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CONTENTS

Cone-Beam Computed Tomography to evaluate changes in trabecular lower jawbone microstructure caused by bone loss and antiresorptive treatment

María E Avendaño[1] , Marina S Bonanno[2] , Estefanía M Zeni Coronel[2] ,
Clarisa Marotte[2] , Susana N Zeni[2] , Mario R Davison[3]

1. Universidad Nacional de Cuyo. Facultad de Odontología. Departamento de Diagnóstico por Imágenes 1, Mendoza, Argentina.
2. Laboratorio de Osteopatías Metabólicas. Instituto de Inmunología, Genética y Metabolismo (INIGEM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad de Buenos Aires (UBA), Facultad de Farmacia y Bioquímica (FFyB), Hospital de Clínicas "José de San Martín", Buenos Aires, Argentina.
3. Universidad Nacional de Río Negro. Carrera de Odontología, Rio Negro, Argentina.

ABSTRACT

*For decades, conventional histomorphometry has been the gold standard for analyzing trabecular bone microarchitecture. In recent years, micro-computed tomography (μCT) devices have been validated and are now considered the gold standard for quantifying bone microstructure. **Aim**: The aim of this preliminary report is to evaluate the usefulness of CBCT to assess trabecular mandible microstructural properties in normal ewes and to compare the quantitative changes associated with ovariectomy and antiresorptive treatment. **Material and Method**: Twelve adult Corriedale ewes (n=4/group) aged 3-4 years were divided into 3 groups and studied for 28 months. Eight ewes were ovariectomized (OVX) and divided into OVX and OVX+ZOL groups (n=4/group) which were treated as follows, by jugular injection: OVX received saline solution and OVX+ZOL received zoledronate (Zol) (Gador SA, CABA, Argentina) (4 mg/month). Another four ewes were subjected to sham surgery (SHAM group) and received saline solution. **Results:** Densitometry showed that jaw mineral content (BMC) and density (BMD) were significantly lower in OVX than in SHAM and OVX+ZOL ewes; no difference was observed between OVX+ ZOL and SHAM groups. CBCT analysis showed that bone volume (BV/TV%); trabecular thickness (TbTh); connectivity density (CD) and anisotropy degree (AD) were significantly lower, and trabecular spacing (TbSp), significantly higher in OVX than in SHAM ewes. AD was significantly higher and TbSp significantly lower in OVX+ZOL than in OVX groups. BV/TV%, TbTh and CD showed a clear tendency to being higher in OVX+ZOL than in OVX groups. No statistical difference was observed between OVX+ZOL and SHAM ewes. CBCT in a nondestructive, fast, very precise procedure for measuring bone morphometric indices without biopsies, which are not indicated for morphometric evaluation in osteoporosis. **Conclusions:** The current study demonstrated the potential of the high-resolution CBCT imaging to assess in vivo quantitative bone morphometry and bone quality of lower jaw cancellous bone under normal conditions and to differentiate changes associated with excessive bone loss induced by estrogen withdrawal and antiresorptive intervention.*

Keywords: cone beam computed tomography - osteoporosis - zoledronic acid

CBCT para evaluar cambios en la microarquitectura ósea de la mandíbula por pérdida ósea y tratamiento antirresortivo

RESUMEN

Objetivo: *El presente informe preliminar evaluó la utilidad de Tomografía Computada de Haz Cónico (CBCT) para analizar las propiedades microestructurales trabeculares del maxilar inferior de ovejas y comparar los cambios cuantitativos asociados con la ovariectomía y tratamiento antirresortivo. Se estudiaron dieciséis ovejas Corriedale adultas de 3-4 años. **Materiales y Método:** Doce ovejas fueron ovariectomizadas (OVX) y divididas en 2 grupos: OVX y OVX+ZOL (n=4/grupo) cuyo tratamiento por inyección endovenosa en la yugular durante 28 meses fue el siguiente: OVX con solución salina y OVX+ZOL con zoledronato (Gador S.A. CABA. Argentina) (Zol) (4 mg/mes); 4 ovejas fueron sometidas a cirugía simulada (grupo SHAM). **Resultados:** La densitometría (Lunar DPX) mostró que el contenido mineral del hueso maxilar (CMO) y la densidad (DMO) fueron significativamente más bajos en OVX que en SHAM y OVX+ZOL; no se observaron diferencias entre los grupos OVX+ZOL y SHAM. El análisis de las imágenes por CBCT (Planmeca Promax 3D Classic) mostró que el volumen óseo (BV/TV%); el espesor trabecular (TbTh); la densidad de conectividad (CD) y el grado de anisotropía (AD) fueron significativamente menores (p<0.05), y el espaciado trabecular (TbSp), significativamente mayor en OVX que en SHAM (p<0.05). AD fue significativamente mayor (p<0.05) y TbSp, significativamente menor en OVX+ZOL que en OVX (p<0.05). BV/TV%, TbTh y CD mostró una clara tendencia a ser mayor en OVX+ZOL que en OVX. No se observaron diferencias estadísticas entre OVX+ZOL y SHAM. **Conclusiones:** En base a nuestros resultados consideramos que CBCT presenta suficiente confiabilidad y validez para evaluar in vivo la morfometría cuantitativa y la calidad del hueso esponjoso del maxilar inferior en condiciones normales, así como para diferenciar los cambios en dichos parámetros asociados a la pérdida ósea excesiva por la caída estrogénica e intervención antirresortiva. Aunque se necesitan estudios futuros, nuestros resultados agregarían una herramienta no invasiva adicional para diferenciar la microestructura del hueso trabecular mandibular en estudios preclínicos , sentando las bases para su futura aplicación en la práctica clínica.*

Palabras clave: *tomografía computarizada de haz cónico - osteoporosis - ácido zoledrónico*

INTRODUCTION

Cancellous bone architecture provides appropriate information about the biomechanical characteristics of bone[1]. For decades, conventional histomorphometry has been the gold standard for analyzing trabecular bone microarchitecture. However, it only enables two-dimensional measurements (2D) of specific, small sections of bone, and is invasive, time consuming, and predisposes to sampling errors. In recent years, micro-computed tomography (μCT) devices have been validated and are now the gold standard for quantifying bone microstructure[2]. The μCT technique enables detailed assessment of 3D trabecular bone microstructure. However, because it causes high radiation exposure, it can be only used *in vitro* for small samples. Cone-beam computed tomography (CBCT) has spatial resolution similar to that of conventional μCT but, particularly for trabecular bone, has relatively low cost and requires a lower dose or effective radiation[3]. In recent years, CBCT has emerged as a feasible, non-invasive, accurate tool for the assessment of several parameters of trabecular bone microarchitecture, including bone volume fraction (BV/TV%), trabecular thickness (TbTh) and trabecular spacing (TbSp)[4]. These parameters are important to evaluating trabecular bone quality, and the CBCT technique enables their quantitative examination[5].

Postmenopausal osteoporosis is a metabolic disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, which increases the risk of bone fractures. The primary clinical diagnostic criteria for osteoporosis are based on the decrease in bone mineral density (BMD)[6]. Nonetheless, bone architecture, too, affects the risk of bone fractures. Bisphosphonates (BPs), which are synthetic analogues of pyrophosphate, have traditionally been used to treat postmenopausal osteoporosis and other metabolic bone diseases[7]. BPs are rapidly cleared from systemic circulation and located on bone mineral surfaces, particularly at sites of high osteoclast activity, because of their high affinity for the calcium of the hydroxyapatite crystal[8]. Nitrogen-containing BPs inhibit the mevalonate pathway in the osteoclasts, decreasing cell activity and the rate at which bone is remodeled. Although they prevent loss of bone mass, their effect on bone quality is still not clear.

Bone remodeling enables bone tissue to adapt to different physiological conditions, and to replace damaged bone with newly formed bone. Even though trabecular bone remodeling is similar throughout the skeleton, jawbone remodeling is faster and constant because of the mechanical loading stimulus (forces) during mastication, particularly in alveolar bone[9,10,11]. The high reduction in bone turnover, especially with the most potent BPs, such as zolendronic acid (Zol), could affect the microarchitecture and biomechanical properties of bone. In this regard, BP-related osteonecrosis of the jaw (BRONJ) has been characterized as one of the main side effects of chronic therapy with BPs[12]. Sheep have long been used in bone research because they are large, docile animals that are easy to house and handle, and have low maintenance costs[13]. They are comparable to humans due to their weight, size and bone similarity, and have often been subject to research in maxillofacial areas[14,15,16]. They have molars and premolars with periodontium similar to humans, experience age-related skeletal problems including tooth loss, and develop periodontitis with similar oral pathogenic bacteria to those found in the human oral environment[17]. Ovariectomized (OVX) ewes showed a remarkable decrease in BMD[18]. As in humans, the OVX ewes treated with high doses of BPs for a long time could develop BRONJ[16].

In dentistry, CBCT is still limited to evaluating bone density in the jaw[19,20]. However there is a strong demand in clinical practice for quantitative analyses of bone microstructure. We hypothesize that CBCT has high potential for the diagnosis and treatment of osteoporosis. It is thus important to determine whether it is sensitive enough to identify changes in jawbone microstructure after bone loss or specific treatments. The aim of this preliminary report was to use CBCT analysis for assessing quantitative trabecular changes in the lower jawbone associated with high bone loss and antiresorptive treatment.

MATERIALS AND METHOD
Animals

Twelve virgin adult Corriedale ewes (3-4 years old), with 35 to 40 kg body weight (BW) were used for this study. They were housed, treated, and then euthanized by veterinarians authorized to handle animals by the "National Service for Health and Agro-Food Quality" (SENASA: "Servicio Nacional de Sanidad y Calidad Agroalimentaria"). BW was recorded once a month till the end of the study[16].

All procedures were performed in compliance with

Buenos Aires University's Hospital de Clínicas "José de San Martín" ethics guidelines approved on December 26, 2016. The animals were housed at the experimental farm of Río Negro National University and fed a daily ration of standard dry sheep feed with hay, wheatgrass, and grasses to meet nutritional recommendations. Water was supplied *ad libitum*.

Drug administration

In humans, intravenous amino-BPs injections are used to decrease bone resorption in several bone pathologies (e.g., osteoporosis, bone metastases, multiple myeloma, hypercalcemia of malignancy). To ensure high accumulation of BP in bone in order to induce a drastic decrease in bone resorption, the ewes were administered the most potent amino-BP at higher doses. The drug administration schedule and dose were in keeping with a published regimen for treating myeloma patients[21]. Fasting ewes were administered 4 mg Zol/month (equivalent to 66 µg/kg for 60 kg of BW) by jugular injection for 28 months. In order to rule out confounding factors and evaluate exclusively the effect of BP accumulation on the jaw, no other drug known to suppress the immune system (e.g., glucocorticoids) or inhibit it (e.g., bevacizumab) was administered.

Experimental design

The experimental design was previously published by our group[16]. Briefly, a veterinarian surgeon performed bilateral OVX on 8 animals and sham surgery (SHAM) on 4 animals under general anesthesia and cardiac and respiratory monitoring, following standard protocols for this type of procedure[22]. Two days post-surgery, OVX ewes were divided into two subsets and treated for 28 months (T=28) as follows:

- OVX: OVX ewes receiving 10 mL of saline solution by jugular injection.
- OVX+ZOL: OVX ewes treated with 10 mL of a Zol solution (4 mg/month) to obtain a high cumulative dose of Zol in the bone.
- SHAM: SHAM ewes receiving 10 mL saline solution and serving as controls

At T=28 animals were euthanized, and hemi-mandibles were excised and cleaned of soft tissue. Immediately after excision, they were analyzed by densitometry and computed tomography. Then, the hemi-mandibles were washed with saline and immersed in ethanol 60 % (v/v) for 72 hours. The alcohol was replaced at 24 hours intervals. The samples were dried in an oven at 100 °C for 24 hours and weighed.

Densitometry

Hemi-mandible BMD and bone mineral content (BMC) were evaluated *ex vivo* by dual energy x-ray absorptiometry (DXA) (Lunar DPX) using a software specifically designed for animals. All bones were scanned using an identical scanning procedure. Hemi-mandibles were analyzed on a bone image on the screen. Software precision for mandible BMD was assessed by measuring one piece of mandible five times after repositioning between scans both on the same and on different days[15]. The hemi-mandible coefficients of variation (CVs) were as follows: BMD=0.8 % and BMC=3.0 %. All analyses were performed by a single blinded technician, to minimize inter-observer variation.

Imaging Studies

The hemi-mandibles were analyzed by computed tomography at 1:1 magnification (Tomograph Gendex CB-500, Cone Beam, Georgia, USA). The digitalized images were analyzed using a 2D Image Analysis Software (Image Pro Plus 4.5, Media Cybernetics, USA) at the Department of Oral and Maxillofacial Radiology at Río Negro National University.

CBCT images were taken at 200 mm field of view, 120 kV, 300 minutes, scanning time 0.5 second/rotation, and slice thickness 0.5 mm with a high-resolution model. The CBCT analysis was performed using Planmeca Promax 3D Classic. Field of view was set at 80x80 mm, and voxel size at 200 μm^3. Each maxilla was scanned with tube current 9 mA, voltage 105 kv, and exposure time 9 seconds. The trabecular bone histomorphometry parameters measured by CBCT were analyzed using ImageJ2[23] with BoneJ2 plug-in[24]. CBCT scans were imported into the software. Images were calibrated in mm and default threshold was applied. Five cuboids were obtained from each hemi-mandible, including only cancellous bone, endeavoring to cover the entire tomography trabecular bone. TbTh, TbSp, BV/TV%, connectivity density (CD) and degree of anisotropy (AD) were calculated.

Statistical analysis

IBM SPSS Statistics for Windows Version 21.0 (IBM Corp. USA) was used. A value of $p < 0.05$ was considered significant. Data were expressed

as mean ± standard deviation (SD). Normality of variables was evaluated using the Shapiro Wilk test, and homogeneity of variances was assessed by Levene's test. Data were analyzed using one-way analysis of variance (ANOVA) to detect differences in percentages and means, followed by multiple comparisons using Bonferroni's post hoc test.

RESULTS

No significant difference in BW was observed among groups, either at the beginning or at T=28 (Table 1). At the end of the study, the significantly lowest hemi-mandible weight was observed in OVX (p<0.05), and the significantly highest value in OVX+ZOL (p<0.05) (Table 1).

Hemi-mandible BMC and BMD values were significantly lower in OVX than in SHAM and OVX+ZOL (p<0.01); no statistical difference was observed between OVX+ZOL and SHAM (Table 1). CBCT analysis showed that BV/TV% (Fig. 1), TbTh, CD and AD were significantly lower (p<0.0075; p<0.0075, p<0.001 and p<0.02, respectively), and TbSp was significantly higher (p<0.0002) in OVX than in SHAM (Fig. 2A-D). AD was significantly higher (p<0.018) and TbSp significantly lower in OVX+ZOL than in OVX (p<0.043) (Figs. 2D and 2B, respectively). BV/TV%, TbTh and CD showed a clear tendency to be higher in OVX+ZOL than in OVX (p=0.055; p=0.061 and p=0.054, respectively) (Figs. 1, 2A, and 2C). No statistical difference in the CBCT parameters was observed between OVX+ZOL and SHAM.

The area of analysis of CBCT is shown in Fig. 3. The tomography of the hemi-mandible shows normal radiodensity in SHAM and OVX+ZOL (Fig. 3A and 3C). The arrows point to the areas of low radiodensity in OVX (Fig. 3B).

DISCUSSION

The present preliminary report evaluated the usefulness of CBCT for assessing the quantitative changes in jawbone microarchitecture after estrogen withdrawal and antiresorptive treatment. The comparative analysis of the results showed that the jawbone quality of the ewes, as occurred in bone density, was seriously affected after 28 months post-OVX, and although the loss of bone density was prevented by the treatment with high doses of Zol, the microstructural parameters were only partially recovered.

Table 1. Body and maxilla weight, bone mineral content and density at the end of the study.

Experimental Groups	BW (Kg)	Maxilla weight (g)	BMC (g)	BMD (g/cm²)
SHAM	29.7±2.8	178.4±4.0[b]	37.2±2.9[b]	0.244±0.016[b]
OVX	30.7±4.6	142.8±2.3[c]	24.0±6.1[a]	0.166±0.040[a]
ZOL	32.0±2.8	185.1±5.7[a]	42.3±3.0[b]	0.254±0.026[b]

Table 1. Body and maxilla weight, bone mineral content and density at the end of 28 months of treatment. *Data were analyzed by one-way ANOVA followed by multiple comparisons using Bonferroni's post hoc test. Results were expressed as mean ± SD. Different letters indicate statistically significant difference. BW: body weight; BMC: bone mineral content; BMD: bone mineral density.*
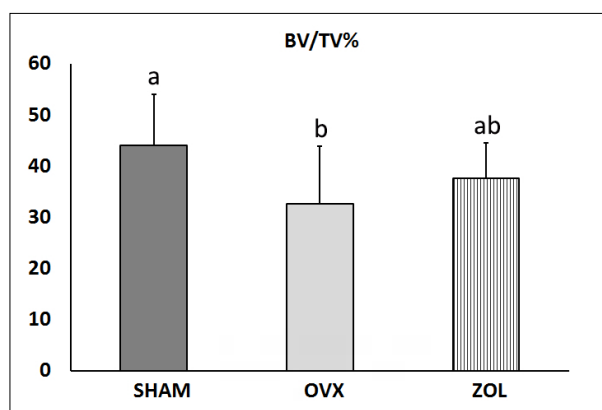
Fig. 1: Change in the jawbone volume (BV/TV%) measured by CBCT in the three experimental groups. Data were analyzed by one-way ANOVA followed by multiple comparisons using Bonferroni's post hoc test. Results expressed as mean ± SD. Different letters indicate statistically significant difference (p<0.05).

Animal models are very important to understanding several aspects of the pathological mechanism of bone diseases, as well as to establishing prevention and management strategies. Most basic investigations in this field have been conducted in rats[25]. Ewes are large animals that provide a suitable experimental model for many reasons. They are closer than rodents to humans in genetic constitution and metabolic rate (0.22 vs. 0.21); they undergo Haversian bone remodeling, and like women, they have menarche and regular, frequent ovulatory cycles. Bone metabolism in the OVX sheep resembles that of women during early postmenopause[26,27].

Estrogen is an important regulator of bone homeostasis. The decline in estradiol levels accelerates bone remodeling characterized by higher bone resorption than bone formation, leading to
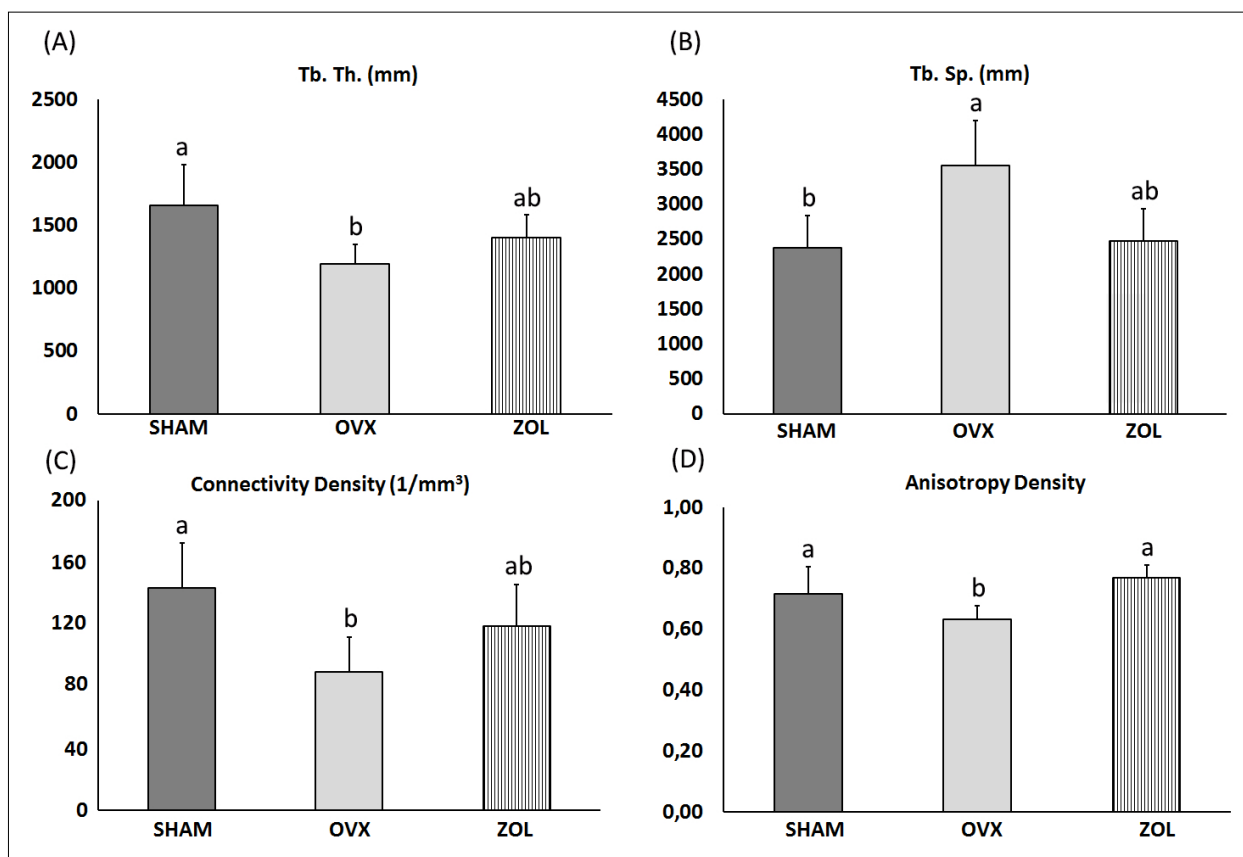
Fig. 2: **A)** *Bar graphs of trabecular thickness (TbTh),* **B)** *trabecular spacing (TbSp),* **C)** *connectivity density, and* **D)** *anisotropy density, measured by CBCT in the three experimental groups. Data were analyzed by one-way ANOVA followed by multiple comparisons using Bonferroni's post hoc test. Results expressed as mean ± SD. Different letters indicate statistically significant difference (p<0.05).*

Fig. 3: *Panoramic tomography of the lower left jaw of SHAM (A), OVX (B) and ZOL (C) groups. The zone delimited in white corresponds to the area of analysis by the CBCT technique. Arrows show the reduced radiodensity area in the OVX group as compared to SHAM and ZOL groups. The latter two show similar levels of radiopacity.*

bone loss over time[28]. The animal model of estrogen withdrawal is ovariectomy. In the present report, as expected, bone mass and density had decreased by 30% at T=28. In addition to bone mass, the composition and microstructure arrangement of the basic components of bone play a critical role in the ability of the jawbone to support mastication. Indeed, the load required for bone deformation is proportional to BMD, and therefore to the degree of bone matrix mineralization. However, the spatial distribution of bone mass (i.e., microarchitecture) influences the biomechanical efficiency of bone to support loads. It has been estimated that bone quantity is responsible for 60 to 80% of the biomechanical resistance of the bone, while the remaining 20-40% depends on bone quality[29].

The term bone quality comprises a set of bone tissue characteristics encompassing multiple aspects of bone physiology, degree of mineralization, morphology and type of trabecular pattern which, in addition to density, contribute to bone strength[30]. According to the literature, BV/TV%, TbTh and TbSp are the most crucial indices for determining the trabecular bone microarchitecture[31]. However, it is important to note that trabecular bone is anisotropic, and that the inclusion of structural anisotropy improves the assessment of bone strength. In the present report, the evaluation of lower jawbone quality included these indices. The reference values were obtained by analyzing the lower jaw of the SHAM control group, which characterizes the physiological bone microarchitectural state. Any changes in these values were considered as having been induced by ovariectomy and anticatabolic treatment.

Under the experimental conditions in the current study, the CBCT technique showed that ovariectomy induced a mean loss of 25% in BV/TV%, and decreases of 30% in TbTh, 50% in TbSp, 40% in CD, and only 12% in AD. All these changes suggest an impairment of lower jaw bone mechanical integrity. According to the literature, BV/TV, which is considered one of the most important parameters in bone quality, shows a high correlation with BMD[32]. In this regard, the significant decrease in BV/TV% following ovariectomy observed in the present study may indicate that the trabecular bone strength of the jaw was highly affected. The changes in the bone morphometric parameters detected by CBCT in the lower jawbone of OVX ewes agree with previous findings in other bones, using a different technique. Conventional histomorphometric analysis of the lumbar vertebrae and the iliac crest showed a decrease in BV/TV% and in TbTh, and an increase in TbSp[33,34]; μ-CT analysis of femoral neck showed a decrease in BV/TV% and TbTh one year after OVX[35,36], while in the spine or distal femur such changes were observed one or two years after OVX[5]. Connectivity estimates the number of connected structures in a network whose maintenance is critical for supporting loads. CBCT measurement of CD showed a significant decrease in OVX, providing evidence that the trabecular network was seriously altered. This finding is crucial because it has been suggested that for the same decline in bone mass, the loss of trabecular connectivity is 2 to 5 times more deleterious to bone strength than is the thinning of the trabeculae[37]. The high reduction in trabecular separation was not unexpected because in 3-D analysis, this parameter is influenced by connectivity. Anisotropy represents the 3D configuration and arrangement of bone, and it is more independent of bone mass than the other 3D morphometric parameters, so may strongly contribute to predicting changes in bone strength. Our CBCT analysis showed that in OVX, both BV/TV% and the heterogeneity of trabeculae were reduced, suggesting a decrease in the load-carrying capacity and mechanical function of the lower jawbone. All these findings indicate that, as expected, densitometry reveals the loss of bone mass and density by ovariectomy, but CBCT analysis adds information showing that ovariectomy seriously jeopardized the structural resistance of the lower jawbone.

Remodeling enables bone tissue to adapt to different physiological conditions and replace damaged bone with newly-formed bone. Although the bone remodeling mechanism in the jawbone is similar to that in other bones, jawbone is remodeled more rapidly because of the effect of mastication[9]. Excessive osteoclastic bone resorption results in an osteoporotic stage characterized by loss of BMD and biomechanical properties of bone as observed in the OVX ewes. Pharmacological strategies for osteoporosis consist of increasing bone strength and improving bone quantity and quality. BPs are the drugs of choice to initiate anticatabolic therapy for osteoporosis. They are incorporated faster in the active areas of osteoclastic bone resorption[6], reducing the rate of bone remodeling and bone loss. In the current study, treatment with Zol was started immediately after surgery (2 days), preventing the bone loss that occurs as a consequence of the estrogen withdrawal induced by the ovariectomy. In this regard, the results of the present report agree with previous findings showing that preventive treatment with Zol protected against OVX-induced loss of BMD and BMC. However, the CBCT technique showed that bone quality was only partially recovered. Indeed, in OVX+ZOL ewes, BV/TV%, TbTh and CD values were 15% lower than in control ewes, while TbSp and AD values were similar. These results provided evidence that although Zol prevented the decay of cancellous bone mass and density induced by OVX, the recovery of bone microstructure was only partial. It must be taken into account that the main

bone quality defect in osteoporosis is the disruption in bone microarchitecture, and that Zol treatment cannot completely restore the mechanical integrity of jawbone because it lacks an anabolic effect.

Traditionally, the evaluation of bone status has depended on bone quantity or bone density; however, if bone architecture is also determined as described in the present report, it is possible to assess bone mass and quality at the same time, enabling a more precise evaluation of bone strength and therefore of the risk of fracture. The results of the present report clearly indicate that high-resolution CBCT is adequate for characterizing and differentiating the microarchitecture of both normal and osteoporotic lower jaws of ewes, before and after antiresorptive treatment. The future standardization of these findings may extend the availability of this technology in basic longitudinal research, laying the groundwork for future use in clinical practice, especially when repetitive follow-up measurements are needed.

Although CBCT technology can be used to evaluate the jawbone *in vivo*, one limitation of the present study is that the measurements were conducted *ex vivo*, in the absence of motion artifacts that could affect the results. We will conduct further studies *in vivo* to determine the potential effects of repeated CBCT scans, and whether it can be used monitor changes accurately and precisely over time.

CONCLUSION

CBCT in a nondestructive, fast, very precise procedure for measuring bone morphometric indices without biopsies, which are not indicated for morphometric evaluation in osteoporosis.

