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Penetration degree of sealer in artificial lateral canal after passive ultrasonic irrigation with EDTA for different times

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ABSTRACT

The aim of this study was to evaluate the degree of penetration of obturation cement in artificial lateral canals after Passive Ultrasonic Irrigation (PUI) with ethylenediaminetetraacetic acid (EDTA) for different times. Fifty upper molar palatine roots were used, in which two artificial lateral canals were made at distances of 7 and 3 millimeters from the root apex. After instrumentation and drying the canal, the final toilet stage was performed on five groups (n = 10), as follows: G1 - EDTA 17% + PUI for 10 seconds; G2 - EDTA 17% + PUI for 20 seconds; G3 - EDTA 17% + PUI for 30 seconds; G4 - EDTA 17% + PUI for 60 seconds; G5 - EDTA 17% + activation by instrument R50 for 5 minutes (Control). The canals were sealed by the single cone technique, and after 72 hours, sectioned in two planes transverse to the artificial canal, to see the degree of penetration of the sealing cement. In the radiographic analysis, there was no statistical difference (p> 0.05) between groups in the two artificial lateral canals. However, PUI of EDTA for 60 seconds produced a significant difference in the degree of penetration of the sealing cement (p <0.05) at 7 mm from the apex. Therefore, PUI with EDTA for 60 seconds promoted a higher degree of penetration of the obturator cement in the artificial lateral canal.

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Keywords: *dentin; EDTA; root canal.*

Grau de penetração do cimento obturador em canais laterais simulados após ativação ultrassônica passiva do EDTA em diferentes tempos

RESUMO

O objetivo deste trabalho foi avaliar o grau de penetração do cimento obturador em canais laterais artificiais, após Irrigação Ultrassônica Passiva (IUP) do ácido etilenodiaminotetracético (EDTA), em diferentes tempos. Foram utilizadas 50 raízes palatinas de molares superiores, e em seguida confeccionados dois canais laterais artificiais a 7 e 3 milímetros do ápice radicular. Após a instrumentação e secagem dos canais, foi iniciada a etapa de toillet final, de acordo com os seguintes grupos (n=10): G1- EDTA 17%+IUP durante 10 segundos; G2 - EDTA 17%+IUP durante 20 segundos; G3 - EDTA 17%+IUP durante 30 segundos; G4- EDTA 17%+IUP durante 60 segundos; G5- EDTA 17%+ativação pelo instrumento R50 durante 5 minutos (Controle). Os canais foram obturados pela técnica do cone único, e após 72 horas, seccionados em dois planos transversais dos canais artificiais, para se visualizar o grau de penetração do cimento obturador. Na análise radiográfica, não houve diferença estatística (p>0,05) entre os grupos, nos dois canais laterais artificiais. Entretanto, a IUP do EDTA por 60 segundos conseguiu um obter resultado significativo, sobre o grau de penetração do cimento obturador (p<0,05) a 7 milímetros do ápice. Portanto, a IUP do EDTA no tempo de 60 segundos promoveu maior grau de penetração do cimento obturador nos canais laterais artifciais.

Palavras-chave: *dentina; EDTA; canal radicular.*

INTRODUCTION

The aim of root canal endodontic treatment is to eliminate microbes and promote periapical tissue health. It involves maintaining aseptic conditions throughout canal biomechanical preparation and filling[1].

During root canal instrumentation, a smear layer forms, which consists of bacteria, debris and

necrotic tissue, with negative impact on dentin tubules and on penetration of drugs and intracanal sealers[2]. Cleaning the root canal enables better adaptation of sealer materials[3] and adhesion of resin-based endodontic cements to the dentin[4], and prevents apical and coronal microbial leakage [5].

Complex root canal anatomy should also be considered, because it prevents complete disinfection of the lateral walls and therefore requires the use of auxiliary substances and methods with physicochemical action [6,-8]. Irrigation solutions should provide antimicrobial action, organic tissue dissolution, root canal debridement, and compatibility with periapical tissues[9]. Among the most frequently used substances are chlorhexidine, sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) [10,11].

In order to improve the smear layer removal technique, the irrigation solution may be associated with ultrasonic devices. Versiani et al. (2015)[6] observed that the conventional mechanical irrigation method (CMIM) was unable to act on all the root walls and the apical area. Passive Ultrasonic Irrigation (PUI) has therefore been proposed as a technique to activate the irrigating solution, since its active tips produce mechanical-vibrating effects on the dentin walls, providing more effective cleaning and better sealing of the dentinal tubules[12,13]. Previous studies have shown that PUI promoted greater debris removal from the lateral canal[14,15] in straight and curved roots[16,17]. However, complete irrigation penetration may not be achieved at all stages of root canal treatment[18].

