are warranted, including the analysis of adverse events, in order to prevent gingival tissue harm.

The tapered-tip toothbrush performed better than the end-rounded toothbrush in terms of efficacy at the first evaluation.

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Association between demarcated enamel hypomineralization on second primary molars and dental caries in childhood

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ABSTRACT

Hypomineralized Second Primary Molars (HSPM) is the name used to describe the presence of demarcated enamel hypomineralization in second primary molars. HSPM has been compared with Molar Incisor Hypomineralization (MIH) as regards its clinical appearance and consequences. The aim of this study was to investigate associations between HSPM and dental caries in childhood. It was a cross-sectional population-based study that included 216 children aged 4-6 years from public schools in Botelhos, Brazil. Children with all second primary molars erupted were eligible for participation. Clinical examination was performed by one calibrated examiner. HSPM was recorded according to modified European Academy of Paediatric Dentistry criteria. The main outcome was dental

caries experience as indicated by the dmft index (%dmft>0) according to WHO criteria. Chi-square test and Logistic Regression Model were used to adjust the results for effects of covariates. Among the 216 children examined, 22.2% presented HSPM. After adjustment for logistic regression, children with HSPM were found to have a 2.28 times greater chance of presenting dental caries. Our results reinforce the importance of HSPM in caries development in children, which should be considered by public health dentists in the process of planning actions aimed at this population.

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Keywords: children - tooth demineralization - dental caries - DMF Index - socioeconomic factors

Associações entre hipomineralização demarcada de esmante nos segundos molares decíduos e cárie dentária na infância

RESUMO

A hipomineralização de segundo molar decíduo (HSMD) é denominação utilizada para descrever a presença de hipomineralização demarcada do esmalte nos segundos molares decíduos. A HSMD foi comparada com a hipomineralização de molares e incisivos (HMI) no que diz respeito à aparência e às consequências clínicas. O objetivo deste estudo foi investigar associações entre HSMD e cárie na infância. Foi realizado um estudo transversal de base populacional, composto por 216 crianças de 4 a 6 anos de idade de escolas públicas de Botelhos, Brasil. As crianças com todos os segundos molares decíduos foram elegíveis para participação. O exame clínico foi realizado por um examinador calibrado. A HSMD foi registrada de acordo com os critérios modificados da European Academy of Paediatric Dentistry. O desfecho principal foi a experiência de cárie

dentária nos dentes decíduos conforme o índice ceo (% ceo>0), segundo os critérios da OMS. Os testes de Qui-quadrado e o modelo de regressão logística foram utilizados para ajustar os resultados dos efeitos das covariáveis. Verificou-se que entre as 216 crianças examinadas, 22,2% apresentaram HSMD. Após o ajuste da regressão logística, as crianças com HSMD tiveram 2,28 vezes mais chances de apresentar cárie dentária. Nossos resultados reforçam a importância do HSMD no desenvolvimento da cárie em crianças, fato que deve ser considerado pelos dentistas de saúde pública no processo de planejamento de ações voltadas a essa população.

Palavras-chave: crianças - hipomineralização dentária - cárie dentária - fatores socioeconômicos

INTRODUCTION

Hypomineralized second primary molars (HSPM), a qualitative developmental defect of enamel (DDE), with a demarcated change in translucency or opacity, are often found in children^{1,2}. HSPM has been

compared with Molar Incisor Hypomineralization (MIH) as regards its clinical appearance and consequences³, and the diagnostic criteria proposed by the European Academy of Paediatric Dentistry

(EAPD) in 2003⁴ have been used in clinical studies to describe the prevalence of HSPM¹.

The exact mechanism and etiological factors underlying MIH and HSPM are not fully understood^{5,6}, however, researchers agree that fluoride intake is not implicated in the aetiology of demarcated enamel opacity⁵. As the formation of second primary molars coincides with that of permanent first molar formation, a common cause for the both MIH and HSPM processes may be considered⁷, which would explain the higher prevalence of MIH among children with HSPM in deciduous dentition^{2,3,7}.

DDE is often associated with higher caries levels among affected children, including dental caries in childhood⁸. In Brazil, for example, a prospective study suggested that the presence of DDE was the main risk factor associated with the development of caries in 36-month-old children of low socioeconomic status, surpassing other classic risk factors such as night-time breast-feeding and tooth-brushing habits⁹. Studies have demonstrated a positive association between demarcated enamel opacity and dental caries in children worldwide^{8,10-12}. However, in spite of such evidence, several confounding factors can interfere in the results of those associations, e.g., access to fluoridated water, oral hygiene status of children, and socioeconomic factors such as education and family income.

Particularly in developing countries such as Brazil, a country marked by strong geographical contrasts regarding children's access to dental care¹³, understanding the relationship between enamel defects and caries could contribute to guiding more effective public health measures. The aim of this study was to investigate associations between HSPM and dental caries in Brazilian children aged 4 to 6 years from Botelhos, Minas Gerais, and to investigate the influence of clinical and demographic factors on the occurrence of caries in the primary dentition. The hypothesis was that even in populations with low socio-economic status, HSPM acts as a risk factor for caries.

MATERIAL AND METHODS

Approval for this study was obtained from the Ethics Committee of the Piracicaba Dental School, São Paulo, Brazil (Protocol 037/2010) and the procedures followed were in accordance with the ethical standards of the Helsinki Declaration of

1975, as revised in 2000. Participants' parents provided informed consent before the study began.

This was a cross-sectional population-based study. Children aged 4 to 6 years from public schools in Botelhos, Minas Gerais (which has a population of just over 15 thousand) were invited to this study. Children were excluded if they were absent on the day of the examination, if their parents declined to provide consent or if they did not present all 4 deciduous second molars at the time of examination¹⁴. Data were collected by means of a structured questionnaire that had been previously verified for clarity of the questions. The questionnaire was used to collect sociodemographic information related to the children and was completed by the parents. The sociodemographic factors were children's age (dichotomized by median) and gender (male or female), place of residence (rural or urban) and access to fluoridated water (yes/no).

The examination was carried out at the schools by the first author (CMCS) in accordance with the World Health Organisation (WHO) criteria¹⁵, under natural light, with the aid of a mouth mirror, wooden spatula and gauze, strictly following biosafety standards.

The presence of visible plaque on the buccal surface of upper incisors, central and lateral incisors (52, 51, 61, 62) was recorded (yes/no) before the children brushed their teeth. If the child had plaque on two or more incisors, it was considered as visible plaque present (yes), otherwise, it was considered absent (no).

Before the examination for dental caries and HSPM, the participating children were given toothbrushes and toothpaste to brush their teeth under the researcher's supervision. Dental caries was assessed using the decayed, missing and filled tooth index (dmft) in accordance with the WHO¹⁵. Children were classified as with HSPM if they had demarcated opacity, atypical restoration or structural loss in one or more deciduous second molars¹⁶.

The median dmft was calculated and information was also collected on the number of primary teeth with treatment needs, in accordance with the international methodological criteria defined by the WHO¹⁵ (one or more teeth need fillings, extraction, pulp care, crown), and after this, the frequency of children in need of dental treatment (yes/no) was assessed. All participants were informed about the screening outcome and referred to public dental care for treatment when oral diseases were identified.

Prior to calibration, the authors adjusted the criteria for the diagnosis of HSPM, using clinical photographs of children with enamel defects. For calibration, 20 children belonging to the same age group were randomly selected and examined. In order to determine intra-examiner agreement, the children were re-examined after an interval of 24 hours, without access to the previous records. Kappa statistics were used to measure concordance. The kappa values for intra-examiner reliability regarding the presence of dental caries, treatment needs and HSPM were 0.92, 0.92 and 0.94, respectively.

All statistical analyses were performed with the software program SAS 9.2 for Windows (SAS Institute Inc. Cary, NC, USA). The presence of HSPM defects (yes/no) was analyzed as a dependent variable. The Odds Ratio (OR) and confidence intervals (CI) were calculated and *p-values* were obtained using the Chi-square test.

The clinical and demographic characteristics of children with and without HSPM were compared. A Logistic Regression Model was used to adjust the OR for the effects of covariates. Only variables with $p < 0.2$ in the bivariate analyses were selected for the model. The variables that remained in the Logistic Regression Model at $p < 0.05$ were considered statistically significant and the result of logistic regression was accepted only if approved by the Hosmer-Lemeshow goodness-of-fit statistics.

RESULTS

A total 216 children (52.7% girls; median age = 5) who were present on the day of examination and whose guardians had filled out and signed the free and informed consent form, were enrolled in this study. Overall, 35 (16.3%) children lived in rural areas and did not have access to fluoridated water. There were no significant differences in the prevalence of HSPM by sex or other sociodemographic characteristics of children (Table 1).

Among the 216 children examined, 22.2% ($n = 48$) presented HSPM. At tooth level, 64 of the 864 primary second molars scored were diagnosed with HSPM (7.4%). No differences were found in the presence of HSPM either between mandible and maxilla or between left and right sides. Demarcated enamel opacities (89%) were the most frequently scored characteristics of HSPM, followed by atypical restorations (6.1%) and post-eruptive enamel loss (4.9%).

Caries prevalence was expressed as the percentage of individuals in each group with $dmft > 0$. Among 216 children, 55.5% ($n = 120$) presented $dmft > 0$ and, of these, 81.16% ($n=101$) presented treatment needs at the time of examination. The maximum $dmft$ score was 19. Mean $dmft$ was 2.45 ($sd = 3.30$), and the overall median $dmft$ was 1. Visible plaque was present in 43.5% of children. The frequency of HSPM, dental caries, treatment needs and visible plaque are presented in Table 2.

The bivariate and multiple analyses at child level using the presence of HSPM as the exploratory factor are shown in Table 3.

Bivariate logistic regression showed that children with HSPM presented more treatment need, visible plaque on the buccal surface of upper incisors, and more experience of $dmft$ than children without HSPM. However, after adjusting by logistic, the model showed that children with HSPM had a 2.28 times greater chance of having dental caries in deciduous teeth.

DISCUSSION

Reports of clinical studies on the prevalence of demarcated enamel hypomineralization in second primary molars and dental caries in primary dentition demonstrate that this enamel defect is associated with a greater experience of caries in deciduous dentition, when compared to children without HSPM^{1,2,7,12}. This study, carried out with Brazilian children, confirms previous results and highlights the importance of public health dentists paying attention to this clinical aspect when planning actions aimed at children.

From a biochemical point of view, teeth with enamel hypomineralization are at higher risk for dental caries, probably due to their higher porosity and lower mechanical resistance factors^{16,17}, which predispose the enamel to post-eruptive tooth breakdown, mainly in teeth with brown and yellow opacities¹⁸, and create a suitable local environment for adhesion and colonization of cariogenic bacteria.

In this population-based study, 22.2% of the children examined, who were all 4-6 years old, were found to have HSPM. The prevalence of HSPM found in this group was higher than the prevalence reported in literature^{1,2,7,12}. Clinical studies on the prevalence of demarcated hypomineralization in deciduous teeth using the DDE index¹⁵ report widely varying prevalence rates. It was difficult to compare the

Table 1. Association between children's general sociodemographic factors and HSPM

Sociodemographic factors	Yes N (%)	No N (%)	Total N (%)	p value*	OR	CI 95%
Sex (n = 216)						
Male	23 (22.55)	79 (77.45)	102 (100)	0.9130	1.04	
Female	25 (21.93)	89 (78.07)	114 (100)		Ref	0.54-1.97
Age in years (n = 216)						
> 5	17 (18.89)	73 (81.11)	90 (100)	0.3193	0.71	
≤ 5	31 (24.61)	95 (76.19)	126 (100)		Ref	0.37-1.39
Place of residence (n = 215)						
Rural	6 (17.14)	29 (82.86)	35 (100)	0.4210	0.70	
Urban	42 (23.33)	138 (76.67)	180 (100)		Ref	0.36-1.75
Access to fluoridated water (n = 209)						
Yes	41 (23.42)	134 (76.58)	175 (100)	0.2613	Ref	
No	5 (14.70)	29 (85.30)	34 (100)		0.56	0.20-1.55

* Chi-square test, $\alpha = 5\%$ **Table 2. HSPM, treatment needs, visible plaque on the buccal surface of upper incisors and experience of caries (dmft) according to age of children in the sample.**

Age in years	HSPM		Treatment needs		Visible plaque		Caries experience			Total
	Yes N (%)	No N (%)	Yes N (%)	No N (%)	Yes N (%)	No N (%)	dmft = 0 N (%)	dmft > 0 N (%)	Mean (SD)	N (%)
4	15 (28.84)	37 (71.16)	21 (40.38)	31 (59.62)	19 (36.53)	33 (63.47)	29 (55.77)	23 (44.23)	1.77(±2.82)	52 (100)
5	16 (21.62)	58 (78.38)	35 (47.30)	39 (52.70)	26 (35.13)	48 (64.87)	32 (43.25)	42 (56.75)	2.55 (±3.58)	74 (100)
6	17 (18.89)	73 (81.11)	45 (50.00)	45 (50.00)	49 (55.45)	41 (45.55)	35 (38.89)	55 (61.11)	2.77 (±3.29)	90 (100)
Total	48 (22.22)	168 (77.78)	101 (46.75)	115 (53.25)	94 (43.52)	122 (56.48)	96 (44.45)	120 (55.56)	2.45 (±3.30)	216 (100)

results of the various studies because of examination variability, methods of recording and different age groups. However, the prevalence of other developmental defects of enamel varies considerably around the world and the same variation has been observed for permanent teeth. In the case of MIH, there was a prevalence of 2.5% among Chinese children¹⁹ while in Brazil, for the same city as the one considered in this study, a prevalence of 19.8% was reported among children aged 6 to 12 years²⁰. HSPM has been compared with MIH regarding several aspects, such as asymmetric distribution in the dentition, and clinical consequences such as post-eruptive enamel breakdown and presence of atypical restorations⁷. The same possible causes of MIH are mentioned for HSPM¹, and researchers agree that fluoride intake is not implicated in the aetiology of MIH⁵. In this study, there was no difference in prevalence of enamel defects in children living in fluoridated and non-fluoridated areas. Hence, the prevalence of HSPM, as in the case

of MIH, seems to be unaffected by environmental fluoride levels. However, it must be considered that access to fluoridated water can contribute to a lower prevalence of caries among children with qualitative enamel defects.

In Brazil, residents in rural areas are at greater risk for the development and aggravation of dental diseases due to their more limited access to public health services and interventions such as community water fluoridation^{13,21}. In addition, some of these factors are linked to unfavourable socioeconomic contexts such as malnutrition and other socially dependent factors that may influence the incidence of enamel defects on a population basis. Although previous studies have shown higher prevalence of MIH in rural areas¹⁹, this study did not corroborate that fact, probably due to its small sample size. Despite being a population-based study, we used a small sample of children, all from public schools, which may interfere in the results referring to sociodemographic data and its relationship with HSPM, since other studies

Table 3. Association between children's clinical factors and HSPM

Clinical variables	HSPM		Total N (%)	P value*	OR	CI 95%	Adjusted OR	CI 95%
	Yes N (%)	No N (%)						
Decayed deciduous teeth								
d = 0	13 (13.54)	83 (86.46)	96 (100)		Ref			
d > 0	35 (29.17)	85 (70.83)	120 (100)	0.0061	2.63	1.30-5.32	2.28	1.04-4.98
Dental caries experience								
Children with dmft ≤ 1	17 (15.04)	96 (84.96)	113 (100)		Ref			
Children with dmft > 1	31 (30.09)	72(69.91)	103(100)	0.0079	2.43	1.25-4.73		
Treatment needs								
Yes	30 (29.70)	71 (70.30)	101 (100)	0.0132	2.28	1.18-4.40		
No	18 (15.65)	97 (84.35)	115 (100)		Ref			
Visible Plaque								
Yes	27 (28.72)	67 (71.28)	94 (100)	0.0437	1.94	1.01-3.71		
No	21 (17.21)	101 (82.79)	122 (100)		Ref			

*Logistic Regression Model; α = 5%

point to a higher prevalence of enamel defects in populations with low socioeconomic status. As Brazil is a country marked by social inequalities, reflected by the levels of oral health of its population, understanding the relationship of enamel defects, caries and socioeconomic conditions can help managers and health professionals in the process of organizing actions and oral health services offered to the population.

DDE is often associated with higher rates of caries experience among affected children^{10,12}. The correlation between enamel defects and childhood caries is an important issue for the polarization of caries experience in underprivileged populations^{9,12}. Brazil is a country with significant socioeconomic discrepancies, which result in an uneven distribution of oral diseases in the population. Despite its small sample size, in this study, after adjustment of the OR for the effects of covariates, it was observed that children with HSPM had a 2.28 higher risk of having dental caries in childhood. This highlights the importance of hypomineralized enamel defects as a

risk indicator for the development of dental caries, and this evidence should be considered by public health dentists when planning health promotion actions aimed at this group.

The high prevalence rates of HSPM and its potential to affect children's oral health suggest the importance of enamel defects being included as an indicator of dental health status in oral epidemiological studies of children. The understanding of the relationship between HSPM and dental caries has implications for public health planners, and the result of this study shows the need for further research into the influence of HSPM on the pattern of dental caries in childhood development.

CONCLUSION

It is concluded that there is a positive association between HSPM and dental caries in children, even after adjustment for clinical and demographic variables. This highlights the importance of HSPM in the development of dental caries, which should be considered when planning public health actions.

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Comprehensive clinics. A comparative study among Dental Schools in Argentina

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ABSTRACT

In this study, comprehensive clinics (CC) are interpreted as a pragmatic projection of the complexity of health. The aim of the study was to analyze the characteristics of the CCs at different schools of dentistry in Argentina. Data were collected from document analysis and interviews with key respondents at 8 universities, classified according to the criteria of Bray and Thomas. Interviews were developed and analyzed according to discourse analysis. The convergences and divergences among curricula were established and analyzed statistically.

Triangulation of results showed that: (a) Most curricula (7/8) included subjects called CC; (b) the permanence of CCs in the

curricula was not stable, with changes recorded in different periods; and (c) in the so-called CCs, the complex clinical approach showed frequent displacements based on teacher values or competence.