In conclusion, the present study demonstrated the potential of the high-resolution CBCT imaging to evaluate *in vivo* the quantitative bone morphometry and bone quality of lower jaw cancellous bone in normal condition, and to distinguish the changes associated with excessive bone loss induced by estrogen withdrawal and antiresorptive intervention. Although further studies are needed, these results provide an additional tool for evaluating trabecular mandibular bone microstructure in preclinical studies, laying the groundwork for its future application in clinical practice.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

REFERENCES

1. He RT, Tu MG, Huang HL, Tsai MT, Wu J, Hsu JT. Improving the prediction of the trabecular bone microarchitectural parameters using dental cone-beam computed tomography. BMC Med Imaging. 2019;19(1):1-9. https://doi.org/10.1186/s12880-019-0313-9

2. Van Dessel J, Huang Y, Depypere M, Rubira-Bullen I, Maes F, Jacobs R. A comparative evaluation of cone beam CT and micro-CT on trabecular bone structures in the human mandible. Dentomaxillofacial Radiol. 2013;42(8):20130145. https://doi.org/10.1259/dmfr.20130145

3. Servais JA, Gaalaas L, Lunos S, Beiraghi S, Larson BE, Leon-Salazar V. Alternative cone-beam computed tomography method for the analysis of bone density around impacted maxillary canines. Am J Orthod Dentofacial Orthop. 2018;154(3):442-9. https://doi.org/10.1016/j.ajodo.2018.01.008

4. Barngkgei I, Al Haffar I, Shaarani E, Khattab R, Mashlah A. Assessment of jawbone trabecular bone structure amongst osteoporotic women by cone-beam computed tomography: the OSTEOSYR project. J Investig Clin Dent. 2016;7(4):332-40. https://doi.org/10.1111/jicd.12170

5. Kreipke TC, Rivera NC, Garrison JG, Easley JT, Turner AS, Niebur GL. Alterations in trabecular bone microarchitecture in the ovine spine and distal femur following ovariectomy. J Biomech. 2014;47(8):1918-21. https://doi.org/10.1016/j.jbiomech.2014.03.025

6. Peck WA. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med. 1993;94(6):646-50. https://doi.org/10.1016/0002-9343(93)90218-e

7. McClung MR. Bisphosphonates. Endocrinol Metab Clin. 2003;32(1):253-71. https://doi.org/10.1016/S0889-8529(02)00079-8

8. Diel IJ, Fogelman I, Al-Nawas B, Hoffmeister B, Migliorati C, Gligorov J, et al. Pathophysiology, risk

factors and management of bisphosphonate-associated osteonecrosis of the jaw: Is there a diverse relationship of amino-and non-aminobisphosphonates? Crit Rev Oncol Hematol. 2007;64(3):198-207. https://doi.org/10.1016/j.critrevonc.2007.07.005

9. Huja SS, Fernandez SA, Hill KJ, Li Y. Remodeling dynamics in the alveolar process in skeletally mature dogs. Anat Rec Part Discov Mol Cell Evol Biol Off Publ Am Assoc Anat. 2006;288(12):1243-9. https://doi.org/10.1002/ar.a.20396

10. Phal PM, Myall RWT, Assael LA, Weissman JL. Imaging findings of bisphosphonate-associated osteonecrosis of the jaws. Am J Neuroradiol. 2007;28(6):1139-45. https://doi.org/10.3174/ajnr.A0518

11. Zahrowski JJ. Bisphosphonate treatment: an orthodontic concern calling for a proactive approach. Am J Orthod Dentofacial Orthop. 2007;131(3):311-20. https://doi.org/10.1016/j.ajodo.2006.09.035

12. O'Ryan FS, Khoury S, Liao W, Han MM, Hui RL, Baer D, et al. Intravenous bisphosphonate-related osteonecrosis of the jaw: bone scintigraphy as an early indicator. J Oral Maxillofac Surg. 2009;67(7):1363-72.https://doi.org/10.1016/j.joms.2009.03.005

13. Reinwald S, Burr D. Review of nonprimate, large animal models for osteoporosis research. J Bone Miner Res. 2008;23(9):1353-68. https://doi.org/10.1359/jbmr.080516

14. Voss PJ, Stoddart M, Ziebart T, Zeiter S, Nelson K, Bittermann G, et al. Zoledronate induces osteonecrosis of the jaw in sheep. J Cranio-Maxillofac Surg. 2015;43(7):1133-8. https://doi.org/10.1016/j.jcms.2015.04.020

15. Voss PJ, Stoddart MJ, Bernstein A, Schmelzeisen R, Nelson K, Stadelmann V, et al. Zoledronate induces bisphosphonate-related osteonecrosis of the jaw in osteopenic sheep. Clin Oral Investig. 2016;20(1):31-8. https://doi.org/10.1007/s00784-015-1468-6

16. Davison MR, Lyardet L, Preliasco M, Yaful G, Torres P, Bonanno MS, et al. Aminobisphosphonate-treated ewes as a model of osteonecrosis of the jaw and of dental implant failure. J Periodontol. 2020;91(5):628-37. https://doi.org/10.1002/JPER.19-0213

17. Ismaiel MO, Greenman J, Morgan K, Glover MG, Rees AS, Scully C. Periodontitis in sheep: a model for human periodontal disease. J Periodontol. 1989;60(5):279-84. https://doi.org/10.1902/jop.1989.60.5.279

18. Li YH, Gao FQ, Cheng LM, Zeng MD, Qiu QY, Ding M. Different changes of microarchitectures of cortical and cancellous bones in sheep femoral head after long-term glucocorticoid interventions. Sci Rep. 2018;8(1):1-6. https://doi.org/10.1038/s41598-018-28433-7

19. Barngkgei I, Al Haffar I, Khattab R. Osteoporosis prediction from the mandible using cone-beam computed tomography. Imaging Sci Dent. 2014;44(4):263-71. https://doi.org/10.5624/isd.2014.44.4.263

20. Gomes CC, de Rezende Barbosa GL, Bello RP, Bóscolo FN, De Almeida SM. A comparison of the mandibular index on panoramic and cross-sectional images from CBCT exams from osteoporosis risk group. Osteoporos Int. 2014;25(7):1885-90. https://doi.org/10.1007/s00198-014-2696-3

21. Clemons MJ, Dranitsaris G, Ooi WS, Yogendran G, Sukovic T, Wong BY, et al. Phase II trial evaluating the palliative benefit of second-line zoledronic acid in breast cancer patients with either a skeletal-related event or progressive bone metastases despite first-line bisphosphonate therapy. J Clin Oncol. 2006;24(30):4895-900. https://doi.org/10.1200/JCO.2006.05.9212

22. Fini M, Giavaresi G, Rimondini L, Giardino R. Titanium alloy osseointegration in cancellous and cortical bone of ovariectomized animals: histomorphometric and bone hardness measurements. Int J Oral Maxillofac Implants. 2002;17(1). PMID: 11858572.

23. Abràmoff MD, Magalhães PJ, Ram SJ. Image processing with ImageJ. Biophotonics Int. 2004;11(7):36-42.

24. Doube M, K\losowski MM, Arganda-Carreras I, Cordelières FP, Dougherty RP, Jackson JS, et al. BoneJ: free and extensible bone image analysis in ImageJ. Bone. 2010;47(6):1076-9. https://doi.org/10.1016/j.bone.2010.08.023

25. Pautke C, Kreutzer K, Weitz J, Knödler M, Münzel D, Wexel G, et al. Bisphosphonate related osteonecrosis of the jaw: a minipig large animal model. Bone. 2012;51(3):592-9. https://doi.org/10.1016/j.bone.2012.04.020

26. Thorndike EA, Turner AS. In search of an animal model for postmenopausal diseases. Front Biosci-Landmark. 1998;3(3):17-26. https://doi.org/10.2741/A260

27. Turner AS. The sheep as a model for osteoporosis in humans. Vet J. 2002;163(3):232-9. https://doi.org/10.1053/tvjl.2001.0642

28. Seifert-Klauss V, Fillenberg S, Schneider H, Luppa P, Mueller D, Kiechle M. Bone loss in premenopausal, perimenopausal and postmenopausal women: results of a prospective observational study over 9 years. Climacteric. 2012;15(5):433-40. https://doi.org/10.3109/13697137.2012.658110

29. Faulkner KG. Bone matters: are density increases necessary to reduce fracture risk? J Bone Miner Res. 2000;15(2):183-7. https://doi.org/10.1359/jbmr.2000.15.2.183

30. Bouxsein ML. Bone quality: an old concept revisited. Osteoporos Int. 2003;14(5):1-2. https://doi.org/10.1007/s00198-003-1467-3

31. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro–computed tomography. J Bone Miner Res. 2010;25(7):1468-86. https://doi.org/10.1002/jbmr.141

32. Kulah K, Gulsahi A, Kamburoğlu K, Geneci F, Ocak M, Celik HH, et al. Evaluation of maxillary trabecular microstructure as an indicator of implant stability by using 2 cone beam computed tomography systems and micro-computed tomography. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019;127(3):247-56. https://doi.org/10.1016/j.oooo.2018.11.014

33. Giavaresi G, Fini M, Torricelli P, Martini L, Giardino R. The ovariectomized ewe model in the evaluation of biomaterials for prosthetic devices in spinal fixation. Int J Artif Organs. 2001;24(11):814-20. https://doi.org/10.1177/039139880102401106

34. Newton BI, Cooper RC, Gilbert JA, Johnson RB, Zardiackas LD. The ovariectomized sheep as a model for human bone loss. J Comp Pathol. 2004;130(4):323-6. https://doi.org/10.1016/j.jcpa.2003.12.007

35. Wu Z xiang, Lei W, Hu Y yu, Wang H qiang, Wan S yong, Ma Z sheng, et al. Effect of ovariectomy on BMD, micro-architecture and biomechanics of cortical and cancellous bones in a sheep model. Med Eng Phys. 2008;30(9):1112-8. https://doi.org/10.1016/j.medengphy.2008.01.007

36. Holland JC, Brennan O, Kennedy OD, Rackard SM, O'Brien FJ, Lee TC. Subchondral trabecular structural changes in the proximal tibia in an ovine model of increased bone turnover. J Anat. 2011;218(6):619-24. https://doi.org/10.1111/j.1469-7580.2011.01376.x

37. Iolascon G, Frizzi L, Di Pietro G, Capaldo A, Luciano F, Gimigliano F. Bone quality and bone strength: benefits of the bone-forming approach. Clin Cases Miner Bone Metab. 2014;11(1):20. https://doi.org/10.11138/ccmbm/2014.11.1.020

Phytotherapy: knowledge, experience and prescription in oral healthcare. A cross-sectional survey of dental practitioners

Rosemary SA Shinkai[1,2] , Tomie T de Campos[2] , Lucas S Mendes[2] , Lena Katekawa[3] ,
Edgard Michel-Crosato[1] , Maria GH Biazevic[1]

1. Universidade de São Paulo, Faculdade de Odontologia, Departamento de Odontologia Social, São Paulo, Brasil.
2. Universidade de São Paulo, Faculdade de Odontologia, Departamento de Prótese, São Paulo, Brasil.
3. Universidade de São Paulo, Faculdade de Odontologia, Curso de Especialização em Prótese, São Paulo, Brasil.

ABSTRACT

Herbal medicine is widely used as an integrative complementary treatment for common chronic diseases, yet potential risks include adverse effects and coprescription. **Aim:** *This exploratory survey assessed Brazilian dentists' knowledge, attitudes, and practices regarding phytotherapy.* **Materials and Method:** *This study used a consecutive sample of registered dentists who were active in clinical practice and attending advanced training for complex oral rehabilitation at a leading dental school in Latin America. Participants responded to a semi-structured 38-item instrument designed to collect data on sociodemographic, professional and health variables, and on their knowledge, use, prescription and opinions of phytotherapy. Quantitative data were analyzed with descriptive and inferential statistics. Qualitative data were described and analyzed with a thematic approach.* **Results:** *A total 53 dentists responded the survey (88.3% response rate). Six dentists reported formal education in phytotherapy and 33 had informal knowledge. Twenty-one dentists (39.6%) reported using herbal medicine, and 17 (32.1%) prescribed phytotherapy to their patients, mostly traditional medicinal plants. A logistic regression model showed that post-graduate degree and personal use of phytotherapy were associated with phytotherapy prescription. Opinions on how to improve the rational use of phytotherapy included the need for research evidence, specific academic education, and knowledge dissemination to clinicians. As a clinical recommendation, it was proposed that questions on herbal medicine should be asked during the dental patient's anamnesis.* **Conclusions:** *Dentists with post-graduate degrees and personal use of phytotherapy prescribe herbal medicines for their patients in real-world clinics, regardless of formal education on the subject. Qualitative findings indicate the need to implement scientific initiatives.*

Keywords: herbal medicine - oral health - perception - phytotherapy

Conhecimento, experiência e prescrição de fitoterapia em saúde bucal: Um levantamento transversal com dentistas clínicos

RESUMO

A fitoterapia é amplamente utilizada como um tratamento integrativo e complementar para doenças crônicas comuns, mas os riscos potenciais incluem efeitos adversos e co-prescrição. **Objetivo:** *Esta pesquisa exploratória avaliou o conhecimento, as atitudes e as práticas de cirurgiões-dentistas brasileiros sobre fitoterapia.* **Materiais e Método:** *Uma amostra consecutiva foi composta por dentistas com registro profissional, que eram ativos em prática clínica e frequentavam treinamento avançado para reabilitação oral complexa em uma das principais escolas de odontologia da América Latina. Os participantes responderam a um instrumento semiestruturado de 38 itens para coletar dados sobre variáveis sociodemográficas, profissionais e de saúde, bem como conhecimento, uso, prescrição e opiniões sobre fitoterapia. Os dados quantitativos foram analisados com estatística descritiva e inferencial. Os dados qualitativos foram descritos e analisados com abordagem temática.* **Resultados:** *Um total de 53 dentistas respondeu à pesquisa (taxa de resposta de 88,3%). Seis cirurgiões-dentistas relataram formação em fitoterapia e 33 possuíam conhecimento informal. Vinte e um dentistas (39,6%) relataram o uso de fitoterápicos e 17 (32,1%) prescreveram fitoterapia para seus pacientes, principalmente plantas medicinais tradicionais. Um modelo de regressão logística mostrou que pós-graduação e uso pessoal de fitoterapia estiveram associados à prescrição de fitoterapia. As opiniões para melhorar o uso racional da fitoterapia incluíram a necessidade de evidências de pesquisa, formação acadêmica específica e disseminação do conhecimento para os clínicos. Como recomendação clínica, foram propostas questões sobre fitoterapia para a anamnese odontológica do paciente.* **Conclusões:** *Cirurgiões-dentistas com pós-graduação e uso pessoal de fitoterapia prescrevem mais medicamentos fitoterápicos para seus pacientes em clínicas do mundo real, independentemente da educação formal sobre o assunto. Os resultados qualitativos indicam a necessidade de iniciativas de implementação científica.*

Palavras-Chave: medicamentos fitoterápicos - saúde bucal - percepções - fitoterapia

INTRODUCTION

Herbal medicines and medicinal plants are widely used for preventive or therapeutic reasons in many ways, including local traditional medicine, prescription by healthcare professionals, self-medication and dietary supplements. A global report by the World Health Organization (WHO) in 2019 showed that 124 Member States (64%) had laws or regulations on herbal medicines within a national policy on traditional, complementary, integrative medicine[1]. Thus, knowledge on traditional medicine and clinical phytotherapy have been incorporated as a complementary practice into public and private health systems to provide comprehensive health care, especially for prevention and management of lifestyle-related chronic diseases in ageing populations[2]. However, natural products are not always safe, and medicinal plants and herbal medicines may negatively impact health due to misuse. There are potential adverse effects and interaction of herbal medicines with other herbal products or synthetic drugs[3,4], resulting in unintentional decreased, exacerbated or null effect of the target pharmacological therapy, with possible health risks. Another common challenge is coprescription with synthetic drugs that could induce polypharmacy[5]. These problems are common in adults, and particularly relevant for elderly patients, who often have comorbidities, use multiple medications, or self-medicate inappropriately[5-7]. Additionally, in contrast to synthetic drugs with isolated bioactive chemicals, herbal medicines have a system of phytocompounds with multi-target and multi-pathway actions, which can be problematic if not recognized. Therefore, the healthcare provider must assess patients' use of medicinal plants and herbal medicines prior to treatment in order to fully understand their clinical conditions.

In dentistry, the literature has shown an increasing interest in herbal medicine for several oral health problems[8,9], but also reported adverse effects of herbal products in the oral cavity[10] and potential interaction with blood coagulation[11] and anesthesia[12]. Nevertheless, the internationally indexed literature is limited regarding the knowledge and opinions on phytotherapy indication, safety, and effectiveness among dentists in different settings of developing countries that have great biodiversity and traditional knowledge[13-15], and developed countries with profitable herbal industries[16,17]. In Brazil, previous studies have interviewed only a few dentists working in multi-professional teams of the public health system[15,18,19], where national programs on integrative and complementary practices in primary care have been available since the late 2000s, though not fully implemented. However, private clinicians involved in oral rehabilitation are usually the ones who treat older patients with complex oral deficiencies and chronic health problems who use multiple medications. After treatment, these patients often return to the same professional for life-long maintenance and oral health care. However, this group of dental clinicians has not yet been assessed regarding their experience with phytotherapy. We hypothesize that sociodemographic (gender, age) and professional factors (post-graduate degrees, education in phytotherapy), and personal use of herbal medicine are associated with phytotherapy prescription by dentists.

The aim of this cross-sectional survey was thus to evaluate the knowledge and perceptions of oral rehabilitation practitioners on medicinal plants and herbal medicine, as well as their experiences with phytotherapy for personal use and clinical prescription in dental practice.

MATERIALS AND METHOD

The research design is an observational, cross-sectional, survey-type, descriptive-analytic, mixed methods study. This exploratory study with dental clinicians is part of an umbrella research project on phytotherapy in dentistry. The research protocol was approved by the university's research ethics committee, in compliance with the Declaration of Helsinki, as revised in 2000, and the Brazilian regulations for research in human beings, and registered prospectively in a national system (Plataforma Brasil, CAAE: 58861222.8.0000.0075, Parecer 5.509.538).

Participants

A consecutive non-randomized sample was obtained from the population of dentists attending clinical courses for professional development at the School of Dentistry of the University of São Paulo, a public state university located in São Paulo, the largest city in Brazil.

Dentists were recruited and selected according to the study eligibility criteria, during the second semester

of 2022. Inclusion criteria were: adults with no restriction of gender and age, who were registered as dentists in the national professional council, active in clinical practice, and attending advanced-level clinical courses to treat adult patients with major oral rehabilitation needs and/or orofacial pain. Dentists were excluded if they were unwilling to participate or unable to respond to a questionnaire.

Survey instrument and procedures

Eligible dentists were recruited in person to participate in the study voluntarily, and those who accepted the invitation signed an informed consent form, after verbal explanation of the research protocol and before the survey. Data were collected while the dentists were attending their in-person clinical courses, from August to November 2022.

Each participant answered a hard copy self-administered, semi-structured, 38-item questionnaire in Portuguese language, in privacy and with no time limit. A three-part research instrument, with closed and open-ended questions, was developed for this research, based on previous literature[15,18]. The content validity was verified by senior dentists and researchers with expertise in complementary and integrative medicine, research methods and ethics.

The first part of the instrument consisted of 13 items to collect quantitative information on demographic and socioeconomic profile, access to and use of health services, and self-perception of general, mental and oral health. Part 2 included 14 items to collect quantitative and qualitative data on the personal use of and perception of synthetic drugs, medicinal plants and herbal medicines. Part 3 contained 11 questions to collect quantitative and qualitative data on dental clinical practices and prescription of medicinal plants and herbal medicines, as well as perceptions of use, effects, benefits and risks. At the end of Part 3, there was an open question for any suggestions to improve the rational use of phytotherapy in dental clinics. Participants could leave any question unanswered for any reason. The researchers were always available to clarify any doubt. Data obtained from completed forms were entered anonymously in a spreadsheet for analysis.

Quantitative Data Analysis

Data were analyzed with the software JASP (Version 0.17.1) (https://jasp-stats.org) (https://github.com/jasp-stats/jasp-desktop). Descriptive statistics were used to summarize quantitative results. Exploratory inferential statistics were used to analyze the outcome measure 'phytotherapy prescription' as a function of selected independent variables. Initially, bivariate tests (chi-squared and Fisher's exact tests) were used to calculate crude odds ratios. Multivariable logistic regression was used to analyze the factors jointly, and all variables potentially associated with 'phytotherapy prescription' were included in the final model after preliminary analysis.

Qualitative Data Analysis

Qualitative data from open-ended questions included participants' personal use and clinical prescription of herbal medicines and medicinal plants, as well as their opinions on how to improve the rational use of phytotherapy in dentistry. Data on reported use of herbal medicines and medicinal plants were described in relation to product/preparation used or prescribed, posology, and target health problem or condition. A semantic analysis was conducted with participants' surface word meanings.

Qualitative data on dentists' opinions were encoded and analyzed using thematic analysis[20,21], with inductive approach of participants' narrative and deductive refinement by researcher's interpretation. Data were analyzed by using a codebook, which was prospectively developed through engagement with the dataset. All themes (shared meanings) were obtained directly from the data for interpretation and analytic narrative. Representative excerpts were identified to illustrate each theme.

RESULTS

A consecutive sample of 60 eligible dentists was recruited, of whom 53 responded to the survey, yielding a response rate of 88.3%. Seven dentists declined the study invitation because they did not wish to or had no time to answer the questionnaire.

Descriptive statistics of sociodemographic, professional and health characteristics of the participants (Table 1) showed that participants' ages ranged from 22 to 70 years (mean age 38.8±13.5 years), and time from dental graduation from 1 to 42 years. Most dentists had excellent or good general health, oral health, and mental health. A total 33 dentists (62.3%) reported taking medications for one or more health problems, such as hypertension, hypercholesterolemia, back pain, diabetes, etc.

Related to the participants' knowledge, attitudes,

Table 1. Respondents' sociodemographic, professional and health characteristics (n=53).

VARIABLE	Frequency	Percent	Mean	SD	95% CI
Sociodemographic variables					
Gender					
Male	21	39.6			
Female	32	60.4			
Age (years)					
22 to 40	28	52.8			
41 to 70	25	47.2			
Ethnic group					
White	33	62.3			
Black/Mulatto	6	11.3			
Asian	14	26.4			
Professional variables					
Time from dental graduation (years)			14.7	12.8	10.7 – 17.3
Dental post-graduate degree					
Yes	29	54.7			
No	24	45.3			
Workplace (as a dentist)					
Private practice only	40	75.4			
Private practice and university	9	17.0			
Private practice and public service	2	3.8			
Only public service or university	2	3.8			
Health variables					
Perception of general health					
Excellent – Good	47	88.7			
Average	6	11.3			
Bad – Very poor	0	0			
Perception of oral health					
Excellent – Good	52	98.1			
Average	1	1.9			
Bad – Very poor	0	0			
Perception of mental health					
Excellent – Good	41	77.4			
Average	7	13.2			
Bad – Very poor	3	9.4			

and practices regarding phytotherapy in dentistry (Table 2), a few dentists reported formal education (courses, classes) in phytotherapy for oral health care (2) or use of herbal medicine in general (4). Most dentists (33) had informal knowledge from family, friends, neighbors, and colleagues. A total 21 dentists reported using phytotherapy for personal health issues. Out of 17 dentists who prescribed phytotherapy, 14 reported doing so occasionally, and only one prescribed herbal medicine frequently. Table 3 shows the results of exploratory inferential statistics for phytotherapy prescription by dentists as a function of the variables 'gender', 'age', 'post-graduate degree', 'formal education in phytotherapy', and 'personal use of phytotherapy'. Bivariate Fisher's exact tests showed significant effect for the binary variables 'age', 'post-graduate

degree', and 'personal use of phytotherapy'. In the final logistic regression model (P=0.002; Cox & Snell R^2=0.312), 'post-graduate degree completed' and 'personal use of phytotherapy' were significant for the outcome 'phytotherapy prescription'. Dentists with post-graduate degrees were 7.2 times more likely to prescribe phytotherapy than those without. In relation to 'personal use of phytotherapy', dentists who used herbal medicine were 6.1 times more likely to be prescribe phytotherapy than those who did not.

A total 21 dentists (39.6%) reported using herbal medicine, and 12 described the specific plant and health indication. The most frequently cited plants for personal use were chamomile, lemongrass and passionfruit. Seventeen dentists (32.1%) reported prescribing phytotherapy for their patients, and seven

Table 2. Respondents' knowledge, attitudes and practices regarding phytotherapy in dentistry.

ITEM	FREQUENCY (%)		
	Yes	No	Do not know / no response
Knowledge			
Formal education in clinical phytotherapy for oral health care (graduate and/or post-graduate courses in dentistry)	2 (3.8)	50 (94.3)	1 (1.9)
Formal education on herbal medicine and medicinal plants (extension courses, events)	4 (7.5)	40 (75.5)	9 (17.0)
Informal knowledge on herbal medicine and medicinal plants (from family, friends, colleagues, etc.)	33 (62.3)	11 (20.7)	9 (17.0)
Self-learning knowledge on clinical phytotherapy (from the literature, internet, videos, etc.)	9 (17.0)	35 (66.0)	9 (17.0)
Knows that there is a potential risk of using herbal medicine or medicinal plant concurrently with synthetic drug	19 (35.85)	19 (35.85)	15 (28.3)
Attitudes			
Personal use of herbal medicines and medicinal plants	21 (39.6)	32 (60.4)	0
Believes that herbal medicine is better than synthetic drug for			
Cost	21 (39.6)	9 (17.0)	23 (43.4)
Ease of procurement	7 (13.2)	22 (41.5)	24 (45.3)
Safety	17 (32.1)	10 (18.9)	26 (49.0)
Efficacy	4 (7.55)	19 (35.85)	30 (56.6)
Speed of effect	1 (1.9)	21 (39.6)	31 (58.5)
Duration of effect	5 (9.4)	16 (30.2)	32 (60.4)
Practices			
Asks dental patients whether they use any medicinal plant or herbal medicine (in clinical anamnesis)	3 (5.7)	50 (94.3)	0
Prescribes phytotherapy for oral health care at the clinics	17 (32.1)	36 (67.9)	0

Table 3. Results of bivariate tests and multivariable logistic regression model for the outcome measure 'phytotherapy prescription' by dentists (n=53).

VARIABLE		Phytotherapy prescription (Yes)		Fisher's exact test		Logistic regression model	
		N	%	Odds ratio	P	Odds ratio (95%IC)	P*
Gender	Male	5	29.4	.		.	
	Female	12	70.6	0.640	0.374	3.022 (-0.460;2.671)	0.166
Age (years)	22 to 40	5	29.4	.		.	
	41 to 70	12	70.6	1.417	0.037	1.417 (-1.256;1.953)	0.670
Post-graduate	No	3	17.6	.		.	
	Yes	14	82.4	1.841	0.008	8.261 (0.266;3.957)	0.025
Education in phytotherapy	No	14	82.4	.		.	
	Yes	3	17.6	0.809	0.379	1.205 (-1.853;2.225)	0.858
Personal use of phytotherapy	No	5	29.4	.		.	
	Yes	12	70.6	1.930	0.002	7.120 (0.444;3.482)	0.011

*Wald test

described some details of herbal prescription. The most frequently prescribed plants were passionfruit and mallow. Only two dentists reported adverse effects when patients used prescribed phytotherapy: one patient had a drop in blood pressure, while the other had stained teeth after treatment for bad breath and aphtha. Table 4 presents the description of herbal medicines and medicinal plants used by the participants for personal reasons and professional prescription.

Table 5 shows the themes obtained from participants' opinions on how to improve the rational use of

Table 4. Respondents' reports on herbal medicines and medicinal plants (in alphabetical order) for personal use and clinical prescription at dental clinics.

HERBAL MEDICINE / MEDICINAL PLANT Popular name (Scientific name)	INDICATION FOR USE	PRODUCT	POSOLOGY
Personal use			
Anise (*Pimpinella anisum*)	Insomnia, anxiety	Infusion	Continuous use
Bilberry (*Peumus boldus*)	Gastritis, reflux, pain	Infusion	During event 5-10 days
Chamomile (*Matricaria chamomilla*)	Insomnia, anxiety Gastritis, reflux, pain	Infusion	Daily, 1 per day Continuous use 5-10 days
Maytenus (*Maytenus ilicifolia*)	Gastritis, reflux, pain	Infusion	During event
Garlic (*Allium sativum*)	Fever, cold, sore throat	Capsule	2 months
Ginger (*Zingiber officinale*)	Fever, flu, cold, sore throat Increase immunity Weight loss	Decoction	During event
Ginseng (*Panax ginseng*)	Increase immunity Weight loss		
Green tea (*Camellia sinensis*)	Increase immunity Weight loss	Tea	Daily, 1 per day Continuous use
Lavender (*Lavandula sp*)	Insomnia	Oil	
Lemongrass (*Melissa officinalis*)	Insomnia, anxiety Gastritis, reflux, pain Fever, cold Increase immunity	Infusion	Daily, 1 per day During event 5-10 days
Linseed (*Linum usitatissimum*)	Xerostomia, hyposalivation	Oil	
Passionfruit (*Passiflora incarnata*)	Insomnia, anxiety	Infusion Industrialized tablet	Daily, 1 per day During event
Sunflower (*Helianthus annuus*)	Cutaneous wound	Oil	
Wolfberry (*Lycium barbarum, Lycium chinense*)	Increase immunity Weight loss		
Prescription for dental patient			
Arnica (*Arnica montana*)	Post-surgery care, toothache	Industrialized tablet	2 per day
Lemongrass (*Melissa officinalis*)	Burning mouth, mucositis, stomatitis, aphtha, ulcer	Infusion (7 leaves in 1 L water)	Mouthwash (cold)
Mallow (*Malva sylvestris*)	Gingival inflammation, mucositis, stomatitis, aphtha, ulcer	Industrialized solution	Mouthwash
Passionfruit (*Passiflora incarnata*)	Pre-treatment anxiety	Industrialized tablet, solution	2 per day; or one tablet the night before and another one hour before the dental session
Sweet potato leaf (*Ipomoea batatas*)	Gingival inflammation, toothache	Infusion	Mouthwash, 2 per day

phytotherapy in dentistry, as well as representative excerpts of data. Twenty participants expressed their opinions on the subject, from which three themes were derived: 1) 'Evidence on effectiveness, benefits and risks', representing the importance of sound evidence from scientific research and traditional practices; 2) 'Specific education in graduate and post-graduate courses', denoting the need for formal education on the topic during academic courses; and 3) 'Improved communication to dental

Table 5. Themes derived from respondents' opinions to improve the rational use of phytotherapy in dentistry and some representative excerpts.

THEMES	Representative excerpts of respondents' opinions
Evidence on effectiveness, benefits and risks (11 code citations)	*(more) Studies, articles that prove the effect, the results.* *Awareness of the benefits* (of phytotherapy) *compared to synthetic drugs.*
Specific knowledge education in graduate and post-graduate courses (8 code citations)	*Need for dental undergraduate classes and postgraduate courses in pharmacology about this topic.* *Address this subject during the academic course, indicating its effectiveness for dental treatment.*
Improved communication to dental practitioners (8 code citations)	*We need greater dissemination about its use and application.* *This subject should be publicized by the Dental Councils, providing more information for us, dental clinicians, in the daily clinics.*

practitioners', meaning that sustained dissemination of qualified information to professionals is a priority.