In view of the controversial data in the literature on the use of EDTA 17% and ultrasound to seal dentinal tubules, the aim of this study was to evaluate the degree of penetration of the obturation cement in artificial lateral canals after passive ultrasonic activation of EDTA for different times. The hypothesis tested was that the degree of penetration of the obturation cement is not influenced by the PUI with EDTA.

MATERIALS AND METHODS

Preparation of teeth

After approval by the Research Ethics Committee of FACIME/UESPI (1,376,991), 50 human teeth with intact, straight roots and fully formed apices (palatine root of the 1st and 2nd upper molars) were obtained from the University's Tooth Bank, Brazilian State of Piauí.

The dental crowns were sectioned with a double-faced diamond disk (KG Sorensen®, Cotia, São Paulo, Brazil), at slow rotation, in order to facilitate canal instrumentation. Canals were measured with a K-file file (Dentsply® Maillefer, Petrópolis, Rio de Janeiro, Brazil) #15 by reaching the apical foramen and withdrawing 1 mm in order to obtain the working length. Subsequently, files #20 and #25 #30 were used with the Reciproc System R 50 instrument (VDW, Germany). Root canal preparation steps included irrigation with 2 mL of 1% sodium hypochlorite (Milton's solution; Biodynamics®, Ibiporã, Paraná, Brazil) each time instruments were changed, with metal vacuum suction (Endo Points®, Rio de Janeiro, Brazil), and absorbent paper points (Denstply®, Petrópolis, Brazil). A total 12 mL of solution were used.

Two lateral canals were prepared perpendicular to the long axis of the root, in the middle third (apex of 7 mm) and in the apical third (apex of 3 mm), using a rotating instrument with a Kerr file (Dentsply®, Petrópolis, Brazil) size 10 (100 micrometers diameter). This instrument was modified by beveling the tip to form a chamfer. In order to adapt it for low rotation, the other end was removed and the instrument was fixed with cyanoacrylate to a mandrel[19].

After instrumentation and drying the canal, the final toilet stage was begun according to the following groups (n=10): G1-EDTA 17% + PUI for 10 seconds; G2-EDTA 17% + PUI for 20 seconds; G3-EDTA 17% + PUI for 30 seconds; G4-EDTA 17% + PUI for 60 seconds; G5- EDTA 17% + activation by instrument R50 for 5 minutes (Control).

PUI was performed using the E-1 insert (Irrisonic, Helse São Paulo, Brazil) coupled to an ultrasound device (CVDentus, São Paulo, Brazil) at a power of 20%, according to the study group. Then the canals were filled using the single cone technique, in which Sealer 26 cement (Dentsply®, Petrópolis, Rio de Janeiro, Brazil) was placed in the canal using a Lentulo spiral No. 40 (Dentsply®, Petrópolis, Rio de Janeiro, Brazil). The canal opening was sealed with light-cured composite resin (Dentsply®, Petrópolis, Rio de Janeiro, Brazil) and the roots were radiographed.

After 72 hours, the teeth were sectioned with diamond discs (KG Sorensen®, Cotia, São Paulo, Brazil) at two transverse planes 1 mm below the

artificial canals, in order to not remove the sealant cement. Then a medium-grain diamond bur (KG Sorensen®, Cotia, São Paulo, Brazil) was used to wear away 1 mm of the cross-sectional area, revealing the degree of sealant cement penetration.

A single examiner trained for this purpose (Kappa = 0.76) used a 10x stereomicroscope to evaluate the degree of cement penetration in the lateral canal. The microscopic and radiographic results were classified into four groups: Grade 0 (without cement penetration); Grade 1 (cement penetration in the proximal third); Grade 2 (cement penetration to the middle third); Grade 3 (cement penetration to the final third).

Statistical analysis

The data were analyzed using the statistical program SPSS (Statistical Package for Social Sciences) version 20.0 specific for Windows. The Shapiro-Wilk and Wilcoxon tests were used to analyze the normality and values of the means between groups. The level of significance was set at 5%.

RESULTS

Table 1 shows the descriptive results of the degree of penetration of the endodontic cement in the lateral canal, based on radiographic and microscopic analyses.

Table 2 shows that for the radiographic analysis, there was no significant difference ($p > 0.05$) between experimental groups. However, under microscopic analysis, a difference was observed between groups ($p < 0.05$), where the highest degree of penetration of the filler cement in the artificial lateral canals was seen in group 4 (Table 3).