It was concluded that there are divergences between theory and practice, and that it would be recommendable to have more rigorous curricular design taking into account current trends regarding the complexity of health and its transfer to educational management.

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Keywords: dental schools - dental education.

Clínicas integradas. Estudio comparado entre diferentes Facultades de Odontología

RESUMEN

En el presente estudio, las clínicas integradas (CI) son interpretadas como una proyección pragmática de la complejidad de la salud. El objetivo del trabajo fue analizar las características de las (CI) desarrolladas en las diferentes facultades de odontología públicas de la República Argentina. Los datos se recuperaron a partir del análisis documental y de entrevistas realizadas a informantes clave en 8 universidades, las cuales fueron categorizadas según criterios de Bray y Thomas. Las entrevistas se desarrollaron y analizaron según el análisis del discurso. Se establecieron las convergencias y divergencias existentes entre los currículos y se analizaron estadísticamente.

La triangulación de los resultados reveló: (a) la mayoría de los

currículos (7/8) incluían asignaturas denominadas CI; (b) la permanencia de las CI en los currículos no fue estable, registrándose cambios en diferentes periodos. (c) en las denominadas CI, el abordaje clínico complejo mostró frecuentes desplazamientos basados en los valores o competencia docente.

Se concluyó que los resultados muestran divergencias entre la teoría y la práctica, resultando recomendable un mayor rigor en la formulación curricular atendiendo las tendencias vigentes acerca la complejidad de la salud y su transferencia a la gestión educativa.

Palabras clave: facultades de odontología - educación odontológica.

INTRODUCTION

Courses at health science universities focus on generating human resources that are technically qualified and able to use strategies aimed at improving individual and community health^{1,2}. The educational process promotes meaningful learning from the standpoint of social demands and personal development, understanding meaningful learning to

mean a personal action that is achieved due to the flexibility of neural networks^{3,4}.

In Argentina, there are 18 schools, faculties or degree programs of dentistry, of which 8 are financed by the state and the rest are privately managed. The curricular organization of degree programs is governed by the criteria established by the National Commission for the Evaluation and Approval of

Universities (CONEAU), which is responsible for the approval and qualification of the different educational options. There are different curricular options, some of which follow a flexnerian model, while others have incorporated modalities based on interdisciplinary integration following a sequence of increasing complexity⁵. Although there are criteria that enable the establishment of equivalence among these alternatives, no valid method has been developed that would enable the interinstitutional circulation of the students, guaranteeing a reasonable equivalence between the competencies certified by each institution⁶.

In the field of health, curricula based on disciplines independent of one another generally determine structures that hinder the integration of knowledge focused on the solution of complex problems. Comprehensive care clinics serve as a pragmatic projection of complexity⁷. Integration is not a consequence of the elimination of disciplines, but the approach to health problems as complex problems^{8,9}.

Comprehensive care clinics are a useful tool in curriculum design, approaching health in its full complexity by using relevant interventions that require generic and subject-specific evidence-based competencies relevant to the context, and favoring meaningful learning for the actors involved.

In the present research, the objects of study are the so-called Comprehensive Clinics in the dental courses at public universities in Argentina. They were analyzed using theoretical triangulation and the comparative method. Triangulation is a heuristic process (principles, rules and strategies) aimed at documenting and contrasting information from different viewpoints. Hence, it is possible to distinguish different types of triangulation depending on the focus of comparison and contrast: techniques, agents, times, methods, or data analysis techniques.

Although there are four basic types of triangulation (data triangulation, theory triangulation, methodological triangulation, and investigator triangulation), Janesick¹⁰ and Cohen and Manion¹¹ included interdisciplinary triangulation and time triangulation based on the simultaneous use of longitudinal and cross-sectional designs¹². Analysis triangulation is the combination of two or more methods of analyzing a single group of data for the purpose of validation.

The comparative method has been applied in the field of education, with contributions based on the grounded theory methodology¹³⁻¹⁶. According to Arnal et al¹⁷, the application of comparative method enables the following to be distinguished: a) incompatibility¹⁸, b) complementarity¹⁹, and c) epistemological unity²⁰ among the comparative institutions or methods.

The aim of the present work was to identify the existing similarities and differences regarding the conceptual and methodological characteristics of the Comprehensive Care Clinic in the different curricula at state universities in Argentina.

MATERIALS AND METHODS

Analytical tools were designed and applied to compare the different comprehensive care clinics.

We compared courses called Comprehensive Care Clinic in the curricula of eight Schools of Dentistry at public Argentine universities and identified congruence and divergence between them. The following institutions were analyzed:

Universidad de Buenos Aires (UBA)

Universidad Nacional de Córdoba (UNC)

Universidad Nacional de Rosario (UNR)

Universidad Nacional de Cuyo (UNCuyo)

Universidad Nacional de Río Negro (UNRN)

Universidad Nacional del Nordeste (UNNE)

Universidad Nacional de La Plata (UNLP)

Universidad Nacional de Tucumán (UNT)

For the purpose of this study, the institutions were anonymized by using numbers to identify them.

The methodology used is summarized in Table 1.

Stage 1

(a) Definition of the theoretical framework and identification of the documentary sources

The information was analyzed following criteria by Bray et al.²¹, which recognize three axes for the analysis. The 1st axis, defined as “**Aspects of education and society**”, involved analyzing the clinical courses identified nominally or functionally as integrated courses providing different levels of care, and approved by the CONEAU (National Committee of University Assessment and Accreditation).

The 2nd axis, “**Level of geographic location**”, involved identifying the corresponding province/district with the aim of establishing epidemiological profiles that could influence prioritization of health problems.

Table 1. Materials and methods

Stage 1	
(a) Definition of the theoretical framework.	
(b) Identification of documentary sources (formal documents presented by the institutions to the CONEAU).	
(c) Selection of key respondents.	Identification of key actors.
	Selection of key respondents in keeping with the inclusion criteria.
(d) Gathering the corresponding documentary data.	
(e) Construction of the methodological tools.	Identification of the categories used to organize a classification of the courses identified as integrated courses in the documentary analysis. The resulting categories distinguished: <ul style="list-style-type: none"> • the existence or lack of non-integrated specialty-specific clinics • whether a general dentist or a specialist was in charge of teaching • the curricular structure in terms of place of the comprehensive care clinics in the curriculum.
	Preparation of an <i>ad hoc</i> matrix using the identified categories.
	Preparation of guidelines for the semi-structured interviews.
Stage 2	
(a) Contextualization of each institution from a historical and social standpoint.	
(b) Categorization of the courses by means of juxtaposition, comparison, and determination of convergences and divergences in the concept of integration applied by each institution.	
(c) Conducting the interviews and analyzing the discourse that arose from the semi-structured interviews.	Audio recording and transcription of the interview.
	Normalization of the text.
	Segmentation to obtain basic or elemental and structural segments of the text and modulators (the terms curriculum, interdisciplinarity, and clinic, as well as modulators were included) appearing in equational sentences (verb TO BE). Texts with a factual function were eliminated, and those in which the segments were related were joined [31].
	Finding contextual definitions.

Analysis of the 3rd axis, “**Demographic characteristics**”, involved identifying clinics/courses defined as comprehensive care clinics but excluding those specific to certain age group, gender or medical risk, as well as those specific to the incumbencies of the specialties.

(b) Selection of key respondents for the test interviews

Participants were selected by applying a proactive criterion, i.e., according to their knowledge of the subject²².

The following criteria were applied:

- Inclusion criteria: having held a different teaching position between 2000 and 2015 and agreeing to participate in the study; participation was anonymous.
- Exclusion criteria: having held a position in the governing body of their institution.

(c) Documentary data collection and analysis from the different institutions

(d) Methodological tool design

- **Identification of the categories** used to classify the courses identified as comprehensive care clinics in the documentary analysis.
- **Construction of *ad hoc* matrices** using the categories.
- Preparation of rules for gathering and recording the data from the **semi-structured interviews**.

Stage 2:

(a) Contextualization of each institution was performed from a historic and social standpoint.

(b) Comparison of the courses proposed by the different dental schools was performed following the process described by Bereday²³ and other authors^{24,25}. Juxtaposition enabled identification of the convergences and divergences between the concepts of integration applied at the different institutions and summarized in the matrices.

(c) **Test interviews** were conducted by a single researcher. From a total 3 teachers per institution, with different degrees of responsibility, one was selected as having more specific knowledge of the subject. Eight teachers with different degrees of responsibility were interviewed, one from each school. They were assured anonymity, and each interview lasted not more than 20 minutes.

(d) **Test interviews were analyzed** according to Magariños de Morentin²⁶. The aim was to identify the different meanings put forth by the respondent and that became apparent in the respondent's discourse during the interview. The following steps were used:

- **Tape-recording** and transcription of the interview.
- **Normalization** of the text.
- **Segmentation**, to obtain the basic/elemental and structural units of the text, in addition to their modulators. The texts containing the terms **curriculum**, **interdisciplinarity**, **clinic**, and modulators in equational sentences (verb TO BE) were identified and included. Texts with a factual function were eliminated, and texts showing a relation between/among segments were joined²⁷.
- **Contextual definitions**, based on which conceptual axes were defined for the purpose of comparing the institutions.

RESULTS

Analysis and categorization arising from the available documents

Historical-contextual analysis

A general approach and an institution-specific approach were used.

Two events that affected all the studied institutions were detected in the general analysis:

- Regulation of national universities linked to the process of democratization of the country.
- Creation and regulation of the CONEAU, in 1995 and 1996 respectively²⁸.

The institution-specific analysis comprising the 1985-1995 period showed the following internal and external events to have had an impact on the curricular design of each institution:

- Change of university authorities.

- Projects with external funding at three institutions (Identified institutions as 1, 2, and 7)
- Definition of the regulations regarding requirements for accreditation of degree programs and subsequent adjustments.

Structural analysis

- The curricula of seven institutions included clinically integrated courses/comprehensive care clinics (Comprehensive Clinic, Social Practice, and so forth).
- All institutions include more than one clinically integrated course or comprehensive clinic in their curricula.

The courses identified as comprehensive care clinics in the study plans corresponded to at least one of the following characteristics:

- a) A single course at the end of the degree program.
- b) Several courses taught at different stages of the degree program.
- c) Courses with increasing levels of complexity, taught at each stage of the degree program.
- d) Courses that were or were not included as a curricular activity, taught at extramural sites, and defined as a link between teaching and health care services.

Descriptive analysis of the courses and the didactic sequence (Table 2)

The following didactic modalities were detected:

- a) The evidence-based theoretical contents were taught in separate courses,
 - Outside the integrated clinic without clinical practice.
 - There was a separate specialty clinic.
 - There was no separate specialty clinic.
- b) The theoretical contents were taught in the clinically integrated course/integrated clinic
 - by specialists from discipline-specific departments (surgery, endodontics, etc.), focusing on specific techniques.
 - by specialists from discipline-specific departments, focusing on a standardized treatment plan or case studies.
 - by general dentists (family, community, etc.), focusing on the problems of the patients seeking care.

Table 2. Categories arising from the documentary analysis**Analysis of each didactic unit**

- *There was a separate discipline-specific clinic.*
- *There was no separate discipline-specific clinic.*

Analysis of the didactic sequences

- *Taught by specialists from discipline-specific departments, and focusing on specialty-specific techniques.*
- *Taught by specialists from discipline-specific departments, and focusing on a standardized treatment plan or on case studies.*
- *Taught by general dentists, and focusing on the problems of the patients seeking care.*

Structural analysis.

The integrated clinic was placed on the curriculum as:

- *a single course at the end of the degree program*
- *courses provided at several stages of the degree program*
- *courses with increasing levels of complexity, provided at each stage of the degree program*
- *curricular activity, provided at extramural sites, defined as a link between teaching and health care services.*

Interviews (Table 3)

- **Text normalization** enabled the detection of subject-specific language resources, without performing normative adjustments.
- **Segmentation** enabled the detection of 246 text segments.
- **Contextual definitions**

The segments obtained enabled the detection of the different meanings/beliefs held by the respondent prior to the interview.

The characterization of the concept “**curriculum**” was identified in the texts and corresponded to one of the following descriptions²⁹⁻³¹:

- An organized structure of knowledge.
- A learning plan focusing on different disciplines/specialties.
- An entire set of learning experiences aimed at solving problems.

The concept “**interdisciplinarity**” was characterized as:

- The fusion of theories.
- The fusion of instruments and/or techniques.
- An approach that takes into account the problem in its full complexity.

The concept “**clinic**” was characterized as:

- A practical course.
- A discipline-specific practice course.
- A comprehensive healthcare service provided by the institution.
- **Comparison of conceptual axes and sequential and contrast networks**

Divergences and convergences were observed in the conceptualization of the term “curriculum”, “interdisciplinarity”, and “clinic”. Comparison of the conceptual definitions from the different institutions showed the following results:

Total convergence (convergence in all three conceptual definitions) was observed between two institutions (1 and 8).

Total divergence (no converge between any of the selected answers) was observed between two pairs:

- Institutions 1 and 3
- Institutions 3 and 4.

Partial convergence in at least one of the three contextual definitions was observed in the following 8 cases:

- Institutions 1 and 4
- Institutions 7 and 2
- Institutions 3 and 7
- Institutions 3 and 6
- Institutions 7 and 5
- Institutions 7 and 6
- Institutions 4 and 2
- Institutions 8 and 4.

Partial divergence in at least one of the three contextual definitions was observed in the following 7 cases:

- Institution 1 vs. Institutions 3, 6, 5 and 7
- Institution 2 vs. Institutions 1, 8, 5 and 3
- Institution 3 vs. Institutions 1, 8, 7 and 2
- Institution 5 vs. Institutions 1, 8, 7 and 2
- Institution 6 vs. Institutions 7; 2, 4, 1 and 8
- Institution 7 vs. Institutions 1, 8 and 4
- Institution 8 vs. Institutions 3, 6, 5 and 7.

Table 3. Analysis of interviews with key respondents

Concept	Conceptual definitions	Key respondents' answers in the test study
		Congruence
Curriculum	A: <i>Organized structure of knowledge</i>	3 - 5 - 6
	B <i>Learning plan focusing on different disciplines/specialties</i>	2 - 7
	C <i>Entire set of learning experiences aimed at solving problems</i>	1 - 4 - 8
Interdisciplinarity	A <i>Fusion of theories</i>	2
	B <i>Fusion of instruments and/or techniques</i>	3 - 5 - 7 - 6
	C <i>Approach that takes into account the problem in its full complexity</i>	1 - 8 - 4
University clinic	A <i>Practical course</i>	2 - 4 - 6 - 7
	B <i>Discipline-specific practice course</i>	3 - 5
	C <i>Comprehensive health-care service of the institution</i>	1 - 8

DISCUSSION

Triangulation of data obtained in the present study showed that Comprehensive Clinics appeared textually in most curricula (7/8 schools), which can only be interpreted as appropriation of discourse. The convergences or divergences found during this study would seem to be the result of:

- the paradigms in place or that serve as reference, and which the involved actors uphold,
- the rationality of the actors involved, i.e., their capacity to think, evaluate, understand and follow a course of action in keeping with principles of consistency,
- local realities governed by intellectual traditions that shape education (epistemological, organizational-institutional, subjective) and are perceived by those who have the *habitus* for recognizing and using such realities,
- the styles and processing codes, whether inherited or emerging³²,

The weight of the hegemonic institutions, the inadequate relation among ideologies (academic rationalism, social economic efficiency, social reconstructionism, orthodoxy, progressivism, cognitive pluralism) and the components of the curriculum (intentions, contents, teaching, learning and assessment methods). For the purposes of an evaluation of the courses, it would seem interesting

to continue questioning the institutions about the significant weight of the “academic tribes”³³ or “the invisible school”¹¹ in the university institutions in dispute for fields of work and allocation of funds. This phenomenon has been observed both in social and natural sciences^{5,9,33}.

The didactic organization of the different curricula shows “who and what” are at the center of the teaching and learning processes:

- The core actor is the discipline-specific teacher, and focus is placed on specific teaching techniques.
- The core actor is the student, and focus is placed on learning standardized treatment plans or case studies, in which the theoretical contents are provided by discipline-specific teachers.
- The core actor is the patient, and focus is placed on the patient’s problems, which are usually complex.

This patient’s problems must be solved according to the priorities that arise from the application of properly contextualized scientific evidence.

As tools for curricular organization, comprehensive care clinics are based on the concept of integrated and comprehensive healthcare services, and must include different integration circuits, i.e. horizontal, vertical, functional and virtual circuits. These

circuits are generated through the relationships among all involved actors, and are not based on the weight of the knowledge contributed by each of the actors/teachers⁷.

It should be noted that the methodological tools became apparent in the test interviews aimed at identifying contextual definitions of basic concepts inherent to the study and the conceptual networks. These methods were useful to identify variables referring to the competencies that may be essential in the evaluation instances that lead to a reasonable comparison between the studied degree programs. The proper implementation of comprehensive clinics, keeping the focus on the patients and recognizing the complexity of the health problems they are going through, is an intervening variable in the achievement of the essential competencies.

This rigorous analysis is considered essential to the construction of questionnaires especially focused on the student learning process and its subsequent validations. The questionnaire or other instruments would enable the broadening of the different categories of actors and identification of the different conceptualizations and approaches used at each institution with regard to integrated learning and its various components.

In view of the characteristics of the conceptual networks identified in the present study, it would seem necessary to perform factorial studies (varimax or oblimin rotation) that enable the identification of the main components and factors that must be taken into account to facilitate the implantation of curricular changes and the identification of the intervening variables that affect the results of curriculum studies. The triangulation method used in the present study was useful to answer to the demands that higher educational institutions face today.

CONCLUSIONS

1. The method used here enables the following conclusions:
 - a) Most curriculum documents stated the existence of clinical integration (7/8 schools).
 - b) There was divergence between the statements in the curriculum documents and the conceptualization on generic competencies to guarantee relevant learning.
 - c) The present qualitative analysis suggests that focusing on the problem/s of the

patient seeking care as an issue relevant to the learning process had shifted, in several cases towards a teaching process which was strongly influenced by the teacher's personal values.