DISCUSSION

This exploratory study found that almost one third of the respondents reported prescribing herbal medicine in their clinical practice. Dentists with post-graduate degrees and personal use of phytotherapy were more likely to prescribe herbal medicine to their patients, regardless of formal education on the subject. Phytotherapy was mostly prescribed to control patients' anxiety before their dental visit, and for painful intraoral complaints in mucosa, gingiva or tongue. In addition, sociocultural factors seemed to drive dentists' attitudes and practices regarding phytotherapy, as most of them had informal knowledge on herbal medicine from family, friends, neighbors and colleagues. These findings are important to understand the use of phytotherapy by dental practitioners since the literature contains little information on prescription of herbal medicine as a first-line or complementary treatment in dentistry. To the best of authors' knowledge, no previous study has conducted an in-depth assessment of clinical dentists' knowledge, attitudes and practices in phytotherapy. Previous surveys have explored the opinions of some dentists on phytotherapy, such as the use of herbal medicine in public primary health care[15,18,19], and awareness of potential adverse drug reactions[13]. Other studies have evaluated dental practitioners' knowledge and practice in complementary and alternative medicine (CAM)[14,17], but not specifically in phytotherapy. In southern India, a country with ancient tradition in herbal medicine, 30% of dentists reported practicing alternative medicine in dentistry, including

phytotherapy[14]. In Germany, where naturopathy and herbal industry are strong areas, medicinal plants were the first four CAM procedures cited by clinical dentists, reaching up to 64% of recommendations[17]. Among the assessed variables, personal use of herbal medicine was the main factor associated with dental prescription of phytotherapy. A preceding study also reported the dominant influence of personal beliefs and practices on the teaching of herbal medicine by full-time faculty of health sciences, including dentistry[16]. In the present sample, almost 40% reported personal use of phytotherapy for common but specific health problems such as insomnia, anxiety, gastritis, colds and low immunity. Likewise, dentists reported prescribing herbal medicine for their patients occasionally, mainly for behavioral and symptomatic reasons. The most frequently cited medicinal plants for dental prescription were chamomile, passionfruit, mallow and lemongrass, which are widely used as traditional medicine by the general public as well[18]. Other traditional medicinal plants recommended by German dentists were *Arnica montana*, clove, *Salvia officinalis*, tea tree and *Aloe vera*[17].

Having a post-graduate degree was another positive factor associated with dental prescription of phytotherapy. Time from dental graduation and formal education in phytotherapy were not significant factors. One possible explanation is that dentists with advanced dental education may be more open to adopting phytotherapy, which has not been regularly taught in academic healthcare courses[15,18] despite the increasing educational efforts in many countries[1]. Few dentists had received formal education in phytotherapy during their academic courses or continuing education, or from

self-learning for general or oral health purposes. On the contrary, informal knowledge on herbal medicine and medicinal plants (from family, friends, colleagues) was the most frequent source. This can be an additional explanation for the fact that most prescribed medicinal plants for dental patients were also commonly used as traditional medicine in the community[18].

Limited specific knowledge on herbal medicine in dentistry was evident from the findings of the present study. This may act as a barrier for the rational use of phytotherapy in clinical practice. Most dentists did not know or did not respond whether they considered herbal medicine to be better than synthetic drugs in relation to cost, ease of procurement, safety, efficacy, or speed or duration of effect. In addition, about 36% of participants did not know that there is a potential risk of using herbal medicine or medicinal plant concurrently with a synthetic drug. This figure is similar to the number of Indian dentists who were not aware of potential adverse reactions to herbal medicines[13], which can be a serious medical problem[3,4] and affect surgical procedures[11,12]. In Brazil, dentists working in the public primary healthcare sector also reported that they were not confident in prescribing phytotherapy because they lacked specific information or training[15,18,19].

In the qualitative part of this study, the respondents offered insight into the problems, needs and solutions involved in improving the rational use of phytotherapy in dentistry. The three themes identified from dentists' opinions reflect a triad of joint, concurrent actions needed: 'Evidence on effectiveness, benefits and risks', 'Specific knowledge education in graduate and post-graduate courses', and 'Improved communication to dental practitioners'. Ideally, reliable content on phytotherapy should be learned during academic education and widely disseminated to professionals. However, there is a translational gap between the production of scientific knowledge and its effective use in clinical practice.

Firstly, most dental schools do not include phytotherapy content systematically in their syllabus. Formal education during academic courses is essential to build the professional foundations of clinical practice, but scientific findings are continuously evolving and changing clinical protocols over time. Therefore, over their careers, clinicians should update their knowledge with evidence-based information from reliable sources. However, regarding the way they seek information, most dental practitioners prefer to fill a gap in their knowledge by resorting to informal sources, mainly consulting a colleague or using a general internet search[22,23]. The indexed literature has provided summarized scientific evidence on the use of herbal medicine in dentistry[8,9], but clinicians may not search for and apply this information in practice. Indeed, most dental practitioners have reported lack of time to keep updated with the latest scientific articles, and low confidence level for using evidence-based search[22,23]. This situation can be more problematic for professionals who do not have access to subscription scholarly journals or are not proficient in the language of the publications.

To bridge the scientific translational gap on a large scale, the third theme 'Improved communication to dental practitioners' focused on dissemination of practical knowledge should be taken up by dental professional organizations, as mentioned by one participant. Official channels of communication can help disseminate evidence-based content to be used directly in dental practice, such as updated news and clinical practice guidelines with rigorous methodology[24]. Dissemination and implementation of research is a top priority in behavioral and social oral health sciences to tackle the evidence-to-practice gap and improve oral health education, promotion and care, as well as continuous professional training[25,26]. It is noteworthy that this sample consisted of dentists who were seeking professional development in clinical courses to treat adult patients with complex oral rehabilitation needs. However, many patients may not disclose the use of over-the-counter medications, herbal medicine, or dietary supplements in medical and dental visits[27], while healthcare professionals may not be aware of the importance of this subject. In fact, 94% of the respondents did not ask their patients whether they used any medicinal plant. To help practitioners improve their routine procedures in dental patient anamnesis, four specific questions on medicinal plants and herbal medicines have been suggested, based on the study findings, scientific literature, and researchers' experience (Table 6). The aims of these questions are to enquire about patients' use of such products, and to raise awareness on the importance of reporting it before and during dental treatment.

Table 6. Clinical practice recommendation of specific questions on use of medicinal plants and herbal medicines for dental patient's anamnesis.

PROPOSED QUESTION	RATIONALE
Do you use any natural product, herbal medicine, medicinal plant (such as leaves, roots, and flowers) or dietary supplement? It can be in any form, such as tea or infusion, extract, oil, pills, etc.	It is important to be specific in asking patients about any form of internal or external use. Some people may not remember during dental anamnesis that they use some traditional medicine.
If YES, please explain WHY, HOW and WHEN you use it.	Indication and posology are important to assess potential risks for dental treatment and further research, if appropriate.
If YES, did you receive a prescription or recommendation from someone or learn by yourself?	To ascertain whether there is a professional prescription, traditional practice of other source of information.
Are you aware that any medication, including herbal medicine and dietary supplements, can have adverse effects or interfere with your dental treatment?	To alert patients about the potential health risks of any medication or traditional product used, as well as the need to report their use to the dentist.

A limitation of this preliminary study is the small number of respondents from a single location, which does not allow the findings to be generalized to the entire population of clinical dentists. However, this sample consisted of real-world dental practitioners and should be more homogeneous than samples obtained in online surveys. Moreover, as far as we know, this was the first study that collected quantitative and qualitative research data on dentists' knowledge, attitudes and practices regarding phytotherapy. This comprehensive information was analyzed by the research team, which consisted of three members specializing in complementary and integrative medicine (clinical phytotherapy and acupuncture), and two experts in health social sciences.

The outcomes of this exploratory study will contribute to refine the research model and instrument for large-scale application among dental practitioners and clinical staff. A further step is to design a participatory research project to allow front-line dental clinicians engage with scientific knowledge production and use in practice. In addition, it would be useful to conduct a prospective study with implementation of education activities on phytotherapy in dentistry using digital technologies. To gain capillarity and reach the end-user clinicians, it will be necessary to build sustained collaboration with dental associations and professional councils to broadly disseminate evidence-based information and good practices on the rational use of phytotherapy in oral healthcare.

CONCLUSION

In summary, the results suggest that dentists with post-graduate degrees and who use herbal medicine for their own health issues also prescribe phytotherapy for dental patients. Most dentists perceive a lack of specific information about herbal medicine during their academic training and from professional sources in dentistry. Therefore, professional behavior and the practice of phytotherapy in dental clinics seem to reflect personal experience with general health rather than formal education and scientific knowledge on the subject. The implementation of scientific initiatives can help solve the evidence-to-practice gap.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

REFERENCES

1. World Health Organization. WHO global report on traditional and complementary medicine 2019. Geneva: World Health Organization; 2019. https://apps.who.int/iris/bitstream/handle/10665/312342/9789241515436-eng.pdf?sequence=1&isAllowed=y

2. World Health Organization. WHO traditional medicine strategy: 2014-2023. Geneva: World Health Organization; 2013. 9789241506090_eng.pdf (who.int)

3. Enioutina EY, Salis ER, Job KM, Gubarev MI et al. Herbal Medicines: challenges in the modern world. Part 5. status and current directions of complementary and alternative herbal medicine worldwide. Expert Rev Clin Pharmacol 2017;10(3):327-338. https://doi.org/10.1080/17512433.2017.1268917 .

4. Williams CT. Herbal supplements: precautions and safe use. Nurs Clin North Am 2021;56(1):1-21. https://doi.org/10.1016/j.cnur.2020.10.001

5. Huang CY, Wu MY, Chang CL, Liao YC et al. Coprescription trends in western medicine, Chinese herbal medicine and dental medicine among older adults in Taiwan from 1997 to 2013. Complement Ther Med 2021; Dec 63:102782. https://doi.org/10.1016/j.ctim.2021.102782

6. Shinkai RS, Hatch JP, Schmidt CB, Sartori EA. Exposure to the oral side effects of medication in a community-based sample. Spec Care Dentist 2006;26(3):116-20. https://doi.org/10.1111/j.1754-4505.2006.tb01434.x

7. Jerez-Roig J, Medeiros LF, Silva VA, Bezerra CL et al. Prevalence of self-medication and associated factors in an elderly population: a systematic review. Drugs Aging 2014;31(12):883-96. https://doi.org/10.1007/s40266-014-0217-x

8. Chinsembu KC. Plants and other natural products used in the management of oral infections and improvement of oral health. Acta Trop 2016; Feb 154:6-18. https://doi.org/10.1016/j.actatropica.2015.10.019

9. Moghadam ET, Yazdanian M, Tahmasebi E, Tebyanian H et al. Current herbal medicine as an alternative treatment in dentistry: In vitro, in vivo and clinical studies. Eur J Pharmacol. 2020 Dec 15;889:173665. https://doi.org/10.1016/j.ejphar.2020.173665

10. Muniz IAF, Campos DES, Shinkai RSA, Trindade TGD et al. Case report of oral mucosa garlic burn during COVID-19 pandemic outbreak and role of teledentistry to manage oral health in an older adult woman. Spec Care Dentist 2021;41:639-643. https://doi.org/10.1111/scd.12605

11. Akram M, Rashid A. Anti-coagulant activity of plants: mini review. J Thromb Thrombolysis 2017;44:406-411. https://doi.org/10.1007/s11239-017-1546-5

12. Vahabi S, Eatemadi A. Phyto-anesthetics: A mini-review on herb-anesthesia drug interactions. Biomed Pharmacother 2016;84:1885-1890. https://doi.org/10.1016/j.biopha.2016.10.100

13. Khan SA, Goyal C, Tonpay SD. A study of knowledge, attitudes, and practice of dental doctors about adverse drug reaction reporting in a teaching hospital in India. Perspect Clin Res 2015;6:144-9. https://doi.org/10.4103/2229-3485.159938

14. Suganya M, Vikneshan M, Swathy U. Usage of complementary and alternative medicine: A survey among Indian dental professionals. Complement Ther Clin Pract 2017;26:26-29. https://doi.org/10.1016/j.ctcp.2016.11.005

15. Fontenele RP, Sousa DMP de, Carvalho ALM, Oliveira F de A. Fitoterapia na Atenção Básica: olhares dos gestores e profissionais da Estratégia Saúde da Família de Teresina (PI), Brasil. (Phytotherapy in Primary Health Care: perspectives of managers and professionals in the Family Health Program of Teresina, Piauí, Brazil). Cien Saude Colet 2013;18:2385-94. https://doi.org/10.1590/s1413-81232013000800023

16. Dougherty K, Touger-Decker R, O'Sullivan Maillet J. Personal and professional beliefs and practices regarding herbal medicine among the full time faculty of the Newark-based schools of the university of medicine and dentistry of New Jersey. Integr Med 2000;2:57-64. https://doi.org/10.1016/s1096-2190(00)00012-3

17. Baatsch B, Zimmer S, Rodrigues Recchia D, Büssing A. Complementary and alternative therapies in dentistry and characteristics of dentists who recommend them. Complement Ther Med 2017;35:64-69. https://doi.org/10.1016/j.ctim.2017.08.008

18. Oliveira SG, de Moura FR, Demarco FF, Nascente Pda S et al. An ethnomedicinal survey on phytotherapy with professionals and patients from Basic Care Units in the Brazilian Unified Health System. J Ethnopharmacol 2012;140:428-37. https://doi.org/10.1016/j.jep.2012.01.054

19. Mattos G, Camargo A, Sousa CA, Zeni ALB. Plantas medicinais e fitoterápicos na Atenção Primária em Saúde: percepção dos profissionais [Medicinal plants and herbal medicines in Primary Health Care: the perception of the professionals]. Cien Saude Colet. 2018 Nov;23(11):3735-3744. Portuguese. https://doi.org/10.1590/1413-812320182311.23572016

20. Braun V, Clarke V. Using thematic analysis in psychology, Qual Res Psychol 2006; 3(2):77-101. https://doi.org/10.1191/1478088706qp063oa

21. Braun V, Clarke V. Is thematic analysis used well in health psychology? A critical review of published research, with recommendations for quality practice and reporting. Health Psychol Rev 2023 Jan 19:1-24. https://doi.org/10.1080/17437199.2022.2161594

22. Isham A, Bettiol S, Hoang H, Crocombe L. A Systematic Literature Review of the Information-Seeking Behavior of Dentists in Developed Countries. J Dent Educ 2016;80:569-77. https://doi.org/10.1002/j.0022-0337.2016.80.5.tb06117.x

23. Isett KR, Doshi AM, Rosenblum S, Eller W et al. Temporal search persistence, certainty, and source preference in dentistry: Results from the National Dental PBRN. PLoS One 2022;17(5):e0264913. https://doi.org/10.1371/journal.pone.0264913

24. London SD, Chamut S, Fontelo P, Iafolla T et al. Assessment of the Quality of Current American Dental Association Clinical Practice Guidelines. JDR Clin Trans Res 2023;8:178-187. es. JDR Clin Trans Res. 2023 Apr;8(2):178-187. https://doi.org/10.1177/23800844221083563

25. McNeil DW, Randall CL, Baker S, Borrelli B et al. Consensus Statement on Future Directions for the Behavioral and Social Sciences in Oral Health. J Dent Res 2022;101:619-622. https://doi.org/10.1177/00220345211068033

26. Randall CL. Dissemination and implementation research for oral and craniofacial health: Background, a review of literature and future directions. Community Dent Oral Epidemiol 2023;51:119-132. https://doi.org/10.1111/cdoe.12841

27. Bakuri S, Lanning SK, Best AM, Sabatini R et al. Dental patients' self-reported use of dietary supplements on medical history questionnaires. Gen Dent 2016;64:72-76.

Efficacy of Roncolab mobile application for diagnosing the primary sign of sleep-disordered breathing (snoring) in children

Gerardo Aragón-Villalba[1] , Gabriel Muñoz-Quintana[1] , Gisela N Rubin de Celis[1] , Jose M Torres-Hortelano[2] , Irene A Espinosa de Santillana[1]

1. Benemérita Universidad Autónoma de Puebla, Estomatología, Terminal Pediatría Maestría en Estomatología, Puebla México.
2. Sociedad Española de Disfunción Craneomandibular y Dolor Orofacial, Barcelona, España.

ABSTRACT

*Sleep-disordered breathing (SDB) is a group of disorders associated with breathing anomalies during sleep. Easily detectable by sound, snoring is one of the most common manifestations and the main sign of SDB. Snoring is characteristic of breathing sound during sleep, without apnea, hypoventilation, or interrupted sleep. It may reduce the percentage of sleep and increase microarousals due to breathing effort or gas exchange. A range of questionnaires have been validated and adapted to the pediatric population to screen for patients who require laboratory testing. The Pediatric Sleep Questionnaire (PSQ) screens for SDB and identifies primary signs such as snoring. RoncoLab is a mobile application that records and measures snoring intensity and frequency. **Aim:** To compare the RoncoLab app and the PSQ regarding how efficiently they diagnose snoring. **Materials and Method:** This was an observational, analytical study of 31 children aged 7 to 11 years who visited the pediatric dental clinic at Benemérita Universidad Autónoma de Puebla, Mexico (BUAP). The PSQ was applied to diagnose SDB. Guardians were then instructed on how to download and use the mobile application to record data while the child was sleeping at home. Agreement between RoncoLab and the PSQ was analyzed statistically by Cohen's Kappa index at 95% confidence level. **Results:** The Kappa index for identification of primary snoring was 0.743 (p<0.05). App sensitivity was 0.92, and specificity 0.82. **Conclusion:** There is good agreement between PSQ and RoncoLab for diagnosing primary snoring, with acceptable sensitivity and specificity.*

Keywords: sleep disorders - children - snoring - validity of results - mobile applications.

Eficacia de la aplicación móvil Roncolab para el diagnóstico de signo primario de trastorno respiratorio del sueño (ronquido) en niños.

RESUMEN

*Los trastornos respiratorios del sueño (TRS) son un grupo de padecimientos asociados con anormalidades respiratorias del sueño. Una de las manifestaciones más comunes es el ronquido; signo fácil de detectar por el ruido que emite y se considera como el signo principal. Este trastorno es característico del ruido respiratorio durante el sueño, sin apneas, hipoventilación, ni interrupciones del sueño. Este puede ocasionar disminución del porcentaje del sueño y aumentar los microdespertares, esto debido al esfuerzo respiratorio o al intercambio de gases. En la actualidad existen cuestionarios validados y adaptados para la población pediátrica útiles como herramienta clínica para el tamizaje y selección de pacientes que requieran pruebas de laboratorio. El Pediatric Sleep Questionnaire (PSQ) permite el cribado de TRS e identificación de signos primarios como el ronquido. RoncoLab es una aplicación móvil que registra y mide la intensidad y frecuencia del ronquido. **Objetivo:** Comparar la eficacia del diagnóstico del ronquido por medio del RoncoLab contrastado con el PSQ. **Materiales y Método:** Estudio observacional, analítico, en el cual se incluyeron 31 niños de 7 a 11 años, que acudieron a la clínica de odontopediatría de la Benemérita Universidad Autónoma de Puebla, México (BUAP) Se aplicó el PSQ a los 31 niños para el diagnóstico de TRS, después se le instruyó al tutor como descargar y utilizar la aplicación móvil para registrar los datos obtenidos en las horas de sueño en casa. El análisis estadístico de concordancia entre los instrumentos diagnósticos se realizó con el Índice Kappa de Cohen a un nivel de confianza del 95%. **Resultados:** El Índice de Kappa para la identificación de los ronquidos primarios fue de 0.743 (p<0.05). La sensibilidad de la aplicación fue de 0.92, mientras la especificidad fue de 0.82. **Conclusión:** Existe buena concordancia entre el PSQ y el RoncoLab en el diagnóstico de ronquido primario, con sensibilidad y especificidad aceptable.*

Palabras clave: trastornos del sueño - niños - validación de resultados - aplicación móvil.

INTRODUCTION

Sleep is a complex physiological and behavioral process that enables people to disconnect from the external environment[1]. Healthy people usually fall asleep 20 minutes after lying down. It has been reported that 20% to 30% of people have sleeping difficulties[2]. Normal sleep is important to development at all stages of life. On average, a person spends one third of their life sleeping, reflecting the fact that sleep is essential to performing and completing important physiological functions that ensure complete physical and mental equilibrium throughout the day. Sleeping is key to physical and psychological development in childhood and adolescence[3,4].

Sleep-disordered breathing (SDB) is a group of different disorders involving breathing anomalies during sleep. Obstructive type SDBs are not a specific disease, but an upper airway dysfunction syndrome. In some cases, the breathing anomaly occurs while the patient is awake[4,5].

Lack of sleep is harmful to cognition, decision making, psychomotor function, mood and immune function. Poor sleep is a risk factor for cardiovascular disease, dementia, obesity, diabetes, depression, pain and mortality, among other disorders.

Snoring, the main sign of sleep-disordered breathing, is one of its most common manifestations. SDB comprises a variety of disorders, ranging from the simplest, which is habitual or primary snoring, all the way to sleep apnea-hypopnea syndrome (SAHS), including intermediate disorders such as upper airway resistance syndrome (UARS) and central hypoventilation syndrome[6].

Primary snoring is a characteristic disorder of breathing sound during sleep, without apneas, hypoventilation or interrupting sleep, although it does reduce sleep percentage and increase microarousals due to breathing effort or gas exchange effort. Snoring is a hoarse sound caused by obstruction of the air flow through the nose and pharynx. Structures with soft tissues (tongue, soft palate, uvula, tonsils, adenoids and pharynx wall) are highly collapsible, which places them in contact with each other and causes them to vibrate when someone breathes during sleep[7].

SDB and sleep apnea are well known in adults. However, they also occur in the pediatric population, with prevalence ranging from 7% to 17%, depending on how they are defined, and the instruments used in each study. In Mexico, the prevalence of child obesity has recently increased to the highest rate in the world, leading to higher prevalence of SDB, including disorders such as obstructive sleep apnea and primary snoring related to obesity. Timely diagnosis and management of SDB in children can prevent associated comorbidities[8-10].

Among the range of sleep-disordered breathing symptoms, primary snoring is the least harmful. Primary snoring is sometimes not considered either for diagnosis or for the possibility of providing treatment. It has been reported that more than one third of children with primary snoring develop sleep apnea-hypopnea within four years, and 7% develop a moderate to severe disease such as central hypoventilation syndrome. The most significant risk factor for progression of the disease is child obesity, for which prevalence is reported as 8% to 12%[11].

Among 3- to 6-year-olds, the main cause of snoring is physiological hypertrophy of adenoids and tonsils, which at these ages are larger in relation to airway diameter. Most snoring incidents during sleep are therefore expected at these ages[12].

The gold standard for diagnosing SDB and sleep apnea is assisted polysomnography (PSG) in the lab. However, it is expensive, time consuming, technically complex, and requires patients to sleep in the laboratory. In Mexico, there may be significant delays in the diagnosis and treatment of STB due to the limited availability of PSG. Nevertheless, simpler tools such as validated sleep questionnaires can also be used to detect SDB and have gained considerable clinical relevance for recording initial symptoms in epidemiological studies[13-15].

Sleep questionnaires are used as a subclinical tool to describe psychometric qualities in the field of sleep medicine to screen for signs, symptoms and factors that foster the development of SDB. Pediatric sleep questionnaires are mainly directed to parents. Most of these questionnaires for SDB screening, such as the *Pediatric Sleep Questionnaire* (PSQ), are long and tedious, so various mobile applications, such as *RoncoLab,* have been proposed for diagnosing SDB signs and symptoms.

The *RoncoLab* app is attractive, innovative and easy for parents to use[16-18]. It shows whether, how much and how long a patient snores; analyzes snoring; can be programmed to activate after a given number of minutes and can play background sound to help the patient fall asleep. It shows the different sleep analyses performed during the night and provides a list of possible remedies to snoring and a list of different factors that may foster the onset of snoring.

By default, *RoncoLab* record samples of the patient's snoring, as well as other sounds occurring during the night. Once the recording has been made, the app provides a graph showing silent and snoring times. It also records total sleep time and how long the patient has been in bed and assigns a score to patient snoring. It can play back the recorded snoring. The main aim of the application is to ascertain rest quality and snoring intensity.

The aim of this study was to compare the efficacy of the *RoncoLab* mobile app to that of the *Pediatric Sleep Questionnaire* for diagnosing the primary sign (snoring) of SDB in the pediatric population.

MATERIALS AND METHOD

This was an observational analytical study. Children aged 7 to 11 years, who had been referred to the postgraduate Pediatric Dentistry clinic at the School of Stomatology at Benemérita Universidad Autónoma de Puebla for a sleep study, were invited to participate. Prior to registration, the Ethics and Research Committee (2021160) obtained informed consent from the parents and oral and written assent from the children. Exclusion criteria were patients (1) under respiratory treatment, (2) under maxillary orthopedic treatment (maxillary expansion), (3) with intellectual disability, (4) under psychiatric treatment, or (5) with craniofacial anomalies. The PSQ for SDB diagnosis, validated and adapted to the pediatric population, was applied to the 31 children. The 22 questions were answered by a parent or legal guardian.

A snorer was defined as any child whose guardian provided affirmative answers to at least one of the 2 following questions in the questionnaire: 'Does your child snore more than half the time?' 'Does your child snore all the time?' Probable sleep-disordered breathing was identified when 8 or more questions were answered affirmatively (score <0.33) (Table 1). Any questions answered "No" o "Don't know" were not counted.

Table 1. Short version of the *Pediatric Sleep Questionnaire*

A. Behavior during the night and while sleeping:

When your child is sleeping, does he/she…

				Do not fill in this column
… snore more than half the time?	Yes	No	D.K.	A2
… snore all the time?	Yes	No	D.K.	A3
… snore noisily?	Yes	No	D.K.	A4
… breathe noisily or deeply?	Yes	No	D.K.	A5
… have problems or difficulty breathing?	Yes	No	D.K.	A6

Have you ever…

… noticed that your child stops breathing during the night?	Yes	No	D.K.	A7

Does your child…

… tend to breathe with his/her mouth open during the day?	Yes	No	D.K.	A24
… have a dry mouth when he/she wakes up in the morning?	Yes	No	D.K.	A25
… occasionally wet the bed?	Yes	No	D.K.	A32

B. Behavior during the day and other possible problems:

Does your child…

… wake up in the morning feeling tired?	Yes	No	D.K.	B1
… fall asleep during the day?	Yes	No	D.K.	B2
Has your child's teacher or any other carer ever mentioned that your child seems to be sleepy during the day?	Yes	No	D.K.	B4
Does your child find it difficult to wake up in the morning?	Yes	No	D.K.	B6
Does your child complain of headache in the morning upon waking up?	Yes	No	D.K.	B7
Has your child ever, since birth, had a "stop" in growth?	Yes	No	D.K.	B9
Is your child overweight (weigh more than normal for his/her age)?	Yes	No	D.K.	B22

C. Please mark the relevant box with an X → Your child…	Never	Some-times	Often	Nearly always	Do not complete this box
…does not seem to hear what people say to him/her.					C3
…has difficulty organizing his/her activities.					C5
…is easily distracted by irrelevant stimuli.					C8
…fidgets, moving hands and feet while seated.					C10
…is constantly on the go, as if drive by a motor.					C14
…interrupts the conversations or games of others.					C18

Table back-translated from *Tomas M. et al. 2007. Versión española del Pediatric Sleep Questionnaire. Un instrumento útil en la investigación de los trastornos del sueño en la infancia*. (Version in Spanish of the Pediatric Sleep Questionnaire. A useful instrument for researching childhood sleep disorders)

Then the parent or guardian was instructed on how to download and use the mobile application to record information while the child was sleeping at home.

The parent or guardian was instructed to use *RoncoLab* for two nights at home in the patient's bedroom, beginning on the day that the PSQ sleep questionnaire was answered. The mobile application was switched on when the patient went to bed and began to rest in a quiet environment isolated from any other area or object that emitted sounds that could alter the records. *RoncoLab* was programmed to activate after the first 20 minutes, corresponding to the beginning of the deep sleep phase. Snoring was recorded throughout all the patient's sleeping hours.

RoncoLab defined as a snorer any child who snored for more than 15 minutes. (Figs. 1 a-b).

Cross tabulation was used to calculate *RoncoLab* sensitivity and specificity.

Statistical analysis was performed on IBM SPSS version 22. Agreement between diagnostic instruments was analyzed using Cohen's Kappa index at 95% confidence interval, and considered insignificant if the result was 0.0 to 0.2, low for 0.2 to 0.4, moderate for 0.4 to 0.6, good for 0.6 to 0.8 and very good for 0.8 to 1.0.

RESULTS

A total 31 children and their parents signed consents and assents, respectively, and joined the study, answered the questionnaire and used the mobile application between August 2022 and December 2022. There were 20 boys (64.5%) and 11 girls. (35.5%). Mean age was 8.8 years.

Screening prevalence for sleep-disordered breathing among Mexican children was 42% according to the Pediatric Sleep Questionnaire (PSQ) validated and adapted for SDB screening in the pediatric population. The *RoncoLab* application identified 51.6% of the patients as snorers, while the PSQ identified 45.2%.