DISCUSSION

Several factors may influence the degree of penetration of the sealant cement, such as the root canal anatomy, the efficacy of smear layer removal, the final irrigation technique and obturation, and the physicochemical properties of the sealing materials. Under microscopic analysis, this study found the greatest degree of sealing cement penetration in lateral canals at 7 mm from the root apex for canals treated with EDTA PUI for 60 seconds. Thus, the null hypothesis was rejected.

Table 1: Descriptive analysis of the degree of penetration of endodontic cement in the lateral canals, according to treatment group and distance from the apex.

Type of Analysis	Groups	Distance from the apex	Score 0	Score 1	Score 2	Score 3
Radiographic	G1	3 mm	10	-	-	-
		7 mm	10	-	-	-
	G2	3 mm	9	-	-	1
		7 mm	8	1	-	1
	G3	3 mm	7	1	-	2
		7 mm	7	2	-	1
	G4	3 mm	8	-	1	1
		7 mm	5	1	-	4
	G5	3 mm	7	-	1	2
		7 mm	8	-	1	1
Microscopic	G1	3 mm	8	-	1	1
		7 mm	10	-	-	-
	G2	3 mm	10	-	-	-
		7 mm	7	1	1	1
	G3	3 mm	6	1	1	2
		7 mm	6	2	-	2
	G4	3 mm	5	1	1	3
		7 mm	4	1	0	5
	G5	3 mm	7	-	1	2
		7 mm	6	-	1	3

G1, EDTA 17% + PUI for 10 seconds; G2, EDTA 17% + PUI for 20 seconds; G3, EDTA 17% + PUI for 30 seconds; G4, EDTA 17% + PUI for 60 seconds; G5, EDTA 17% + activation by instrument R50 for 5 minutes (Control).

Table 2: Radiographic analysis of the degree of penetration of the endodontic cement in the lateral canals (apical third).

Distance from the apex	Groups	Mean	p
Apex	G1	21.00	
	G2	23.65	
	G3	28.40	0.36
	G4	25.90	
	G5	28.55	
Middle	G1	19.50	
	G2	24.35	
	G3	26.45	0.11
	G4	32.60	
	G5	24.60	

p <0.05 is statistically significant. G1, EDTA 17% + PUI for 10 seconds; G2, EDTA 17% + PUI for 20 seconds; G3, EDTA 17% + PUI for 30 seconds; G4, EDTA 17% + PUI for 60 seconds; G5, EDTA 17% + activation by instrument R50 for 5 minutes (Control).

Table 3: Microscopic analysis of the degree of penetration of the endodontic cement in the lateral canals (apical and middle third).

Distance from the apex	Groups	Mean	p
Apex	G1	22.10	
	G2	23.90	
	G3	26.80	0.69
	G4	29.70	
	G5	25.00	
Middle	G1	17.50	
	G2	22.15	
	G3	26.80	0.04
	G4	33.15	
	G5	27.90	

p <0.05 is statistically significant. G1, EDTA 17% + PUI for 10 seconds; G2, EDTA 17% + PUI for 20 seconds; G3, EDTA 17% + PUI for 30 seconds; G4, EDTA 17% + PUI for 60 seconds; G5, EDTA 17% + activation by instrument R50 for 5 minutes (Control).

EDTA has chelating and disinfecting properties[20]. It removes calcium from the crystalline phosphate network, causing superficial demineralization and exposure of collagen fibers in the dentin tubules. Galler et al [21] suggest that irrigation with EDTA may potentiate cell differentiation by exposing growth factors trapped in the dentin matrix.

There was greater penetration in the middle third than in the apical third, as observed in previous studies[22,23]. Other authors further state that the EDTA solution is able to remove the smear layer only in the coronal and middle thirds due to the presence of a greater amount of dentin tubules and their diameter, which enable greater contact between the solution and the dentin tissue[24-26]. However, some studies report that the association of EDTA with sodium hypochlorite may potentiate its cleaning action and enable it to reach the apical third[10,27].

Another strategy used is activation of the EDTA using the memory instrument or ultrasonic tip. This procedure induces an increase in temperature and rupture of the surface tension of the solution[13], causing a high activity energy release. Thus, this mechanism acts on the smear layer, promoting its removal and greater diffusion of drugs and sealing of the dentinal tubules[28]. Thus, several root canal irrigation techniques and systems have been developed to improve final canal irrigation[29].

Some studies, such as Jiang et al,[28], Stamos et al[30] and Sabins et al[31] concluded that the ultrasonic system is more effective than the sonic system for cleaning root canals; however, it can damage the dentinal tissue. On the other hand, Van Der Sluis et al[32] report that there is no consensus in the literature to support the idea that one form of energy is better than the other.