- d) It seems recommendable to seek greater rigor in the different components of curricular integration, in line with international trends of university quality.
- e) The present study enables us to recommend the application of this methodology in baseline institutional studies to identify the definitions and conceptual networks that are necessary to develop useful questionnaires for educational research and decision making.

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Self-reported habits regarding dental bur use and conditioning among academic and non-academic argentinian dentists

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ABSTRACT

The removal of tissues affected by caries lesions is one of the most frequent procedures in daily dental practice. The aim of this study was to collect information about the habits of members of the academic and non-academic dental community in Argentina regarding the use and conditioning of burs employed in the removal of dentin during the treatment of carious lesions. A 14-item questionnaire was prepared, and once validated in small groups, it was sent to three lists of dental subscribers through the computer services area of the School of Dentistry, University of Buenos Aires, Argentina (FOUBA). By the deadline for receiving responses, which was set as four weeks after the sending date, 294 replies were received. Results: percentage (95%CI): 47.93% (42.18-53.68%) of the respondents were faculty members, of whom 37.24% (31.68-42.81%) claimed to belong to FOUBA. Of the respondents, 26.90% (21.79-32.00%) reported being under 35 years old, 37.59% (32.01-43.16%) 36 to 45 years old, 22.07% (17.30-26.84%) 46 to 55 years and 11.38% (7.72-15.03%) over 55 years. Regarding bur type, 68.07% (62.31%-73.44%) claimed to use round burs to remove carious dentin and 27.72% (22.60%-23.31%) round + others.

Regarding rotary speed, 22.07% (17.30-26.84%) use medium speed, 32.76% (27.36-38.16%) use high speed, and 32.76% (27.36-38.16%) use medium + super high. Only 17.93% (13.52-22.35%) indicated that they know how to quantify the number of times they use their burs, while 50.34% (44.59-56.10%) of the respondents said that they use burs "until they no longer cut", 23.79% (18.89-28.69%) use them 1 to 5 times. 98.27% (96.77-99.77%) said they clean their burs once used. When the answers regarding the use of rubber dam were compared with area of specialization (Chi-square), it was found that faculty members mention the use of burs under rubber dam always or almost always 74.82% (67.60-82.04%) while outside the academic field, 51.01% (42.98-59.03%) of dentists say that they never or almost never do so ($p < 0.001$). Conclusion: Most respondents state that they sterilize their rotary instruments in some way after use. About half of them do not keep track of the number of uses and use burs "until they no longer cut".

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Keywords: dental instruments - disinfection - sterilization - surveys and questionnaires.

Autoreporte sobre hábitos de uso y acondicionamiento de fresas de odontólogos argentinos en ámbitos académicos y no académicos

RESUMEN

La eliminación de los tejidos afectados por las lesiones de caries es uno de los procedimientos más realizados en la práctica diaria de la odontología. El objetivo de este estudio fue recolectar información acerca de los hábitos de miembros de la comunidad odontológica de la Argentina en cuanto a la selección, utilización y acondicionamiento de fresas para la remoción de dentina en el tratamiento de lesiones cariosas. Se elaboró un cuestionario de 14 reactivos que fue validado en pequeños grupos. Una vez definido el formato final se lo envió a tres listas de suscriptores odontólogos a través del área de Informática de la Facultad de Odontología de la Universidad de Buenos Aires (FOUBA). El plazo para la recepción de respuestas se estableció en cuatro semanas luego del cual se obtuvieron 294 réplicas. Resultados: porcentaje (IC95%), el 47,93% (42,18-53,68%) eran docentes, de los cuales el 37,24% (31,68-42,81%) afirmó ser docente de la UBA. El 26,90% (21,79-32,00%) refirió te-

ner menos de 35 años, el 37,59% (32,01-43,16%) entre 36 y 45 años, el 22,07% (17,30-26,84%) entre 46 y 55 años y el 11,38% (7,72-15,03%) más de 55 años. El 68,07% (62,31%-73,44%) afirmó utilizar fresas redondas y el 27,72% (22,60%-23,31%) redondas + otras. En cuanto a la velocidad utilizada: media velocidad: 22,07% (17,30-26,84%), superalta: 32,76% (27,36-38,16%), media + superalta: 32,76% (27,36-38,16%). Sólo el 17,93% (13,52-22,35%) indicó que sabe cuantificar la cantidad de usos que les dan a sus fresas, en tanto que el 50,34% (44,59-56,10%) de los consultados afirmó que utiliza las fresas "hasta que no corten más", el 23,79% (18,89-28,69%) le da entre 1 y 5 usos. El 98,27% (96,77-99,77%) afirmó que limpia sus fresas una vez utilizadas. Cuando se contrastaron las respuestas respecto al uso de aislamiento con el área de desempeño (Chi-cuadrado) se encontró que los docentes refieren utilizar las fresas con aislamiento absoluto siempre o casi siempre 74,82% (67,60-82,04%) mientras que fuera del ámbito acadé-

mico el 51,01% (42,98-59,03%) respondieron que nunca ó casi nunca lo hacen ($p < 0,001$). Conclusión: la mayoría afirma que acondiciona de alguna manera el instrumental rotatorio luego de su uso, alrededor de la mitad no lleva registro del número de

usos y sostiene que utiliza las fresas "hasta que no cortan más".

Palabras clave: instrumentos dentales - desinfección - esterilización - encuestas y cuestionarios.

INTRODUCTION

The removal of caries-affected tissues is one of the most frequent procedures in daily dental practice. The aim of using different combinations of speed, pressure and type of cutting instrument is to achieve maximum cutting power without causing biological damage to the pulp. The hydroxyapatite in the dentine is a fragile material, so it requires less energy to produce fracture, while the collagen matrix is a soft material and the way it is cut is guided by different principles, like those of ductile materials. Although this maneuver can be performed using either manual or rotary instruments, the literature currently recommends the use of plain cut round burs, whose size is chosen in relation to the size of the lesion at low, medium or high speed¹⁻³.

There are different forms and brands of dental burs as well as commercial recommendations, detailed information about cleaning, disinfection and sterilization. However, data about the recommended number of uses is often unreported. Some authors such as Morrison and Corond⁸, report that the life of a dental bur is limited to no more than five or six sterilization cycles because it becomes distempered and loses its cutting edge, thus beginning to require greater working pressure with the consequent generation of heat and deleterious effect on the pulp. Sterilization of dental instruments, including burs, for reuse occurs regularly in all dental offices in order to minimize the risk of cross contamination between patients. The methods used for both cleaning and decontamination and for sterilization significantly reduce the durability and resistance of burs^{5,6}.

Regarding the use of the rubber dam, some authors indicate that the rate of its usage by general dentists is 37% - 43%^{7,8}, while Siegel and von Fraunhofer⁹ report that 63% refer to using it. Another publication found that professionals with 5 to 10 years' work experience had the highest rate (76.3%), while professionals with more than 20 years of practice had the lowest rate (53.2%). Endodontists had the highest and most frequent use rate⁹.

Two different studies analyzed the behavior and attitudes of students from the United States of

America and health professionals in India regarding the use of different types of burs for tissue removal during caries treatment^{8,10,11}, finding that most professionals in India preferred diamond burs, while students in different dental schools in the United States used tungsten carbide burs. In these studies, most dentists said they use the burs until they wear out (85%), sterilize them once a day (35%) or after each patient (35%).

The aim of this study was to collect information about the habits of members of the dental community in Argentina regarding the use and conditioning of dentin removal burs in the treatment of carious lesions.

MATERIALS AND METHODS

A 14-item questionnaire was prepared in Google Forms platform, with an informed consent inserted at the beginning and anonymity set as selection criteria. Once validated in small groups, the relevant authorizations were processed and the questionnaire was sent to three lists of dental subscribers through the computer services area of the School of Dentistry, University of Buenos Aires, Argentina (FOUBA). According to information provided by the aforementioned area, the lists consisted of 8478 dentists, of whom 1555 were also faculty members and 27 were full professors. By the deadline for receiving responses, which was set as four weeks after the sending date, 294 replies were received. Data was analyzed by rates with 95% confidence intervals and chi-square test (significance level: $P < 0.05$).

RESULTS

After four weeks, 294 replies were received, of which 47.93% (42.18-53.68%) claimed to be members of faculty and 37.24% (31.68-42.81%) claimed to teach at FOUBA (Fig. 1).

Regarding area of specialization, most respondents reported disciplines related to the indispensable use of burs for caries treatment such as restorative dentistry, endodontics or prosthetic dentistry, as well as pediatric dentistry.

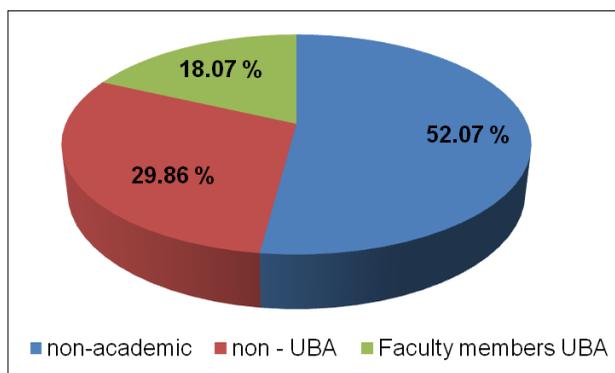


Fig. 1: Distribution of respondents by academic affiliation

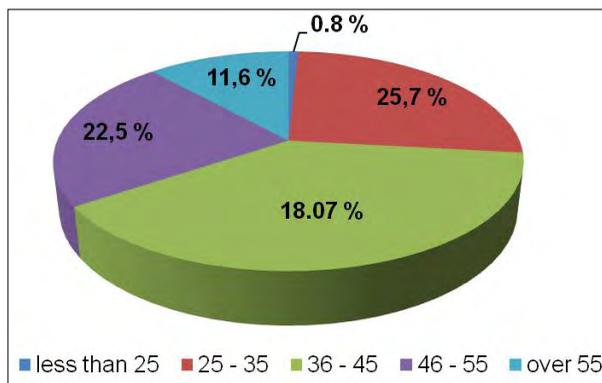


Fig. 2: Distribution of respondents according to their age

In terms of age, 26.90% (21.79-32.00%) reported being under 35 years old, 37.59% (32.01-43.16%) 36 to 45, 22.07% (17, 30-26.84%) 46 to 55 and 11.38% (7.72-15.03%) over 55 years (Fig. 2).

About 22.07% (17.30-26.84%) reported that they remove carious dentin with medium speed rotary instruments, while 32.76% (27.36-38.16%) reported using super-high speed and 32.76% (27.36-38.16%) a combination of both (Fig. 3). This item had significantly different responses as a function according to academic involvement ($P < 0,01$). Around 31% (23.55%-39.59%) of the dentists who also identified themselves as faculty members claimed to employ medium speed, 20.59% (13.93%-27.97%) ultra-high speed and 34.56% (26.21%-42.61%) both speeds. On the other hand, non-academic respondents answered 14.19%, (9.00%-20.87%), 44.59% (36.43%-52.98%) and 32.43% (24.98%-40.61%), respectively.

In terms of main use, removal of carious dentin was the most frequently mentioned. Among surveyed dentists, 68.07% (62.31%-73.44) declared that they use round burs and 27.72% (22.60%-23.31%) associate round burs with another means. This was not found to depend on whether or not the respondents belonged to the academia ($P > 0.05$).

Faculty members referred to using dental burs with absolute isolation always or almost always in 74.82% (67.60-82.04%) of the cases, while amongst respondents from outside the academic field, 51.01% (42.98-59.03%) answered that they never or almost never do so ($P < 0.001$) (Fig. 4).

Only 17.93% (13.52-22.35%) indicated that they keep a record of the number of times they use burs, while 50.34% (44.59-56.10%) of the respondents said that they use burs "until they no longer cut", and 23.79% (18.89-28.69%) of total respondents claim they use burs 1 to 5 times.

Table 1 summarizes the quantity and quality of devices and solutions claimed to be used during cleaning, decontamination and sterilization of burs. Overall, 98.27% (96.77-99.77%) said they clean their burs once used, 44.68% (38.80-50.70%) reported using three conditioning methods for burs, while 28.72% (23.50-34.40%) stated using two, and 19.15% (14.70-24.20%) four of them. Only about 4% selected only one 3.90% (2.00-6.90%) or five methods 3.55% (1.70-6.40%). The two most frequently selected single cleaning, decontamination or sterilization methods were metal brush, 94.33% (90.90-96.70%) and enzymatic solution, 92.55% (88.80-95.30%). Of the 28.72% (23.50-34.4%) that selected 2 modes, mechanical brush, 96.30% (89.60-99.20%) and enzymatic solution, 88.89% (80.00-94.80%) were the most frequently chosen methods to combine. Within the 44.68% (38.80-50.70%) that selected 3 modes, mechanical brush, 99.21% (95.70-100.00%), enzymatic solution, 98.41% (94.40-99.80%), autoclave, 34.92% (26.60-43.90%) and stove, 29.37% (21.60-38.10%) were the most frequently indicated to be used in association.

Of the 19.15% (14.70-24.20%) that selected 4 modes, metal brush, 98.15% (90.10-100.00%), enzymatic solution, 98.15% (90.10-100.00%), autoclave, 51.85% (37.80-65.70%), sodium hypochlorite, 40.74% (27.60-55.00) and stove, 38.89% (25.90-53.10%) were the most frequently indicated.

DISCUSSION

Although the removal of dentin affected by caries is one of the most frequent procedures in the daily practice of dentistry, the literature contains few studies about the attitudes of dental practitioners and faculty members regarding selection and

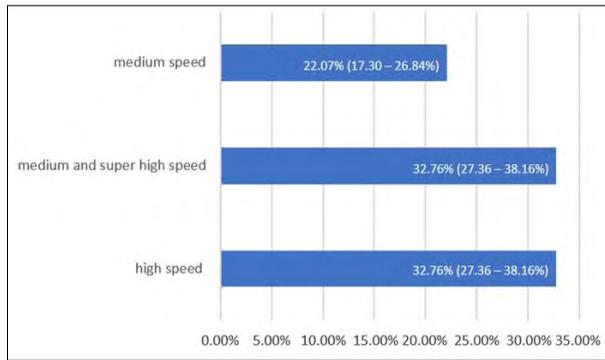


Fig. 3. Use of speeds reported by respondents

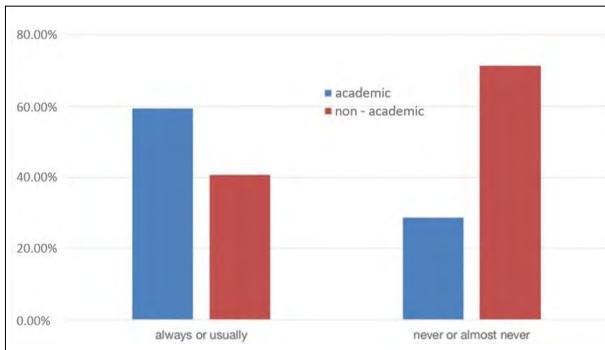


Fig. 4. Use of burs with absolute isolation reported by faculty members and non-academic dentists.

management methods of instruments used for removal of infected dentin. Of the dentists who responded the survey in this study, 97.28% said

they clean or condition the burs, in contrast to 70% of the participants in the study by Sharma et al¹⁰. Regarding how many times a bur is used, although there is evidence in the literature that supports that an optimal limit is within five times, most of the respondents declared that they use them “until they no longer cut” in agreement with the paper by Sharma¹⁰ that explored the same variable in a group of Hindu dentists. With respect to the use of rubber dam, 62.1% of our respondents stated that they always or almost always use it, unlike the findings of Imberly and Carrico⁸, who indicate that 37 - 43% of dentists use it. A separate analysis comparing academic and non-academic respondents shows that the rates reported by non-academics are similar to those found by Imberly and Carrico⁸. Metal brush and enzymatic solutions were the most frequently mentioned in one and two mode cleaning methods. When respondents referred to use three or more cleaning methods, stove, autoclave and ultrasound were the devices added to the protocols.

CONCLUSIONS

Most respondents state that they somehow condition the rotary instruments after use. About half of the respondents do not keep track of the number of uses and maintain that they use burs “until they no longer cut”.