RoncoLab sensitivity was .92, and specificity was .82. Data were recorded on the presence of the primary sign of SDB (Table 2). The Kappa index for identification of primary snoring between the PSQ
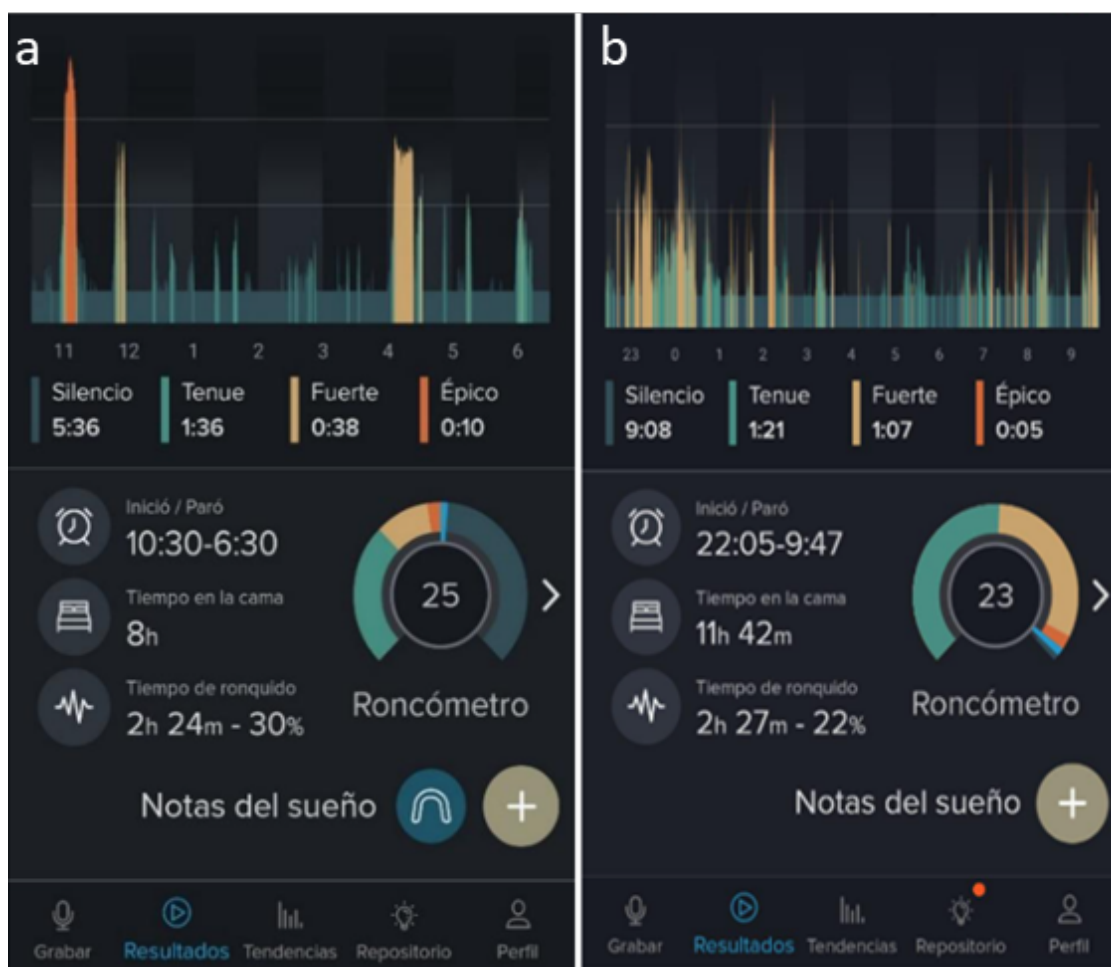

Fig. 1a-b: Screenshots of the RoncoLab application provided by parents.

Table 2. Screening for primary sign with RoncoLab. Snoring.

Snores		Frequency	Percentage (%)
	Yes	16	51.6
	No	15	48.4
	Total	31	100

Table 3. Agreement in diagnosis of primary snoring. *Pediatric Sleep Questionnaire vs. RoncoLab*

		PSQ		Total	Kappa Value	P Value
		YES	NO			
RoncoLab	YES	13	3	16	.743	<.000
	NO	1	14	15		
Total		14	17	31		

and the *RoncoLab* mobile application was 0.743 with p<0.05 (Table 3).

DISCUSSION

Although validation is required, the *RoncoLab* mobile application may be implemented in diagnosis and screening for Sleep-Disordered Breathing, since it has been found to be efficient, useful and practical for diagnosing snorers.

Among Mexican children, there is a high percentage of possible cases of sleep-disordered breathing compared to reports in the worldwide literature. In Argentina, Frairea A. et al.[10] used the PSQ to study a pediatric population and reported 7% SDB prevalence and 9.7% snorers.

It is therefore important to raise awareness and conduct further studies on SDB in the Mexican pediatric population using validated, adapted instruments to standardize figures. It is suggested to consider other factors such as body mass index, high blood pressure, place of origin, and ethnicity, among others, to help decipher the high percentage of probable SDB in Mexican children.

To conclude, agreement between PSQ and the *RoncoLab* application for diagnosing primary snoring in the pediatric population was good, displaying acceptable sensitivity and specificity.

BIBLIOGRAFÍA

1. Siegel JM. REM sleep: a biological and psychological paradox. Sleep Med Rev. 2011; 15(3):139–142. Siegel JM. REM sleep: a biological and psychological paradox. Sleep Med Rev. 2011; 15(3):139–142. https://doi.org/10.1016/j.smrv.2011.01.001
2. Hillman DR, Lack LC.Public health implications of sleep loss: the community burden. Med J Aust. 2013; 199(8): 7-10.https://doi.org/10.5694/mja13.10620
3. Sung V, Hiscock H, Sciberras E, Efron D. Sleep problems in children with attention-deficit/hyperactivity disorder: prevalence and the effect on the child and family. Arch Pediatr Adolesc Med. 2008 Apr;162(4):336-42. https://doi.org/10.1001/archpedi.162.4.336
4. Cruz Navarro I. El niño roncador (SAHS). Rev Pediatr Aten Primaria. 2014 ;(23):89-100. https://doi.org/10.4321/S1139-76322014000200013
5. Valarelli LP, Corradi AMB, Grechi TH, Eckeli AL, et al. Cephalometric, muscular and swallowing changes in patients with OSAS. J Oral Rehabil. 2018; 45:692-701. https://doi.org/10.1111/joor.12666
6. Shi L, Chen SJ, Ma MY, Bao YP, et al. Sleep disturbances increase the risk of dementia: A systematic review and meta-analysis. Sleep Med Rev. 2018 Aug; 40:4-16. https://doi.org/10.1016/j.smrv.2017.06.010

7. Yi Hua, How How Ch, Hwee Chan Y, et al. Approach to the snoring child. Singapur Med J. 2020;61(4):170-17. https://doi.org/10.11622/smedj.2020054
8. Eitner S, Urschitz MS, Guenther A, et al. Sleep problems and daytime somnolence in a German population-based sample of snoring school-aged children. J Sleep Res. 2007;16(1):96–101. https://doi.org/10.1111/j.1365-2869.2007.00560.x
9. Goodwin JL, Babar SI, Kaemingk KL, et al. Symptoms related to sleep-disordered breathing in White and Hispanic children: the Tucson children's assessment of sleep apnea study. Chest. 2003;124(1):196–203. https://doi.org/10.1378/chest.124.1.196
10. Frairea, J, Deltettoa, N, Catalanic F, Beneitez, E, et al. Prevalencia de trastornos respiratorios del sueño en adolescentes y su relación con la presencia de obesidad e hipertensión arterial. Arch Argent Pediatr. 2021; 119(4): 245-250. http://dx.doi.org/10.5546/aap.2021.eng.245
11. Chawla J, Waters KA. Snoring in Children. J Paediatr Child Health. 2015; 51:847–851. https://doi.org/10.1111/jpc.12976
12. Massicotte C, Al-Saleh S, Witmans M, et al. The utility of a portable sleep monitor to diagnose sleep-disordered breathing in a pediatric population. Can Respir J. 2014;21(1):31–35. https://doi.org/10.1155/2014/271061

13. Collop NA, Anderson WM, Boehlecke B, et al. Clinical guidelines for the use of unattended portable monitors in the diagnosis of obstructive sleep apnea in adult patients. Portable monitoring task force of the American Academy of Sleep Medicine. J Clin Sleep Med. 2007;3(7):737–747. https://doi.org/10.5664/jcsm.27032

14. Masoud AI, Jackson GW, Carley DW. Sleep and airway assessment: A review for dentists. CRANIO. 2017;35(4):206–222. https://doi.org/10.1080/08869634.2016.1228440

15. Chervin RD, Weatherly RA, Garetz SL, et al. Pediatric Sleep Questionnaire: prediction of sleep apnea and outcomes. Arch Otolaryngol Head Neck Surg. 2007;133(3):216–222. https://doi.org/10.1001/archotol.133.3.216

16. Spruyt K, Gozal D. Pediatric sleep questionnaires as diagnostic or epidemiological tools: a review of currently available instruments. Sleep Med Rev. 2011;15(1):19–32. https://doi.org/10.1016/j.smrv.2010.07.005

17. Chervin RD, Hedger K, Dillon JE, et al. Pediatric Sleep Questionnaire (PSQ): validity and reliability of scales for sleep-disordered breathing, snoring, sleepiness, and behavioral problems. Sleep Med. 2000;1(1):21–32. https://doi.org/10.1016/S1389-9457(99)00009-X

18. Tomas M. Millares A. Beseler B. Versión española del Pediatric Sleep Questionnaire. Un instrumento útil en la investigación de los trastornos del sueño en la infancia. Análisis de su fiabilidad. An Pediatr (Barc). 2007; 66(2):121-8. https://doi.org/10.1157/13098928

Langerhans cell histiocytosis oral lesions in pediatric patients

Verónica H Pavan[iD], Virginia Fernández de Preliasco[iD], Melisa Ienco[iD],
Carolina Benchuya[iD]

Universidad de Buenos Aires, Facultad de Odontología, Cátedra Odontologia Integral Niños, Ciudad Autónoma de Buenos Aires, Argentina.

ABSTRACT

*Langerhans cell histiocytosis (LCH) is a disease with unknown etiology. It presents as single-system (affecting a single organ or tissue) or as multisystem (with or without risk organ involvement). The oral cavity may be involved or be the site of the first manifestation. **Aim:** To describe, group, and determine the frequency of oral lesions in pediatric patients with LCH, and to relate these lesions to age and the different disease subtypes. **Materials and Method:** Clinical and radiographic examinations were used to evaluate 95 patients diagnosed with LCH, aged 0 to 16 years, who were referred to the Department of Comprehensive Pediatric Dentistry at the School of Dentistry, University of Buenos Aires. Clinical histories were prepared and informed consents obtained. Lesions were diagnosed by observation, palpation and biopsies, and grouped according to affected tissues into bone, mucosal, and bone-mucosal. **Results:** 42.1% presented oral lesions, and in 14.73%, these lesions were the first manifestation of LCH. Ninety percent presented only bone lesions, while the remaining 10% presented bone-mucosal and mucosal lesions. In the single-system subtype, 52.5% presented bone lesions. In the multisystem subtypes (with or without risk organs), all three types of lesions were found. The association between age at which LCH was diagnosed and oral tissue involvement showed that bone-mucosal lesions occur in young children (average age 1.4 years) diagnosed with multisystem LCH. Oral mucosa was only affected in reactivations of the disease. **Conclusions:** A high frequency of oral lesions was observed, which were sometimes the first manifestation of the disease, most often affecting bone tissue. Dentists can play an active role in the initial diagnosis of the disease.*

Keywords: Histiocytosis, Langerhans Cell - oral lesions, children

Lesiones bucales de Histiocitosis de Células de Langerhans en pacientes pediátricos

RESUMEN

*La Histiocitosis de células de Langerhans (LCH) (Langerhans cell histiocytosis) es una enfermedad de etiología aún desconocida. Se presenta en forma unisistémica (afecta un solo órgano o tejido) o multisistémica (con o sin órganos de riesgo afectados). La cavidad bucal puede estar comprometida o ser el sitio de la primera manifestación. **Objetivo:** describir, agrupar y determinar la frecuencia de las lesiones bucales de pacientes pediátricos con LCH, relacionarlas con la edad y los diferentes subtipos de la enfermedad. **Materiales y Método:** se evaluaron mediante exámenes clínicos y radiográficos 95 pacientes entre 0 y 16 años con diagnóstico de LCH, derivados a la Cátedra de Odontología Integral Niños, Facultad de Odontología, Universidad de Buenos Aires. Se confeccionaron historias clínicas y se obtuvieron los consentimientos informados. Las lesiones fueron diagnosticadas a través de observación, palpación y biopsias, y se agruparon según los tejidos afectados en óseo, mucoso y óseo-mucoso. **Resultados:** el 42.1% presentó lesiones bucales y en el 14.73% estas fueron la primera manifestación de LCH. El 90% mostró solo lesiones óseas, mientras que en el 10 % restante se observaron lesiones óseo-mucosas y mucosas. En el subtipo unisistémico el 52.5% presentó lesiones óseas. En los subtipos multisistémicos, "con" o "sin" órganos de riesgo, se hallaron los tres tipos de lesiones. La relación entre la edad de diagnóstico de LCH y el compromiso de tejidos bucales evidenció que las lesiones óseo-mucosas ocurren en niños pequeños (edad promedio 1.4 años) con diagnóstico de LCH multisistémica. La mucosa bucal solo se vio afectada en las reactivaciones de la enfermedad. **Conclusiones:** Se observó una alta frecuencia de lesiones bucales, siendo en ocasiones la primera manifestación de la enfermedad, afectando con mayor frecuencia al tejido óseo. El odontólogo puede desempeñar un rol activo en el diagnóstico inicial de la enfermedad.*

Palabras clave: Histiocitosis de células de Langerhans - lesiones bucales, niños

INTRODUCTION

Langerhans-cell histiocytosis (LCH) is an infrequent disease. Its pathogenesis is unknown but may be a dysregulation of the immune response as a result of inappropriate stimulation of the immune system or a myeloid neoplasm. It has been associated to a mutation of the gene BRAF 600 E and activation of ERK[1-3]. In 50 % to 90 % of the cases diagnosed, patients are between less than one year old to 15 years old[4], with an estimated annual incidence of 2 to 10 cases per million children[5].

LCH is characterized by the abnormal accumulation and proliferation of histiocytes, eosinophiles and pathological Langerhans cells, and may involve one or multiple tissues and systems. The cell infiltrate destroys the affected tissues[6].

In contrast to usual skin cells, pathological Langerhans cells appear round, functionally and immunologically immature, surrounded by eosinophiles, macrophages, lymphocytes, and sometimes giant multinucleate cells[5]. Diagnosis is performed by biopsy of the lesion to ascertain presence of Birbeck granules, and immunohistochemical confirmation by the markers CD1a, S100 or CD207. CD1a is specific to and distinctive of Langerhans cells[7].

Clinical manifestations include asymptomatic lesions, single lesions that remit, or multisystem disorders[8]. Bone lesions have been reported in 82% of patients with LCH. Lesions were generally associated to painful swelling, which could be the most frequent initial sign. The bones in which LCH was most frequently found were skull (27%), mandible and maxilla (11%), and the long bones femur (13%), humerus (5%) and tibia (3%)[9]. In the oral cavity, there may be lytic bone lesions in one or several areas, pathological mandibular fractures, pain, sores, periodontal involvement with marked tooth mobility, early tooth eruption, or mobility and premature loss of primary or permanent teeth[10-12].

According to the organs involved, the disease is grouped into three subtypes:

- Single-system (SS LCH): a single organ or tissue is involved.
- Multisystem with risk organs involved (MS LCH RO$^+$): Risk organs include liver, bone marrow, and any involved in the central nervous system such as skull and face bones (orbit, malar or maxillary bone). These lesions may extend to the soft intracranial tissue and are often associated with diabetes insipidus.
- Multisystem without risk organs involved (MS LCH RO$^-$): Lesions are located on skin, bone or lymph nodes[13].

New LCH lesions at the same site as was previously affected or at a different site from the original one is called reactivations. Their seriousness may be the same as or different from the initial lesion, and they may occur in the oral cavity[14,15].

There is no report in the literature on the grouping of lesions, association with age, or LCH subtypes. The aims of this study are therefore to group lesions according to the tissue involved (bone, bone-mucosa and mucosa), determine the frequency of oral lesions in pediatric patients with LCH, and relate them to age and the different subtypes of the disease.

MATERIALS AND METHOD

This study evaluated 95 patients with confirmed LCH diagnosis who were referred from different hospitals to the Department of Comprehensive Pediatric Dentistry at the School of Dentistry at Buenos Aires University (FOUBA).

Inclusion criteria were newborn to 16-year-old children with LCH diagnosis confirmed by pathohistological study, by presence of Birbeck granules and positive immunological makers CD1a and S100 or CD207 in cells from the lesion. Exclusion criterion was patients with non-Langerhans histiocytosis.

The following information was recorded in each patient's clinical history: personal data, medical and family background, including date of diagnosis and status of the disease (activity or remission). Informed consent was obtained and the procedures to be followed were explained to patients. This study was approved by the FOUBA Ethics Committee (CETICA N°006/2019), in keeping with the principles in the Declaration of Helsinki.

Diagnosis and location of oral lesions: Each patient underwent extraoral and intraoral clinical diagnosis. Panoramic radiographs were taken to detect any osteolytic lesions of the jaws and the degree of dental involvement in patients whose age and cooperation so allowed. Periodical radiographs were taken in cases where radiolucency or tooth mobility was observed. Axial or volumetric CT

scans were requested to ascertain lesion size and extension towards surrounding tissues.

Oral lesions were grouped according to affected tissue as follows: *1) bone lesions; 2) mucosal lesions,* and *3) bone-mucosal lesions.*

When oral lesions were present, biopsies were taken and the tissue sent to the Surgical Pathology Laboratory at the Department of Pathological Anatomy, FOUBA.

Statistical analysis

This was a retrospective, observational, cross-sectional study. Descriptive statistics were calculated, CI 95%, chi-square test and Kruskal Wallis test to compare LCH type groups, different types of oral lesions and ages.

RESULTS

The sample consisted of 95 patients, 56.84% male, average age at time of consultation with dentist 5.4 ± 3.7 years, average age at diagnosis of the disease 2.7 ± 2.9 years, while average ages at diagnosis for the different subtypes SS LCH, MS-RO$^+$ LCH and MS-RO$^-$ LCH were 4.06, 0.6 and 1.42 years, respectively; *(p < 0.001)*. Table 1 shows the distribution of patients according to disease subtypes and whether oral lesions were present. In the group with oral lesions, 35.0 % had SS LCH, 7.5 % had MS-RO$^+$ LCH, and 57.5 % had MS-RO$^-$ LCH (p <0.001).

Oral lesions were present in 42.1% of the patients:

1) Bone lesions: Closed osteolytic lesions within mandibular or maxillary bone that do not involve the oral mucosa. These lesions may produce facial swelling or pain (Figs. 1A and B).
2) Mucosal lesions: Pericoronitis in first permanent molars. During permanent tooth eruption there may be inflammation and pain in the marginal gum (Fig. 1C) with presence of a surrounding reddish halo. Pericoronitis persists until the tooth is fully erupted.
   2.a) Palatal mucosal lesion: Rash-like, flat erythematous lesion on palatal mucosa (Fig. 1D).
   2.b) Labial mucosal lesion: Lesion on labial mucosa with persistent, erosive or ulcerous loss of substance (Fig. 1E).

Table 1. Distribution of patients according to LCH subtype and presence of oral lesions

LCH Subtype	Patients with LCH (n=95)					
	Without oral lesions (n = 55) 57.8% CI 47.3 – 67.9			With oral lesions (n = 40) 42.2% CI 32.0- 52.7		
	N	%	CI 95%	N	%	CI 95%
SS LCH	30	54.5	40.7 - 67.8	14	35	20.5 – 51.7
MS-RO$^+$ LCH	3	5.5	1.4 - 16.1	3	7,5	1.5 – 20.4
MS-RO$^-$ LCH	22	40	27.3 - 54.1	23	57.5	40.8-72.9

3) Bone-mucosal lesions: Extensive lesions involving both jaws and compromising alveolar processes. Teeth lacking bone support present severe mobility, pain, pathological root resorption and/or exposed cement. These conditions may lead to spontaneous tooth loss. The osteolytic alveolar bone lesion may induce primary or permanent tooth eruption earlier than the normal sequence, without adequate crown calcification or with little root formation. Radiographs show radiolucent areas surrounding affected teeth (Figs. 1F and 1G).

Table 2 shows that, regarding lesions, 52.5 % were in bone tissue, 40 % in bone-mucosal tissues, and 7.5 % involved only mucosal tissues (p = 0.002).

Table 3 shows children's average age at the time of LCH diagnosis according to the oral tissue involved (p=0.212).

Bone and bone-mucosal lesions were found in lower jaw, followed by upper jaw. Mucosa of the palate, lip and jugal zone were less frequently affected.

Oral lesion was the first LCH manifestation detected in 14.73% of patients. Table 4 shows the oral tissues involved. The most frequent was bone tissue, with 85.8 % (p= 0.008). Within this group, 85.71 % of patients had SS LCH and 14.29 % had MS-RO$^-$ LCH. No initial lesions of the disease were recorded in the mouths of MS-RO$^+$ LCH patients (p = 0.008). Table 5 shows that in 10.52 % of total patients (n=10), oral lesion was the only manifestation of LCH, with bone tissue involvement in 90 %, bone-mucosal in 10 %, and mucosal alone in none (p = 0.011).
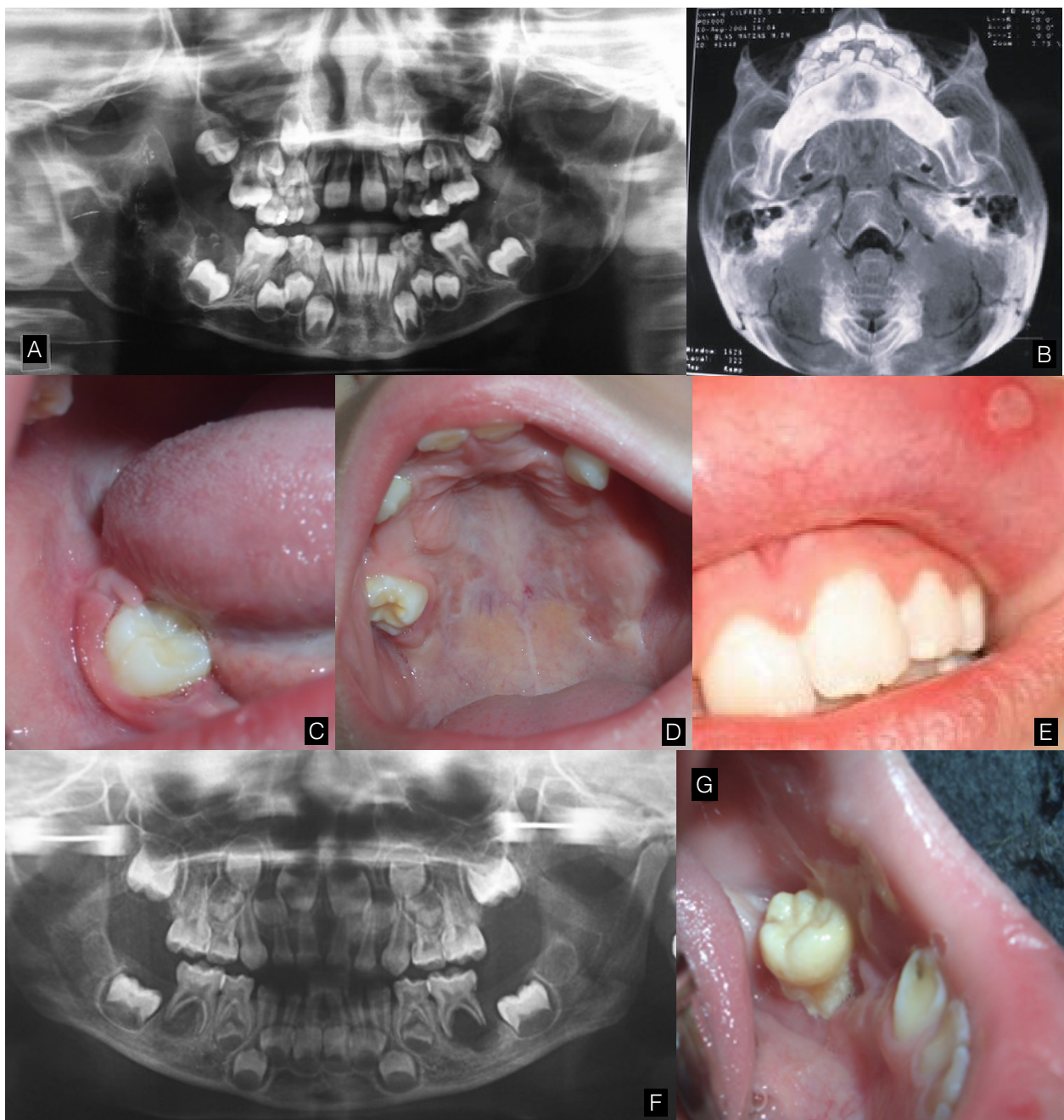
Fig. 1: HCL oral lesions. A) Bone lesions. Panoramic radiograph showing radiolucent areas compatible with osteolytic lesions on both jaws. B) CT scan: osteolysis visible in the mental area. Lesions on mucosa: C) Pericoronitis of tooth 4.6; D) Rash and erosion on palate, and E) Persistent erosive lesion on upper lip mucosa. F) Bone-mucosal lesions. Panoramic radiograph with radiolucent zones compatible with osteolytic lesions of the alveolar bone surrounding teeth 7.5 and 8.5. G) Photograph of tooth 7.5 without bone support, showing exposed root portion.

Table 2. Patients with oral lesions according to LCH subtype

Lesion type	N	%	CI 95	SS LCH	MS RO⁺ LCH	MS RO⁻ LCH
Bone	21	52.5	36.0-68.5	13	2	6
Bone and mucosal	16	40	24.8-56.7	1	1	14
Mucosal	3	7.5	1.5-20.4	0	0	3
Total	40	42.1	32.0-52.7			

Table 3. Association between ages and tissues involved

Type of oral tissue involved	Average age at time of diagnosis
Bone	2.85 years
Bone-mucosal	1.46 years
Mucosal	2.67 years

Table 4. First lesion in the mouth (n = 14) 14.73% CI 8.2 – 23.5 (n=95)

Type of LCH lesion	N	%	CI 95	SS LCH	MS RO⁺ LCH	MS RO⁻ LCH
Bone	12	85.8	57.7- 98.3	11	0	1
Bone-mucosal	2	14.2	1.6- 42.8	1	0	1
Mucosal	0	0	0	0	0	0

Table 5. Single LCH lesion in oral cavity, without systemic involvement (n=10), 10.52 %, CI 5.1 – 18.5

Tissue involved	N	%	CI
Bone	9	90	55.4 –99.8
Bone-mucosal	1	10	0.12 –44.5
Mucosal	0	0	0

DISCUSSION

LCH has a broad range of systemic manifestations according to the tissue or organ involved. The current study surveyed and grouped oral lesions in pediatric LCH patients. The results agree with other studies that report that oral lesions may be the first manifestations of LCH, and that the oral cavity is often the only site involved[16-22]. Oral lesions may also be reactivations of the disease. Sigala et al. (1972)[23] studied 50 cases of LCH in which 36 % of the patients presented oral lesions, and 16 % corresponded to the first manifestation of LCH.

In the current study, 40 out of the total 95 patients (42.1 %) presented LCH lesions in the oral cavity. In 10.52 %, they were the only lesions, while in others, they were part of a broader systemic process. Most lesions involved the lower jaw, in agreement with Pippa Vallejo (2016)[10] regarding lytic bone lesions present in the ascending branch and posterior portion of the mandibular body, which may be single or multiple.

According to Nakamura[24], the loss of alveolar bone produces gingival retraction, destruction of keratinized gum, periodontal pockets, swelling and pain, and facilitates early ectopic eruption of permanent molars. In children younger than one year of age, primary teeth erupt prematurely and there is possible loss of permanent tooth buds[21]. In the current study, in patients whose first histiocytosis lesion was in the oral cavity, it involved bone tissue in 85.8%, and bone-mucosa in 14.2%, while mucosa alone was not involved as first LCH lesion.

Rios[25] and Erdem[20] report that the radiographic image looks like a periodontal cyst, apical granuloma, localized form of periodontitis, or even a neoplastic process, so it is essential to conduct a pathohistological study. When osteolysis involves the alveolar process, the loss of supporting bone tissue leaves the tooth "floating" within the lesion produced by LCH. In some cases, the gum accompanied the bone resorption with loss of insertion level and consequent exposure of the root[10]. Lytic lesions observed radiographically in the alveolar bone had a flat base and rounded walls, as if shaped by a hole puncher[26]. Milán reports that LCH lesions in oral mucosa are ovoid, erythematous, painful upon palpation, and located mainly at the bottom of the vestibular sulcus and jugal mucosa[27].

LCH oral lesions are not considered high-risk; nevertheless, when they occur on the alveolar processes of the maxilla, they may invade the orbit, malar, or maxillary sinus, and access the base of the skull, with possible risk to the central nervous system[14]. Patients with lesions in the upper jaw therefore have different prognosis and treatment[13]. In the current study, oral lesions in the single-system subtype involved bone exclusively, while in the multisystem subtypes, they were varied, involving bone, or mucosa, or both.

When the only evidence of LCH was in the oral zone (edema, teeth with mobility, or pain unrelated to changing teeth), the disease was diagnosed by a pediatric dentist.

At the time of birth, the jaws hold 20 primary teeth

soon to erupt, and 28 permanent teeth in formation. Any alteration in the jawbones may produce sequels in both dentition sets. Age of diagnosis differs according to the different presentation types of the disease. In the current study, children aged 0 to 2 years presented bone-mucosal lesions and corresponded to the multisystem type (MS-RO⁺ LCH and MS-RO⁻ LCH), while in children older than 2 years, the most frequent lesions were bone or mucosal. Preliasco et al.[28] agree that younger children would be at higher risk when the oral cavity is involved.

CONCLUSIONS

The oral lesions observed in 40 children differed in extension, and in clinical and radiographic appearance. The aim of this study was therefore to group them based on the type of tissue involved in the lesion. The tissues affected were bone, oral mucosa, or both. A high frequency of oral lesions was observed, which may be one of the first manifestations of the disease, with bone lesions being the most frequent.

Patients with bone-mucosal lesions, with multisystem LCH with or without risk organ involvement were the youngest at the time of diagnosis. No evidence was found of LCH subtype influencing the likelihood of developing oral manifestations.

Lesions on the maxilla may spread to the central nervous system. Thus, pediatric patients with oral involvement, and according to their systemic situation, may have different prognosis and need to receive different courses of treatment.