When comparing PUI and CMIM, Rodig et al[33] and Paragiola et al[34] observed that PUI showed greater efficacy in root canal cleansing. Other authors compared the degree of penetration of the filling cement through these two techniques, and showed that ultrasonic activation of EDTA 17% presented a greater number of sealed dentinal tubules[35,36].

Contradicting the microscopic analysis, the radiographic analyses revealed that even using PUI, there was no difference between degree of penetration in the apical and middle thirds, as observed in previous studies[37,38]. In addition, Costa et al[39] and Ciucchi et al[40] emphasize that this can occur due to the greater amplitude of displacement that occurs at the tip of the insertion, where the apical zone would probably be. These contrasts may be related to differences in the methodology, such

as quantity of irrigating solution, activation time, selection of activator tip, and canal conicity after instrumentation.

Considering the foregoing discussion, further studies should be conducted combining chelating and disinfecting chemicals applied for different times and using different techniques, with the aim of increasing the cleaning and degree of penetration of the obturator materials in both the middle and apical thirds.

In view of the results, it may be concluded that the PUI with EDTA for 60 seconds promoted a higher degree of penetration of the obturation cement in the artificial lateral canals.

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REFERENCES

1. Zöllner NA, Ferreira MCA, Carvalho PL, Rodrigues JD, Clemente RGP, Medeiros JMF. Análise da remoção do smear layer pelo uso de três soluções irrigantes. RGO 2007; 55:349-356. URL:http://www.revistargo.com.br/viewarticle.php?id=697
2. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old Enterococcus faecalis biofilms in dentin canals. J Endod 2012; 38:1376-1379.
3. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: Mode of action and indications for their use. Int Endod J 2003; 36:810-830.
4. Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The effect of diferent irrigating solutions on bond strength of two root canal-filling systems. J Endod 2009; 35:537-540.
5. Economides N, Kokorikos I, Kolokouris I, Panagiotis B, Gogos C. Comparative study of apical sealing ability of a new resin-based root canal sealer. J Endod 2004; 30:403-405.
6. Versiani MA; De-Deus G; Vera J; Souza E, Steier L, Pécora JD, Sousa-Neto MD. 3D mapping of the irrigated areas of the root canal space using micro-computed tomography. Clin Oral Investig 2015;19:859-866.
7. Haapasalo M, Qian W, Portenier I, Waltimo T. Effects of dentin on the antimicrobial properties of endodontic medicaments. J Endod 2007; 33:917-925.
8. De-Deus G, Murad C, Paciornik S, Reis CM, Coutinho-Filho T. The effect of the canal-filled area on the bacterial leakage of oval-shaped canals. Int Endod J 2008; 41:183-190.
9. Cheung GS, Stock CJ. In vitro cleaning ability of root canal irrigants with and without endosonics. Int Endod J 1993; 26:334-343.
10. Ballal NV, Kandian S, Mala K, Bhat KS, Acharya S. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: a scanning electron microscopic study. J Endod 2009; 35:1573-1576.
11. Prado M, Gusman H, Gomes BP, Simão RA. Scanning electron microscopic investigation of the effectiveness of phosphoric acid in smear layer removal when compared with EDTA and citric acid. J Endod 2011; 37: 255-258.
12. Guerisoli DM, Marchesan MA, Walmsley AD, Lumley PJ, Pecora JD. Evaluation of smear layer removal by EDTAC and sodium hypochlorite with ultrasonic agitation. Int Endod J 2002; 35:418-421.
13. Kuah HG, Lui JN, Tseng PS, Chen NN. The Effect of EDTA with and without Ultrasonics on Removal of the Smear Layer. J Endod 2009; 35:393-396.
14. Van der Sluis LW, Vogels MP, Verhaagen B, Macedo R, Wesselink PR. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. J Endod 2010; 36: 737-740.
15. Castelo-Baz P, Varela-Patiño P, Cantatore G, Domínguez-Perez A, Ruíz-Piñón M, Miguéns-Vila R, Martín-Biedma B. In vitro comparison of passive and continuous ultrasonic irrigation in curved root canals. J Clin Exp Dent 2016; 8:437-441.
16. Al-Jadaa A, Paque F, Attin T, Zehnder M. Acoustic hypochlorite activation in simulated curved canals. J Endod 2009; 35: 1408-1411.
17. Malki M, Verhaagen B, Jiang LM, Nehme W et al. Irrigant flow beyond the insertion depth of an ultrasonically oscillating file in straight and curved root canals: visualization and cleaning efficacy. J Endod 2012; 38:657-661.
18. Vera J, Arias A, Romero M. Effect of maintaining apical patency on irrigant penetration into the apical third of root canals when using passive ultrasonic irrigation: an in vivo study. J Endod 2011; 37:1276-1278.
19. Barbosa THM, Silva MS, Silva DP, Moura ACM, Ferraz MAAL, Falcão CAM. Influence of the ultrasonic activation of irrigating solutions on sealer penetration into lateral root canals. RGO 2018; 66: 117-121. URL: http://www.scielo.br/pdf/rgo/v66n2/1981-8637-rgo-66-02-00117.pdf
20. Marending M, Paqué F, Fischer J, Zehnder M. Impact of irrigant sequence on mechanical properties of human root dentin. J Endod 2007; 33:132-135.
21. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, Schmalz G. Influence of root canal