Table 1. Responses regarding number of cleaning modes employed and devices/solutions preferred. Rates and IC95%

chosen elements	single mode	two modes	three modes	four modes
metal brush	94.33 (90.90 - 96.70)	96.30 (89.60 - 99.20)	99.21 (95.70 - 100.00)	98.15 (90.10 - 100.00)
common brush	1.78 (0.60 - 4.10)	0.00	0.79 (0.00 - 4.30)	7.41 (2.10 - 17.90)
enzymatic solution	92.55 (88.80 - 95.30)	88.89 (80.00 - 94.80)	98.41 (94.40 - 99.80)	98.15 (90.10 - 100.00)
alcohol	12.77 (9.10 - 17.20)	3.70 (0.80 - 10.40)	9.52 (50.00 - 16.60)	25.93 (15.00 - 39.70)
sodium hypochlorite	17.08 (12.90 - 22.00)	3.70 (0.80 - 10.40)	9.52 (5.00 - 16.60)	40.74 (27.60 - 55.00)
autoclave	28.01 (22.80 - 33.60)	1.23 (0.00 - 6.70)	34.92 (26.60 - 43.90)	51.85 (37.80 - 65.70)
stove	23.76 (18.90 - 29.20)	4.94 (1.40 - 12.20)	29.37 (21.60 - 38.10)	38.89 (25.90 - 53.10)
ultrasound	19.50 (15.00 - 24.60)	1.23 (0.00 - 6.70)	18.25 (11.90 - 26.10)	38.89 (25.90 - 53.10)
rates (CI95%)				

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Cognitive impairment related to arterial stiffness in cardiovascular disease patients with severe periodontitis

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ABSTRACT

Severe periodontal disease (SPD) associated with systemic peripheral inflammation, cognitive impairment (CI) and arterial stiffness (AS) has been recognized. The aim of this study was to investigate whether CI and arterial stiffness (AS) occur in cardiovascular disease (CVD) patients with SPD. A cross-sectional case-control study included hospitalized patients with CVD. Demographic characteristics, CVD and atherogenic risk factors were recorded. SPD was diagnosed by clinical and radiographic dental examinations. Dental clinical attachment level (CAL) and CAL % were recorded. A Mini-Mental State Examination test (MMSE) assessed cognition, a MMSE score of < 27 was set as the cut-off point of CI; a score \geq 27 was considered as no CI. Patients were categorized into: MMSE<27 (cases) and MMSE \geq 27 (controls). AS was evaluated by pulse wave velocity (PWV). Serum VCAM-1 levels were determined in a random sample. Results: This study comprised 91 patients (cases, n=26; 29.6%; controls, n=65, 71.4%); aged 73 \pm 8 vs. 73 \pm 7 years, respectively (p=0.73), of whom 53.8% and 36.9%

respectively, were women; SPD was found to be a risk factor for CI; the presence of SPD increased the risk for MMSE <27 by an average 5.39 times (model 1). PWV was associated with MMSE < 27 in the three models. The risk of having MMSE < 27 increased an average of 2.404-fold for each 1-unit increase in PWV. SPD and AS had significant and independent associations on the risk for development CI. MMSE negatively correlated with CAL% (r=0.69) and PWV (r=0.70). PWV positively correlated with CAL% (r=0.67). Serum VCAM-1 levels were higher in SPD with lower MMSE scores. In conclusion, SPD increases the risk of development of cognitive decline in CVD patients. PWV was directly associated with the risk of cognitive decline.

These findings denote a significant opportunity to improve periodontal health in order to avert CI in CVD patients.

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Keywords: arterial stiffness - cognitive impairment - cardiovascular diseases - severe periodontitis - VCAM-1.

Deterioro cognitivo relacionado a la rigidez arterial en pacientes con enfermedad cardiovascular y periodontitis grave

RESUMEN

La enfermedad periodontal severa (EPS) podría estar asociada a la rigidez arterial (RA) y al deterioro cognitivo (DC). Se realizó un estudio transversal de casos y controles y se investigó la presencia de RA y DC en pacientes con enfermedad cardiovascular (ECV) y EPS. En pacientes hospitalizados con ECV se registraron las características demográficas y factores de riesgo aterogénicos. El DC se diagnosticó a través del Mini-Mental State Examination (MMSE). Punto de corte: MMSE<27 (casos); puntaje \geq 27 ausencia de DC (controles). La EPS fue diagnosticada clínica y radiográficamente. Se registraron el nivel inserción clínica (NIC) y NIC %. La RA fue evaluada a través de la velocidad de onda de pulso (VOP). VCAM-1 sérico se determinó en una muestra aleatoria. Se incluyeron 91 pacientes (casos, n=26; 29.6%; controles, n=65, 71.4%); edad promedio: 73 \pm 8 vs. 73 \pm 7 años, respectivamente (p=0.73); % de mujeres: 53.8 vs. 36.9, respectivamente y EPS (n=54) y ausencia de EP

(noEP) en 37. MMSE< 27 en 26 pacientes; 23 de ellos, con EPS. La presencia de EPS aumentó el riesgo de MMSE< 27 en 5.39 veces (modelo 1). La VOP se asoció a MMSE< 27 (Modelo 1, 2 y 3). El riesgo de MMSE< 27 incrementó en promedio en 2.40 veces por cada aumento de unidad de VOP. EPS y RA mostraron asociaciones significativas e independientes sobre el riesgo de DC. MMSE se correlacionó negativamente con NIC % (r=0.69) y POV (r=0.70); y POV, positivamente con NIC % (r=0.67). Los niveles séricos de VCAM-1 fueron más elevados en presencia de EPS y puntajes bajos de MMSE. Puede concluirse que en pacientes con ECV y EPS, el aumento en RA incrementaría el riesgo de DC. Estos hallazgos enfatizan la necesidad de promover y mantener la salud bucal para evitar el DC en pacientes con ECV.

Palabras clave: rigidez arterial - deterioro cognitivo - enfermedad cardiovascular - Periodontitis severa - VCAM-1.

INTRODUCTION

The World Health Organization¹ has documented that about 50 million people have dementia worldwide, and epidemiological estimates show an increased incidence of dementia of nearly 10 million new cases every year. Currently, there are no effective approaches to prevent, slow, stop or reverse the dementia process, but there is increasing interest in identifying risk factors for primary prevention or to delay the onset of this disease². Risk reduction of modifiable factors is currently the fundamental strategy to reduce the number of individuals affected by dementia³.

Periodontal disease (PD) is an infectious chronic inflammatory disease, and one of the most frequent human chronic disorders that may be amendable by oral hygiene. PD is characterized by subgingival infection with chronic inflammatory processes leading to destruction of supportive connective tissues surrounding the roots of teeth⁴. Clinical and experimental studies have demonstrated that chronic peripheral inflammation, as present in PD, could be a key determinant of the pathophysiology of cognitive decline associated with dementia⁵. The odds ratio (OR) for dementia was three times higher in individuals with atherosclerotic CVD than in those without⁶.

The low-grade inflammation occurring in PD may be involved in the pathophysiology of atherosclerosis and cardiovascular disease (CVD). PD patients exhibit alterations in arterial wall thickness, arterial wall stiffness⁷, arterial distensibility and endothelial function⁸. Arterial stiffness (AS) and vascular endothelial dysfunction may be associated with subclinical cerebrovascular damage, detectable through structural brain alterations and impaired cognition⁹. AS exposes the small brain vessels to highly pulsatile pressure and flow, which may result in cerebral microvascular alterations. Moreover, AS could compromise cerebral perfusion, modifying the blood-brain barrier and causing subcortical brain lesions¹⁰. The mechanical damage caused by AS may also lead to periventricular white matter lesions, and silent brain infarcts also associated with an increased risk of dementia¹¹.

The aim of the present study was to assess whether there is a relationship between AS and cognitive impairment (CI) in CVD patients with severe PD (SPD). In this study we demonstrated that the presence AS in CVD patients with SPD was

associated with a risk more than five times higher of developing CI.

MATERIALS AND METHODS

Study population

An exploratory cross-sectional, case-control study was conducted in 91 patients with CVD admitted to the Department of Cardiology, Spanish Hospital of Buenos Aires, Argentina, from July-2016 to July-2017. This study population was representative of the older population with CVD at large. The current investigation was conducted in compliance with the STROBE Statement guidelines after securing informed consent from the participants and approved by the Research Ethics Committee of the Hospital. Inclusion criteria: Patients with CVD, aged more than 60 years, having SPD or No periodontal disease (NoPD) assessed by clinical and radiographic dental examination. The CVD diagnosis included the following conditions: heart failure, ischemic heart disease, heart conduction block, peripheral arteriopathy, surgical myocardial revascularization, percutaneous transluminal coronary angioplasty, and valvular heart disease. Risk factors included: hypertension (blood pressure above 140/90mm/Hg), diabetes, smoking, hypercholesterolemia (>200mg/dl)¹². Ongoing treatment of chronic CVD and CVD risk factors at the time of the study was recorded. Diabetes was present in 25 of the 91 patients studied.

Exclusion criteria: Patients with I) fewer than 10 teeth (in order to ensure that periodontal diagnosis was representative of the clinical dental status of the patient and to avoid overestimation of diagnosis of periodontal disease), II) previous stroke, III) congenital or acquired psychiatric diseases or brain development disorders, IV) chronic inflammatory diseases such as rheumatoid arthritis, v) less than 7 years of elementary education, VI) kidney dysfunction, VII) prior neoplastic pathology or undergoing chemotherapy or radiation therapy, VIII) patients taking antipsychotic medication and/or anti-inflammatory drugs and IX) mild or moderate PD.

Periodontal examination

The radiographic study included a digital panoramic X-ray using a CS9000-3D extraoral imaging system (Carestream Health, Inc., Rochester, NY, USA) which was digitally analyzed using Kodak Dental Imaging Software, version 6.12.10.0. All permanent

teeth, except for the third molars, were evaluated. Clinical periodontal examination was performed by a single trained operator. Periodontal measurements were performed at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual/palatal, mid-lingual/palatal and disto-lingual/palatal) on all present teeth, using a manual Marquis Type periodontal probe (Hu-Friedy Co., Chicago, IL, USA).

The periodontal parameters measured included probing depth and clinical attachment level (CAL) assessed by a standardized protocol¹³. Average periodontal parameters were calculated by the arithmetic mean of the six determinations performed on each tooth. This method precludes overdiagnosis of periodontal disease, as patients may have high CAL values at one periodontal site and no alterations at other sites, due to trauma or other associated factors i.e. harmful oral habits, tooth brushing, removable prosthesis, among others. CAL was expressed as CAL% which represents the percentage of total number of sites with ≥ 5 mm CAL with respect to the total sites examined per participant.

Intra-examiner reproducibility of probing depth and CAL measurements was assessed before the study. The weighted K coefficient was 0.96 for probing depth and 0.91 for clinical attachment level. Alveolar bone loss, determined on the radiographic images, was assessed as an adjuvant parameter to confirm diagnosis of PD, as described below. The diagnosis of SPD was made according to Caton *et al.*¹⁴ jointly by the American Academy of Periodontology and the European Federation of Periodontology. PD was established when: 1. Average CAL values were ≥ 5 mm, 2. Average CAL values < 5 mm, but with values ≥ 5 mm at $> 30\%$ of studied sites, 3. Horizontal alveolar bone loss $> 1/3$ of the root length in at least 30% of teeth, as measured on the radiographic images.

Measurement of Arterial Stiffness

AS was evaluated by pulse wave velocity (PWV) using Doppler ultrasound synchronized with carotid-femoral electrocardiogram. Quantification of carotid-femoral PWV was assessed as a measure of AS. Simultaneous electrocardiogram (ECG) and the pulse wave of the carotid, and femoral arteries (Toshiba Nemio) were recorded.

All subjects were studied in the supine position, the carotid artery was located at the suprasternal node

supraclavicular in mode B. Doppler flow and ECG were recorded simultaneously (Toshiba Nemio ultrasound). Subsequently, PWV was measured and the data expressed as m/sec. The time delay between the feet of simultaneously recorded pulse waves and the carotid and the femoral artery was measured. The same procedure was carried out with the femoral artery at the level of the inguinal crease. The average of 6 determinations was taken at each site. Mean femoral time was subtracted from the carotid average and divided by 80% of the distance between the two points¹⁵.

However, due to the strong dependence of PWV on age, each value was compared with the reference range obtained by others in a healthy non-hypertensive population who had no CV risk factors, independently of age¹⁶.

Assessment of cognitive impairment

Cognitive functioning was assessed using the Mini-Mental State Examination (MMSE). This test consists of 11 items to assess orientation in time and place, attention, memory, language, and visual construction. The maximum obtainable score of cognitive function was the sum total points of all the items, yielding a single total score ranging from 0 to 35 (35 being the maximum score), with lower scores denoting impaired cognition.

A cut-off point for the MMSE, equal to or above 27 defined normal cognitive function¹⁷. This cut-off point score was also applied to the 25 patients who had diabetes mellitus, as defined by Yamazaki *et al.*¹⁸. Lower scores defined various degrees of mental cognition deficits (26–25, borderline cognitive deficit; 24–21, mild/early impaired cognition; 20–10, moderate impairment; less than 9, severely impaired cognition).

Two certified trained professionals carried out the cognitive evaluation, recording the cognitive domains with their respective scores¹⁹.

Biochemical determinations

Serum VCAM-1 levels were determined for a random sample of 37 of 91 patients. Concentrations of soluble vascular cell adhesion molecule VCAM-1 (CD106) in serum were measured by Enzyme-Linked Immunosorbent Assay (abcam187393, UK). It employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which capture the sample analyte in solution. Signal is

generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. The assay has a sensitivity of 637pg/ml and specificity of 97%. The results were expressed in ng/ml.

Statistical analysis

Statistical analyses were performed using the statistic program SPSS, version 24.0 IBM Corp., Armonk, NY, USA. Descriptive statistics and frequency distribution were performed for each variable according to type of variable. Demographic, vascular risk factors and SPD characteristics were compared between groups using independent Student's t test or Chi square test, as appropriate. Significant associations were investigated using multivariate logistic regression analysis. The magnitude of association was expressed using odds ratios (ORs) and 95% confidence intervals (95% CI) obtained from the multivariate logistic regression analysis.

Analyses were adjusted for the following groups of potential confounders: i. demographic conditions (age and gender) and ii. vascular risk factors (diabetes, cholesterolemia, hypertension, heart failure and ischemia heart disease).

Pearson's coefficient correlation analyses were conducted between the following continuous variables: MMSE vs. CAL%, MMSE vs. PWV and PWV vs. CAL%.

p value of less than 0.05 was considered statistically significant.

RESULTS

The characteristics of the patients studied according to the presence or absence of CI are shown in Table 1. There were 26 patients with MMSE scores, < 27 (28.6%) and 65 with MMSE \geq 27 (71.4%). There were no significant differences in average age of patients between groups (Table 1). There were more women (53.8%) among the patients with MMSE < 27 scores as compared to women (36.9%) in the MMSE \geq 27 group. No differences were observed in the percentage of men and women between groups (Chi square=1.546; p = 0.214). Mean age of women in the MMSE < 27 group was 75 ± 7 years, as compared to a mean age of 72 ± 7 years observed in the women in the MMSE \geq 27 group; this three-year difference was not significant.

There were no significant differences among the two MMSE score groups and the atherogenic risk factors

or CVD types (Table 1). The prevalence of cognitive decline in patients with arteriopathy (ischemia heart disease and peripheral arteriopathy) was 73% with MMSE score < 27 and 50.79 % with MMSE score \geq 27, differences were not significant.

Patients with MMSE < 27 had higher prevalence of SPD than those with MMSE \geq 27 (n=23, 88.5% vs. n=31, 47.7 %; Chi square = 30.012; p =0.008). Only three of the 26 patients with MMSE < 27 (11.5%) did not have PD, whereas 34 of the 65 patients with MMSE \geq 27 (52.3%), had noPD. Distribution of scores in the MMSE <27 group showed that 6 (23.07%) subjects scored between 26 and 25, 15 (57.70%) subjects scored 24 to 21, and 5 (19.23%) subjects scored 20 or < 20. The MMSE negatively correlated with CAL% (Fig. 1A). There was a significant difference in CAL% > 5, (5.8 ± 0.42 vs. 1.94 ± 0.20 m/sec; p=0.001) between case and control groups.

The associations between CI, PWV and SPD are shown in Table 2. The multivariate logistic regression analysis for MMSE < 27 showed that PWV and SPD had significant and independent effects on development CI. After adjustment for PWV, the presence of SPD was associated with increased risk of having MMSE < 27 by an average 5.39 times. Meanwhile, after adjustment for SPD, the risk of having MMSE < 27 increased an average of 2.404-fold for each 1-unit increase in PWV. After adjustment for CAL% > 5, the risk for MMSE < 27 increased an average of 2.44 and 2.60 for 1-unit increase in PWV (Model 2 and Model 3), respectively. However, MMSE < 27 associations with demographics (age and gender) (Model 2) or vascular risk factors (diabetes, total serum cholesterol, hypertension, heart failure and ischemia heart disease) (Model 3) were not significant.

AS determined by elevated PWV rates was significantly higher in patients with SPD than in those with NoPD (11.72 ± 0.76 vs. 10.63 ± 0.85 m/sec, respectively; p= 0.001; 95% CI, 1.59- 0.57). PWV was positively correlated with CAL% (p = 0.001) (Fig. 1B). MMSE score was negatively correlated with AS measured by the PWV (Fig. 1C, p=0.01). Lower MMSE scores were found in patients with SPD (27.53 ± 3.18 vs. 30.68 ± 2.12 p = 0.003 [d 3.15; 95% CI 1.14 - 5.16]).

The VCAM-1 levels in patients with MMSE < 27 (2385 ± 468 pg/ml, n=10) were similar to those with MMSE \geq 27 (2219 ± 462 pg/ml, n=27; p=0.17).

Table 1. Characteristics of the study population

Variables	MMSE < 27 (n=26)	MMSE ≥ 27 (n=65)	p
Age (years) ^a	73 ± 8	73 ± 7	0.73
Male ^b	12 (46)	41 (63)	0.21
Hypertension ^b	18 (69)	42 (65)	0.92
Total cholesterol (mg/dl) ^a	229 ± 18	232 ± 22	0.67
LDL cholesterol > 130 (mg/dl) ^b	16 (62)	42 (65)	0.97
Diabetes ^b	7 (27)	18 (28)	0.99
Smoking ^b	2 (8)	4 (6)	0.84
Heart failure ^b	17 (65)	47 (72)	0.69
Ischemia heart disease ^b	14 (54)	28 (43)	0.48
Heart conducted block ^b	3 (12)	4 (6)	0.66
Valvular disease ^b	8 (31)	21 (32)	0.91
Peripheral arteriopathy ^b	5 (19)	5 (8)	0.22
Other pathologies ^b	1 (4)	4 (6)	0.94
Severe Periodontal Disease ^b	23 (88)	31 (48)	0.008

MMSE, Mini Mental State Examination. MMSE < 27 = cases. MMSE ≥ 27 = controls.
^a Values are Means ± Standard Deviation
^b Number of patients; in brackets, number of patients/total number of patients of each group, expressed as percentage.
 p-value for the comparison between groups. p < 0.05 was considered significant.

However, serum VCAM-1 concentrations were higher among patients with SPD who had MMSE < 27 versus those with SPD and MMSE ≥ 27 (2385 ± 468pg/ml, n=10 vs. 2024 ± 457pg/ml, n=12; p= 0.02).