These results show that interdisciplinary work from the beginning of the disease may improve LCH patient wellbeing. Pediatricians and pediatric dentists should check the oral cavity thoroughly to identify any clinical or radiographic manifestations of LCH. If the pediatric dentist is the first to see a child with LCH lesion confirmed by biopsy, they must immediately confer with an oncologist in order to locate any other systemic lesions that might be present. It is recommendable to take panoramic radiographs periodically to monitor evolution or diagnose any new lesions.

REFERENCES

1. Hutter C, Minkov, M. Insights into the pathogenesis of Langerhans cell histiocytosis: the development of targeted therapies. Immunotargets Ther. 2016; 5: 81–91. https://doi.org/10.2147/ITT.S91058

2. Murakami I, M, Iwasaki T, Kuwamoto S, Morimoto A et al. Interleukin-1 loop model for pathogenesis of Langerhans cell histiocytosis. Cell Commun Signal. 2015 Feb 22; 13:13. https://doi.org/10.1186/s12964-015-0092-z

3. Rabassa-Blanco J, Brunet-Llobet L, Marcote-Sinclair P, Balsells-Mejía S et al. Prevalence of, and risk factors for, dental sequelae in adolescents who underwent cancer therapy during childhood. Oral Dis. 2022 Jul; 16. https://doi.org/10.1111/odi.14317

4. DiCaprio MR, Roberts TT. Diagnosis and management of Langerhans cell histiocytosis. J Am Acad Orthop Surg. 2014 Oct;22(10):643-52. https://doi.org/10.5435/JAAOS-22-10-643

5. Casanovas, A.; Elena, G.; Rosso, Diego; Histiocitosis de células de Langerhans; Sociedad Argentina de Hematología; Revista Argentina de Hematología 18; 1; 2014; 60-66 oai:ri.conicet.gov.ar:11336/16172

6. Bechan GI, Egeler RM, Arceci RJ. Biology of Langerhans cells and Langerhans cell histiocytosis. Int Rev Cytol. 2006; 254:1-43. https://doi.org/10.1016/S0074-7696(06)54001-X

7. Kumar N, Sayed S, Vinayak S. Diagnosis of Langerhans cell histiocytosis on fine needle aspiration cytology: a case report and review of the cytology literature. Patholog Res Int. 2011; 2011:439518. https://doi.org/10.4061/2011/439518

8. Minkov M, Grois N, Braier J, Rosso D, Arico M, Broadbent V, Gadner H, Ladisch S, et al. Immunosuppressive treatment for chemotherapy-resistant multisystem Langerhans cell histiocytosis Med Pediatr Oncol. 2003;40(4):253-6. https://doi.org/10.1002/mpo.10167

9. Morimoto A, Ishida Y, Suzuki N, Ohga S, Shioda Y, Okimoto Y, Kudo K, Ishii E; HLH/LCH Committee of the Japanese Society of Pediatric Hematology. Nationwide survey of single-system single site Langerhans cell histiocytosis in Japan. Pediatr Blood Cancer. 2010;54(1):98-102. https://doi.org/10.1002/pbc.22224

10. Pipa Vallejo A, Pipa Muñiz M, Pipa Muñiz C, Gangoiti Moratinos Zet al. Histiocitosis de células de Langerhans con implicación oral: a propósito de un caso. Av.

Odontoestomatol 2016; 32(4):187-193., ISSN 02131285 http://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0213-12852016000400002&lng=es.

11. Spodzieja K, Olczak-Kowalczyk D. Premature Loss of Deciduous Teeth as a Symptom of Systemic Disease: A Narrative Literature Review. International Journal of Environmental Research and Public Health2022; 19(6):3386. https://doi.org/10.3390/ijerph19063386

12. Cisternino A, Farah Fusco N, Ferrero S, Rasperini G. Role of multidisciplinary approach in a case of Langerhans cell histiocytosis with initial periodontal manifestations Int J ClinExpPathol 2015;8(10):13539-13545. PMCID: PMC4680515

13. Emile J F, et al., Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages, Blood 2016;127(22):2672-81. https://doi.org/10.1182/blood-2016-01-690636

14. Braier J, Pollono D, Rey G, Latella A, De Socio S, Tomarchio S, Rosso D, Goldberg J. Reactivations in Langerhans cell histiocytosis (LCH). Histiocyte society 18 th Annual Meeting Porto, Portugal. Med Pediatr Oncol 2003;(40):3;180

15. Felstead AM, Main BG, Thomas SJ, Hughes CW. Recurrent Langerhans cell histiocytosis of the mandible. Br J Oral Maxillo fac Surg. 2013;51(3):264-265. https://doi.org/10.1016/j.bjoms.2012.10.005

16. Shirley J, Thornton J. Oral manifestations of Langerhans´ cell histiocytosis: Review and report of case. ASDC J Dent Child 2000; July-August:293-6. PMID: 10997248.

17. Hernández Juyol M, Boj Quesada J R, Gallego Melcon S. Manifestaciones orales de la Histiocitosis de células de Langerhans a propósito del caso de un niño de dos años. Med Oral 2003;8:19-25. Medicina Oral S. L. C.I.F. B 96689336–ISSN 1137–2834

18. Hicks J, Flaitz CM. Langerhans cell histiocytosis: Current insights in a molecular age with emphasis on clinical oral and maxillofacial pathology practice. Oral Surgery, Oral Med Oral Pathol Oral RadiolEndodontology. 2005;100 (2 SUPPL.):42-66 https://doi.org/10.1016/j.tripleo.2005.06.016

19. Madrigal-Martínez-Pereda C, Guerrero-Rodríguez V, Guisado-Moya B, Meniz-García C, Langerhans cell histiocytosis: Literature review and descriptive analysis of oral manifestations. Med Oral Patol Oral Cir Bucal. 2009;1;14 (5):E 222-8. PMID: 19218906

20. Erdem AP, Kasimoglu Y, Sepet E, Gencay K, Sahin S, Dervisoglu S. Oral manifestations may be the first sign of Langerhans cell histiocytosis. Oral Health Prev Dent. 2013;11(1):57-59. http://www.ncbi.nlm.nih.gov/pubmed/23507682.

21. Merglová V, Hrusak D, Boudová L, Mukensnabl P, Valentová E, Hosticka L. Langerhans cell histiocytosis in childhood–Review, symptoms in the oral cavity, differential diagnosis and report of two cases. J Cranio-Maxillofacial Surg. 2014;42(2):93-100. https://doi.org/10.1016/j.jcms.2013.03.005

22. Fernández de Preliasco V, Benchuya C, Pavan V. Lesiones bucales asociadas con la enfermedad de Histiocitosis de Células de Langerhans en odontopediatría. Revista de la Asociación de la Academia Nacional de Odontología. 2011;9(9):12-17.

23. Sigala JL, Silverman S Jr, Brody HA KJ. Dental involvement in histiocytosis. Oral Surg Oral Med Oral Pathol. 1972;33(1):42-48. https://doi.org/10.1016/0030-4220(72)90206-X

24. Nakamura S, Bessho K Nakao K, Iizuka T, Scott RF. Langerhans' cell histiocytosis confined to the jaw. J Oral MaxillofacSurg 2005; 63:989-95. https://doi.org/10.1016/j.joms.2004.07.029

25. Rios C, Gonzalez Diaz M, Bello Rodriguez R, Torrens de la Nuez R. Granulomatosis de células de Langerhans. Presentación de un caso. Rev. Cubana Estomatológica 1998;35(3):124-6. ISSN 1561-297X

26. Benchuya C. Estudio Básico Clínico de Pacientes Pediátricos con Histiocitosis de células de Langerhans. Tesis de Doctorado,2015. Facultad de Odontología Universidad de Buenos Aires.

27. Milian MA. Langerhans's cell histiocytosis. Med Oral Patol Oral Cir Bucal 2005 Mar-Apr;10(2):185. English, Spanish. PMID: 15735553.

28. Preliasco V F; Benchuya C; Pavan V; de la Cal C; Ganzinelli S Sterin_Borda L. IL-1βeta and PGE2 levels are increased in the saliva in children with Langerhans cell histiocytosis. J Oral Pathol Med 2008; 37(9): 522-7. https://doi.org/10.1111/j.1600-0714.2008.00675.x

Morphological assessment of the isthmus in mesial root canals of first mandibular molars

Thiago S Guimarães[1], Marilia FV Marceliano-Alves[2], José C Provenzano[2],
Patricia P Olivares[2], Ana GS Limoeiro[3], Flavio RF Alves[2], Luciana A Dias[2]

1. Universidade Estácio de Sá, Departamento de Endodontia, Rio de Janeiro, Brasil
2. Universidade Nova Iguaçu, Programa de Pós-Graduação em Odontologia (Endodontia), Nova Iguaçu, Brasil
3. Faculdade São Leopoldo Mandic, Campinas, São Paulo, Brasil

ABSTRACT

Root canal morphology and its anatomical variations pose a great challenge to endodontists. **Aim:** *The aim of this in silico study was to perform a qualitative and quantitative analysis of the three-dimensional morphological characteristics of the isthmus in the mesial root canals of mandibular molars using microcomputed tomography (micro-CT).* **Material and Method:** *Six hundred first mandibular molars were selected, including 317 with two mesial canals with isthmuses between the canals, and fully formed root. Isthmus morphology was determined in 3D longitudinal sections using Fan et al. (2010) classification. Root length, and the volume and area of apical and coronal level were measured. Additionally, the structural model index (SMI) of the canals were also assessed.* **Results:** *The prevalence of isthmuses in the mesial root canals was 32% type II, 29% type III, 22% type IV, and 17% type I. The root length was found to be 9.1±0.5 mm, the volume and area, of all root canal system, were 41.8±40.1 mm³ and 63.6±24.2 mm² respectively. The isthmi volume and area alone were 11.06±9.03 mm³ and 30.02±11.02 mm². The study confirmed that isthmuses are present in mesial canals of mandibular first molars, being more frequent in the apical third.* **Conclusion:** *The high prevalence of isthmuses with complex morphological features underscores the importance of using intracanal medications to disinfect areas unprepared by instruments.*

Keywords: endodontics - root canal therapy - X ray microtomography.

Avaliação morfológica do istmo nos canais radiculares mesiais dos primeiros molares inferiores

RESUMO

A morfologia do canal radicular e suas variações anatômicas representam um grande desafio para os endodontistas. O objetivo deste estudo ex vivo foi realizar uma análise qualitativa e quantitativa das características morfológicas tridimensionais do istmo nos canais mesiais de molares inferiores por meio de microtomografia computadorizada (micro-CT). **Material e Método:** *Foram selecionados 600 primeiros molares inferiores, incluindo 317 com dois canais mesiais com istmos e raiz totalmente formada. A morfologia do istmo foi determinada em cortes longitudinais 3D usando a classificação Fan et al. (2010). Foram mensurados o comprimento da raiz, o volume e a área apical e coronal e da cavidade pulpar. Adicionalmente, também foram avaliados o structure model index (SMI) dos canais.* **Resultados:** *A prevalência de istmos nos canais mesiais foi de 32% tipo II, 29% tipo III, 22% tipo IV e 17% tipo I. O comprimento da raiz foi de 9,1±0,5 mm, o volume e a área de todo o sistema de canais radiculares foram de 41.8±40.1 mm³ e 63.6±24.2 mm², respectivamente. O volume e área do istmo isoladamente foram 11.06±9,03 mm³ e 30.02±11.02 mm². O estudo confirmou que os istmos estão presentes em canais mesiais dos primeiros molares inferiores, sendo mais frequentes no terço apical.* **Conclusão:** *A alta prevalência de istmos com características morfológicas complexas ressalta a importância do uso de medicação intracanal para desinfecção de áreas não tocadas por instrumentos.*

Palavras-chave: endodontia - tratamento de canal radicular - microtomografia por Raio X.

INTRODUCTION

Root canal morphology and its anatomical variations pose a great challenge to endodontists[1]. Although success rates for endodontic treatments are generally high, cases of failure have been attributed to lack of knowledge of root canal internal anatomy[2], the understanding of which is essential for performing effective endodontic treatments and ensuring proper cleaning and disinfection. Some of the most common teeth requiring endodontic treatment are mandibular first molars because emerge at about six years of age in the oral cavity and are more exposed to endodontic treatment[3]. They present diverse anatomy and configuration, with the mesial root being particularly complex due to the presence of isthmuses[4].

Isthmuses are anatomical variations found in roots with two canals, primarily in the mesial roots of mandibular and maxillary first molars. The incidence of isthmuses is particularly high in mesial roots of mandibular molars[5]. Larger isthmuses, especially in mandibular molars, may harbor necrotic debris, pulp remnants, or organic matter that promotes microbial growth. This may interfere with proper cleaning and disinfection of the root canal system[6] and lead to endodontic treatment failure[7].

Accurate morphological mapping of the root canal system is essential to achieve the best possible outcome during endodontic therapy. Micro-CT has been used for *in vitro* and *ex vivo* morphological evaluations based on qualitative and quantitative three-dimensional images, and is considered the gold standard in endodontics[8,9]. It is an advanced method that reveals the internal anatomy of root canal systems through high-resolution images[10].

Numerous studies have been performed to classify the morphology of mesial root canals. A classic study that has undergone modifications over the years is Vertucci's classification[11-15]. The classification of isthmuses was established by Fan et al.[5], who identified different shapes such as foliar, separated, mixed, and cannular. In addition, a ribbon-shaped isthmus was described as an oval junction formed between two merging and then dividing root canals, with its own isthmus roof and floor[16]. The current study adopted Fan's classification for isthmuses.

The aim of this *ex vivo* study was to analyze the three-dimensional (3D) morphological characteristics of isthmuses in mesial root canals of mandibular molars using micro-CT. This approach enabled us to make both qualitative and quantitative assessments of the isthmus structures.

MATERIALS AND METHOD

This project was approved by the local research ethics committee (Iguaçu University Research Ethics Committee's approval N° 3.422.230). Six hundred extracted mandibular first molars with straight or curved mesial roots were selected from the Iguaçu University's institutional tooth bank. Criteria were met in 317 teeth that had two single mesial canals with isthmuses present between them and complete root formation. Isthmus morphology was determined in 3D longitudinal images. Root length, volume and total area of the root canal, as well as of the apical and coronal level, volume and area of the isthmus, and structural model index (SMI) were analyzed.

Morphometric analysis

Samples were scanned using a micro-scanner CT (Skyscan 1174v2; Bruker micro-CT, Kontich, Belgium), and reconstructed for 3D evaluation of the area, volume, and surface of the root canals using the software CTan V.1.14.4.1 (Bruker micro-CT, Kontich, Belgium) for qualitative evaluation of the canal configuration analyses. The first assessment is the isthmus morphology according Fan's[5] classification, in which the Type I, is the sheet connection: narrow sheet and complete connection existing between 2 canals from the top to bottom of the isthmus. In some cases, 1 or more small dentin fusions were discerned in the isthmus area. Type II, separate is the narrow but incomplete connection existing between 2 canals from the top to bottom of the isthmus. Type III, mixed: incomplete isthmus existing above and/or below a complete isthmus. Type IV, cannular connection: narrow cannular communication between 2 canals[5].

The specimens were analyzed through the longitudinal aspect using the CTan V.1.14.4.1 sotware (Bruker micro-CT) to assess the following aspects of the mesial roots: root length, apical level, coronal level, volume and total area of the pulp cavity, volume and area of the isthmus, and structural model index (SMI) analysis.

On the longitudinal sections, the measure tool option was used to measure from the cervical and apical portions to determine root length, distance from the isthmus to the apex of the mesial roots and also to determine the distance from the isthmus (mm) to the cervical part of the mesial roots, and also to identify the presence of an isthmus.

Volume and total area of the pulp cavity and isthmus

With the top and bottom root dimensions already determined, the volume and total area of the pulp cavity were evaluated using the Region of Interest tool (ROI). Then, the binary selection tool was used to preview the selected area, observing the histogram bar to obtain two colors: white for solid parts and black for empty parts. Then, the Morphometry tool was used to perform a 3D analysis. The Data were calculated, and the values were automatically obtained by the software.

SMI analysis

The SMI indicates the relative prevalence of regions of trabecular bone and plaques in a 3D model. Total volume and area as well as the volume and area of the isthmuses were obtained from 3D analyses. These measurements were made separately by two calibrated evaluators.[5] Any cases in which measurements differed between evaluators were analyzed by a third evaluator. At the end of the evaluation, the kappa coefficient (κ) was used to analyze the agreement between them.

RESULTS

The morphological analysis data of mesial roots in first mandibular first molars showed that root mean root length was 9.1±0.5 mm. The volume and area, of all root canal system, were 41.8±40.1 mm^3 and 63.6±24.2 mm^2 respectively. The isthmi volume and area alone were 11,0.6±9,03 mm^3 and 30,02±11,02 mm^2 (Table 1).

Micro-CT analysis of the 317 cases identified isthmuses showed higher prevalence in the apical third, especially between 3 and 5 mm from the apex. The distribution of isthmus types and their respective prevalence rates were as follows: Type I - 17%, Type II - 32%, Type III - 29%, and Type IV - 22%, showing separate type and mixed types (Figs. 1 and 2).

Mean SMI of the studied isthmuses was 1.7 mm, close to 0, which is a flat format. A value of 2.2 mm was determined for canal SMI, indicating a more cylindrical shape (values close to 3 indicate cylindrical) (Table 1)

DISCUSSION

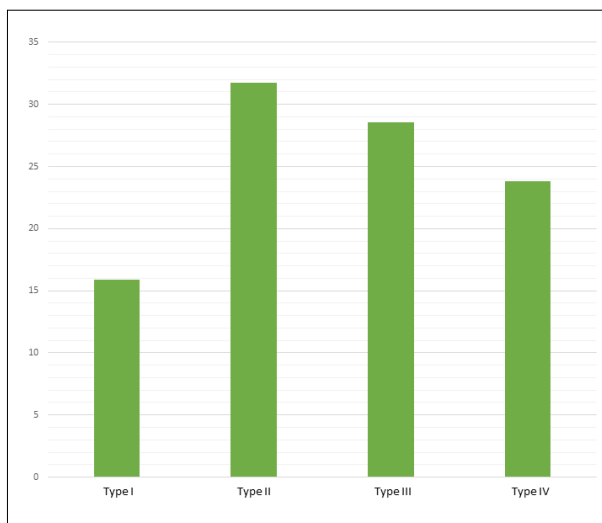The mandibular first molars assessed in this study show the expected dominance of two mesial and

Fig. 1: Isthmus prevalence found in mesial roots of mandibular molars according to Fan et al.5 classification.
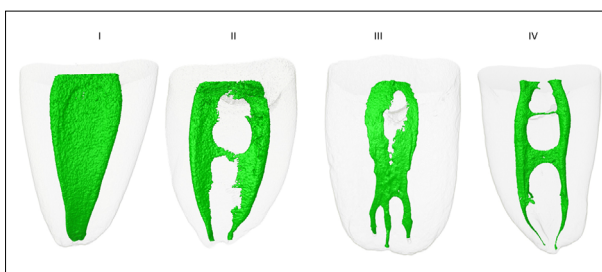

Fig. 2: Representative images of Fan's isthmus types found in mandibular molars assessed in the present study.

Table 1. Morphometric analysis of mesial roots of mandibular first molars in the study.

Parameter	Mean ± SD	Range
Total Root Length (mm)	9.1±0.5	14.3-5.3
Total Volume of Pulp Cavity (mm^3)	41.8±40.1	74.9-20.7
Total Area of Pulp Cavity (mm^2)	63.6±24.2	141-35.7
Isthmus Volume (mm^3)	11.6±9.3	17.3-0.96
Isthmus Area (mm^2)	30.2±11.2	124-2.7
Canal SMI	1.7±0.7	2.9-1.55
Isthmus SMI	2.2±0.8	2.8-0.3

one distal canal, which is consistent with previous reports[4,17]. The mesial roots were used in this study because of their complexity and frequent connections between canals[7].

The apical third of the root is a critical area in endodontic treatment because of the presence of ramifications that can serve as bacterial reservoirs[18]. Other complexities along the canal may also exist in this risk area, contributing to treatment

failure[19]. Therefore, the aim of this study was to evaluate complexities of the root canal isthmi from mandibular molars, using micro-CT.

Isthmus prevalence ranges from 10%[20] to 100%[21], and may be age-related, as it seems to be higher in the younger than in elderly age groups[7]. An isthmus may occur anywhere along the root, though they are more common in the middle and apical thirds of the mesial roots of mandibular molars[22-24]. The current study found high incidence of isthmuses along the root canal system, especially in the apical third[4], which is consistent with a study that found isthmuses within 3-5 mm from the apical foramen of maxillary and mandibular molars[25].

In line with other studies[5,7,26], our findings showed that Type II was slightly more common than other types in mandibular first molars. This highlights the importance of using intracanal medications for disinfection in areas not reached by the instruments. These findings are consistent with studies conducted on Turks[27] and Iranians[28], but contradicts results reported on Yemenis[29] and Venezuelans[30].

A systematic review has shown that anatomical variability in mandibular molars can be attributed to population differences, different methods of analysis, inclusion and exclusion criteria, and sample sizes[8]. The most frequently found root canal isthmus configuration is Vertucci type IV[23,26], which is described as two independent canals. This configuration is the most common in Egyptians[31].

Many methods such as clearing techniques, micro-CT and CBCT can be employed to assess root canal anatomy[32]. Micro-CT has been used as an advanced research tool to assess the *ex vivo* anatomy of root canals due to its high-resolution and ability to preserve the specimens[33]. Despite its limited clinical applicability, micro-CT has become established as the reference method for *ex vivo* studies of root canal anatomy. Its adoption facilitates future comparisons and continuous improvements of the exploration of root canal structures[33].

Regarding morphological analysis of mesial roots in first mandibular first molars, the results showed that root mean root length was 9.1±0.5 mm, similar to other studies[5,7,23,24,34]. The volume and area, of all root canal system, were 41.8±40.1 mm^3 and 63.6±24.2 mm^2 respectively, in accordance to previous studies[12,13,20,23]. The isthmi volume and area alone were 11.6±9,.3 mm^3 and 30.2±11.2 mm^2 [22,24] (Table 1).

This study identified some limitations, including the lack of information on age and gender of the patients from whom teeth had been extracted, potentially affecting the generalizability of the findings.

In conclusion, this study confirms the presence of isthmuses in the mesial canals of mandibular first molars, with higher frequency in the apical third. Although the present study was conducted *ex vivo*, its clinical implications highlight the significance of carefully selecting an appropriate root canal disinfection protocol to achieve a higher success rate in the treatment of mandibular first molars. Based on that findings, important to consider their complex anatomy and difficult-to-access areas during endodontic treatment[34,39-41].

It has been shown that there is an association between untreated canals and isthmuses and apical periodontitis[34-37], so, regardless of whether an isthmus is detected, it is recommended to improve the irrigation of all root canal system in these areas as well[36,38,40,41]. Micro–computed tomography is the gold standard for research of root canal anatomy because it provides nondestructive three-dimensional microscopy data with high spatial resolution that enables accurate assessment of morphological features. Knowledge of anatomical features such as lateral canals and isthmuses is important to guide clinical protocols to ensure adequate disinfection and contribute to the healing of periradicular tissues[34,38]. However, micro-computed tomography has hardly any clinical value and can only be used for *in vitro* and *ex vivo* studies[35-37].

For clinical practice, preoperative Cone Beam Computed Tomography (CBCT) scanning may play a significant role in revealing complex anatomy[39-41]. CBCT settings include multiple FOVs and voxel sizes that can better address a variety of specific tasks[40,41]. However, CBCT imaging cannot detect and measure apical isthmus length accurately, even with the highest-resolution settings. This highlights the importance of efficient, rigorous endodontic cleaning, even when such variations are not detected in CBCT exams[39].

REFERENCES

1. Pineda F, Kuttler Y. Mesiodistal and buccolingual roentgenographic investigation of 7,275 root canals. Oral Surg Oral Med Oral Pathol 1972;33:101-10. https://doi.org/10.1016/0030-4220(72)90214-9

2. Seelig A, Gillis R. Preparation of cleared specimens for pulp cavity studies. J Dent Res 1973;52:1154. https://doi.org/10.1177/00220345730520053101

3. Hull TE, Robertson PB, Steiner JC, del Aguila MA. Patterns of endodontic care for a Washington state population. J Endod 2003;29:553-6. https://doi.org/10.1097/00004770-200309000-00002

4. Vertucci F, Seelig A, Gillis R. Root canal morphology of the human maxillary second premolar. Oral Surg Oral Med Oral Pathol 1974;38:456-64. https://doi.org/10.1016/0030-4220(74)90374-0

5. Fan B, Pan Y, Gao Y, Fang F, et al. Three-dimensional morphologic analysis of isthmuses in the mesial roots of mandibular molars. J Endod 2010;36:1866-9. https://doi.org/10.1016/j.joen.2010.08.030

6. Urban K, Donnermeyer D, Schäfer E, Bürklein S. Canal cleanliness using different irrigation activation systems: a SEM evaluation. Clin Oral Investig 2017;21:2681-7. https://doi.org/10.1007/s00784-017-2070-x

7. Gu L, Wei X, Ling J, Huang X. A microcomputed tomographic study of canal isthmuses in the mesial root of mandibular first molars in a Chinese population. J Endod 2009;35:353-6. https://doi.org/10.1016/j.joen.2008.11.029

8. Al-Rammahi HM, Chai WL, Nabhan MS, Ahmed HMA. Root and canal anatomy of mandibular first molars using micro-computed tomography: a systematic review. BMC Oral Health 2023;29;23:339. https://doi.org/10.1186/s12903-023-03036-5

9. Piasecki L, José Dos Reis P, Jussiani EI, Andrello AC. A Micro-computed Tomographic Evaluation of the Accuracy of 3 Electronic Apex Locators in Curved Canals of Mandibular Molars. J Endod 2018;44:1872-7. https://doi.org/10.1016/j.joen.2018.09.001

10. Keleş A, Keskin C. A micro-computed tomographic study of band-shaped root canal isthmuses, having their floor in the apical third of mesial roots of mandibular first molars. Int Endod J 2018;51:240-6. https://doi.org/10.1111/iej.12842

11. Gulabivala K, Aung TH, Alavi A, Ng YL. Root and canal morphology of Burmese mandibular molars. Int Endod J 2001;34:359-70. https://doi.org/10.1046/j.1365-2591.2001.00399.x

12. Peiris R, Takahashi M, Sasaki K, Kanazawa E. Root and canal morphology of permanent mandibular molars in a Sri Lankan population. Odontology 2007;95:16-23. https://doi.org/10.1007/s10266-007-0074-8

13. Sert S, Aslanalp V, Tanalp J. Investigation of the root canal configurations of mandibular permanent teeth in the Turkish population. Int Endod J 2004;37:494-9. https://doi.org/10.1111/j.1365-2591.2004.00837.x

14. Kartal N, Cimilli HK. The degrees and configurations of mesial canal curvatures of mandibular first molars. J Endod 1997;23:358-62. https://doi.org/10.1016/S0099-2399(97)80182-3

15. Al-Qudah AA, Awawdeh LA. Root and canal morphology of mandibular first and second molar teeth in a Jordanian population. Int Endod J 2009;42:775-84. https://doi.org/10.1111/j.1365-2591.2009.01578.x

16. Keleş A, Keskin C, Çiftçioğlu E, Alak G. Evaluation of the band-shaped isthmuses in the mesiobuccal root canal system using micro-computed tomography. Clin Oral Investig 2022;26:5909-14. https://doi.org/10.1007/s00784-022-04548-0

17. Gulabivala K, Aung TH, Alavi A, Ng YL. Root and canal morphology of Burmese mandibular molars. Int Endod J 2001;34:359-70. https://doi.org/10.1046/j.1365-2591.2001.00399.x

18. Nair PNR, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:231-52. https://doi.org/10.1016/j.tripleo.2004.10.005

19. Carr GB, Schwartz RS, Schaudinn C, Gorur A, et al. Ultrastructural Examination of Failed Molar Retreatment with Secondary Apical Periodontitis: An Examination of Endodontic Biofilms in an Endodontic Retreatment Failure. J Endod 2009;35:1303-9. https://doi.org/10.1016/j.joen.2009.05.035

20. Keleş A, Keskin C, Alqawasmi R, Versiani MA. Micro-computed tomographic analysis of the mesial root of mandibular first molars with bifid apex. Arch Oral Biol 2020 Sep;117:104792. https://doi.org/10.1016/j.archoralbio.2020.104792

21. Harris SP, Bowles WR, Fok A, McClanahan SB. An anatomic investigation of the mandibular first molar using micro-computed tomography. J Endod [Internet]. 2013;39:1374-8. https://doi.org/10.1016/j.joen.2013.06.034

22. Hsu YY, Kim S. The resected root surface. The issue of canal isthmuses. Dent Clin North Am. 1997;41:529-40. https://doi.org/10.1016/S0011-8532(22)00066-0

23. Marceliano-Alves MF, Lima CO, Bastos LG do PMN, Bruno AMV, et al. Mandibular mesial root canal morphology using micro-computed tomography in a Brazilian population. Aust Endod J 2019;45:51-6. https://doi.org/10.1111/aej.12265

24. Mannocci F, Peru M, Sherriff M, Cook R, et al. The isthmuses of the mesial root of mandibular molars: a micro-computed tomographic study. Int Endod J 2005;38:558-63. https://doi.org/10.1111/j.1365-2591.2005.00994.x

25. Teixeira FB, Sano CL, Gomes BPFA, Zaia AA, et al. A preliminary in vitro study of the incidence and position of the root canal isthmus in maxillary and mandibular first molars. Int Endod J 2003;36:276-80. https://doi.org/10.1046/j.1365-2591.2003.00638.x

26. Martins JNR, Marques D, Silva EJNL, Caramês J, et al. Prevalence Studies on Root Canal Anatomy Using Cone-beam Computed Tomographic Imaging: A Systematic Review. J Endod [Internet]. 2019;45:372-386.e4. https://doi.org/10.1016/j.joen.2018.12.016

27. Demirbuga S, Sekerci AE, Dinçer AN, Cayabatmaz M, et al. Use of cone-beam computed tomography to evaluate root and canal morphology of mandibular first and second molars in Turkish individuals. Med Oral Patol Oral Cir Bucal 2013;18:e737-44. https://doi.org/10.4317/medoral.18473

28. Janani M, Rahimi S, Jafari F, Johari M, et al. Anatomic Features of C-shaped Mandibular Second Molars in a Selected Iranian Population Using CBCT. Iran Endod J [Internet]. 2018;13(1):120-5.