disinfectants on growth factor release from dentin. J Endod 2015; 41:363-368.
22. Moon YM, Shon WJ, Baek SH, Bae KS, Kum KY, Lee W. Effect of final irrigation regimen on sealer penetration in curved root canals. J Endod 2010; 36:732-736.
23. De Deus GA, Gurgel-Filho ED, Maniglia-Ferreira C, Coutinho-Filho T. The influence of filling technique on depth of tubule penetration by root canal sealer: a study using light microscopy and digital image processing. Aust Endod J 2004; 30:23-28.
24. Generali L, Prati C, Pirani C, Cavani F, Gatto MR, Gandolfi MG. Double dye technique and fluid filtration test to evaluate early sealing ability of na endodontic sealer. Clin Oral Investig 2017; 21:1267-1276.
25. Akcay M, Arslan H, Durmus N, Mese M, Capar ID. Dentinal tubule penetration of AH Plus, iRoot SP, MTA fillapex, and guttaflow bioseal root canal sealers after different final irrigation procedures: A confocal microscopic study. Lasers Surg Med 2016; 48:70-76.
26. Kara Tuncer A, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. J Endod 2012; 38: 860-863.
27. Mancini M, Armellin E, Casaglia A, Cerroni L, Cianconi L. A comparative study of smear layer removal and erosion in apical intraradicular dentine with three irrigating solutions: a scanning electron microscopy evaluation. J Endod 2009; 35:900-903.
28. Jiang, LM; Verhaagen, B; Versluis, M; van der Sluis, LW. Influence of the oscillation direction of an ultrasonic file on the cleaning efficacy of passive ultrasonic irrigation. J Endod 2010; 36:1372-1376.
29. Boutsioukis C, Lambrianidis T, Kastrinakis E. Irrigant flow within a prepared root canal using various flow rates: a computational fluid dynamics study. Int Endod J 2009; 42:144-155.
30. Stamos DE, Sadeghi EM, Haasch GC, Gerstein H. An in vitro comparison study to quantitate the debridement ability of hand, sonic, and ultrasonic instrumentation. J Endod 1987; 13:434-440.
31. Sabins RA, Johnson JD, Hellstein JW. Comparison of the cleaning efficacy of short-term sonic and ultrasonic passive irrigation after hand instrumentation in molar root canals. J Endod 2003; 29:674-678.
32. Van der Sluis L, Wu MK, Wesselink P. Comparison of 2 flushing methods used during passive ultrasonic irrigation of the root canal. Quintessence Int 2009; 40(10): 875-879.
33. Rodig T, Dollmann S, Konietschke F, Drebenstedt S, Hülsmann M. Effectiveness of different irrigant agitation techniques on debris and smear layer removal in curved root canals: a scanning electron microscopy study. J Endod 2010; 36:1983-1987.
34. Paragliola R, Franco V, Fabiani C, Mazzoni A, Nato F, Tay FR, Breschi L, Grandini S. Final rinse optimization: influence of different agitation protocols. J Endod 2010; 36:282-285.
35. Kanter V, Weldon E, Nair U, Varella C, Kanter K, Anusavice K, Pileggi R. A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112:809-813.
36. Hedge V, Arora S. Effect of advanced irrigation protocols on self-expanding Smart-Seal obturation system: A scanning electron microscopic push-out bond strength study. Contemp Clin Dent 2015; 6:26-30.
37. Uroz-Torres D, Gonzalez-Rodriguez MP, Ferrer-Luque CM. Effectiveness of the EndoActivator system in removing the smear layer after root canal instrumentation. J Endod 2010; 36:308-311.
38. Klyn SL, Kirkpatrick TC, Rutledge RE. In vitro comparisons of debris removal of the EndoActivator system, the F file, ultrasonic irrigation, and NaOCl irrigation alone after hand-rotary instrumentation in human mandibular molars. J Endod 2010; 36:1367-1371.
39. Costa WF, Antoniazzi JH, Campos MNM, Pécora JD, Robazza, CRC. Avaliação comparativa. Sob microscopia ótica, da capacidade de limpeza da irrigação manual convencional versus ultra-sônica dos canais radiculares. Rev. Paul. Odont. 1986; 8:50-60. URL:http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=LILACS&lang=p&nextAction=lnk&exprSearch=108650&indexSearch=ID
40. Ciucchi B, Khettabi M, Holz J. The effectiveness of different endodontic irrigation procedures on the removal of the smear layer: a scanning electron microscopic study. Int Endod J 1989; 22:21-28.