DISCUSSION

This study showed that CVD patients who had SPD were also likely to present AS and CI. The patients with SPD showed increased arterial stiffness as measured by PWV, and poorer cognitive function as detected by lower MMSE scores. The current study contributes to the body of literature by focusing on CVD patients with SPD. The study is important since the data denote a significant opportunity to improve periodontal health in order to decrease some of the very negative consequences of SPD by adopting simple oral hygiene measures. Regular tooth brushing plus other modifiable risk factors such as minimizing sugary food intake may reduce the risk of SPD and of developing AS and/or CI. In real life, risk factors coexist and interact with one another, significantly increasing the chance of developing other alterations. SPD has also

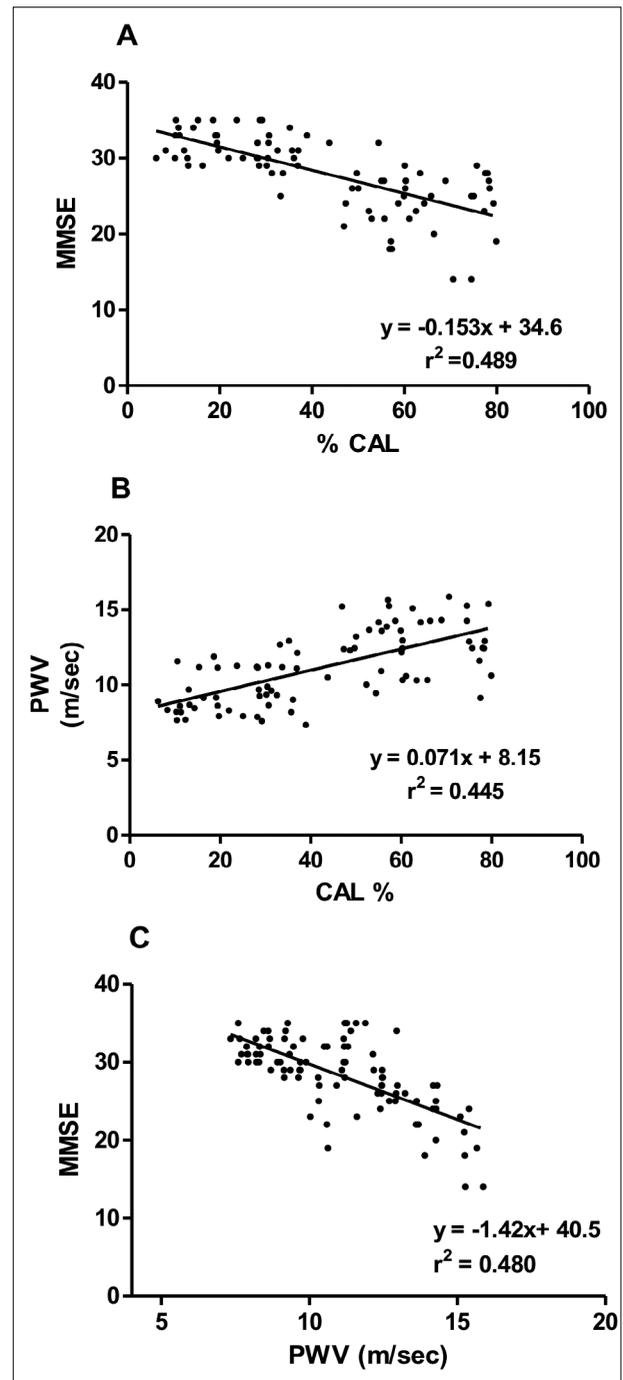


Fig. 1: Correlations between A: MMSE vs. CAL%; B: PWV vs. CAL% and C: PWV vs. MMSE.

MMSE: Mini Mental State Examination; PWV: Pulse wave velocity; CAL %: Mean Clinical attachment level %.

been known to affect other structures, including the cardiovascular²⁰ and nervous systems³. The inflammatory processes that occur with SPD could account for the alterations found in distal areas of the dental and buccal regions. Several systematic reviews have supported the contribution risk of SPD

Table 2. Multivariate logistic regression analyses for MMSE < 27

Factors	Model 1			Model 2			Model 3		
	OR	95%CI		OR	95%CI		OR	95%CI	
PWV	2.404 **	1.509	3.829	2.447 **	1.473	4.066	2.605 **	1.486	4.567
SPD	5.397 **	1.084	26.856	0.944	0.044	20.103	1.002	0.043	23.312
CAL >5%				1.066	0.985	1.153	1.062	0.979	1.153
Demographics									
Age (years)				0.965	0.862	1.080	0.965	0.850	1.096
Sex (female/male)				0.167	0.031	0.890	0.191	0.033	1.104
Vascular risk factors									
Diabetes mellitus (yes/no)							1.332	0.205	8.652
Cholesterolemia (mg/dl)							0.881	0.146	5.323
Hypertension (yes/no)							0.505	0.075	3.391
Heart Failure (yes/no)							0.663	0.103	4.265
Ischemia heart disease (yes/no)							0.882	0.162	4.788

A total three models were run (Table 2). These models included: PWV and SPD (Model 1); PWV, SPD and CAL >5% in addition to demographics and vascular risk factors (Model 2) and PWV, SPD and CAL >5% in addition to demographics and vascular risk factors (Model 3). PWV, Pulse Wave Velocity; SPD, Severe Periodontal Disease; CAL, Clinical Attachment Level; MMSE, Mini Mental State Examination; OR: odds ratio; CI confidence interval - ** p < 0.01

in the development of dementia²¹. Furthermore, SPD has also been associated with increased brain amyloid- β load²², while lipopolysaccharides from periodontal bacteria have been demonstrated in Alzheimer disease brain specimens²³. Peripheral infectious diseases and low-level systemic inflammation have also been proposed as potential etiological factors involved in dementia²⁴.

Recent studies have reported that PD and infection with *Porphyromonas gingivalis* constitute significant risk factors for developing amyloid- β plaques, dementia and Alzheimer's disease²⁵. Patients with Alzheimer's disease also had neuroinflammation consistent with infection by *P.gingivalis*, DNA and gingipains antigens in the brain. It has been postulated that blocking the gingipains diminishes neurodegeneration and the host response to *P.gingivalis* infection. Other authors reported that patients with CVD had 100% arterial colonization by *P.gingivalis*²⁶, whereas *P.gingivalis* is usually found at low levels in healthy individuals without periodontitis²⁷. Oral bacteria may escape into the bloodstream to other tissues, reaching arteries during and after brushing, chewing or dental interventions. The link between periodontitis and CVD is also well established. An association between childhood periodontitis with CVD risk factors and subclinical atherosclerosis in adulthood has been documented²⁸. Observational studies showed periodontitis to be a

risk indicator for myocardial infarction and stroke²⁹. As periodontitis and CI share risk factors, it raises the possibility of a causal biological path linking both disorders. A pro-inflammatory phenotype most likely constitutes a common factor that links PD and CI³⁰. However, there are studies suggesting that these diseases are the independent result of the atherogenic process³¹.

Our data are consistent with recent findings from a systematic review and meta-analyses³². They demonstrated that independently of the heterogeneity of the patients with severe periodontitis, they exhibited higher AS assessed by PWV, a cardiovascular risk factor that cannot be underestimated⁹. The abnormal PWV in patients with SPD was likely due to endothelium dysfunction in response to a chronic inflammatory disease, which can be detected before the occurrence of clinically apparent CVD. Endothelial dysfunction induces an imbalance between vasodilator and vasoconstrictor synthesis that may trigger structural and functional vascular remodeling⁶. A systematic review undertaken by Paraskevas et al.³³ provided additional evidence that PD induces an elevation of plasma C-reactive protein (CRP) levels; this acute-phase response reactant may also be a marker of predisposition to a higher risk for CVD.

It is clear that AS is determined by the proportion of elastin and collagen that form the extracellular matrix of the artery. The inflammatory response to a fibro-

proliferative response is associated with breakdown of elastin and increases in collagen. This mechanism is superimposed on the decrease of nitric oxide (NO), a powerful regulator of AS which enhances hemodynamic imbalance; the bioavailability of NO contributes to the physiological regulation of arterial elasticity. The loss of endothelial NO produces a detrimental effect on clearance of the major cytotoxic peptides and amyloid- β which are responsible for the initiation and progression of cognitive decline³⁴.

The increase in AS denoted by PWV and the % CAL could lead to a rise in brain central pulse pressure and impact large and small vessels, blood pressure and flow dynamics³². The brain is rich in vascularization and has low resistance to blood flow; thus, it is particularly susceptible to hemodynamic changes, generating greater arterial stiffness from the direct transmission of the pulse wave. As a result, cerebral microvascular disease may result from the damaging forces of abnormal flow pulsations extending into small cerebral arteries. CI may also be the direct consequence of an ischemic brain. Altogether, the data are consistent with the association of AS and CI observed in the current study.

As a result of the inflammatory response, the vascular wall is remodeled, with increased AS and VCAM-1 serum level changes. The expression of adhesion molecules, such as VCAM-1 and ICAM-1, reduce the permeability of the microvasculature by interaction with leucocytes, diminish vasodilation and exacerbate cytokines production with increased production of reactive oxygen that lead to the development of oxidative stress status³⁵. In our study, there were higher serum VCAM-1 concentrations in patients with CI.

Other authors have shown that even in non-inflamed tissues, the level of VCAM-1 on a given endothelial cell in brain micro-vessels was greater than in other tissues³⁶. Thus, we could infer that elevated serum VCAM-1 levels in patients with MMSE scores below

normal may be more representative of a cerebral inflammatory state with an impaired microvascular endothelium³⁷. Elevated VCAM-1 might reflect defects of the vascular system as reported by Zuliani et al.³⁸, who found elevated plasma VCAM-1 in patients with dementia. VCAM-1 may thus reflect cerebral endothelial inflammation, and may be a suitable marker for the detection of early cognitive decline.

Finally, the present study has limitations. For example, folic acid intake and folic acid levels were not measured, although folate deficiency has been associated with increased oxidative stress, endothelial dysfunction and progression of dementia³⁹. Additionally, the MMSE was the tool used to assess CI; this test has been validated as an accurate instrument for screening, and it facilitates an early detection of CI. However, other methods with high specificity and sensitivity were not used in our study⁴⁰.

Although the sample size potentially constituted a limitation for carrying out some specific statistical analyses, the CI observed in CVD patients with SPD in the current study represents an improvement over other studies. Extension of the study time should be considered in future research to replicate these data.

CONCLUSIONS

The current study showed that AS in CVD patients increased the risk of CI. The presence of SPD, in addition to atherogenic risk factors, probably contributes to the increase in AS. The inflammatory process induced by SPD and detected by the increase in serum VCAM-1 levels, could contribute to the progression of endothelial dysfunction and arterial stiffness, as a substrate for CI. The presence of severe periodontal disease in CVD patients needs to be considered as a contributing factor to CI. These findings highlight the need to promote oral health assessment in order to prevent CI in CVD patients with SPD.

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Prevalence and severity of early childhood caries in malnourished children in Mendoza, Argentina

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ABSTRACT

The aim of this correlational, descriptive, cross-sectional study was to describe the pathological profile of Early Childhood Caries (ECC) in malnourished children who attended two centers for prevention and treatment of child malnutrition in Mendoza, Argentina. The study included 145 children aged 12 to 71 months, with prior parental consent, and recorded dmft and dmfs according to ICDAS II 2 to 6 and 4 to 6 active caries categories, age and severity of caries experience. Parametric and non-parametric statistics were applied with $p=0.05$. Caries prevalence for enamel and dentin lesions was 48.2%, declining to 35.2% when only dentin lesions were considered (dmft 4-6). The following means were found for dmft and dmfs: dmft 2-6 2.10 ± 3.31 , with "d" 2.03 ± 3.24 ; dmfs 3.07 ± 6.1 with "d" 2.91 ± 6.1 ; dmft 4-6 1.21 ± 2.46 with "d" 1.14 ± 2.37 ; dmfs 4-6 1.98 ± 5.14 and "d" 1.86 ± 5.06 . There were statistically

significant differences between dmft 2-6 and dmft 4-6. Active enamel lesions (ICDAS II active categories 2 and 3) accounted for 37% of total lesions. Active lesions type 5 were the most frequent. The indicators for dental status and severity of caries experience increased with age, both with moderate positive correlations. Conclusions: Malnourished children under six years old studied in Mendoza presented comorbidity with ECC. Dental status worsened with age. The values for caries indicators demonstrated the weight of caries diagnoses in early stages and the importance of providing preventive measures and systematic monitoring during these children's early years of life.

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Keywords: early childhood - caries - malnutrition.

Prevalencia y severidad de caries de la infancia temprana en niños con desnutrición infantil de Mendoza, Argentina

RESUMEN

El presente estudio descriptivo transversal correlacional tuvo como objetivo describir el perfil de patología de la Caries de Infancia Temprana (CIT) en niños con desnutrición infantil asistidos a dos centros de prevención y tratamiento de la desnutrición infantil, en Mendoza, Rep. Argentina. Sobre un total de 145 niños entre 12 y 71 meses de edad, previo consentimiento parental, se registraron dmft y dmfs según categorías de caries activas ICDAS II 2 a 6 y 4 a 6; edad, y severidad de experiencia de caries. Se aplicó estadística paramétrica y no paramétrica con nivel de $p=0,05$. La prevalencia de caries para lesiones de esmalte y dentina fue del 48,2% disminuyendo a un 35,2% cuando se consideraron solo lesiones en dentina (dmft 4-6). Se hallaron las siguientes medias para dmft y dmfs: dmft 2-6 $2,10 \pm 3,31$, con "c" de $2,03 \pm 3,24$; dmfs de $3,07 \pm 6,1$ con "c" de $2,91 \pm 6,1$; dmft 4-6 de $1,21 \pm 2,46$ con "c" de $1,14 \pm 2,37$;

dmfs 4-6 de $1,98 \pm 5,14$ y "c" de $1,86 \pm 5,06$. Las diferencias observadas entre dmft 2-6 y dmft 4-6 fueron estadísticamente significativas. Las lesiones activas de esmalte (categorías 2 y 3 activas de ICDAS II) constituyeron 37% del total de lesiones. Las lesiones activas tipo 5 fueron las más frecuentes. Los indicadores de estado dental y de severidad de experiencia de caries aumentaron con la edad, hallándose correlaciones positivas moderadas para ambas variables. Conclusiones: Los niños menores de seis años con desnutrición infantil estudiados en Mendoza, presentaron co-morbilidad con CIT. El estado dental empeoró con la edad. Los valores encontrados de los indicadores de caries demostraron el peso del diagnóstico de caries en sus estadios iniciales y la importancia de arribar con medidas preventivas y seguimiento sistemático en los primeros años de vida de estos niños.

Palabras clave: infancia temprana - caries - desnutrición.

INTRODUCTION

Early childhood caries (ECC) is a relevant public health problem¹. It has been defined as the presence of one or more decayed, missing (due to caries) or filled teeth in children 71 months of age or younger². With the addition of an unfavorable systemic condition such as child malnutrition, ECC acquires further meaning in terms of risk factors and complications.

Child malnutrition, according to the United Nations Children's Fund (UNICEF), is "the outcome of insufficient food intake (in quantity and quality), the lack of adequate care and infectious diseases"³. Deficient nutritional status impacts stature (chronic malnutrition) and weight (acute malnutrition), causing delayed growth and affecting the child's physical and mental development³.

Child malnutrition contributes new forms of severity to ECC. Family and community socioeconomic variables have been included as potentiators of the severity and course of early childhood caries^{1,4-6}, and it has been suggested that socioeconomically disadvantaged children are more vulnerable. In situations of poverty, the variable "malnutrition" is often added to the complex etiological panorama of ECC, thus affecting a disadvantaged social group that has barriers to access to both preventive and curative healthcare^{7,8}. Malnutrition causes alterations in tooth development, leading to defects in enamel development and saliva composition through alteration of protein synthesis or mineral availability⁹⁻¹¹. This is why, in 2012, a new category was proposed for ECC associated to hypoplasia caused by nutritional deficiencies in early childhood stages¹². The comorbidity of ECC and childhood malnutrition has been studied, defining nutritional status by means of several anthropometric measurements and caries prevalence with the dmft index. Different studies have reported varying results:

A study in India in 2014 related body mass index (BMI) to dmft, and although it found no significant association between them, the group of children with low weight had the highest caries prevalence¹³. Another study in Brazil in 2014 used WHO 2006 growth standards and found a statistically significant association between ECC and severe and mild malnutrition, though not between ECC and moderate malnutrition¹⁴. In Cartagena, Colombia in 2009, children with chronic childhood malnutrition were found to have 82% caries prevalence and increased

fluorosis¹⁵. In Argentina, there are few studies on populations of malnourished children. One study relating saliva flow to nutritional status in overweight children analyzed a sample of 60 preschoolers in areas adjacent to Buenos Aires city, reporting that 5% of children were underweight according to BMI¹⁶. In Barranqueras, Chaco, in 2007, 59 children were studied, finding differences between malnourished and normally nourished children regarding caries indices, delayed eruption chronology and increase in frequency of hypoplasia¹⁷.

Many studies do not include the diagnosis of enamel lesions in the "decayed" component of indicators. As systematic review of caries in relation to body mass index reports this problem and, in order to avoid discrepancies in caries diagnostic criteria, recommends the inclusion of more sensitive systems such as ICDAS II (International Caries Detection and Assessment System)¹⁸ and more studies in children under 6 years old¹⁹. There are data on caries prevalence in low socioeconomic level preschoolers for Greater Mendoza, reporting 50.2% caries prevalence (including enamel lesions) for children under 6 years old²⁰. To date, no study has related ECC to child malnutrition in Mendoza Province.

Accordingly, the aim of this study is to describe the frequency and severity of ECC and possible associations among variables in children with chronic and acute child malnutrition, aged 12 to 71 months in Greater Mendoza, Argentina, including both dentin and enamel lesions.