29. Senan E, Alhadainy H, Madfa AA. Root and Canal Morphology of Mandibular Second Molars in a Yemeni Population: A Cone-beam Computed Tomography. Eur Endod J 2021;6:72-81. https://doi.org/10.14744/eej.2020.94695

30. Gomez F, Brea G, Gomez-Sosa JF. Root canal morphology and variations in mandibular second molars: an in vivo cone-beam computed tomography analysis. BMC Oral Health 2021;21:424. https://doi.org/10.1186/s12903-021-01787-7

31. Saber SM, Seoud MA El, Sadat SMA El, Nawar NN. Root and canal morphology of mandibular second molars in an Egyptian subpopulation: a cone-beam computed tomography study. BMC Oral Health 2023;23:217. https://doi.org/10.1186/s12903-023-02939-7

32. Ahmed HMA, Rossi-Fedele G. Preferred Reporting Items for Root and Canal Anatomy in the Human Dentition (PROUD 2020) - A Systematic Review and a Proposal for a Standardized Protocol. Eur Endod J 2020;5:159-76. https://doi.org/10.14744/eej.2020.88942

33. Ordinola-Zapata R, Bramante CM, Versiani MA, Moldauer BI, et al. Comparative accuracy of the Clearing Technique, CBCT and Micro-CT methods in studying the mesial root canal configuration of mandibular first molars. Int Endod J 2017;50:90-6. https://doi.org/10.1111/iej.12593

34. Susin L, Liu Y, Yoon JC, Parente JM, et al. Canal and isthmus debridement efficacies of two irrigant agitation techniques in a closed system. Int Endod J 2010;43:1077-90. https://doi.org/10.1111/j.1365-2591.2010.01778.x

35. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36:1277-88. https://doi.org/10.1016/j.joen.2010.04.007

36. Paque F, Laib A, Gautschi H, et al. Hard-tissue debris accumulation analysis by highresolution computed tomography scans. J Endod 2009;35:1044-7. https://doi.org/10.1016/j.joen.2009.04.026

37. Karabucak B, Bunes A, Chehoud C, et al. Prevalence of apical periodontitis in endodontically treated premolars and molars with untreated canal: a cone-beam computed tomography study. J Endod 2016;42:538-41. https://doi.org/10.1016/j.joen.2015.12.026

38. Tahmasbi M, Jalali P, Nair MK, et al. Prevalence of middle mesial canals and isthmi in the mesial root of mandibular molars: an in vivo cone-beam computed tomographic study. J Endod 2017;43:1080-3. https://doi.org/10.1016/j.joen.2017.02.008

39. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008 Nov;34(11):1291-1301.e3. https://doi.org/10.1016/j.joen.2008.07.028

40. Tolentino ES, Amoroso-Silva PA, Alcalde MP, Honório HM, Iwaki LCV, Rubira-Bullen IRF, Húngaro-Duarte MA. Accuracy of High-resolution Small-volume Cone-beam Computed Tomography in Detecting Complex Anatomy of the Apical Isthmi: Ex Vivo Analysis. J Endod. 2018 Dec;44(12):1862-1866. https://doi.org/10.1016/j.joen.2018.08.015

41. Kamburoglu K, Onder B, Murat S, et al. Radiographic detection of artificially created horizontal root fracture using different cone beam CT units with small fields of view. Dentomaxillofac Radiol 2013;42:20120261. https://doi.org/10.1259/dmfr.20120261

Fluoride in drinking groundwater and prevalence of fluorosis in children and adolescents: A systematic review

Francineudo Oliveira Chagas[2] , Lidia A Rocha Valadas[1] , Ana Sorazabal[1] ,
Adeyinka Dayo[3] , Thereza CF Botelho Dantas[4,5] , Aldo Squassi[1]

1. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Odontología Preventiva y Comunitaria, Buenos Aires, Argentina.
2. Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Fortaleza, Brasil.
3. University of Pennsylvania, School of Dental Medicine, Department of Oral Medicine, Philadelphia, United States.
4. Faculdade Paulo Picanço, Curso de Odontologia, Fortaleza, Brasil.
5. Centro Universitário Christus, Curso de Odontologia, Fortaleza, Brasil.

ABSTRACT
Fluorosis is a worldwide public health problem. One of the factors related to it is the type of water consumed, such as groundwater. High fluoride concentration in groundwater may be explained by contamination from local industries. Since fluoride and arsenic are the main pollutants of groundwater, some studies correlate groundwater consumption with high prevalence of fluorosis. **Aim:** *The aim of this study was to conduct a systematic review to determine whether children's risk of fluorosis is related to drinking groundwater.* **Materials and Method:** *The protocol for this systematic review was registered at the National Institute of Health Research Database (CRD42021227298). A comprehensive search was conducted to identify potentially relevant studies by exploring a range of electronic databases (Medline via PubMed, Scopus, Cochrane Library, Science Direct, Web of Science Core Collection, Medline via Ovid, Lilacs, Embase, and grey literature).* **Results:** *A total 2189 articles were found. After reading titles and abstracts, 63 were selected for screening, and the final data was extracted from 15 articles.* **Conclusion:** *A relationship was identified between drinking fluoridated water from wells and the prevalence of fluorosis in individuals up to 18 years old. This is the first study to assess the issue systematically worldwide.*

Keywords: fluoride - groundwater - fluorosis - children

Fluoruro en el agua subterránea potable y prevalencia de fluorosis en niños y adolescentes: Una revisión sistemática

RESUMEN
La fluorosis es un problema de salud pública a nivel mundial y el tipo de agua consumida es uno de los factores relacionados con ella, como el consumo de aguas subterráneas. La alta concentración de fluoruro en estas aguas puede justificarse por la contaminación por industrias locales y las características del suelo, donde algunos estudios correlacionan el consumo de aguas subterráneas con una alta prevalencia de fluorosis, ya que el fluoruro, junto con el arsénico, se consideran los principales contaminantes de estas aguas. **Objetivo:** *El objetivo es realizar una revisión sistemática que relacione el riesgo de fluorosis en niños expuestos al consumo de agua procedente de pozos.* **Materiales y Método:** *El protocolo de esta revisión sistemática fue registrado en el National Institute of Health Research Database (CRD42021227298). Se realizó una búsqueda bibliográfica de estudios primarios explorando diversas bases de datos electrónicas (Medline vía PubMed, Scopus, Cochrane Library, Science Direct, Web of Science Core Collection, Medline vía Ovid, Lilacs, Embase y literatura gris).* **Resultados:** *Se encontraron 2189 artículos, tras la lectura de títulos y resúmenes se seleccionaron 63 referencias para examinar y, finalmente, se extrajeron los datos de 15 artículos.* **Conclusiones:** *Se identificó una relación entre el consumo de agua fluorada de pozo y la prevalencia de fluorosis en individuos de hasta 18 años, siendo este estudio el primero en evaluarlo sistemáticamente a nivel mundial.*

Palabras clave: fluoruro - aguas subterráneas - fluorosis – niños - adolescentes.

INTRODUCTION

The use of fluoride in dental caries management is one of the great milestones in dentistry, and has been recognized as the main factor responsible for the significant decline in caries prevalence around the world[1,2]. Epidemiological data from communities with access to a fluoridated public water supply provided the initial evidence that fluoride promotes oral health[3,4].

Caries prevention mechanisms through the action of fluorides are post-eruptive, occurring through their topical cumulative effect, and acting on the dynamics of demineralization/remineralization[5]. However, excessive fluoride intake during the tooth development period can cause dental fluorosis, which is the only proven relevant side effect of fluoride use[2].

Fluorosis is a worldwide public health problem and, like caries, has also been related to socioeconomic factors. The type of water consumed is one of these factors, and the consumption of well water is one of the consequences of people's socioeconomic condition[6]. Many communities still drink groundwater, often with fluoride concentration greater than the 1.5 ppm recommended by the World Health Organization. This increases the risk of fluorosis, thereby constituting a serious public health problem[7].

The high fluoride concentration in well water may be caused by lithology or by contamination from local industries or agrobusiness operations that increase the risk of heavy metals, pesticides, nitrates, radon and fluoride in the water[8,9]. Other factors are the amount and duration of precipitation, infiltration rate, level of groundwater exploitation in the area, etc.[10]. The increasing uptake of groundwater resources can also affect the distribution and concentration of fluoride[11].

Some studies correlate the consumption of groundwater with high prevalence of fluorosis because fluoride and arsenic are the main pollutants in groundwater. Fluoride concentration in the water increases with depth, with wells deeper than 30 m containing the highest amounts. The risk of fluorosis is proportional to the amount of fluoridated water the child is exposed to.

Fluorosis is classified as mild, moderate or severe[6-8,12]. Plasma and urine fluoride concentration is proportional to the fluoride concentration in the water consumed[10,11].

In view of the growing concern about this issue, the aim of this study was to conduct a systematic review to determine whether children's risk of fluorosis is related to drinking fluoridated groundwater.

MATERIALS AND METHOD

This systematic review was conducted in accordance with the guidelines of the Cochrane handbook for systematic review of Interventions, following the four-phase diagram of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). The study protocol was registered at the National Institute of Health Research Database (CRD42021227298).

The research question was adapted from the PECO framework for the systematic review of clinical studies:

 – Population (P): Patients up to 18 years
 – Exposure (E): Groundwater
 – Comparison (C): Exposure to water from supply network or commercial mineral water
 – Outcome (O): Fluorosis

In terms of research questions, based on the PECO model, the review aimed to assess the current knowledge and literature for the effect of patient exposure to groundwater on the outcome of fluorosis. The criteria followed in this study are described in Table 1.

Table 1. Eligibility criteria.

Inclusion Criteria	Exclusion Criteria
• Studies including patients up to 18 years old • Peer-reviewed studies • Quantitative randomized controlled trials • Retrospective, prospective, or concurrent cohort studies • Cross-sectional studies • Studies published in any language • Studies published on any date	• Qualitative opinions • Editorials • Literature reviews • Expert opinions • Newsletters/opinion letters • Case reports

2.1 Literature search strategy

A comprehensive search was conducted to identify potentially relevant studies by exploring a range of electronic databases (Medline via PubMed, Scopus, Cochrane Library, Science Direct, Web of Science Core Collection, Medline via Ovid, Lilacs, and Embase). Additionally, a Google scholar and

reference search on grey literature was undertaken to identify any other relevant published work. The search was conducted without applying any date limits or language restrictions.

The search strategy included the terms: "Children", "child" and "Fluoride", "Fluorine", "Water Well*", and "Water Ground*"; and "Fluoridated water", "water fluoridation" and "Enamel defect*", "Fluorosis".

Trade names of various classes of sealers were also used as a part of the search strategy. Boolean operators ("OR" and "AND") were used to join search terms related to the search question (Table 2). Table 3 shows the literature searches and results found in Pubmed, Scopus, Cochrane, Web of Science Core Collection, Medline via Ovid, BVS, and Embase.

Table 2. Search terms.	
Search 1	"Children", "child"
Search 2	"Fluoride", "Fluorine", "Water Well*", "Water Ground*"
Search 3	"Fluoridated water", "water fluoridation", "drinking water"
Search 4	"Enamel defect*", "Fluorosis"

2.2 Study Selection

Literature search results were de-duplicated by using Mendeley software (Thomson Reuters, New York, NY). Articles were initially screened based on the title and abstract according to the scope (i.e., articles that do not report fluorosis and exposure to groundwater) and publication type (i.e., reviews, comments, letters, or abstracts).

2.3 Data Extraction and Quality Assessment

Based on the selection criteria, two examiners (LV, FC) examined the titles and abstracts independently, and any disagreements were resolved according to a predefined strategy, using consensus and arbitration as appropriate. If the disagreement could not be resolved, then a third investigator (AS) agreed to help reach a consensus. Furthermore, a manual search of the reference lists of relevant studies was performed.

The seven domains of ROB-2 instrument were scored to quantify the risk of bias: confounding bias, measurement of exposure bias, selection bias, post-exposure interventions bias, missing data bias, measurement of the outcome bias and selection of the reported result. Subsequently, an overall judgement was made to mark each study as low risk of bias, high risk of bias or some concerns.

Table 3. Literature searches and number of articles found.

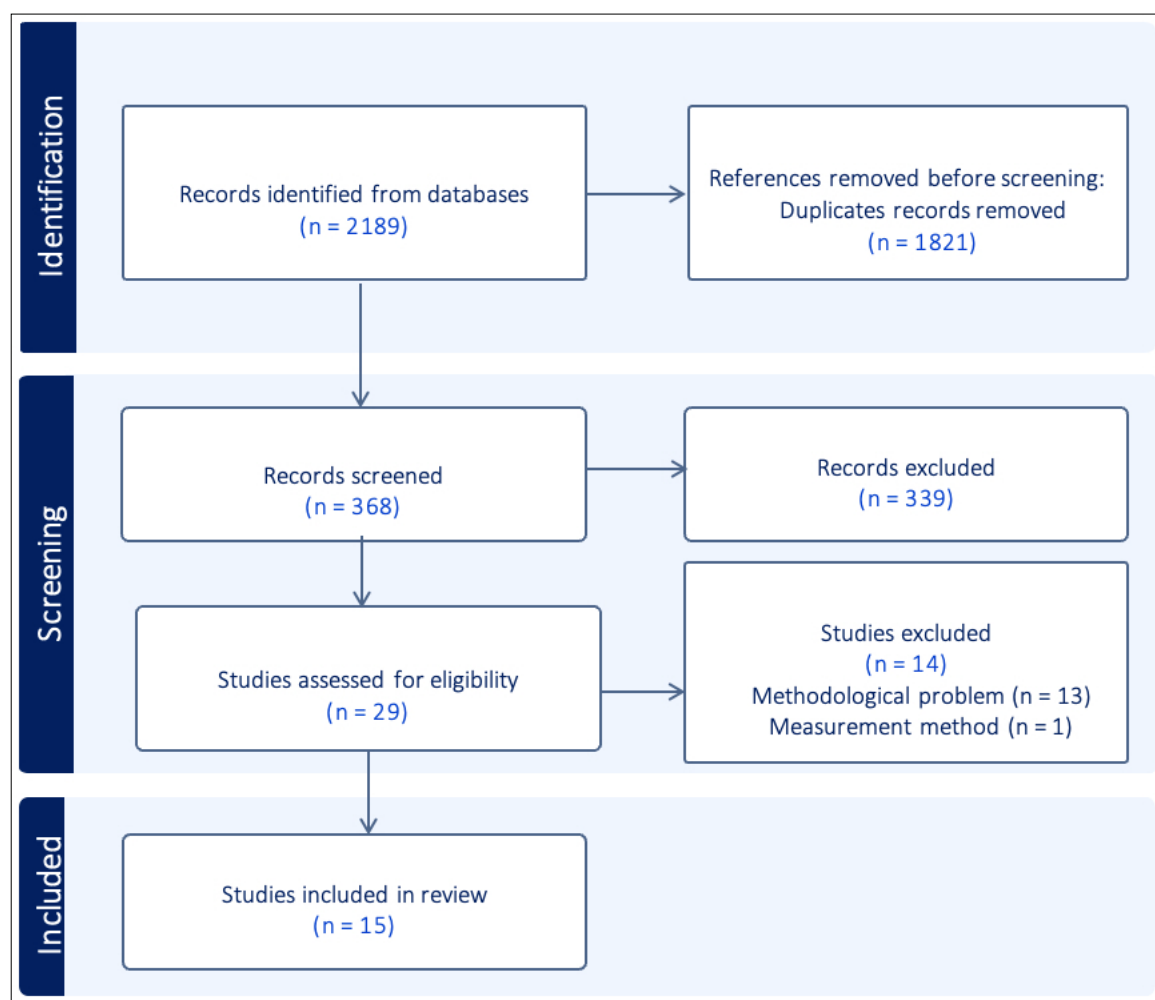|	Searches related to the descriptors				
	"Children" OR "child" #1	"Fluoride" OR "Fluorine" OR "Water Well*" OR "Water Ground*" #2	"Fluoridated water" OR "water fluoridation" OR "drinking water" #3	"Children" OR "child" #4	#1 AND #2 AND #3 AND #4
Pubmed	1,313,471	60,645	51,561	4,240	388
Scopus	3,169,927	246,326	1,185	4,935	-- 55
Cochrane	144,754	10,081	3,828	539	15
Web of Science	2,263,174	691,017	114,744	7,714	-- 665
Medline via Ovid	2,239,364	61,373	48,523	1,946	1
Virtual Health Library (BVS/VHL)	2,510,968	351,664	83,293	8,888	564
Embase	1,679,224	67,398	67,435	4,438	501
Total					2,189

Fig. 1: Literature searches and results.

RESULTS

The literature search after the identification period found 2189 articles (Table 3), of which 368 were screened according to the selection criteria, and 15 were selected, as shown in Figure 1.

The main data extracted from the selected studies are shown in Table 4. Data came from 4 continents, including Asia with 53.3% of studies, Africa with 20%, and Europe and America with 13.3% each. Participant ages were 14 to 16 years. Regarding the index used for diagnosing dental fluorosis, Dean's index was the most frequent, being adopted in 60% of the studies, followed by the Thylstrup-Fejerskov Index (TFI) in 20%. Other indices used were WHO, Pendry's and Horowitz's, with 6.7%.

Table 5 shows the main data on fluoride concentration in the water (0.1 to 18mg/L), and prevalence of fluorosis (4.25 to 100%), which were both highly heterogenous.

Figure 2 shows the quality assessment of the studies considered. In general, the domains presented a low risk of bias.

DISCUSSION

This study performed a systematic review of the literature to assess the risk of fluorosis in children exposed to drinking groundwater. The studies evaluated reported high fluoride concentration in the water consumed.

Fluoride intake has beneficial effects, as a complement to prevent and control dental caries, as well as adverse effects, mainly tooth enamel and skeletal fluorosis following prolonged exposure to high concentrations[1-2]. Excessive fluoride intake usually occurs through the consumption of groundwater that is naturally rich in fluoride. The assessment of the distribution of high groundwater

Table 4. Summary of the studies selected for this systematic review.

Study ID	Country	Subjects' age in years	Total number of children	Index used for diagnosing dental fluorosis
Akpata et al.[13]	Saudi Arabia	12-15	2355	Modified Thylstrup-Fejerskov Index (TFI)
Alarcon-Herrera et al.[14]	Mexico	6-12	333	Dean's index
Arif et al.[15]	India	5-13	1136	Dean's index
Gautam et al.[16]	India	4–16	90	Dean's index
Ibrahim et al.[17]	Sudan	7-16	113	Dean's index
Indermitte et al. [18]	Estonia	7-15	2,627	Dean's index
Ismail et al. [19]	Canada	0-7	48	Pendrys' Fluorosis Risk Index (Modified Fluorosis Risk Index)
Mandinic et al. [20]	Serbia	12	164	Dean's index
Narwaria et al. [21]	India	5-12	750	Dean's index
Rango et al. [22]	Main Ethiopian Rift Valley	10-15	491	Thylstrup and Fejerskov index (TFI)
Ray et al. [23]	India	1-5 and 11-15	2,159	WHO
Shanthi et al. [24]	India	9-12	1,500	Dean's index
Shomar et al. [25]	Gaza Strip	5–16	353	Dean's index
Tobayiwa et al. [26]	Zimbabwe I	8-15	200	Thylstrup and Fejerskov index (TFI)
Zhu et al. [27]	China	8–12	9,030	Horowitz's Tooth Surface Index of Fluorosis (modification of Dean's index)

Table 5. Results of the studies selected for this systematic review.

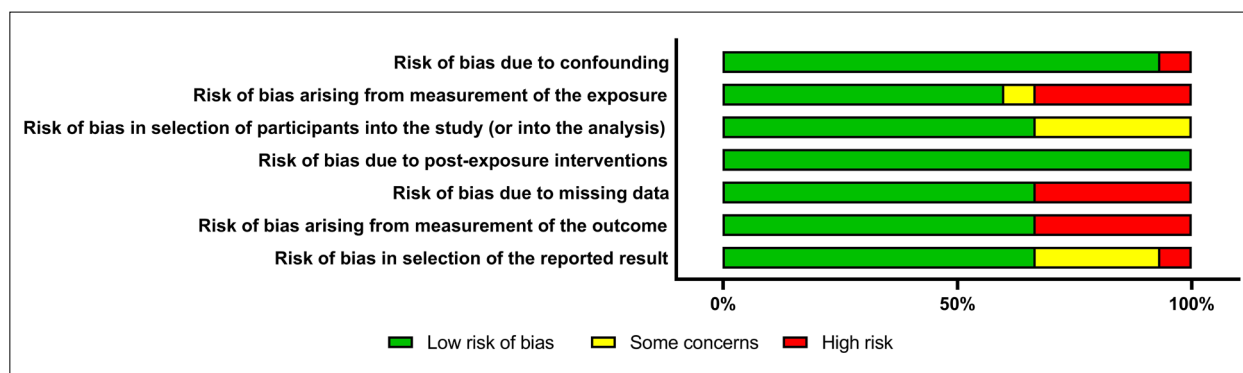| Study ID	F- concentration informed by the authors, in ground water analyzed	Daily Water Consumption	Prevalence (total) of fluorosis	Prevalence of dental fluorosis (mild)	Prevalence of fluorosis (moderate)	Prevalence of fluorosis (severe)
Akpata et al[13]	0.543 to 2.848 ppm	-	90.7%	14.73%	31.25%	44.67%
Alarcon-Herrera et al[14]	< 1.5 to 16 mg/L	-	86.4%	67.88%	10.8%	7.8%
Arif et al[15]	0.5 to 8.5 mg/L.	>4 mg/day	69.3%	31.9%	58.6%	9.4%
Gautam et al[16]	0.64 to 14.62 mg/L	-	90.8%	-	-	-
Ibrahim et al[17]	0.25 to 2.56 ppm	-	95.5%	63.7%	27.4%	4.4%
Indermitte et al[18]	0.01 to 7.20 mg/L	-	17.5%	-	-	-
Ismail et al[19]	< 3.8 ppm	-	66.7 %	-	-	-
Mandinic et al[20]	0.10 to 11 ppm	-	4.2%	3.65	0	0.6
Narwaria et al[21]	1.5 to 3 ppm	-	45.4%	20.8 %	19.47 %	5.2 %
Rango et al[22]	1.1–18 mg/L (8.5 ± 4.1 mg/L)	1.2 ± 0.4 L/d	100%	17%	29%	45%
Ray et al[23]	0.2 to 2.1 ppm	-	24.9%	8.33%	2.17%	-
Shanthi et al[24]	< 0.7 to 3.5 ppm	-	48.3%	35%	9.2%	4.1%
Shomar et al[25]	0.7 to 2.6 ppm	-	60%	-	-	-
Tobayiwa et al[26]	5-10 ppm	-	64%	-	-	-
Zhu et al[27]	1.0 to 2.0 mg/L	-	38.6%	25.6%	10.0%	3.0%

Fig. 2: Quality assessment of the studies evaluated.

fluoride is therefore of great practical significance for drinking water safety[28]. The WHO recommends a fluoride concentration in drinking water in the range of 0.5 to 1.5 mg/L[29]. The current review only included research papers reporting consumption of groundwater with fluoride concentration above 1.5 mg/L.

Fluorides are naturally ubiquitous in the environment (water, soil, air, etc.). The amount of fluoride in water can be georeferenced, and high levels have been reported in certain places, especially in Asia and Africa[20].

Several studies conducted around the world provide strong evidence of association between fluoride concentration in drinking water and prevalence of fluorosis [13,14,16,18,21,22]. To facilitate data collection, the current study evaluated the risk in children because most primary studies consider school-age children, who are easily screened.

Increasing fluoride intake with fluoridated water can increase the risk of dental fluorosis in a situation of high exposure in children under 8 years of age. Nevertheless, in adults, such exposure is not a problem, but provides protection against dental caries[30].

The only way to prevent dental fluorosis in children is to ensure that the fluoride concentration in the water they consume is within safe limits[18]. It should be noted that water is used in food preparation as well as for drinking. The current review found that in places all over the world where people drink groundwater with high fluoride concentration, there is higher prevalence of dental fluorosis. Even though public health officers are aware of the negative impact of dental fluorosis, a condition affecting much of the population in developing countries, governments do not address the issue[28]. The inability to find alternative water sources is a challenge to the reduction of exposure to high fluoride levels.

Some studies assess the risk and/or prevalence of fluorosis in populations exposed to groundwater, most of which consider water from wells or natural sources. The concentration of fluoride in spring water is lower than in groundwater. Only two systematic reviews addressed this issue, both of which were based on local data[7,31]. A study with data at global level was therefore considered necessary.

The selected studies indicate that the prevalence of dental fluorosis increases with the concentration of fluoride in groundwater. To prevent dental fluorosis, it is suggested that groundwater wells should be routinely analyzed for fluoride concentration, and if necessary, treated appropriately with fluoridation or defluoridation, to ensure an anti-caries effect with minimum risk of fluorosis.

CONCLUSIONS

A relationship was identified between the consumption of fluoridated groundwater and the prevalence of fluorosis in individuals up to 18 years of age, this study being the first to systematically assess the situation in different continents.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

REFERENCES

1. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res. 2004 May-Jun;38(3):182-91. https://doi.org/10.1159/000077753

2. Buzalaf MAR, Pessan JP, Honório HM, Ten Cate JM. Mechanisms of action of fluoride for caries control. In Buzalaf MAR (ed): Fluoride and the Oral Environment. Monogr Oral Sci. Basel, Karger, 2011, vol 22, pp 97-114. 2.

3. Narvai PC, Castellanos RA, Frazão P. Prevalência de cárie em dentes permanentes de escolares do Município de São Paulo, SP, 1970-1996 [Dental caries prevalence in permanent teeth of schoolchildren in the Municipality of Sao Paulo, Brazil, 1970-1996]. Rev Saude Publica. 2000 Apr;34(2):196-200. Portuguese. https://doi.org/10.1590/S0034-89102000000200015

4. Rompante P. Mecanismos preventivos do flúor e cárie dentária. Instituto Superior de Ciências da Saúde Norte, Sociedade Portuguesa de Pediatria. Acta Pediatr Port. 2009;40(5):223-8. https://doi.org/10.25754/pjp.2009.4511

5. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A. Dental caries. Nat Rev Dis Primers. 2017 May 25;3:17030. https://doi.org/10.1038/nrdp.2017.30

6. Mohammadi AA, Yousefi M, Yaseri M, Jalilzadeh M, Mahvi AH. Skeletal fluorosis in relation to drinking water in rural areas of West Azerbaijan, Iran. Sci Rep. 2017 Dec 11;7(1):17300. https://doi.org/10.1038/s41598-017-17328-8

7. Demelash H, Beyene A, Abebe Z, Melese A. Fluoride concentration in ground water and prevalence of dental fluorosis in Ethiopian Rift Valley: systematic review and meta-analysis. BMC Public Health. 2019 Oct 16;19(1):1298. https://doi.org/10.1186/s12889-019-7646-8

8. Mohammadi AA, Yousefi M, Yaseri M, Jalilzadeh M, Mahvi AH. Skeletal fluorosis in relation to drinking water in rural areas of West Azerbaijan, Iran. Sci Rep. 2017 Dec 11;7(1):17300. https://doi.org/10.1038/s41598-017-17328-8

9. Yousefi M, Asghari FB, Zuccarello P, Oliveri Conti G et al. Spatial Distribution Variation and Probabilistic Risk Assessment of Exposure to Fluoride in Ground Water Supplies: A Case Study in an Endemic Fluorosis Region of Northwest Iran. Int J Environ Res Public Health. 2019 Feb 15;16(4):564. https://doi.org/10.3390/ijerph16040564

10. Bhagavan SV, Raghu V. Utility of check dams in dilution of fluoride concentration in ground water and the resultant analysis of blood serum and urine of villagers, Anantapur District, Andhra Pradesh, India. Environ Geochem Health. 2005 Feb;27(1):97-108. https://doi.org/10.1007/s10653-004-0786-4

11. Chuah CJ, Lye HR, Ziegler AD, Wood SH, Kongpun C, Rajchagool S. Fluoride: A naturally-occurring health hazard in drinking-water resources of Northern Thailand. Sci Total Environ. 2016 Mar 1;545-546:266-79. https://doi.org/10.1016/j.scitotenv.2015.12.069

12. Chouhan S, Flora SJ. Arsenic and fluoride: two major groundwater pollutants. Indian J Exp Biol. 2010 Jul;48(7):666-78. https://nopr.niscpr.res.in/bitstream/123456789/9735/1/IJEB%2048(7)%20666-678.pdf

13. Akpata ES, Fakiha Z, Khan N. Dental fluorosis in 12-15-year-old rural children exposed to fluorides from well drinking water in the Hail region of Saudi Arabia.