Dynamics of the medical-dental relationship in a University Hospital in Buenos Aires, Argentina

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ABSTRACT
Lack of communication between the medical and dental professions impacts healthcare quality, especially in hospitals. Different authors have described the oral status of inpatients. Following that line of research, the current study set the following aims: to characterize the dynamics of medical-dental healthcare interaction at a university hospital and to describe oral status and identify need for dental treatment in a sample of 150 inpatients at a hospital in Buenos Aires City, Argentina.A descriptive study was conducted on patients who were referred to dentistry by their physicians. The following variables were surveyed: personal data, medical history, oral health status, need for dental treatment and oral self-care habits.Patient median age was 60 years, 60.7% were male, 68.7% had diseases of the circulatory system, average number of medications per day was 7, of which 28.1% were for the cardiovascular system. Seventy percent of the referrals came from the Cardiology Service and 48% were requested for preoperative evaluation. Percentage of visible plaque was 73.6% and bleeding on probing 75.4%. DMFT was 19.9; 57.3% of patients had periodontal pockets deeper than 4 mm, and 97.2% required surgery, endodontic or prosthetic rehabilitation treatments. The frequency of daily brushing decreased during hospitalization: 28.7% reported not brushing daily and only 5.3% reported brushing 3 times a day.
Referrals to dentistry came mainly from the cardiology service in pre-surgical situations. Inpatients presented high levels of oral pathology and need for dental treatment.
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Keywords: *referral and consultation; inpatients; oral health.*

Dinámica de la relación médico-odontológica en un Hospital Universitario

RESUMEN
La desarticulación entre la profesión médica y odontológica es un fenómeno que impacta en la calidad de atención de las personas, especialmente en ámbitos hospitalarios. Diferentes autores describieron el estado bucal de pacientes internados. Siguiendo esta línea de investigación, este trabajo estableció los siguientes objetivos: caracterizar la dinámica de la interacción medicina-odontología en un hospital universitario, conocer el estado bucal e identificar la necesidad de tratamiento odontológico en una muestra de 150 pacientes internados en un hospital de la Ciudad de Buenos Aires, Argentina. Se realizó un estudio descriptivo de los pacientes que los médicos derivaron a odontología y relevaron variables de las siguientes dimensiones: datos personales, antecedentes médicos, estado de salud bucal, necesidad de tratamiento odontológico y hábitos de autocuidado bucal. La mediana de edad fue de 60 años, el 60,7 % pertenecían al género masculino. El 68,7 % tenían enfermedades del sistema circulatorio, la media de medicamento administrada fue de 7 y el 28,1 % correspondía a medicación del sistema cardiovascular. El 70% de las derivaciones provino del servicio de Cardiología y el 48 % respondieron a evaluación prequirúrgica. El porcentaje de placa visible alcanzó 73,6 % y la hemorragia al sondaje 75,4 %. El CPOD fue de 19,9. El 57,3 % de los pacientes tenía bolsas periodontales superiores a 4 mm, y el 97,2 % requerían tratamientos de cirugía, endodoncia y rehabilitación de prótesis. La frecuencia de cepillado diario bajó durante la internación: el 28,7 % refirió no realizar cepillado diariamente y solamente el 5,3 % mencionó cepillarse 3 veces/día.
Las derivaciones a odontología provinieron principalmente del servicio de cardiología en situaciones prequirúrgicas. Los pacientes internados presentaron elevados niveles de patología bucal y necesidad de tratamiento odontológico.

Palabras clave: *interconsultas; derivación médica; pacientes internados; salud bucal.*

INTRODUCTION

A number of factors contribute to the complexity of healthcare, including technology, supplies, professional qualification, habits of the population, and ways in which healthcare is organized. These components are all involved in a scenario characterized by two types of fragmentation, one of which is clearly recognized by healthcare managers and

public health experts, while the other is masked by the particular interests of the professions, creating divides between professions, and even within professions.