MATERIALS AND METHOD

A descriptive, cross-sectional, correlational study was performed using an intentional sample of 145 children diagnosed with chronic or acute child malnutrition, aged 12 to 71 months, who attended the Las Heras Center for Human Promotion and Prevention of Child Malnutrition, (*Centro de Prevención de Desnutrición Infantil y Promoción Humana de Las Heras*) and the Teresa de Calcuta Hospital of the Cooperative Foundation for Child Malnutrition (*Fundación Cooperadora de la Desnutrición Infantil [CONIN]*) in Mendoza during 2017 and 2018. Nutritional status diagnosis was obtained as secondary data contained in institutional Clinical Histories, determined as z-score for weight-for-height, weight-for-age, height-for-age, BMI-for-age and head circumference-for-age, and processed in the ANTRHO system (WHO)²¹.

The study included all children whose parents or legal guardians provided written consent to participate in the project and excluded from the sample any children with reported comorbidity with diagnosed systemic diseases or whose behavior made it impossible to perform the practices. For ethical reasons, all children in the sample were included in an oral healthcare program. This project was approved by the Ethics and Research Committee of the University Hospital at Cuyo National University, under the Minutes of said Committee dated March 3, 2017. The following variables were considered:

Age categories in months: 12 to 23 months; 24 to 35 months; 36 to 47 months; 48 to 71 months.

Dental status: recorded according to dmft and dmfs indices²², but including within the category “decayed” (“d”) non-cavitated enamel lesions (visible without drying) and cavitated enamel lesions, according to ICDAS II 2 to 6 active categories¹⁸. Two levels of analysis were used: 1) including only dentin lesions: dmft 4-6 and dmfs 4-6, and 2) considering enamel and dentin lesions: dmft 2-6 and dmfs 2-6.

Severity of caries experience was recorded by dividing it into four categories for the dmft index, as follows: dmft=0; dmft: 1, 2, 3; dmft: 4, 5, 6; dmft: 7 or more, both for “d” 2-6 and “d” 4-6.

Clinical examination was conducted by two calibrated researchers (Kappa= 81%) using the visual method applying the ICDAS II criterion for diagnosis and activity. Data were analyzed statistically using SPSS software version 17.0. Central tendency and frequency distribution measures were applied. Non-parametric tests were used with significance level $p=0.05$: Wilcoxon rank test to compare dmft 2-6 to dmft 4-6 and dmfs 2-6 to dmfs 4-6, and categories of severity of caries experience dmft 2-6 and dmft 4-6; Kruskal-Wallis test and Spearman correlation coefficient to establish associations and correlation between results of dmft 2-6 and age; and chi-square test and Spearman correlation coefficient to study severity of caries experience according to age categories.

RESULTS

Distribution according to age groups was even ($\chi^2=2.44$; $p=0.485$). The Kolmogorov-Smirnov test showed that the variables dmft and dmfs did not follow normal distribution, therefore non-parametric tests were applied to contrast the variables (dmft 2-6: $Z_{KS}=3.17$, $p=.000$; and dmfs 2-6: $Z_{KS}=3.73$, $p=.000$)

Dental status indicators

Caries prevalence found for malnourished children was **48.2%** when enamel and dentin lesions were considered. This prevalence declined to **35.2%** when only dentin lesions were considered (dmft 4-6).

Mean dmft 2-6 was 2.10 ± 3.31 with very high disease burden denoted by a “d” component of 2.03 ± 3.24 . The indicator behaved similarly when analyzed per surface, with dmfs 3.07 ± 6.1 and “ds” 2.91 ± 6.1 . For dmft 4-6, values were 1.21 ± 2.46 with a “d” component of 1.14 ± 2.37 . For dmfs 4-6, values were 1.98 ± 5.14 and its “d” component was 1.86 ± 5.06 (Table 1).

Wilcoxon rank test was used to compare dmft 2-6 to dmft 4-6 and dmfs 2-6 to dmfs 4-6, finding a significant difference between both pairs of variables, which shows that indicators that include enamel lesions are significantly higher than those that only consider dentin lesions (dmft: $Z=-6.69$; $p=.000$; dmfs: $Z=-6.67$; $p=.000$).

Types of caries lesions according to ICDAS II active categories

Active enamel lesions observed in this study (ICDAS II active categories 2 and 3) accounted for 37% of total lesions. Active lesions type 5 were the most frequent (32.1%) (Fig. 1). In the first age category, 80% corresponded to enamel lesions.

Severity of caries experience

For both levels of analysis, severity of caries experience showed the highest percentages for the categories dmft 2-6 and 4-6 =0 and dmft 2-6 and 4-6: 1, 2 and 3.

Significant differences were found between the categories for severity of caries experience dmft 2-6 and dmft 4-6, increasing the values = 0 when only dentin lesions were considered, and increasing the values 4, 5, 6 and 7 or + when enamel lesions were included in the indicator (Wilcoxon rank test $Z=-6.04$; $p=.00$) (Table 2).

Dental status according to age categories: Dental status indicator was taken as dmft 2-6 in order to include in the analyses all categories of caries lesions. Values for dmft were 0.11 ± 0.39 from 12 to 23 months; 1.38 ± 2.36 from 24 to 35 months; 2.41 ± 2.44 from 36 to 47 months and 4.53 ± 4.62 from 48 to 71 months (Table 3).

Table 1. Descriptive statistics for dmft and dmfs 2-6 and 4-6 in malnourished children in Mendoza

	Mean	Standard Error	Standard Deviation	Minimum	Maximum
decay t 2-6	2.03	.270	3.248	0	17
indicated extraction t 2-6	.01	.007	.083	0	1
filled t2-6	.06	.025	.307	0	2
dmft 2-6	2.10	.275	3.317	0	17
decay s 2-6	2.95	.507	6.101	0	40
indicated extraction s2-6	.03	.034	.415	0	5
filled s 2-6	.07	.032	.385	0	3
dmfs 2-6	3.07	.514	6.188	0	40
decay t 4-6	1.14	.197	2.371	0	14
indicated extraction t4-6	.01	.007	.083	0	1
filled t4-6	.06	.025	.307	0	2
dmft 4-6	1.21	.205	2.469	0	14
decay s 4-6	1.86	.421	5.064	0	34
indicated extraction t 4-6	.03	.034	.415	0	5
filled s 4-6	.07	.032	.385	0	3
dmfs 4-6	1.98	.427	5.146	0	34

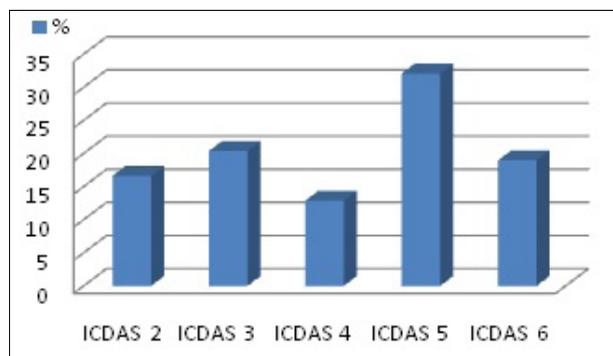


Fig. 1: Percentage of active lesion categories according to ICDAS II in malnourished children in Mendoza.

The Kruskal-Wallis test (KW=45.79; $p=0.00$) confirmed that there are significant differences between the results of dmft 2-6 and children's age in the study.

The Spearman correlation coefficient between dmft 2-6 and age categories in months was positive but moderate (Spearman's $Rho = 0.561$; $p=0.00$), corroborating the tendencies described previously.

Severity of caries experience according to age categories

A statistically significant association was found between the categories of dmft 2-6 and age of malnourished children in the study ($\chi^2=54.578$; $p=0.00$), with a positive though moderate correlation

between severity of caries experience and age (Spearman's $Rho = 0.559$; $p=0.00$). The contingency table for the two variables shows that at higher ages, there is an increase in cases of the categories with caries, and reduction in cases without caries (Table 4).

DISCUSSION

This study brings to light the problem of ECC in children under six years old diagnosed with child malnutrition in Mendoza, Argentina.

Caries prevalence was 48.2% when examination included early enamel lesions and 38.2% when only dentin lesions were considered. Other studies in different parts of the world report different prevalence values. Tsang et al. (2019) report caries prevalence of 58.2% and a mean value of 3 for the "decayed" component of dmft in populations of malnourished children under 6 years old in Nepal²³. Janikaram et al. (2019) report caries prevalence of 33.2% in similar populations²⁴.

It should be highlighted that the values found in the current study are similar to those reported in a previous study on caries prevalence in children under six years old in Mendoza Province. Said study on children with low socioeconomic level in Greater Mendoza reported caries prevalence of 50.2 for enamel and dentin lesions and 35.2 for dentin lesions²⁰. Malnourished children constitute

Table 2. Frequency distribution for severity categories of caries experience for dmft 2-6 and dmft 4-6 in malnourished children in Mendoza.

	Frequency	%	Valid %	Cumulative %
dmft 2-6= 0	75	51.7	51.7	51.7
dmft 2-6: 1. 2 and 3	39	26.9	26.9	78.6
dmft 2-6: 4. 5 and 6	15	10.3	10.3	89.0
dmft 2-6: 7 or more	16	11.0	11.0	100.0
dmft 4-6= 0	93	64.1	64.1	64.1
dmft 4-6:1. 2 and 3	37	25.5	25.5	89.7
dmft 4-6: 4. 5 and 6	8	5.5	5.5	95.2
dmft 4-6: 7 or more	7	4.8	4.8	100.0

Table 3. Dental status expressed through dmft 2-6 and dmfs 2-6 according to age groups in malnourished children in Mendoza.

Age categories in months	Statistics	d t 2-6	m t 2-6	f t 2-6	dmft 2-6	d s 2-6	m s 2-6	f s 2-6	dmfs 2-6
12-23 months	Mean	.11	.00	.00	.11	.11	.00	.00	.11
	Standard error	.066	.000	.000	.066	.066	.000	.000	.066
	Standard Deviation	.398	.000	.000	.398	.398	.000	.000	.398
	Rank (Min-max)	0-2	0	0	0-2	0-2	0	0	0-2
	N	36	36	36	36	36	36	36	36
24-35 months	Mean	1.38	.00	.00	1.38	1.62	.00	.00	1.67
	Standard error	.365	.000	.000	.365	.501	.000	.000	.499
	Standard Deviation	2.368	.000	.000	2.368	3.246	.000	.000	3.236
	Rank (Min-max)	0-12	0	0	0-12	0-15	0	0	0-15
	N	42	42	42	42	42	42	42	42
36-47 months	Mean	2.41	.00	.00	2.41	2.72	.00	.00	2.72
	Standard error	.454	.000	.000	.454	.555	.000	.000	.555
	Standard Deviation	2.442	.000	.000	2.442	2.987	.000	.000	2.987
	Rank (Min-max)	0-8	0	0	0-8	0-10	0	0	0-1'
	N	19	19	19	19	19	19	19	19
48-71 months	Mean	4.29	.03	.21	4.53	7.29	.13	.26	7.68
	Standard error	.739	.026	.094	.750	1.598	.132	.117	1.604
	Standard Deviation	4.555	.162	.577	4.625	9.853	.811	.724	9.889
	Rank (Min-max)	0-17	0-1	0-2	0-17	0-40	0-5	0-3	0-40
	N	38	38	38	38	38	38	38	38

part of the population with low resources, so it is expectable that they would share risk factors with the previously studied population in Mendoza. The similar prevalence values found in the mentioned study can be considered as a reference framework to understand the severity of the problem in this particular epidemiological scenario.

Discrimination of the dmft index shows that there was a main burden of the decayed component, with very little intervention or treatment, in agreement with other studies^{24,25}. This reflects the low demand

for dental treatment, and it may be assumed that there are barriers to access to healthcare in common with other child populations, regarding ECC⁷.

Values for dmft and dmfs tended to increase with age, being four times higher in the 48- to 71-month age group than in the 24- to 35-month age group. One study in Nigeria found a similar relation in children with and without malnutrition²⁶. In the current study, the difference between dmft 2-6 and dmft 4-6 was statistically significant. It is interesting to note that 31% of the affected surfaces were enamel lesions,

Table 4. Contingency table for caries experience severity and age groups in malnourished children in Mendoza.

Age categories in months	Severity dmft 2-6				Total
	dmft 2-6= 0	dmft 2-6: 1. 2. 3	dmft 2-6: 4. 5. 6	dmft 2-6: 7 or +	
12-23 months	33	3	0	0	36
24-35 months	24	13	4	1	42
36-47 months	10	10	6	3	29
48-71 months	8	13	5	12	38
Total	75	39	15	16	145

and that in the first year of life, 80% of the lesions were enamel lesions. Although the caries indicators are not high (as expected for the age), caries onset occurred early in life for the malnourished children in the study.

The severity of caries experience, measured through 4 dmft score categories, for both dmft 2-6 and dmft 4-6, showed that the most frequent categories were dmft=0 and dmft= 1, 2, 3. However, evaluation of differences between dmft 2-6 and dmft 4-6 showed that when enamel lesions were considered, there was a significant increase in the categories dmft= 4, 5, 6 and dmft= 7 or more. One of the aforementioned studies in Nepal also described severity categories according to mean dmft, but considering that among children who only experienced caries, 50% presented 1 to 4 decayed teeth, 35.3% had 5 to 9 decayed teeth, and 14.6% had 10 or more decayed teeth²³.

It was also found that severity of caries experience increased with age, with a positive though moderate correlation between severity of caries experience and children's age.

These results emphasize the importance of early prevention, detection and treatment of ECC in malnourished children. Timely action is needed to prevent both, pathological and quality of life

complications caused by ECC in children with malnutrition²⁷.

The pathological profile for ECC in malnourished children has been described by other authors according to conventional dmft indicators. One of the contributions of the current study is that addresses the importance of active enamel lesions diagnosed using the ICDAS II system. It also contributes to understanding the behavior of ECC in malnourished children under six years old in Mendoza Province. Because this is a descriptive study focusing only on malnourished children, the limitations in its design do not enable the establishment of possible associations between ECC and nutritional status. These are questions to be addressed in further studies including eutrophic children and other caries risk variables for comparison.

It may be concluded from the results presented that malnourished children under six years old studied in Mendoza had comorbidity with ECC. Dental status worsened with age, showing a high disease burden with little treatment intervention. The values found for caries indicators showed the weight of caries diagnosis in initial stages and the importance of providing systematic preventive and monitoring actions during the early years of malnourished children.

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A comparative analysis between atraumatic and conventional anesthetic techniques in surgical removal of upper third molars

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ABSTRACT

Pain control is essential in dental practice, and can be accomplished through various techniques. This study seeks to compare atraumatic and conventional anesthetic techniques, applied during surgeries to remove upper third molars. The endpoints evaluated were pain indices, patient satisfaction and anesthetic efficacy. A random parallel split-mouth clinical trial was conducted with 14 patients. Group A received atraumatic anesthesia without a needle (Comfort-in®) and group B received conventional anesthesia by blocking the posterior superior alveolar nerve (PSAN) and Greater Palatine Nerve (GPN). A Visual Analogue Scale (VAS) was used to assess pain.

A significantly ($p < 0.001$) lower perception of pain was observed among individuals who received the atraumatic technique. In 71% of cases, it was necessary to supplement the anesthesia during the procedure. Even considering the need for additional anesthesia, the Comfort-in® technique was more accepted by patients with regard to pain perception than the conventional manual technique.

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Keywords: anesthesia, dental - pain management - patient satisfaction.

Análise comparativa entre a técnica anestésica atraumática e convencional em cirurgia para remoção dos terceiros molares superiores

RESUMO

O controle da dor é fundamental na prática odontológica, podendo ser feito a partir de várias técnicas. Este estudo visa comparar as técnicas anestésicas atraumática e convencional, aplicadas durante cirurgias para remover terceiros molares superiores. Os pontos avaliados foram índice de dor; satisfação do paciente e eficácia anestésica. Um ensaio clínico randomizado, paralelo, boca dividida, foi conduzido com 14 participantes. O grupo A recebeu anestesia atraumática sem agulha (Comfort-in®) e o grupo B anestesia convencional por meio de bloqueio do nervo alveolar superior posterior (NASP) e nervo palatino maior (NPM). Uma Escala Visual Analógica (EVA)

foi utilizada para avaliar a dor. Uma significativa ($p < 0,001$) menor percepção de dor entre os indivíduos que receberam a técnica atraumática foi observada. Em 71% dos casos, fez-se necessária a complementação da anestesia durante a realização do procedimento. Mesmo considerando a necessidade de anestesia adicional, a técnica empregando o sistema Comfort-in® teve maior aceitação dos pacientes quando comparado à técnica manual convencional, no que se refere a percepção de dor; ainda que considerando a necessidade de complementação da anestesia.

Palavras-chave: anestesia dentária - manejo da dor - satisfação do paciente.

INTRODUCTION

Surgical procedures are common in daily dental practice, and even with the advances in technique, equipment, drugs and materials in recent decades, there are still patients who are resistant to these types of treatment due to anxiety and fear¹. Surgical

trauma can cause acutely painful symptoms in tissues, with patients reacting in different ways². Pain control through dental anesthesia is essential during dental practice. Local anesthetic solutions correspond to reversible blocking of nerve

conduction, causing loss of sensation without altering the level of consciousness, with reversibility being a principal characteristic³⁻⁵.

Conventional anesthetic techniques are widely used in dental routine for their simplicity in application and great assertive power. However, they present a greater chance of patient anxiety and fear, mainly due to exposure to needles, which in most cases is associated with pain^{6,7}.

The Comfort-in® system (*Mika Medical; Busan, Korea*) was created with the aim of achieving more comfortable anesthetic infiltration. In this system, defined as atraumatic, anesthetic is administered without a needle by pressure dosed in a fraction of a second. According to the manufacturer⁸, the absence of a needle during the execution of anesthetic technique in dental treatment can be associated with pain reduction, thereby reducing a patient's anxiety. Considering that pain and anxiety can contribute to the avoidance or delay of dental treatment in patients of all ages⁹, studies are needed to evaluate and compare painless anesthetic techniques with conventional techniques¹⁰. Therefore, this study seeks to compare atraumatic and conventional anesthetic techniques with respect to pain indices, patient satisfaction and anesthetic efficacy, in surgeries to remove upper third molars.