Community Dent Oral Epidemiol. 1997 Aug;25(4):324-7. https://doi.org/10.1111/j.1600-0528.1997.tb00947.x

14. Alarcon-Herrera MT, Martin-Dominguez IR, Trejo-Vázquez R, Rodriguez-Dozal S. Well, water fluoride, dental fluorosis, and bone fractures in the Guadiana Valley of Mexico. Fluoride. 2001;34(2), 39-149. https://www.fluorideresearch.online/342/files/FJ2001_v34_n2_p139-149fig.pdf

15. Arif M, Husain I, Hussain J, Kumar S. Assessment of fluoride level in groundwater and prevalence of dental fluorosis in Didwana block of Nagaur district, Central Rajasthan, India. Int J Occup Environ Med. 2013 Oct;4(4):178-84. https://www.semanticscholar.org/paper/Assessment-of-fluoride-level-in-groundwater-and-of-Arif-Husain/1cb61615a0d37f655d06fcdbe9456e9dab38cc0f

16. Gautam R, Bhardwaj N, Saini Y. Study of fluoride content in groundwater of Nawa Tehsil in Nagaur, Rajasthan. J Environ Biol. 2011 Jan;32(1):85-9. https://pubmed.ncbi.nlm.nih.gov/21888237/

17. Ibrahim YE, Affan AA, Bjorvatn K. Prevalence of dental fluorosis in Sudanese children from two villages with 0.25 and 2.56 ppm fluoride in the drinking water. Int J Paediatr Dent. 1995 Dec;5(4):223-9. https://doi.org/10.1111/j.1365-263X.1995.tb00183.x

18. Indermitte E, Saava A, Karro E. Exposure to high fluoride drinking water and risk of dental fluorosis in Estonia. Int J Environ Res Public Health. 2009 Feb;6(2):710-21. https://doi.org/10.3390/ijerph6020710

19. Ismail AI, Messer JG. The risk of fluorosis in students exposed to a higher than optimal concentration of fluoride in well water. J Public Health Dent. 1996 Winter;56(1):22-7. https://doi.org/10.1111/j.1752-7325.1996.tb02390.x

20. Mandinic Z, Curcic M, Antonijevic B, Carevic M et al. Fluoride in drinking water and dental fluorosis. Sci Total Environ. 2010 Aug 1;408(17):3507-12. https://doi.org/10.1016/j.scitotenv.2010.04.029

21. Narwaria YS, Saksena DN. Prevalence of dental fluorosis among primary school children in rural areas of Karera Block, Madhya Pradesh. Indian J Pediatr. 2013 Sep;80(9):718-20. https://doi.org/10.1007/s12098-013-1082-z

22. Rango T, Vengosh A, Jeuland M, Tekle-Haimanot R et al. Fluoride exposure from groundwater as reflected by urinary fluoride and children's dental fluorosis in the Main Ethiopian Rift Valley. Sci Total Environ. 2014 Oct 15;496:188-197. https://doi.org/10.1016/j.scitotenv.2014.07.048

23. Ray SK, Ghosh S, Tiwari IC, Kaur P, Reddy DCS, Nagchaudhuri J. Dental fluorosis in Ledhepur and Rustampur villages near Varanasi. Indian Journal of Medical Research. 1983;77(1):112-188. https://www.ircwash.org/sites/default/files/245.4-1876.pdf

24. Shanthi M, Reddy BV, Venkataramana V, Gowrisankar S et al. Relationship Between Drinking Water Fluoride Levels, Dental Fluorosis, Dental Caries and Associated Risk Factors in 9-12 Years Old School Children of Nelakondapally Mandal of Khammam District, Andhra Pradesh, India: A Cross-sectional Survey. J Int Oral Health. 2014 Jun;6(3):106-10. https://www.ispcd.org/userfiles/rishabh/jioh-06-03-106.pdf

25. Shomar B, Müller G, Yahya A, Askar S, Sansur R. Fluorides in groundwater, soil and infused black tea and

the occurrence of dental fluorosis among school children of the Gaza strip. J Water Health. 2004 Mar;2(1):23-35. Erratum in: J Water Health. 2004 Sep;2(3):215. https://doi.org/10.2166/wh.2004.0003

26. Tobayiwa C, Musiyambiri M, Chironga L, Mazorodze O, Sapahla S. Fluoride levels and dental fluorosis in two districts in Zimbabwe. Cent Afr J Med. 1991 Nov;37(11):353-61.

27. Zhu C, Bai G, Liu X, Li Y. Screening high-fluoride and high-arsenic drinking waters and surveying endemic fluorosis and arsenism in Shaanxi province in western China. Water Res. 2006 Sep;40(16):3015-3022. https://doi.org/10.1016/j.watres.2006.06.026

28. Onipe T, Edokpayi JN, Odiyo JO. A review on the potential sources and health implications of fluoride in groundwater of Sub-Saharan Africa. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2020;55(9):1078-1093. https://doi.org/10.1080/10934529.2020.1770516

29. Bårdsen A, Klock KS, Bjorvatn K. Dental fluorosis among persons exposed to high- and low-fluoride drinking water in western Norway. Community Dent Oral Epidemiol. 1999 Aug;27(4):259-67. https://doi.org/10.1111/j.1600-0528.1998.tb02019.x

30. Satou R, Oka S, Sugihara N. Risk assessment of fluoride daily intake from preference beverage. J Dent Sci. 2021 Jan;16(1):220-228. https://doi.org/10.1016/j.jds.2020.05.023

31. Islam MS, Mostafa MG. Meta-analysis and risk assessment of fluoride contamination in groundwater. Water Environ Res. 2021 Aug;93(8):1194-1216. https://doi.org/10.1002/wer.1508

Unprepared areas and centralization of oval canals prepared with WaveOne Gold or XP-endo Shaper: microcomputed tomographic analyses

Marília FV Marceliano-Alves[1], Vivian Ronquete[1], Thais MC Coutinho[1], Tchilalo Boukpessi[2], Amanda LF Salvioni[1], Paula ASR Goulart[1], Ana GS Limoeiro[1], Flavio RF Alves[4], Pablo A Amoroso-Silva[4]

1. Universidade Iguaçu, Departmento de Endodontia, Nova Iguaçu, Rio de Janeiro, Brazil
2. Université Paris Cité, Laboratory of Orofacial Pathologies, Imaging and Biotherapies, Laboratoire d'Excellence INFLAMEX, URP 2496, Montrouge, France
3. Private Practice, Jequié, Brazil.
4. Universidade Grande Rio, Programa de pós-graduação em Odontologia, Duque de Caxias, Brazil

ABSTRACT

*Current instrumentation systems cannot fully prepare oval root canal systems. This may cause accumulation of hard tissue debris and fail to eliminate bacteria from areas inaccessible to instrumentation, which could perpetuate periapical inflammation and jeopardize the success of endodontic treatment. **Aim:** To evaluate the performance of two endodontic systems in oval canals by investigating the changes in volume, unprepared areas, and centering ability of XP-endo Shaper (XPS) and WaveOne Gold (WOG) in oval canals using microcomputed tomography (micro-CT). **Materials and Method:** Thirty mandibular canines were scanned before and after preparation with WOG (25/.07 and 35/.06) or XPS (30/.01) to evaluate the volume, surface area, and canal centralization at 4 mm and 10 mm from the apical foramen. **Results:** Volume and surface area increased significantly after preparation with both systems (p < 0.05). However, no significant difference was observed in the unprepared areas, regarding either the entire canal (26.21% for WOG and 30.10% for XPS), or the apical segment (18.82% for WOG and 14.63% for XPS) (p >0.05). **Conclusions:** Both systems maintained canal centralization, with no difference between them. XPS and WOG had similar shaping abilities in the mandibular canine, but left almost one third of the unprepared areas.*

Keywords: endodontics - root canal preparation - X ray microtomography.

Areas não preparadas e centralização de canais ovais preparados com WaveOne Gold ou XP-endo Shaper: análises tomográficas microcomputadorizadas

RESUMO

*Os sistemas de instrumentação atuais são incapazes de preparar completamente os sistemas de canais radiculares do canal oval, o que pode levar ao acúmulo de detritos de tecido duro e manter micro-organismos em áreas inacessíveis à instrumentação. Essas bactérias poderiam perpetuar a inflamação periapical e comprometer o sucesso do tratamento endodôntico. **Objetivo:** Para avaliar o comportamento de dois sistemas endodônticos em canais ovais, esse estudo investigou as alterações no volume, áreas não preparadas e capacidade de centralização do XP-endo Shaper (XPS) e do WaveOne Gold (WOG) em canais ovais usando microtomografia computadorizada (micro-CT). Material e métodos: Trinta caninos inferiores foram escaneados antes e depois do preparo com WOG (25/.07 e 35/.06) ou XPS (30/.01) para avaliar o volume, a área de superfície e a centralização do canal a 4 mm e 10 mm do forame apical. **Resultados:** O volume e a área de superfície aumentaram significativamente após o preparo com ambos os sistemas (p < 0,05). No entanto, não foram observadas diferenças significativas nas áreas não preparadas, não apenas em todo o canal (26,21% para WOG e 30,10% para XPS), mas também no segmento apical (18,82% para WOG e 14,63% para XPS) (p >0,05). **Conclusão:** Ambos os sistemas mantiveram a centralização do canal, sem diferenças entre eles. O XPS e o WOG tiveram habilidades de modelagem semelhantes no canino mandibular, mas deixaram quase um terço das áreas do canal sem preparo.*

Palavras-chave: endodontia - preparo do canal radicular - microtomografia por raio-X.

INTRODUCTION

The main goal of infected root canal treatment is maximum intracanal microbial reduction, primarily by irrigation, chemical debridement, and mechanical action of instruments[1], to allow healing of the periradicular tissues. However, these steps can be challenging due to the complexity of the root canal anatomy[2].

Mandibular canines usually have high incidence of oval root canals[3], which various instrumentation systems cannot clean completely[4], leaving unprepared areas[4,5]. In addition, anatomic complexity may complicate infection control by allowing accumulation of hard tissue debris and failing to eliminate bacteria from inaccessible areas to instrumentation[2,4,5]. Remaining microorganisms can perpetuate periapical inflammation and jeopardize the success of endodontic treatment[6]. Therefore, instruments with different kinematics and heat treatment have been developed to treat root canals with complex anatomy, such as oval root canals[6].

The WaveOne Gold (WOG) (Dentsply-Sirona, Ballaigues, Switzerland) is a reciprocating single-file system made of a heat-treated metal alloy (M-wire). It has a parallelogram-shaped cross-section with two cutting edges, resulting in one or two points of contact between the instrument and the dentin walls. These features provide increased flexibility and improved cyclic fatigue resistance compared to conventional NiTi alloys[7,8].

The XP-endo Shaper (XPS) system (30/0.1) (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) uses the Max-Wire alloy (Brasseler, Savannah, GA). It is a single-use file with rotating, eccentric motion to treat the canal three-dimensionally, and it responds to temperature variations. It has a triangular cross-section and a booster tip with six sharp edges and a reduced diameter that facilitates advancement into the apical region of the root canal while maintaining the original curvature. According to the manufacturer, it can expand from its original taper of 0.1 to 0.4 at the end of preparation, enabling extended horizontal action. The manufacturer states two mechanisms of action: agitation of the irrigation fluid, which increases cleaning power, and mechanical contact with the inner walls of the canal, which increases the removal of microorganisms by reaching areas of the canal not reached by the usual instruments.

Microcomputed tomography (micro-CT) is a nondestructive method that provides high-resolution, three-dimensional images for assessment of internal tooth anatomy, and considered the gold standard for assessment ot the root canal preparation[2,10].

The ability of WOG and XPS to clean and disinfect canines has been studied previously, but to our knowledge, this is the first work comparing centering ability[11]. The aim of this study was to evaluate the changes in volume and area, the percentage of unprepared canal areas and the centering ability of the XPS and WOG systems in oval root canals, using micro-CT.

MATERIAL AND METHOD

The project for this study was submitted to and approved by the Institutional Research Ethics Committee (CAAE: 53653621.2.0000.8044).

Sample selection and initial micro-CT scanning

G*Power 3.1 software (Heinrich Heine College, Duesseldorf, Germany) was used to calculate power with $\beta = 95\%$ and $\alpha = 5\%$, and a t test for independent samples was applied. The ideal sample size for each group was determined to be at least 10 teeth. Five additional samples per group were added to compensate for possible loss.

Thirty mandibular canines with moderately curved roots (10° to 20°) were selected from a pool of 432 teeth. All specimens were scanned in micro-CT scanner (SkyScan 1173, Bruker, Kontich, Belgium) at 70 kV and 114 mA, with a 1-mm-thick aluminum filter, exposure time 320 milliseconds, pixel size 12.1 μm, rotation step 0.8°, and rotation 360° along the vertical axis. The files were reconstructed into a three-dimensional data set using the program NRecon v1.6.1.0 (Bruker micro-CT), with beam hardening correction of 50%, ring artifact correction of 10, and smoothing of 5. The volume of interest extended from the cementoenamel junction to the root apex, resulting in the acquisition of 600 to 700 axial slices per sample.

CTAn v.1.14.4 and CTVol v.2.2.1 software (Bruker Micro-CT) were used to evaluate the morphology of the root canal in terms of volume, surface area, and 3D configuration. Teeth were paired based on the anatomical similarities of preoperative canal volume, canal length of approximately 12 mm, canal surface area, and 3D configuration. One specimen from each pair was randomly assigned to each of the 2 experimental groups (n=15): XPS and WOG.
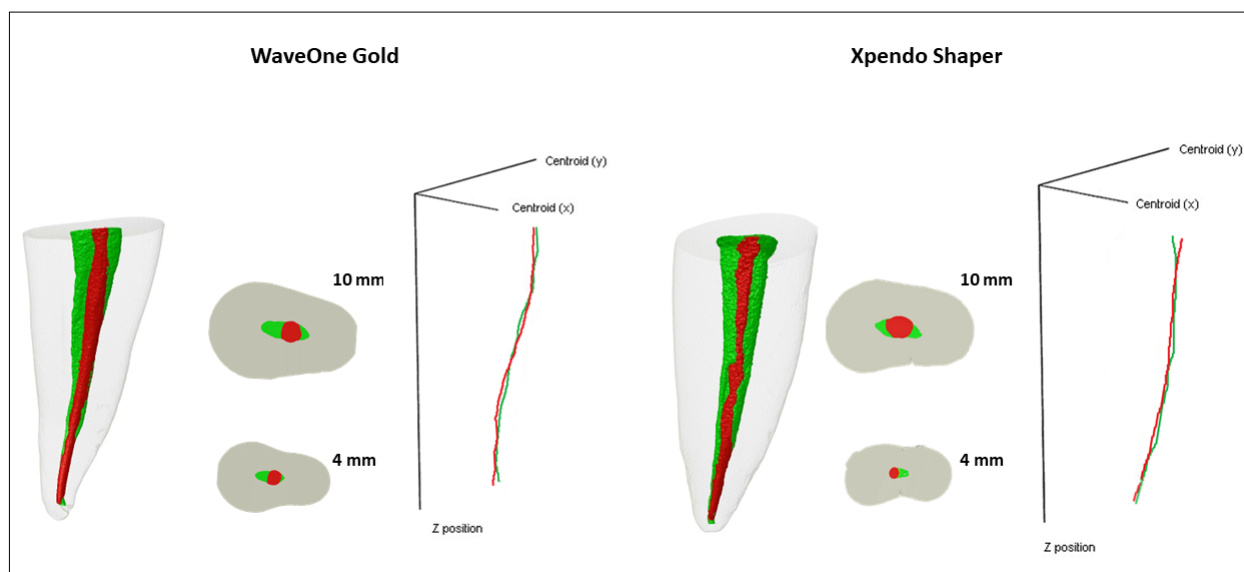
Fig. 1: Representative images show the unprepared areas highlighted in green. Diagram shows the centralization after preparation (red) compared to the centralization of the sound canal (Z-axis-green) along the entire canal (10 mm segment).

Root canal preparation

The canals were rinsed with 1 mL of 2.5% NaOCl and examined with a #10 K manual file (Dentsply-Maillefer, Ballaigues, Switzerland) until the tip of the instrument was visible in the apical foramen under 8× magnification. The working length (WL) was determined to be 1.0 mm based on this measurement. To simulate the resistance imposed by the periodontal ligament and allow the formation of vapor lock, the apexes were sealed with Top Dam (FGM, Joinville, Brazil).

The XPS 30/.01 instrument (promoting a 30/.04 preparation) was used with a 6:1 contra-angle handpiece coupled to an electric motor (VDW Silver, VDW GnbH, Munich, Germany) set at 800 rpm and 100 g/cm. The instrument was used with gentle strokes of 5 to 7 mm to WL.

The WOG system was driven by an electric motor (VDW GmbH, Munich, Germany). The preparation started with the primary instrument (25/.07) and was completed with the medium instrument (35/.06) in reciprocating motion with gentle apical pressure in slow in and out movements with slight apical advancements of 2-3 mm to the WL.

All procedures were performed in a cabinet maintained at 37 °C by a heater (800-Heater; PlasLabs, Lansing, USA) to simulate body temperature. For both groups, after 3 instrumentation cycles (1 cycle equal to 3 back-and-forth movements), the canal was flushed with 1 mL of 2.5% NaOCl, patency was checked with a #10 K file, and the instrument was cleaned with sterile gauze. Irrigation was performed with a 30G NaviTip needle (Ultradent Products Inc, South Jordan, USA) 3 mm from WL using a 5 mL disposable syringe. The irrigant was simultaneously aspirated at the canal opening with a cannula connected to a 90 mL/s suction pump.

Micro-CT images and analyses after instrumentation

After instrumentation, all teeth were rescanned in a micro-CT (Skyscan 1173, Bruker) under the same acquisition and reconstruction parameters. The volume (mm³) and surface area (mm²) at 4 mm (apical canal) and 10 mm (entire canal) from the apical foramen were calculated using Image J 1.50d software (National Institutes of Health, Bethesda, MD). The same software was used to evaluate the amount of unprepared root canal areas (%) by calculating the number of static voxels by the total number of voxels on the canal surface and expressing it as a percentage. CTVol v.2.3.1 software (Bruker, micro- CT) was used to define a color-coded pattern for the canal templates (green for before instrumentation and red for after instrumentation) (Fig. 1). This enabled comparison of the recorded pre- and postoperative scan canal models. Centering ability was assessed by the center of gravity of the canal (Z-axis) compared to before and after preparations along the entire canal (10 mm segment).

Statistical analysis

Statistical analysis was performed using R16 software. Normality of the data was checked using the Shapiro-Wilk test. The Mann-Whitney test was used to compare the differences between the XPS and WOG groups, both for the entire canal and for the apical canal. The Wilcoxon test was used for within-group analyses. A significance level of 5% ($p < 0.05$) was considered for all tests.

RESULTS

Volume and area increased significantly after preparation with both systems ($p < 0.05$). However, no significant difference was observed in the unprepared areas, either in the entire canal (26.21% for WOG and 30.10% for XPS), or in the apical segment (18.82% for WOG and 14.63% for XPS) ($p > 0.05$) (Table 1). Both systems maintained the centralization (centroid analysis) of the canal with no difference between them ($p > 0.05$) (Table 1).

Table 1. Variations of canal parameters after canal preparation with the tested systems.

Micro-CT canal parameters	WaveOne Gold mean (SD)	XP-endo Shaper mean (SD)
4 mm		
Surface area (mm²)	18.82 (21.27)	14.63 (11.93)
Volume (mm³)	5.19 (8.47)	3.28 (11.36)
Unprepared areas (%)	14.19 (17.63)	12.51 (9.08)
10 mm		
Surface area (mm²)	32.02 (33.60)	28.75 (48.48)
Volume (mm³)	16.88 (14.82)	13.07 (16.96)
Unprepared areas (%)	26.21 (29.05)	30.10 (19.29)

DISCUSSION

This multi-analytic study of oval canals evaluated changes in volume and area, as well as the percentage of unprepared areas and the centralization of root canal preparation with XPS and WOG. Separate analyses were performed for the entire canal and the apical canal to better understand the shaping ability of these two new systems. Strict tooth pairing was performed based on micro-CT anatomical parameters to minimize selection bias, even when the same tooth type was used, because anatomical variability within the same group of teeth, including mandibular canines, can be significant[12].

Cleaning and shaping oval canals is considered challenging in clinical practice[1,2]. Root canal preparation is affected not only by the characteristics of the instruments, but also by the variability of dental anatomy. In mandibular canines, canals may have varying lengths and cross-sectional shapes throughout the root, but they are generally oval in the cervical and middle thirds, whereas they may be more round or slightly oval in the apical third[4]. The major challenges in preparing these teeth include maintaining the original canal shape, apical foramen and curvature, and preparing them evenly in all directions[13–15].

Unprepared canal areas and their potential impact on endodontic prognosis are a matter of concern. A histologic and scanning electron microscopy study showed that unprepared areas still contain residual pulp and/or bacterial cells despite an extensive irrigation protocol[1]. Instruments with expansion capacity, such as the XPS, have been proposed to reduce unprepared canal areas, especially in oval canals. The rotary eccentric motion combined with contraction of the instrument at body temperature has the potential to contact more areas than conventional rotary/reciprocating instruments, as shown by some studies on distal roots of mandibular molars[8,16] and mandibular canines[17]. However, these results were not confirmed in the present study, in which the WOG system showed similar results. Whereas in the previously mentioned study, XPS was compared to the Mtwo 35.05 instrument in canines, in the present study, XPS was compared to the WOG 35.06 instrument. This difference in taper could explain the similarity of the unprepared areas between the systems tested. In addition, the choice of the final apical instrument in our study was based on the median apical diameters (0.30 to 0.40 mm) reported in previous studies[18].

According to other studies, XPS and WOG perform better in round or small oval canals[2,8,17]. In contrast, some studies have reported that unprepared canal surfaces after instrumentation can range from 10.9% to 59.6%, especially in long oval canals[8,19,20].

The current study found that unprepared canal surfaces as a percentage of total canal length were 26% for WOG and 30% for XPS, suggesting that XPS cannot reach all areas of the oval root canal despite its properties[8,21,22]. Therefore, in teeth with complex canal anatomy, including oval canals, additional strategies are recommended to optimize root canal shaping, such as circular filling with Hedströem files or ultrasonic tips[23,24].

Apical transportation after canal preparation depends

on the degree and radius of curvature, canal anatomy, and the instrument used. Some accidents may weaken and tear the dentin wall, compromising success[25]. In the present study, no significant deviation was observed for either system, both of which were similar. This result is consistent with another study on mesial canals of mandibular molars[26].

The tested instruments produced well-centered preparations, which can be attributed to their mechanical behavior as a result of their good flexibility provided by the alloys of which they are manufactured[8].

CONCLUSIONS
XPS and WOG had similar shaping abilities in the oval canals of mandibular canines and maintained the centralization of the canals. However, almost one-third of the oval canal areas remained unprepared.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

REFERENCES
1. Siqueira JF, Pérez AR, Marceliano-Alves MF, Provenzano JC, et al. What happens to unprepared root canal walls: a correlative analysis using micro-computed tomography and histology/scanning electron microscopy. Int Endod J. 2018;51:501-8. https://doi.org/10.1111/iej.12753

2. Lacerda MFLS, Marceliano-Alves MF, Pérez AR, Provenzano JC, et al. Cleaning and Shaping Oval Canals with 3 Instrumentation Systems: A Correlative Micro-computed Tomographic and Histologic Study. J Endod. 2017;43:1878-84. https://doi.org/10.1016/j.joen.2017.06.032

3. Wu MK, R'oris A, Barkis D, Wesselink PR. Prevalence and extent of long oval canals in the apical third. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89:739-43. https://doi.org/10.1067/moe.2000.106344

4. Versiani MA, Leoni GB, Steier L, De-Deus G, et al. Micro-computed tomography study of oval-shaped canals prepared with the self-adjusting file, Reciproc, WaveOne, and ProTaper universal systems. J Endod. 2013;39:1060-6. https://doi.org/10.1016/j.joen.2013.04.009

5. Peters OA, Laib A, Göhring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod. 2001;27:1-6. https://doi.org/10.1097/00004770-200101000-00001

6. Versiani MA, Alves FRF, Andrade-Junior C V, Marceliano-Alves MF, et al. Micro-CT evaluation of the efficacy of hard-tissue removal from the root canal and isthmus area by positive and negative pressure irrigation systems. Int Endod J. 2016;49:1079-87. https://doi.org/10.1111/iej.12559

7. Özyürek T. Cyclic Fatigue Resistance of Reciproc, WaveOne, and WaveOne Gold Nickel-Titanium Instruments. J Endod. 2016;42:1536-9. https://doi.org/10.1016/j.joen.2016.06.019

8. Poly A, Marques F, Moura Sassone L, Karabucak B. The shaping ability of WaveOne Gold, TRUShape and XP-endo Shaper systems in oval-shaped distal canals of mandibular molars: A microcomputed tomographic analysis. Int Endod J. 2021;54:2300-6. https://doi.org/10.1111/iej.13625

9. Gavini G, Santos M Dos, Caldeira CL, Machado ME de L, et al. Nickel-titanium instruments in endodontics: a concise review of the state of the art. Braz Oral Res. 2018 18;32(suppl 1):e67. https://doi.org/10.1590/1807-3107bor-2018.vol32.0067

10. Peters OA, Arias A, Paqué F. A Micro-computed Tomographic Assessment of Root Canal Preparation with a Novel Instrument, TRUShape, in Mesial Roots of Mandibular Molars. J Endod. 2015;41:1545-50. https://doi.org/10.1016/j.joen.2015.06.007

11. Krokidis A, Nicola B, Antonio C, Panopoulos P. Comparison of Two Reciprocating and Anatomical Single File Techniques in Cleaning Oval Anatomies. Iran Endod J. 2023;18:41-6. https://doi.org/10.22037%2Fiej.v18i1.33623

12. Versiani MA, Pécora JD, Sousa-Neto MD. Microcomputed tomography analysis of the root canal morphology of single-rooted mandibular canines. Int Endod J. 2013;46:800-7. https://doi.org/10.1111/iej.12061

13. Versiani MA, Carvalho KKT, Mazzi-Chaves JF, Sousa-Neto MD. Micro-computed Tomographic Evaluation of the Shaping Ability of XP-endo Shaper, iRaCe, and EdgeFile Systems in Long Oval-shaped Canals. J Endod. 2018;44:489-95. https://doi.org/10.1016/j.joen.2017.09.008

14. Ronquete V, Martin AS de, Zuim K, Coutinho TM de C, et al. Microtomographic Evaluation of Canal Centralization and Dentine Removal after Canal Preparation with Two Rotary Systems: HyFlex EDM and ProTaper Next. Eur J Dent. 2022;16:663-8. https://doi.org/10.1055/s-0041-1739440

15. Pacheco-Yanes J, Gazzaneo I, Campello AF, Marceliano-Alves MF, et al. Planned Apical Preparation Using Cone-Beam Computed Tomographic Measures: A Micro-Computed Tomographic Proof of Concept in Human Cadavers. J Endod. 2022;48:280-6. https://doi.org/10.1016/j.joen.2021.11.016

16. Poly A, Tseng WJL, Marques F, Setzer FC, et al. Micro-Computed Tomographic Analysis of the Shaping Ability of XP-Endo Shaper in Oval-Shaped Distal Root Canals of Mandibular Molars. Eur Endod J. 2021;6:271-7. https://doi.org/10.14744/eej.2021.44153

17. Xavier SR, de Lima CO, Marceliano-Alves MFV, Lacerda

MFLS, et al. Shaping ability of two root canal instrumentation systems in oval-shaped canals: A microcomputed tomography study. Aust Endod J 2021;47:252-9. https://doi.org/10.1111/aej.12469

18. Green D. A stereomicroscopic study of the root apices of 400 maxillary and mandibular anterior teeth. Oral Surg Oral Med Oral Pathol.. 1956;9:1224-32. https://doi.org/10.1016/0030-4220(56)90192-X

19. Paqué F, Balmer M, Attin T, Peters OA. Preparation of oval-shaped root canals in mandibular molars using nickel-titanium rotary instruments: a micro-computed tomography study. J Endod. 2010;36:703-7. https://doi.org/10.1016/j.joen.2009.12.020

20. Siqueira Junior JF, Rôças I das N, Marceliano-Alves MF, Pérez AR, et al. Unprepared root canal surface areas: causes, clinical implications, and therapeutic strategies. Braz Oral Res. 2018 18;32(suppl 1):e65. https://doi.org/10.1590/1807-3107bor-2018.vol32.0065

21. Velozo C, Silva S, Almeida A, Romeiro K, et al. Shaping ability of XP-endo Shaper and ProTaper Next in long oval-shaped canals: a micro-computed tomography study. Int Endod J. 2020;53:998-1006. https://doi.org/10.1111/iej.13301

22. Thomas JP, Lynch M, Paurazas S, Askar M. Micro-computed Tomographic Evaluation of the Shaping Ability of WaveOne Gold, TRUShape, EdgeCoil, and XP-3D Shaper Endodontic Files in Single, Oval-shaped Canals: An In Vitro Study. J Endod. 2020;46:244-251.e1. https://doi.org/10.1016/j.joen.2019.11.001

23. Gazzaneo I, Amoroso-Silva P, Pacheco-Yanes J, Alves FRF, et al. Disinfecting and Shaping Type I C-shaped Root Canals: A Correlative Micro-computed Tomographic and Molecular Microbiology Study. J Endod.2021;47:621-30. https://doi.org/10.1016/j.joen.2020.11.007

24. Amoroso-Silva P, Alcalde MP, Hungaro Duarte MA, De-Deus G, et al. Effect of finishing instrumentation using NiTi hand files on volume, surface area and uninstrumented surfaces in C-shaped root canal systems. Int Endod J. 2017;50:604-11. https://doi.org/10.1111/iej.12660

25. Bürklein S, Mathey D, Schäfer E. Shaping ability of ProTaper Next and BT-RaCe nickel-titanium instruments in severely curved root canals. Int Endod J. 2015;48:774-81. https://doi.org/10.1111/iej.12375

26. Pérez Morales M de LN, González Sánchez JA, Olivieri JG, Elmsmari F, et al. Micro-computed Tomographic Assessment and Comparative Study of the Shaping Ability of 6 Nickel-Titanium Files: An In Vitro Study. J Endod. 2021;47:812-9. https://doi.org/10.1016/j.joen.2020.12.021

Core curriculum in cariology for dentistry degrees in the Republic of Argentina

Fabiana PM Carletto-Körber[1], Ana Sorazabal[2], Silvia Medici[2], Sofía Ali[3], Alejandra Barrionuevo[4], Juana Cardozo[3], Analía Cucchi[5], Luciana D'Eramo[2], Jorge Escandriolo[6], Pablo Fontanetti[1], Shirley García[7], Adriana Lombardo[8], Alejandra Mardenlli[9], Carla Masoli[2-8], Patricia Mulbany[10], Lía Pereira[7], Silvia Pérez[3], Mariana Preliasco[10], Glenda Rossi[2], Aldo Squassi[2], Jorgelina Ulloque[11], Patricia Vaculik[3], Verónica Ventrera[4], Noemí Bordoni[2], Rodrigo A Giacaman[12].