The first type of fragmentation characterizes healthcare in Argentina. There is a public sector in which the national, provincial and municipal levels present a high degree of autonomy; a large number of diverse "obras sociales" (trade union-managed health insurance) reflecting the development of healthcare linked to social security in Argentina, and finally, a large private sector including a wide range of mutual benefit schemes, private insurance and private facilities. There is little coordination between and even within all of these. This is true regarding both medical and dental healthcare.

The second type of fragmentation or "divide", which has high impact on personal and populational healthcare, arises from the lack of communication between medical and dental professionals. This is seen in concrete individual healthcare, in professional human resource training, and in the implementation of the concept of comprehensive health so often stated by the World Health Organization[1].

The US Public Health Service has published a critical view of emerging associations between oral diseases and systemic diseases, especially HIV and AIDS, diabetes, osteoporosis, endocarditis, adverse effects of medications, immunosuppression and nutritional deficiencies, among others[2]. There is a lack of systematic communication between medical and dental healthcare providers under the concept of comprehensive healthcare[3]. These problems in healthcare structure and dynamics may constitute one of the variables impacting patient health, taking into account the oral health problems in hospitalized patients.

The needs for dental treatment in hospitalized patients have been analyzed by different authors:

- Hanne et al. found in a hospital in Denmark that 91% of hospitalized patients needed treatment of at least one or more oral problems (dental plaque biofilm, dental caries, dry mouth) [4].
- Carrilho et al. reported that 98.1% of the patients had por oral hygiene, 74.5% had gingival inflammation, 60% periodontal disease and 19.8% candidiasis[5]. They also found the following associations:
  a. Dental caries associated to smoking and to deficient oral hygiene
  b. Gingival index and bacterial plaque index associated to hospitalization time and patient age.
  c. Increase in plaque biofilm and worsening of gingival inflammation associated to hospitalization.
- Needelman et al. found that after 14 days hospitalization in critical care units at a University Hospital in London, patients showed an increase in oral biofilm accumulation, deterioration in oral status, and increased risk of systemic infections associated to pneumonia[6].
- Tereazakis et al. conducted a systematic review which included five studies, concluding that oral health deteriorated during hospitalization in terms of plaque biofilm accumulation, gingival inflammation and alterations of the mucosa[7].
- Donatsky et al. found that 82% of inpatients at a university hospital in Denmark required dental treatment, especially surgical treatment and rehabilitation[8].

As a result of our interest in exploring the causes of these observations, we established the following aims for the current study:

- To characterize the connection between medical and dental healthcare, as expressed by referrals requested by different medical services.
- To ascertain the oral health status of patients referred to dentistry.
- To identify the needs for dental care of inpatients at a University Hospital in Buenos Aires City, Argentina.

MATERIALS AND METHODS

The study design was approved by the Research Protocols and Ethics Committee of the Department of Research and Teaching at the Hospital Italiano in Buenos Aires (N1944) and the Bioethics Committee of the School of Dentistry of Buenos Aires University (27/03/2013-50). A descriptive study was conducted on inpatients at Hospital Italiano in Buenos Aires who were referred by treating physicians for dental evaluation, from March 2013 to May 2015. From the hospital electronic medical record (EMR), 203 patients with referrals to the Dental department were identified. Requests for

referrals are submitted by physicians, generating automatic emails to the dental department. Exclusion criteria were: patients hospitalized in the psychiatry department, patients who did not speak Spanish, patients with neurological diagnosis, patients who were unconscious and/or intubated, and patients isolated by the Infections Committee due to:

- diseases with airborne transmission or transmission by suspended particles (TBC and chicken pox)
- diseases transmitted by respiratory droplets (influenza),
- diseases transmitted by direct or indirect contact (vancomycin-resistant enterococci, *Clostridium dificile, Klebsiella pneumoniae).*

Following the above criteria, 53 patients were excluded. The sample for evaluation included 150 patients.

Data were collected according to the following procedure:

1. Email received requesting referral to dental department.
2. Patient duly identified, following the standard of the Joint Commission International[9] in the area where patient was hospitalized.
3. Clinical diagnosis performed in the hospitalization areas, using disposable dental examination instruments and a lamp protected with a disposable glove.
4. Data recorded in an *ad hoc* dental clinical history.
5. Data added to the Hospital Italiano electronic medical record.

Table 1 shows the indicators used and operationalization of the variables analyzed.

Statistic treatment for independent samples was done as follows:

- For qualitative variables, frequency distribution (%) was established.
- For quantitative variables, means, SD and SE, median, minimum and maximum were established.
- For comparison of quantitative variables, Student's test for independent samples was applied.