MATERIALS AND METHODS

This was a randomized parallel split-mouth clinical trial with the objective of comparing the Comfort-in® system (*Mika Medical; Busan, Korea*) with conventional technique using carpule syringe and short needle (30 gauge) for local anesthesia, prior to surgery to remove upper third molars. The study was approved by the ethics committee of the Centro de Ensino Superior Maceió (Cesmac), under the CAAE protocol 91178518.9.0000.0039, with code RBR-9qm2y5 (Brazilian Registry of Clinical Trials).

All participants were informed of research content and signed a free and informed consent form; they were also informed of the risks and benefits involved in participation in the research and were told that they could drop out of the research at any time without loss of treatment. The research took place at the Campus I Dentistry Clinic at Centro Universitário Maceió (Cesmac) located in the city of Maceió in the state of Alagoas, Brazil. A non-probabilistic sample was selected from March to May 2019.

Fourteen individuals participated in the study, in which the posterior superior alveolar nerve and the greater palatine nerve of the right and left sides of palatal arch were anesthetized, totaling 56 blocks. Participants were chosen based on the following criteria: 18 to 45 years old, needing removal of the right and left third molars in symmetrical position, healthy, without any systemic pathologies, and without a history of anxiety and depression disorder. Participants were excluded when it was not possible to perform the projected anesthetic technique due to the specificities of surgical planning.

Immediately before surgery, a person not directly involved with the research project randomly determined which technique would be applied to each side of the arch by tossing a coin. Both upper third molars were removed during the same surgical session.

Each patient received an anesthetic solution (Articaine 4% with epinephrine 1: 100.000, DFL) through the needle-free syringe with pressure and dosage controlled by the Comfort-in® atraumatic anesthesia system (group A). On the contralateral side, the participant received the same anesthetic solution through a conventional carpule syringe and 30G gingival needle under manual pressure (group B). The procedures for blocking the posterior superior alveolar and greater palatal nerve (Fig. 1) were performed in all patients by the same operator (FH).

Immediately after the anesthetic procedure, the patient was asked to rate their pain using a Visual Analogue Scale (VAS)¹¹ followed by measurement of vital signs. The VAS consists of a line representing a continuous quality of intensity and verbal data – no pain to maximum pain. The size of the line may vary, but it is often 10 cm. This scale produces level data at intervals, enabling the use of statistical parameters in the analysis.

Patient satisfaction level after an anesthetic block was assessed by asking the patient to evaluate the statements “I am satisfied after anesthesia” and “I think I'm anesthetized enough” on a 5-point Likert scale¹² by selecting one of the following: strongly agree, agree, no opinion, disagree, or strongly disagree. The responses were recorded according to a consecutive number scale using scores from 1 to 5. The need to supplement the anesthetic solution, the amount of anesthetic used, infiltration time, and the need for additional anesthesia while the surgery was performed were considered.

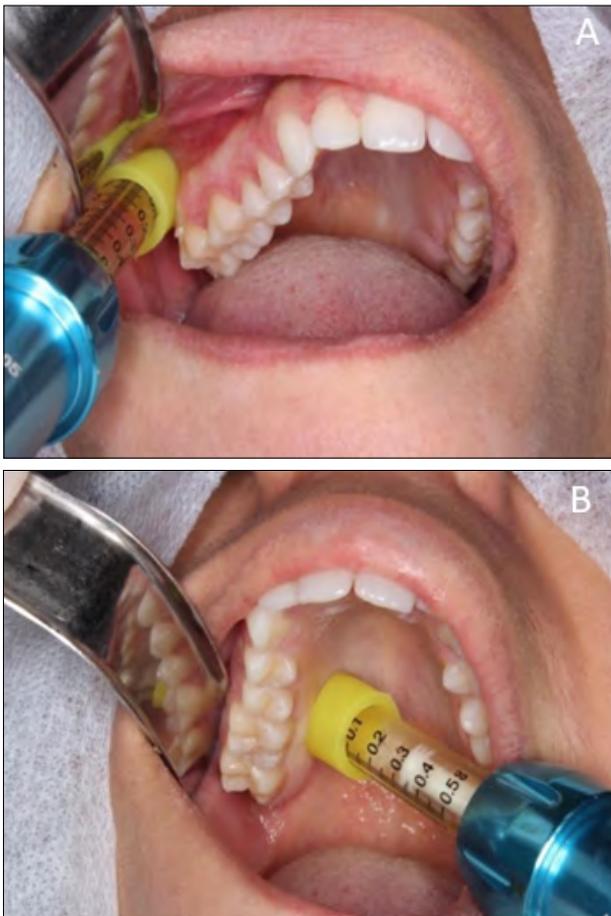


Fig. 1: PSAN Anesthesia with Comfort-in® System A: posterior superior alveolar nerve. B: Greater palatal nerve.

Data analysis

A database was generated and analyzed using Microsoft Excel 2010 software, with the analysis including Student's t test and Pearson's chi-squared test for nominal variables. p values lower than .05 were considered to be statistically significant.

RESULTS

A total 14 participants were selected for the study, with 64% being female and 36% male. Mean age of the sample was 23.42 years. VAS pain values show statistically significant differences between groups, with the group treated with the atraumatic technique (group A) reporting less pain than the group treated with the conventional manual technique (group B), both for superior alveolar nerve and greater palatine nerve.

Comparison of satisfaction values using the Likert Scale showed no significant difference between group A and group B (Table 1).

All participants treated with the conventional

manual technique (Group B) reported sufficient anesthesia while patients anesthetized with the atraumatic technique (group A) needed an anesthetic supplement ($p < 0.0001$). Of the fourteen cases anesthetized using this technique, ten (71%) required supplemental anesthesia: two in NASP, three in NPM and five cases in both nerves.

DISCUSSION

Of the fourteen survey participants, 64% were female and 36% male. Average age was 23.4 years. As in the study by Aragão et al.⁶, there was a higher prevalence of female individuals with similar average age. Females tend to have higher levels of anxiety regarding dental treatment², which can interfere with the patient's perception of pain¹³.

The purpose of the Comfort-in® atraumatic system is to reduce discomfort during anesthesia. It is relatively new on the market, which explains the small number of published papers evaluating its effectiveness. In a recent publication, Oliveira et al.¹⁴ evaluated the effectiveness of anesthesia after the use of this atraumatic system, compared to the conventional technique, finding no statistically significant difference in relation to pain assessment using VAS after injection. The results of the present study, on the other hand, showed that participants who were anesthetized using the atraumatic system reported significantly less pain than those anesthetized using the conventional technique. This can be explained by the differences between the anesthetic techniques used in the two studies.

In the study by Oliveira et al.¹⁴, infiltrative anesthesia was used prior to the restoration of first permanent molars, which may have resulted in greater pain perception caused by the expansion of tissues after the infiltration of the anesthetic solution, even with slow administration speed. In this study, NASP and GPN regional blocking techniques were employed. There was no statistically significant difference between groups regarding patient satisfaction with the anesthetic techniques. Both were satisfied with the level of anesthesia achieved. All patients anesthetized using the conventional technique (Group B), considered it to be sufficient. When anesthetized using the atraumatic technique, on the other hand, 71% of cases requested supplementation of anesthesia.

In a study by Areias et al.¹⁵, comparing the efficacy of a classic local infiltration anesthesia with a

Table 1. Pain perception and degree of satisfaction ($\bar{X} \pm SD$; n=14)

		Atraumatic technique (group A)	Conventional technique (group B)	p value
Pain Perception Visual Analog Scale (VAS)	Posterior Superior Alveolar Nerve (PSAN)	1.50 \pm 1.40	2.57 \pm 2.43	0.041*
	Greater Palatine Nerve (GPN)	1.28 \pm 1.58	4.21 \pm 1.84	0.001*
Degree of Satisfaction (Likert Scale)		4.43 \pm 0.64	4.63 \pm 0.49	0.095*

* Student t test, significant difference

needle-free anesthetic system – Injex® (Rösch, Berlin, Germany), in which 100 pediatric patients were treated, it was observed that in 68% of the cases in which Injex® was used, it was necessary to repeat the anesthesia, as in the present study.

The need to supplement the anesthetic solution of the Comfort-in® system may be related to the divergence between techniques in the amount of anesthetic used. Malamed¹⁶ recommends that the minimum amount of local anesthetic for the greater palatine nerve be 0.45 to 0.6 ml, while for the posterior superior alveolar nerve, it should be 1.8 ml (1 tube). Each ampoule of the Comfort-in® system has a maximum capacity of 0.5ml; for blocking the posterior superior alveolar nerve, a 0.5 ml ampoule with a straight cap was used, while for the greater palatine nerve block, a 0.3 ml ampoule with curved cap was used.

Another divergence between the techniques is related to the proximity at which the anesthetic liquid is placed. Malamed¹⁶ suggests that anesthetic liquid should be placed close to the main nerve trunk to be successful, with a penetration depth of approximately 16 mm for the posterior superior alveolar nerve and approximately 5 mm for the greater palatine nerve. The conventional technique probably enabled placement of a greater volume of anesthetic solution next to the main nerve trunk.

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None.

Another negative point observed relates to the difficulty for the dentist to obtain a 90° angle with the point to be anesthetized with the Comfort-in® system, which the manufacturer recommends during the execution of the technique.

As shown in the studies by Faraco Junior et al.¹⁷, Areias et al.¹⁵, Aragão et al.⁶ and Silveira et al.¹⁸ who used Morpheus® (São Paulo, São Paulo, Brazil), Injex® and The Wand® (Livingston, New Jersey, United States) atraumatic anesthetic injection systems respectively, the Comfort-in® system showed a good level of acceptance by the participants, with a low level of pre-anesthetic anxiety and a low level of pain from the injection. Further studies are needed to for extra testing of the anesthetic effectiveness of the system.

CONCLUSION

Despite the limitations of this study, the data show that the technique employing the Comfort-in® system had a higher level of acceptance by patients than the conventional manual technique with regard to pain perception, even considering the need for supplementing anesthesia.

Conflict of interest

The authors have no conflict of interest to declare.

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Dimensional stability of alginate molds scanned at different storage times

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ABSTRACT

The aim of this study was to evaluate the precision and accuracy of molds made with four commercial brands of alginate scanned at different times using digital model analysis. Eighty molds of a standard Typodont were made using 4 types of alginate (CCC: Cavex Color Change; IA: Identic Alginate; HY5: Hydrogum 5 and JP: Jeltrate Plus). The molds were scanned at four times: immediately (T1), 24h (T2), 72h (T3) and 120h (T4) after molding. Measurements were taken in three dimensions: anteroposterior, transverse and vertical. Significant differences in dimensional changes were noted between the materials over time ($p < 0.05$). Anteroposterior dimensional variation was noted between times, especially for IA and JP. For transverse

and vertical variables, a difference was found between the groups, especially at 24 h, 72 h and 120 h. CCC presented significant dimensional shrinkage only at T120 (transverse). IA and JP presented larger dimensional distortions in the vertical measurements. The molding materials used were not dimensionally stable when evaluated after 120 hours of molding. However, such evidence suggests that alginates with longer storage time, such as Cavex Color Change, are more accurate than conventional alginates.

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Keywords: dental impression materials - dental models - three dimensional imaging.

Estabilidade dimensional de moldes de alginato digitalizados em diferentes tempos de armazenamento

RESUMO

O objetivo do presente estudo foi avaliar a precisão e acurácia por meio de análise de modelos digitais de moldes digitalizados obtidos com quatro marcas comerciais de alginato em diferentes tempos. Oitenta moldes de um Typodont padrão foram obtidos utilizando 4 tipos de alginato (CCC: Cavex Color Change; IA: Identic Alginate; HY5: Hydrogum 5 e JP: Jeltrate Plus). Os moldes foram escaneados em quatro tempos: imediatamente (T1), 24h (T2), 72h (T3) e 120h (T4) após a moldagem. As medidas foram feitas em três dimensões: ântero-posterior, transversal e vertical. Diferenças significativas nas mudanças dimensionais foram observadas entre os materiais ao longo do tempo ($p < 0,05$). Variação dimensional ântero-posterior foi ob-

servada entre os tempos, principalmente para IA e JP. Para as variáveis transversal e vertical houve diferença entre os grupos, principalmente em 24h, 72h e 120h. CCC apresentou contração dimensional significativa apenas em T120 (transversal). IA e JP apresentaram maiores distorções dimensionais na vertical. Os materiais de moldagem utilizados não foram dimensionalmente estáveis quando avaliados após 120 horas de moldagem. No entanto, tais evidências sugerem que os alginatos com maior tempo de armazenamento, como Cavex Color Change, são mais precisos do que os alginatos convencionais.

Palavras-chave: materiais para moldagem odontológica - modelos dentários - imageamento tridimensional.

INTRODUCTION

Digital technology is spreading throughout all sectors of society. There has been noticeable modernization over the years, especially in diagnostic procedures, in which digital virtualization techniques of dental models are increasingly available, providing quality

3D images and fully computerized orthodontic documentation¹.

Plaster models, though considered standard and being widely used by orthodontists for diagnosis and treatment planning, have disadvantages compared to

digital models, which appear to be a good option to replace traditional methods. The speed in obtaining data and measurements, easy storage and access to information, make digital models an alternative to plaster models². This convenience of archiving data on the computer enables data exchange information with colleagues from other centers via the web, contributing to diagnosis, especially when case planning requires an integrated multidisciplinary approach^{3,4}.

One of the methods for producing a digital model is scanning moldings and plaster models. Scanners collect data on shape and appearance, transforming it into a 3D scanned file, in which measurements are obtained with the aid of specific software⁵. Currently, 3D imaging is obtained by directly scanning the alginate or silicone mold. These molds and models can be used for virtual setup, aesthetic aligners and indirect bonding trays⁶. Another alternative for obtaining digital models is through intraoral scanning, which reduces image acquisition time and eliminates the need for molding. On the other hand, this is a new technology, with high cost, making it difficult to use and it is a very sensitive technique, requiring high operator calibration in order to avoid errors in image obtention⁷. In addition, studies are needed to evaluate the precision and accuracy of the measurements obtained by this technique⁸.

The accuracy of digital models is closely linked to the printing process, and molding failures can compromise procedures. Studies show that alginate prints undergo dimensional changes related to time and temperature^{3,4,6,9}. Due to the recognition that the main limitation of alginate is the volumetric change in the mold after it is removed from the mouth, materials with better stability characteristics and longer handling time are being produced¹⁰. The aim of this study was to evaluate the precision and accuracy of dimensional stability of four alginate types scanned at different times.

MATERIALS AND METHODS

Eighty upper arch molds were obtained from a standard Typodont with four different alginate materials: CCC: Cavex Color Change (Cavex Holland BV, Haarlem, The Netherlands); IA: Identic Alginate (Dux Dental, Oxnard, Calif); HY5: Hydrogum 5 (Zhermack, Badia Polesine, RO, Italy) and JP: Jeltrate Plus (Dentsply Caulk, Milford, DE, USA). After the molding procedure,

the trays were placed on the R700™ scanner (3Shape, Copenhagen, Denmark) for their scanning process and digital model production. Scanning was performed at 4 time intervals: immediately (T1), 24 h (T2), 72 h (T3) and 120 h (T4) after molding. For each material, 5 molds were produced at each time interval, generating 5 digital models per material, and 80 models altogether. The Typodont upper arch was also scanned and it served as the gold standard for evaluations (OrthoAnalyzer, 3Shape, Copenhagen, Denmark).

Obtaining digital models

Molding materials were handled according to manufacturers' instructions in an environment with controlled temperature and relative humidity (23 ± 2 °C and $50 \pm 10\%$) to minimize factors that could cause dimensional change. After manipulation, it was placed over the entire inner part of the tray that was later seated over the upper Typodont arch. All molding steps were performed by a single operator. Immediately after obtaining the molds, they were scanned by a R700™ scanner (3Shape, Copenhagen, Denmark) generating the digital models (Fig. 1). The molds which would be stored and scanned after 24, 72 and 120 hours were placed in a humidifier with 100% relative humidity at room temperature (23 °C to 25 °C)^{11,12}.

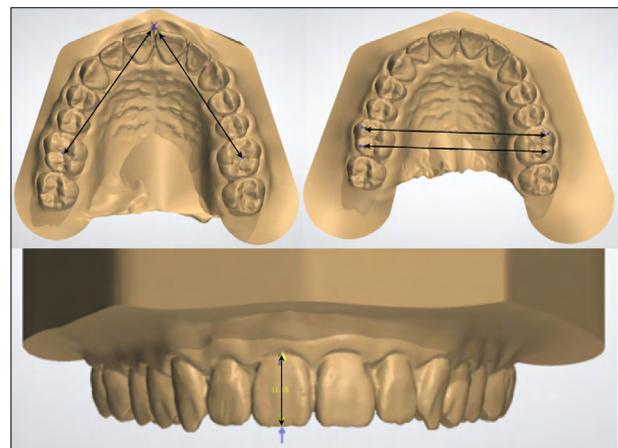


Fig. 1: Measurements performed: anteroposterior distance, transverse distance and vertical distance

Evaluation of digital models

Evaluations were performed by a calibrated evaluator in OrthoAnalyzer software (3Shape, Copenhagen, Denmark). The following measurements were recorded: anteroposterior distance, transverse distance and vertical distance (Fig. 1).

- Anteroposterior distance: the models were measured in occlusal view from the occlusal-central point of the right first molar to the incisal mesial angle of the ipsilateral central incisor. The same measurement was performed from the first molar on the opposite side to the respective central incisor, and then the two measurements were divided by 2.4
- Transverse distance: reference points were established for the first molar buccal cusp tips (right and left). The distance between these points determined the transverse distance.
- Vertical distance: The vertical distance from the right central upper incisor was measured straight from the center of the incisal edge to the gingival margin^{4,13}.