1. Universidad Nacional de Córdoba. Facultad de Odontología. Departamento de Odontología Preventiva y Comunitaria. Córdoba-Argentina
2. Universidad de Buenos Aires. Facultad de Odontología. Buenos Aires-Argentina
3. Universidad Nacional del Nordeste. Facultad de Odontología. Corrientes-Argentina.
4. Universidad Nacional de Cuyo. Facultad de Odontología. Mendoza-Argentina.
5. Universidad Maimonides. Facultad de Odontología. Buenos Aires-Argentina.
6. Universidad Adventista del Plata. Facultad de Odontología. Entre Ríos-Argentina.
7. Universidad Abierta Interamericana. Facultad de Odontología. Buenos Aires-Argentina.
8. Universidad del Salvador. Facultad de Odontología. Buenos Aires-Argentina.
9. Universidad Nacional de Rosario. Facultad de Odontología. Santa Fé-Argentina
10. Universidad Nacional de Río Negro. Facultad de Odontología. Río Negro-Argentina
11. Universidad Católica de Córdoba. Facultad de Ciencias de la Salud. Córdoba-Argentina.
12. Universidad de Talca, Facultad de Odontología, Unidad de Cariología, Departamento de Rehabilitación Buco Maxilofacial. Talca-Chile

ABSTRACT
Aim: To prepare a consensus document of the cariology contents and competences included in the curriculum for the dentistry degree at Argentine dental faculties/schools. **Materials and Method:** Planning the process in stages: Stage **1**- Diagnosis of the situation (Google Form) and invitation to participate extended to the 20 academic units (AU) that offer dentistry degrees. Stage **2**- Assessment process of Content (C) and Competences (I) agreements in the five domains: D1 Basic Sciences, D2 Risk and diagnosis of dental caries, D3 Decision-making for non-invasive treatments, D4 Decision-making for invasive treatment, and D5 Evidence-based cariology at community level. Stage **3**- Consensus. Stages 2 and 3 were held in Workshop format in virtual mode (W). **Results:** Stage 1- Of the total 20 Google forms sent to the AU, 13 responses were received: 7 from National Universities and 6 from Private Universities. All participants agreed to be part of the consensus. Stage 2- W: 20 representatives from 10 AU participated. It began with a contextualizing conference, after which the representatives were divided into 5 groups to assess the agreements of each D. Stage 3- The Cariology Curriculum document was organized into 5 Domains, and 23 C and 31 I of clinical application were defined for teaching cariology. The contents and competences for each domain were agreed upon. The final document was sent to all W participants for their approval and dissemination in each AU involved. **Conclusion:** Cariology contents were defined for dentistry students at Universities in the Argentine Republic.

Key words: dental caries - cariology - education - consensus - cariology teaching - Argentina.

Contenidos Mínimos de Cariología en el Currículo de las Carreras de Odontología en la República Argentina

RESUMEN
Objetivo: Elaborar un documento de consenso de los contenidos y competencias de cariología en el currículo para las facultades/escuelas de odontología argentinas, a nivel del grado. **Materiales y Método:** El proceso de creación se efectuó en etapas: 1- Diagnóstico de situación (Formulario electrónico) e invitación a participar a las 20 unidades académicas (UA) donde se imparte la carrera de odontología. 2- Proceso de valoración de acuerdos de Contenidos (C) y Competencias (I) en los cinco dominios (D): D1: Ciencias Básicas; D2: Riesgo y diagnóstico de caries dental; D3: Toma de decisiones tratamientos no invasivos; D4: Toma de decisiones para el tratamiento invasivo y D5: Cariología basada en la evidencia a nivel comunitario. 3- Etapa de consenso. Las etapas 2 y 3 se efectuaron en formato de Workshop en modalidad virtual (W). **Resultados:** Etapa 1: Del total de 20 formularios enviados a las UA se recibieron 13 respuestas (65%), 7 (53,8%) de Universidades Nacionales y 6 (46,1%) de gestión Privada. El 100% de los participantes estuvieron de acuerdo en formar parte del consenso. Etapa 2: W: Participaron 20 representantes de 10 UA. El proceso se inició con una conferencia contextualizadora, posteriormente los representantes fueron divididos en 5 grupos para la valoración de acuerdos de cada D. Etapa 3: El documento de658. 658.l Currículo en Cariología se organizó en 5 Dominios. Se definieron 23 C y 31 I de aplicación clínica para la enseñanza de la Cariología. Se consensuaron los contenidos y las competencias para cada uno de los dominios. El documento final fue enviado a todos los participantes del W para su aprobación y difusión en cada una de las UA involucradas. **Conclusión:** En base al trabajo realizado se determinó un consenso de competencias y contenidos en cariología para estudiantes de grado de Odontología, de las Universidades de la República Argentina.

Palabras clave: caries dental - cariología - educación - consenso - enseñanza de cariología - Argentina.

INTRODUCTION

Dental caries (DC) is a multifactorial, dynamic, non-transmissible, biofilm-mediated, diet-modulated disease that leads to loss minerals from tooth hard tissues. It is determined by biological behavioral, psychosocial and environmental factors[1]. Caries experience has declined noticeably in developed countries[2]. However, available data suggest that the population in Argentina is far from achieving a balanced oral health status and good quality of life regarding oral health. DC mainly affects children and the elderly, with a marked social gradient and greater impact on persons with high social vulnerability[3].

Until not long ago, it was assumed that the only treatment for DC was surgical, and cariology was taught accordingly, being restricted to the restoration and limitation of damage to tooth hard tissues. In recent decades, however, it has been accepted that preventive measures are also an important part of dentistry. Thus, students of dentistry need to receive thorough, systematic training in cariology.

In Europe, the Association for Dental Education in Europe (ADEE) prepared a document called "Profile and competences for the European dentist"[4], describing the general competences that dentists should have.

In 2011, the European Organization for Caries Research (ORCA) published the first consensus for developing the Core Curriculum in Cariology[5], which in turn provided a basis for the same to be done in Colombia in 2014[6], USA in 2015[7], Chile in 2018[8] and the Caribbean region in 2021[9].

Given that in Argentina there is no consensus among public and private faculties/schools of dentistry regarding cariology contents, and no previous national process to agree upon criteria on the subject, there is a need to define the contents and competences of cariology in the curriculum for undergraduate dentistry faculties/schools in the country.

AIM

To develop a consensus document on the contents and competences of cariology in the curriculum for Argentine faculties/schools of dentistry, at undergraduate level.

MATERIALS AND METHOD

The Organizing Committee (OC) of the Cariology and Public Health Group (GCySP, by its acronym in Spanish) of the Argentine Society of Dental Research (SAIO, by its acronym in Spanish) planned the process in stages (Fig. 1).

Stage 1. Diagnosis of the situation and acceptance of the invitation to participate

The GCySP OC identified the 20 academic units that offer dentistry degrees in the Republic of Argentina (RA) and defined the list of contents and their clinical application, based on the 5 domains initially established by the European Union[5], and subsequently adapted by the USA[7], Colombia[6], Chile[8] and the Caribbean region[9]. The domains were defined as: 1- Basic knowledge; 2- Determination of risk, diagnosis of caries and detection of caries lesions; 3- Decision-making for non-operative/non-surgical preventive management; 4- Decision-making for operative/surgical treatment, and 5- Evidence-based cariology in clinical practice and public health.

The OC prepared a list of all the Schools of Dentistry in the Republic of Argentina and sent a letter of
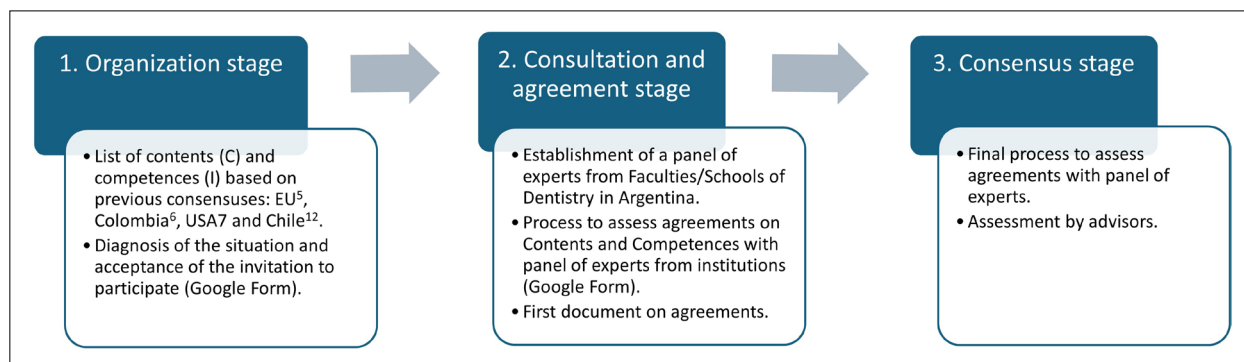
Fig. 1: Consensus process

invitation to the Deans, Academic Secretaries or Directors of the degrees, as relevant to each higher education institution, asking them to participate in the discussion and definition of contents and their corresponding clinical indicators. For such purpose, the people in charge of academic affairs were asked to designate cariology representatives from their respective faculties/schools. This process of invitation, constitution and consent of expert representatives lasted two months. The first step in this stage was to send a Google Form with 11 questions to identify the institution and its representative, and the cariology curriculum at each academic unit.

The purpose of this stage was to conduct an analysis within the Faculties/Schools of Dentistry on some curricular aspects of the teaching of cariology to undergraduates, and to evaluate interest in working towards a consensus for teaching cariology in Argentina.

The inclusion criterion was Academic Units with at least one graduation class.

Stage 2. Content and competence agreement assessment process

During the second stage, the first virtual workshop was organized. It took place in two phases: the first phase included a conference providing context regarding the importance of a consensus curriculum and the need to generate a list of contents and their indicators for clinical application. This conference was given by the project's foreign advisor. During the second phase, the participants from the different academic units were distributed in a heterogeneous and balanced manner into five working groups. There were 19 participating representatives. Work was conducted on content and competence agreement assessment in the five domains: D1- Basic Sciences; D2- Dental caries risk and diagnosis; D3- Decision-making for non-invasive treatments; D4- Decision-making for invasive treatment, and D5- Evidence-based cariology at community level. Each group was assigned one domain to analyze for 40 minutes in separate virtual rooms, and the items discussed were entered into electronic forms (Google Forms). After group work, a plenary session was held during which each group presented its proposals, agreements, contributions or modifications regarding contents and indicators, as part of the feedback process.

The OC drafted the document: "Cariology in the Curriculum of Schools of Dentistry in the Republic of Argentina", which included the observations made at the workshop. The draft was sent to all participants, who then individually analyzed each domain, and returned the draft with or without modifications.

The purpose of this stage was to prepare a document to submit for consensus and validation of the proposal.

Stage 3. Consensus stage

During the third stage of the consensus, the second virtual workshop was held. It was a plenary session to work on the assessment of agreements with the panel of experts. Agreements and consensuses were submitted for approval or rejection to all attendees, taking into account the experience, assessment and relevance of the domains in future curricular plans. Contents and indicators were approved unanimously. The OC wrote the final document for publication, which was copyedited by members of the OC and the national and foreign advisors.

RESULTS
Stage 1

Of the total 20 forms sent to the Academic Units, 13 responses were received (65%) − 7 from National Universities in the provinces of Tucumán, Mendoza, Rio Negro, Corrientes, Santa Fe, Córdoba and Buenos Aires, and 6 privately managed universities: 3 from Buenos Aires City and 3 from the provinces of Mendoza, Córdoba and Entre Ríos (Fig. 2).

All participants agreed to be part of the consensus. Regarding analysis of whether cariology contents were adequately included in the curriculum, 84.6% of the representatives agreed. In response to the question of whether the contents were included in one or more subjects, 92.3% of the participants reported that they were included in several subjects. Learning goals in the different curricula were analyzed. Academic unit representatives identified the following contents in the basic and clinical subjects: risk-based approach (evaluation and control criteria), decisions for preventive/non-operative caries management, cariology in public health and community dentistry, evidence-based dentistry and analysis of social determinants.

All academic units identified cariology contents in the clinical subjects. However, in basic subjects, contents were variable, with 92.3% for risk-

Fig. 2. Distribution of Schools of Dentistry that participated in the consensus in Argentina.

Table 1. Cariology contents in curricula

	Basic Subjects	Clinical Subjects
Risk-based approach	92.30%	100%
Decision-making for non-operative management of caries	92.30%	100%
Decision-making for operative management	76.90%	100%
Evidence-based dentistry	84.60%	100%
Analysis of social determinants	84.60%	100%
Cariology in public health/ community dentistry	76.90%	100%

courses, the two last years were considered). Analysis of each content shows that risk-based approach (criteria for evaluation and control) is taught with equal intensity throughout the course of studies, i.e., during all three periods described. Decision-making for non-operative preventive management of caries is taught to an equal extent in the early and final periods of the course of studies. Evidence-based dentistry is more intensively taught during the first years (early period) and declines as the course of studies progresses. Decision-making for operative management is taught equally during the early and intermediate periods. Cariology and public health is taught almost constantly throughout the course of studies. Analysis of social determinants is taught in the early and final periods, and less intensively during the intermediate period (Fig. 3a-f)

Stages 2 and 3

With the participation of 20 representatives from 10 academic units in the country, corresponding to 50% of the Faculties/Schools of Dentistry at Universities in Argentina, the list of core contents and clinical application indicators for teaching cariology was approved unanimously.

A total 23 core contents and 31 clinical application indicators were agreed upon for teaching cariology in Argentina. The number of contents (C) and indicators (I) per domain were: Domain 1-: Basic Knowledge: 5 C and 7 I; Domain 2: Risk, Detection and Diagnosis: 6 C and 6 I; Domain 3: Decision-making for non-operative and preventive management (considering patient risk and diagnosis): 5 C and 5 I; Domain 4: Decision-making for operative treatment: 4 C and 9 I; Domain 5: Evidence-based cariology in clinical practice and public health: 3 C and 4 I (Tables 2-6).

based approach and decisions for non-operative management of caries, 76.9% for decisions for operative management, 84.6% for evidence-based dentistry and analysis of social determinants, and 76.9% for cariology and public health/community dentistry (Table 1).

Analysis of the opinions of the academic unit representatives regarding criteria for evaluating students showed that grades were preferably given to operatory and preventive interventions. Exploration of the stage of the course of studies at which the aforementioned contents were incorporated showed that the reference people agreed that they were taught throughout the course of studies in different periods, considering 1st and 2nd year as early, 3rd and 4th year as intermediate, and 5th and 6th year as final (for 5-year courses, 5th year was taken as final, while for 6-year

Fig. 3a: Risk-based approach (criteria, evaluation and control).
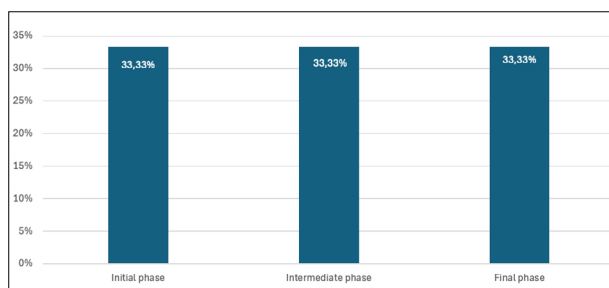
Fig. 3b: Decision-making for preventive/non-operative management of caries.
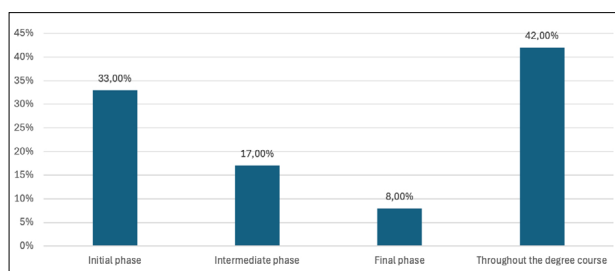
Fig. 3c: Evidence-based dentistry.
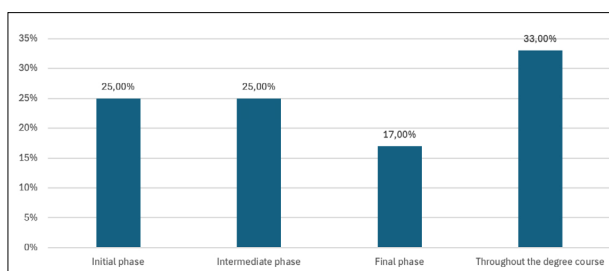
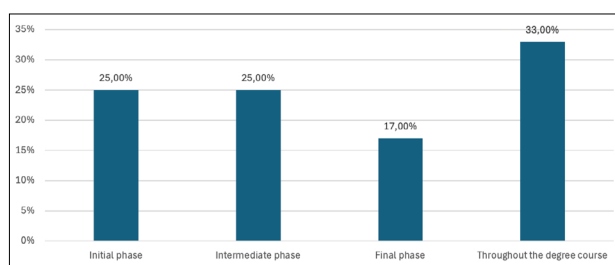Fig. 3d: Decision-making for operative management of caries (with preservation of tooth structure).
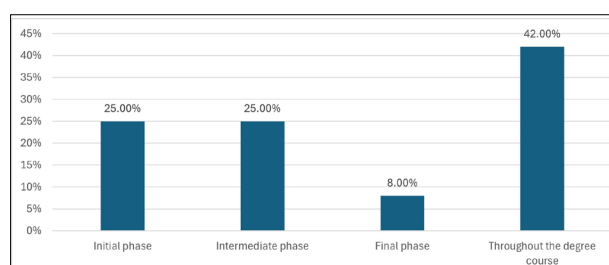

Fig. 3e: Cariology in public health/community dentistry

Fig. 3f: Analysis of social determinants

Table 2. Domain 1. Basic knowledge: List of Contents and Indicators for clinical/community application

DOMAIN 1	Contents	Clinical/community application indicators
Basic knowledge	D1 C1 Anatomy and development of tooth tissues and support structures at macroscopic, microscopic and molecular/ionic levels	I.1 Describe, understand and analyze the development and morphology of dental and periodontal tissues
	D1 C2 Concept of caries disease, caries lesions and non-carious lesions. Role of dental biofilm, diet, hygiene habits, saliva and progression of the disease in the individual and social context	I.2 Identify and use protocols to evaluate risk or factors associated to individual risk of the patient
	D1 C3 Chemical, physical and biological bases of changes in hard tissues, related to clinical and radiological detection. Evaluation of caries lesions and other non-carious lesions	I.3 Detect carious and non-carious lesions at clinical and radiographic level using a validated criterion
		I.4 Assess the activity of caries lesions at clinical level
	D1 C4 Basic principles of professionalism: ethics, bioethics and social responsibility	I.5 Provide dental care respecting bioethical principles
	D1 C5 Psychological theories and techniques to change behaviors and habits in order to improve oral health. Basic tools for effective communication	I.6 Detect needs for changing behavior and habits in order to improve oral health
		I.7 Refer patient to a relevant health professional in a timely manner

Table 3. Domain 2: Risk, Detection and Diagnosis: List of Contents and Indicators for clinical/community application

DOMAIN 2	Contents	Clinical/community application indicators
Risk, detection and diagnosis	D2 C1 Patient-centered diagnosis	I.8 Evaluate and integrate demographic, socio-cultural, behavioral, systemic, clinical and radiographic background to issue diagnostic judgment
	D2 C2 Evaluation of the dental caries disease in the patient considering a life course approach	I.9 Identify and apply indicators, risk factors and prognostic factors at individual level, by determining patient social and community context, applied over time
	D2 C3 Evaluation of patient's systemic health and the oral effects deriving from patient's condition and/or treatments	I.10 Relate systemic diseases and their treatment to dental caries disease
	D2 C4 Criteria for clinical detection, severity, activity and effects of carious lesions on the pulp	I.11 Apply standardized systems for detection and clinical evaluation of carious lesions and pulp health status
	D2 C5 Detection and radiographic evaluation of caries lesions	I.12 Apply criteria to determine radiographic severity of caries lesions.
	D2 C6 Differential diagnosis of caries lesions	I.13 Apply criteria for detection and differential diagnosis of enamel and dentin effects; hypoplasia, amelogenesis imperfecta, dental crack, fluorosis, MHI, non-carious lesions (erosion, abrasion, abfraction, attrition), dental malformations, and evaluate their origin

Table 4. Domain 3: Decision-making for non-operative and preventive management: List of Contents and Indicators for clinical/community application

DOMAIN 3	Contents	Clinical/community application indicators
Decision-making for non-operative and preventive management (consider patient risk and diagnosis)	D3 C1 Behavioral changes to promote oral health	I.14 Apply evaluation instruments associated to behavioral changes
	D3 C2 Diet advice and monitoring. Effects of eating disorders on oral health	I.15 Identify and use instruments to evaluate and monitor diet-related behavior
	D3 C3 Mechanical, chemical and biological control of dental biofilm	I.16 Impart instructions on oral hygiene, monitor indicators, and define maintenance intervals. Professional removal of biofilm
	D3 C4 Fluorides: action mechanism, presentations and rational use according to risk level and age.	I. 17 Properly indicate, prescribe and clinically apply fluorides according to risk level and age
	D3 C5 Other anticaries and remineralization agents	I.18 Indicate agents other than fluoride according to risk level and age

DISCUSSION

The list of cariology contents for the degree, as defined in Argentina, aligns with the six previous consensuses, with the basis provided by European consensus (European core curriculum in cariology, 2011)[5] prepared by the ADEE (Association for Dental Education in Europe) and ORCA (European Organisation for Caries Research) and 75 participants invited from 24 European countries and three countries in North and South America. The following consensuses were subsequently developed: Colombia (2014), Chile (2018), and Caribbean (2021) in LAC, in the USA (2016) and in Spain (2020). These documents propose 5 domains in the teaching of cariology, arranged in Principal and Specific Competences. These curricula focus on competences, whereas our consensus analyzed core contents.

In Argentina, there is a broad range of sociodemographic conditions, and the academic units are distributed according to the situation in each region. In the Northwest region, both La Rioja and Tucumán Provinces have a public university.

Table 5. Domain 4: Decision-making for operative treatment: List of Contents and Indicators for clinical/community application

DOMAIN 4	Contents	Clinical/community application indicators
Decision-making for operative treatment	D4.C1. Rationale for minimally invasive treatment of caries lesions	I.19 Perform micro- and minimally invasive treatment on cavitated and active lesions
		I.20 Prioritize repair and sealing of defective restorations over total replacement of the restoration
	D4. C2. Designing biological preparations for the treatment of caries lesions determined according to activity, location, extension and depth	I.21 Perform minimum sufficient access to the lesion, using appropriate instruments
		I.22 Selectively remove carious tissue
	D4. C3. Surgical management of caries lesions to ensure maintenance of oral health	I.23 Perform actions for pulp-dentin protection
		I.24 Perform morphological reconstruction of proximal contacts, dental contours, surface texture and functional occlusal contacts
	D4. C4. Implementation of individual control and maintenance programs according to evaluation of risk and prognosis	I.25 Determine risk and prognosis at individual and/or community level
		I.26 Establish frequency for control and maintenance according to individual risk level, considering patient's community context
		I.27 Apply control program in maintenance patients and monitor them

Table 6. Domain 5: Evidence-based cariology in clinical practice and public health practice: List of Contents and Indicators for clinical/community application

DOMAIN 5	Contents	Clinical/community application indicators
Evidence-based cariology in clinical and public health practice	D5. C1. Development of programs for promotion, prevention y maintenance of oral health	I.28 Know, design and apply programs for promotion, prevention and maintenance of oral health
	D5. C2. Epidemiology of caries in relation to determining factors of health	I.29 Use the qualitative and quantitative tools for epidemiology
	D5. C3. Base clinical tasks on the available scientific evidence	I.30 Apply the concepts of dentistry based on available scientific evidence
		I.31 Apply validated clinical guides for managing dental caries

In the Northeast region, in Corrientes City, there is only one public university. The provinces of Córdoba, Santa Fe and Mendoza each have both a public and a private university. Entre Ríos Province has only one private university, while Buenos Aires Province has one public and two private universities. The Autonomous City of Buenos Aires has one public and five private universities. In the Patagonian region, Río Negro Province has one public university. A study conducted at some schools of dentistry in Latin America describes cariology as part of the curricula but reports high variability in terms of planning and achievements[10].

During the discussion of this proposal, none of the contents was discarded. However, some of the contents were combined because they addressed knowledge, skills and behaviors in common. The items which were most discussed were the contents associated to the concept of caries disease, caries lesions and non-carious lesions. The role of dental biofilm, diet, saliva and disease progression (D1-C2), mechanical, chemical and biological control of dental biofilm (D3-C3), and implementation of control and maintenance programs according to evaluation of risk and prognosis (D4-C4) pose a challenge to dental student training. During the plenary session, discussion and reflection addressed a range of factors such as changes in prevalence of the disease, development of materials and technology, and competences for managing behavioral and communicational variables, establishing the possibility of achieving a student/professional who

is competent in planning intervention strategies in the community in order to act upon what has been diagnosed. There was general agreement on the need for the curriculum to develop competences in risk evaluation, preventive management of caries lesions, and the way in which this knowledge is applied at individual and community levels.

In agreement with León et al.[11] and Giacaman et al.[12], the main advantages of this proposal for contents and indicators are its flexibility and the gradual insertion of contents across the degree. It can thus be included in the curricula of different educational models – both competence-based and learning outcome- or objective-based. Indeed, the core contents in the consensus need not be delivered exclusively through a single subject, but may be distributed over the curricular network as content

in various subjects. The contents may be included in the three learning cycles: basic, pre-clinical and clinical, and their indicators enable evaluation of the transfer of theoretical content to clinical practice.

CONCLUSIONS

Cariology contents for undergraduates at both public and private faculties/schools of dentistry in the Republic of Argentina refer to the learning objectives expressed in the different subjects across the curriculum. To conclude, an unprecedented consensus in Argentina was able to lay out the core contents and clinical application indicators for cariology for undergraduate students at universities across the country, providing an important contribution to quality education in dentistry.

REFERENCES

1. Machiulskiene V, Campus G, Carvalho JC, Dige I et al. Terminology of Dental Caries and Dental Caries Management: Consensus Report of a Workshop Organized by ORCA and Cariology Research Group of IADR. Caries Res 2020; 54(1):7-14. https://doi.org/10.1159/000503309

2. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B et al. Global burden of untreated caries: a systematic review and metaregression. J Dent Res 2015 May; 94(5):650-658. https://doi.org/10.1177/0022034515573272

3. Fort A, Fuks AJ, Napoli AV, Palomba S et al. Distribución de caries dental y asociación con variables de protección social en niños de 12 años del partido de Avellaneda, provincia de Buenos Aires [Distribution of dental caries and its association with variables of social protection in children 12 years of age in the county of Avellaneda, Province of Buenos Aires]. Salud Colect 2017 Jan-Mar; 13(1):91-104. Spanish. https://doi.org/10.18294/sc.2017.914

4. Plasschaert AJM, Holbrook WP, Delap E, Martinez C, et al. Profile and competences for the European dentist. Eur J Dent Educ 2005; 9:98-107. https://doi.org/10.1111/j.1600-0579.2005.00369.x

5. Schulte AG, Pitts NB, Huysmans MC, Splieth C, et al. European core curriculum in cariology for undergraduate dental students. Caries Res 2011; 45(4):336-45. https://doi.org/10.1159/000330006

6. Martignon S, Marín LM, Pitts N, Jácome-Liévano S. Consensus on domains, formation objectives and contents in cariology for undergraduate dental students in Colombia. Eur J Dent Educ 2014 Nov. 18(4):222-33. https://doi.org/10.1111/eje.12091

7. Fontana M, Guzmán-Armstrong S, Schenkel AB, Allen KL, et al. Development of a Core Curriculum Framework in Cariology for U.S. Dental Schools. J Dent Educ 2016 Jun; 80(6):705-20. https://doi.org/10.1002/j.0022-0337.2016.80.6.tb06133.x

8. Díaz-Yokens M, González S, Giacaman RA, Araya-Bustos F, et al. Cariology Curriculum in Chilean Universities. Rev. Clin. Periodoncia Implantol. Rehabil. Oral 2018. 11(2), 98-101. https://doi.org/10.4067/S0719-01072018000200098

9. Abreu-Placeres N, Grau-Grullón P, Naidu R, García-Godoy F, et al. Cariology consensus for undergraduates at dental schools in the Caribbean region. Eur J Dent Educ 2021 Nov; 25(4):717-732. https://doi.org/10.1111/eje.12651

10. Martignon S, Gomez J, Tellez M, Ruiz JA, et al. Current cariology education in dental schools in Spanish-speaking Latin American countries. J Dent Educ 2013; 77:1330-7. https://doi.org/10.1002/j.0022-0337.2013.77.10.tb05607.x

11. León S, Bravo-Cavicchioli D, Correa-Beltrán G, Giacaman RA. Validation of the Spanish version of the Oral Health Impact Profile (OHIP-14Sp) in elderly Chileans. BMC Oral Health. 2014; 14:95. https://doi.org/10.1186/1472-6831-14-95

12. Giacaman RA, Aranguiz V, Botelho J, Bustos IP, Cabello R, Candiales Y et al. Creación de un Currículo de Competencias en Cariología para Estudiantes de Pregrado de Odontología en Chile. Rev. Clin. Periodoncia Implantol Rehabil Oral 2018; Apr 11(1): 58-68. https://doi.org/10.4067/S0719-01072018000100058

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