Statistical analysis

To verify the examiner's calibration, 30 days after the first evaluation, the measurements of 30% of the sample were repeated, totaling 384 measurements performed on 24 randomly selected digital models. Errors were evaluated by Intraclass Correlation Coefficient (ICC) and Bland-Altman. Statistical analysis was performed using Statistical Package for Social Sciences version 20.0 and Minitab 17.0, with a 95% confidence interval and a significance level of 5% ($p < 0.05$) for all tests. One-way analysis of variance (ANOVA) was used to compare groups and times for different outcome variables. Subsequently, factorial ANOVA (Two-way ANOVA, Post-Test: Bonferroni) was used to identify the influence of each of these factors on the model, and to evaluate any possible interaction between them.

RESULTS

Table 1 presents the results for intra-examiner error. The ICC ranged from 0.79 to 1.0, showing excellent agreement and reliability, and the Bland-Altman, with very little variation in the upper (0.01 to 0.20) and lower (0.00 to 0.06) limits (Table 1). These results demonstrate that all variables studied were accurate and consistent, certifying the examiner's calibration.

The mean values of all measurements (different times and materials) are shown in Table 2. For anteroposterior distance, IA and JP presented a significant decrease at T120, while for HY5 this decrease was observed at T24 and T72. For transverse distance, the largest discrepancy was observed

in HY5 and JP from T0 to T120. In the vertical dimension (Table 2), IA and JP showed significant differences at T120. At T24, CCC and HY5 obtained higher values than IA and JP. Moreover, at T120, IA and JP presented discrepant average values (10.92 and 10.86, respectively), with $p < 0.009^*$. Figs. 2 and 3 explain the interaction between groups in relation to APD and TD, respectively, at different times. The mean difference between the molds obtained from the different materials and the measurements at T0 and T120 from the Typodont (gold standard) are shown in Table 3. A slight reduction of all measurements was observed, with exception of IA for transverse distance, when compared to the gold standard. The material with the greatest discrepancy was JP (-3.46%), followed by IA (-3.02%), CCC (-1.6%) and HY5 (-1.42%) at T120 for vertical distance.

DISCUSSION

Alginate is an irreversible hydrocolloid¹¹ that provides sufficiently detailed reproduction to make it suitable for routine use in dentistry, such as for obtaining diagnostic and working models for therapeutic appliances¹⁴. Despite its frequent use, concerns about its performance include dimensional instability when plaster casting is delayed, and inability to produce accurate molds upon re-casting. It is common for the dimensional stability of irreversible hydrocolloids to decrease with increasing storage time¹ due to the gain or loss of water from the mold and the resulting dimensional changes. These can be minimized by immediate plaster filling^{11,12}. In this study, four types of alginate were used: Cavex Color Change, Identic Alginate, Hydrogum 5 and Jeltrate Plus. The first three are irreversible hydrocolloids, which their manufacturers ensure will provide satisfactory results if the mold is filled with plaster within 5 days. Jeltrate Plus, which recommends only a few hours' storage, preferably in a humidifier, was used to compare the accuracy and dimensional accuracy of the models manufactured with these materials. When anteroposterior distance was evaluated in IA and JP, there was a decrease in the mean values at T120, whereas for HY5 the highest decrease was observed at T72. Higher values for JP were only observed at T72. Although a previous study⁹ showed no significant differences when evaluating Jeltrate Plus within 5 days, a different methodology was

Table 1. Mean, standard deviation (SD) and difference from the first and second evaluation (millimeters). Intraclass Correlation Coefficient (ICC) and Bland-Altman

Variables (mm)	1st Evaluation		2nd Evaluation		Diff.	ICC	Bland-Altman	
	Mean	SD	Mean	SD			Upper	Lower
Anteroposterior Distance	40.72	0.46	40.71	0.46	-0.01	1.00	0.08	0.02
Transverse distance	53.87	0.16	53.87	0.15	0	0.79	0.19	0.04
Vertical Distance	11.09	0.16	11.06	0.13	-0.03	0.81	0.20	0.06

Table 2. Comparison of mean values of DAP, DT and DV between the different times and impression materials (Anova, Post-Test: Bonferroni) and multiple comparisons (two-way ANOVA).

	T0 (n=5)		T24 (n=5)		T72 (n=5)		T120 (n=5)		Difference (time)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Anteroposterior Distance										
CCC	40.67	0.24	40.68	0.05	40.74A	0.07	40.74	0.07	0.064	
IA	40.70	0.09	40.60	0.17	40.70A	0.03	40.38	0.14	0.0001*	T0 x T72 T0 x T120
HY5	40.77	0.04	40.57	0.06	40.53A	0.06	40.66	0.12	0.004*	T0 x T24 T0 x T72
JP	40.80	0.08	40.78	0.30	41.50B	0.68	40.28	0.12	0.004*	T0 x T72 T0 x T120
	0.459		0.300		0.002*		0.001*			
Transverse distance										
CCC	54.04	0.10	54.07A	0.03	54.00A	0.06	53.71	0.06	0.0001*	T0 x T120
IA	54.12	0.08	53.91A.B	0.17	53.79B	0.03	53.83	0.09	0.0001*	T0 x T24 T0 x T72 T0 x T120
HY5	54.00	0.02	53.96A.B	0.06	53.87B	0.02	53.61	0.08	0.0001*	T0 x T72 T0 x T120
JP	54.00	0.02	53.90B.C	0.04	53.82B	0.16	53.57	0.072	0.0001*	T0 x T72 T0 x T120
	0.542		0.035*		0.006*		0.0001*			
Vertical Distance										
CCC	11.08	0.17	11.25A	0.04	11.13A	0.09	11.07A	0.09	0.173	
IA	11.22	0.05	11.03B.C	0.05	11.17A	0.06	10.92B	0.14	0.0001*	T0 x T120
HY5	11.20	0.06	11.16A.C	0.07	11.19A	0.03	11.09A	0.07	0.0001*	T0 x T120
JP	11.16	0.07	10.98B	0.08	10.97B	0.09	10.86B	0.12	0.0001*	T0 x T120
	0.172		0.000*		0.001*		0.009*			

* statistically significant difference (p <0.05)

Different capital letters represent a statistically significant difference in the vertical column

used, which did not involve digital molds. Mold shrinkage was observed between T0 and T24 in IA and HY5, and an increase in anteroposterior distance was recorded for JP between T24 and T72. All of these changes were also found in a previous study⁴, which stated that some materials are stable only when analyzed immediately and noted significant contraction of traditional alginates within 72 hours and within one week. Between T0 and T120, IA and JP presented higher contraction values, following the

same explanations as in previous studies^{14,15}, which suggested that traditional alginates tend to show greater distortions than alginates with extended filling period over time.

For all transverse measurements and vertical distances, there was a difference between groups, especially at 24h, 72h and 120h. For TD, there was a decrease in IA, HY5 and JP after 72h and 120h. CCC was the most stable, with a significant decrease only after 120h. A previous study¹⁶ analyzing Cavex

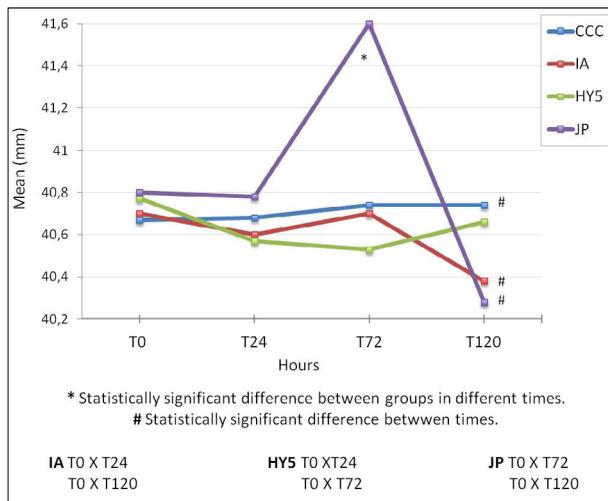


Fig. 2: Anteroposterior distance interaction graph

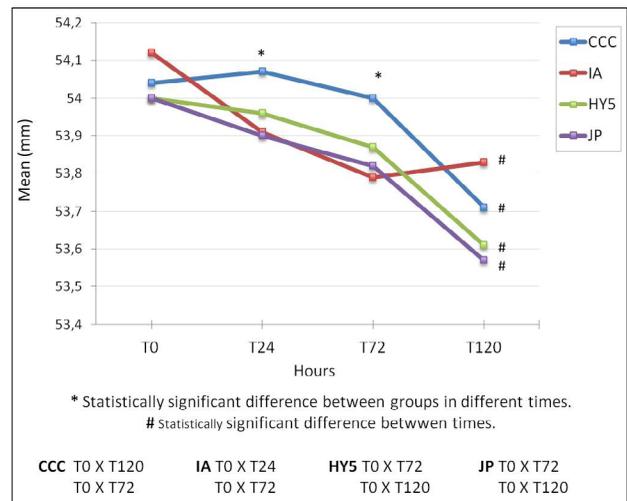


Fig. 3: Transverse distance interaction graph

Color Change dimensions for four days found a greater contraction for TD1 from the fourth day. In contrast, Guiraldo et al¹² concluded that Cavex Color Change did not show significant changes in its dimensions within one week.

These changes at T120 can be explained by the material's characteristics, as it allows longer storage time than conventional alginates^{3,4,16}. For IA, this decrease in TD was observed from T24 onwards. In HY5 and JP, changes in TD demonstrate an increasing contraction of materials, which is more significant at T72 and T120 (Table 2), with larger discrepancies at T120. In contrast to previous studies^{11,12} with different methodologies, this material did not suffer dimensional deformities for a period of five days. The changes observed in the present study may denote shrinkage of alginate molds when they are stored for many hours, as a result of water loss¹² and changes due to the influences of storage temperatures³.

When the vertical distance was evaluated, there was a significant difference in IA and JP at T120 (Table 2), presenting the lowest values, in agreement with Torassian et al.⁴, who found that traditional alginates suffer greater dimensional distortion as from 72 hours, as they are less resistant to the action of time¹⁴. At T72 (JP) and T120, IA and JP also presented the lowest mean values, indicating the possibility of presenting greater distortions over time^{3,4,14}.

The percentage change in material dimensions at T0 and T120 (Table 3), obtained from the molding of the upper arch standard typodont, which served as the gold standard in this study, shows a slight, non-significant difference in measurements between materials. Although significant differences were found between some measurements, differences less than or equal to 0.5mm are considered clinically insignificant¹⁷, and only differences greater than or equal to 1.5mm are considered clinically significant¹⁵. According to the American Board of Orthodontics Objective Grading System (ABO OGS), vertical, transverse and anteroposterior discrepancies greater than 0.5mm are considered significant¹⁸. In this study, the differences ranged from 0.2mm to 0.6mm for measurements and are therefore considered clinically acceptable without compromising treatment planning and control.

Based on the present results it can be concluded that the dimensional accuracy of the molds produced is dependent on the time and material used. Molding materials are not dimensionally stable when evaluated within 120 hours of molding. The most acceptable alginate material with the least distortion when analyzed for up to 120 hours was Cavex Color Change, while the material that suffered the most significant distortions at the time established in this study was Jeltrate Plus.

Table 3. Variation coefficient (Difference between the mean values at T0 and T120 and the gold standard).

	Typodont	CCC	IA	HY5	JP
T0					
Anteroposterior Distance					
Mean	40.81	40.67	40.7	40.77	40.8
Difference		-0.14	-0.11	-0.04	-0.01
%		-0.34%	-0.26%	-0.09%	-0.02%
Transverse distance					
Mean	54.05	54.03	54.12	53.99	53.99
Difference		-0.02	0.07	-0.06	-0.06
%		-0.03%	0.12%	-0.11%	-0.11%
Vertical Distance					
Mean	11.25	11.08	11.22	11.2	11.16
Difference		-0.17	-0.03	-0.05	-0.09
%		-1.5%	-0.26%	-0.44%	-0.8%
T120					
Anteroposterior Distance					
Mean	40.81	40.50	40.38	40.66	40.28
Difference		-0.302	-0.43	-0.15	-0.53
%		-0.7%	-1.05%	-0.36%	-1.2%
Transverse distance					
Mean	54.05	53.70	53.82	53.61	53.56
Difference		-0.35	-0.23	-0.44	-0.49
%		-0.64%	-0.42%	-0.81%	-0.90%
Vertical Distance					
Mean	11.25	11.07	10.91	11.09	10.86
Difference		-0.18	-0.34	-0.16	-0.39
%		-1.6%	-3.02%	-1.42%	-3.46%

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LIII REUNIÓN CIENTÍFICA ANUAL SAIO TUCUMÁN - ARGENTINA VIRTUAL



On November 5, 6 and 7, the 53rd Annual Meeting of the Argentine Society for Dental Research (SAIO) -the Argentine Division of the IADR- was held virtually. The President of the Meeting, Dr Luis Wuscovi, and the Organizing Committee worked remotely from the City of San Miguel de Tucumán, located in Tucumán Province.

Two hundred participants, including researchers, graduate students and alumni attended the meeting. During the three-day meeting, we shared lectures, round tables and group sessions. Virtuality allowed for the presence of Dr Christopher Fox and Dr María del Carmen López Jordi on behalf of International Association for Dental Research (IADR) and Latin American Region (LAR), respectively.

We were honored by the presence of Doctors Francisco Vizoso and Luis Costa. Special features of the 53rd Anniversary meeting included the following lectures:

- "Therapeutic possibilities of the mesenchymal stem cells secretome" by Francisco Vizoso
- "Use of stem cells and their derivatives in dentistry" by Luis Costa.

A dissertation panel of Secretaries of Science and Technology of National Dental Schools was held with the participation of Doctors Juan Carlos Elverdin (Universidad de Buenos Aires), Adriana Actis (Universidad Nacional de Córdoba), María Inés Borjas (Universidad Nacional de Cuyo), Alina Peláez (Universidad Nacional del Nordeste) and María Elena López (Universidad Nacional de Tucumán).

The executive meetings of the following Research Groups were held:

- Periodontal & Implantology Research
- Dental Materials
- Orthodontics
- Education Research
- Oral Medicine & Pathology
- Cariology & Public Health Research

Proposals for research projects were presented during the executive meeting of the Research Group related to the subject of the project in order to encourage discussion and closer collaboration among experts and peers in each dental discipline.

Thanks to the 200 registered participants, the "Virtual" LIII Annual Scientific Meeting of SAIO 2020 have had an excellent scientific level.

We are grateful to Doctor Luis Wuscovi -President of the Organizing Committee-, and his great work team, for paying attention to all the details for this scientific meeting to succeed.

We look forward to seeing you at the LIV Annual Scientific Meeting 2020 to be held with the VIII Latin American Region Meeting in Buenos Aires City. We wish Doctor Analía Garrofé, President of the Meeting the greatest success!

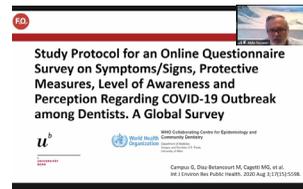
November 2020

**LIII REUNIÓN CIENTÍFICA
ANUAL SAIO
TUCUMÁN - ARGENTINA
VIRTUAL**



Opening ceremony.

From left to right: Christopher Fox (Executive Director of the IADR), María del Carmen López Jordi (President of the IADR), Gabriel Sánchez (President of the SAIO), Luis Wuscovi (President of the 53rd Annual Scientific Meeting).

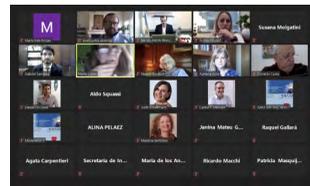


Scientific Conferences.

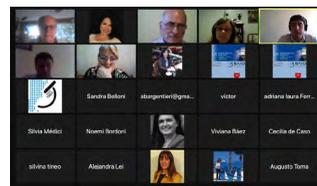
From left to right: Francisco Vizoso (Spain), Luis Costa (Spain-Argentina), Aldo Squassi (Argentina), Gabriel Sánchez (Argentina)



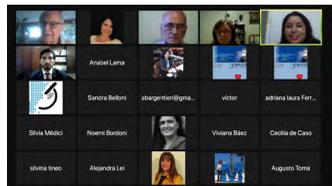
Organizing Committee of the 53rd Annual Meeting: Drs. Luis Sotomayor, María Florencia Moreno García, Sandra Iturre, Mercedes Jorraj, Juan Ángel Medina and Luis Wuscovi (President of the 53rd Annual Meeting).



Dissertation panel of Secretaries of Science and Technology of National Dental Schools.



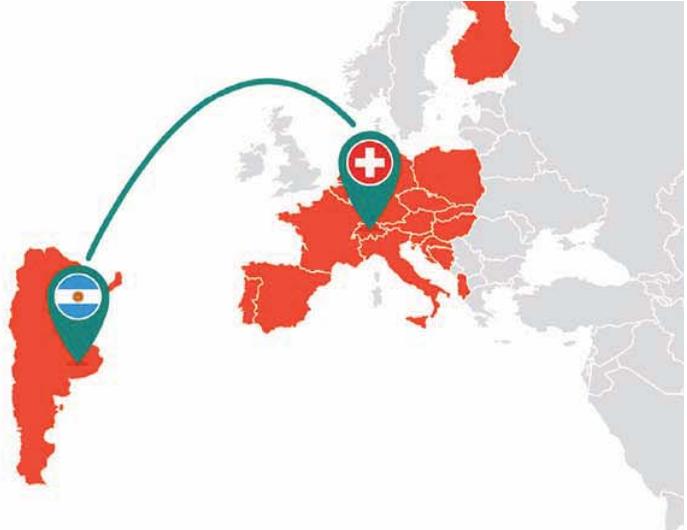
Competition IADR Divisional Award to compete for the "Unilever Hatton Divisional Award".



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La principal causa de la biocorrosión dental en la actualidad proviene de la dieta rica en bebidas y alimentos ácidos. En estos casos, el flujo salival cambia y afecta directamente la función remineralizante en el esmalte, haciéndolo más vulnerable. Debido a esto, todos somos susceptibles al daño del esmalte. Por lo tanto, el secreto es protegerlo y fortalecerlo diariamente, evitando la degradación temprana de los dientes y manteniéndolos saludables durante toda la vida.

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