Table 1: Data surveyed in clinical history.

Field	Indicators	Operationalization of variables
Personal details	Patient sex	Male/female according to patient electronic clinical history.
	Patient age	In years at the time of evaluation.
	Medical coverage	According to electronic clinical history.
Medical history	Main diagnosis	According to electronic clinical history. Grouped according to International Classification of diseases (WHO/ICD 9).
	Medication taken at time of diagnosis	According to electronic clinical history. Grouped according to criteria of Anatomical, Therapeutic and Clinical classification (WHO/ATC).
	Service requesting referral	According to affiliation of requesting physician to the Hospital Italiano service through an electronic Clinical History. Grouped according to HI organizational chart.
	Reason for referral	According to the form prepared by the physician requesting referral.
	Time from hospitalization to dental examination	Time (in days) from date of hospitalization to date of dental diagnosis.
Oral health status	Percentage of visible plaque	Absence (0) and presence (1) of visible plaque on 4 faces of teeth present (mesial, distal, buccal, lingual/palatine) observed and recorded.
	Percentage of bleeding on probing	Absence (0) and presence (1) of bleeding on probing observed and recorded.
	DMFT Index	Sum of decayed, missing and filled teeth.
Need for dental treatment	CPITN	Periodontal examination expressed as a score in mm of probing depth, evaluated with periodontal probe, from the gingival margin to the bottom of the periodontal pocket. Not applicable to completely edentulous patients.
	TNI for dental caries	Based on dental caries process established from dental chart.
Oral hygiene habits	Date of last visit to dentist	Direct report from patient.
	Frequency of oral hygiene prior to and during hospitalization	Direct report from patient.

- For comparison of qualitative variables, Chi square test (independence test) was employed.
- For comparison of percentages between groups, independent proportions test with approximation to normal or binomial, according to the case, was applied.
- For comparison of oral hygiene methods and frequencies before and during hospitalization, Wilcoxon's test was employed.

A significance level lower than 5% was used for all cases to reject the null hypothesis.

RESULTS

Socio-demographic characteristics of the sample included in the study

The study included a purposive sample of 150 patients hospitalized in different departments at the Hospital Italiano in Buenos Aires. Median age was 60 years (range 21-84 years) and 60.7% were male. There was no significant difference for mean age between males and females. Ninety-eight percent of the patients had health coverage through public or private healthcare systems.

Healthcare of referred patients

According to the ICD9 classification, main diagnoses were: diseases of the circulatory system in 68.7% of the patients (Table 2), diseases of the respiratory system in 14% and neoplasms in 9.3%. Other diseases affected 0.7% to 1.3% of the patients.

The following findings were recorded for medications taken:

- Number of medications taken daily per patient ranged from 0 to 16, with mean 7.
- Out of the total medications administered, 28.1% were for treatment of the cardiovascular system (Table 3).
- The Cardiology service was responsible for 70% (Fig. 1) of the referrals to Dentistry.
- 48% of referrals were for pre-surgical checks for cardiac vascular surgery, followed by reports required prior to transplants (Table 4).
- 10.68% of the referrals were for dental problems described with different levels of precision (dental pain, dental trauma, dental prosthesis check, Ludwig's angina, broken tooth, gum bleeding, etc.) (Table 4).
- Median time from hospitalization to dental examination was 3 days, and ranged from 1 to 123 days.

Results of dental examination

Median was 73.6% for percentage of visible plaque and 75.4% for bleeding on probing (Table 5).

Table 2: Main diseases grouped according to ICD9 (*).

	Frequency	Percent
Circulatory system	103	68.7
Respiratory system	21	14.0
Neoplasms	14	9.3
Endocrine, nutritional and metabolic	2	1.3
Central nervous system and sensory organs	2	1.3
Digestive system	2	1.3
Injury and poisoning	2	1.3
Diseases of the blood and blood-forming organs	1	0.7
Genitourinary system	1	0.7
Musculoskeletal System and Connective Tissue	1	0.7
Symptoms, signs that define disease	1	0.7
Total	**150**	**100.0**

(*) International Statistical Classification of Diseases and Related Health Problems.

Table 3: Medication administered according to ATC classification.

Medication administered	Percent
Cardiovascular system	28.1%
Digestive system and metabolism	19.6%
Blood and blood-forming organs	18.5%
Nervous system	10.5%
Anti-infective for general for systemic use	5.3%
Dermatological medications	5.2%
Respiratory system	4.1%
Systemic hormonal preparations, excl. sexual hormones	4.0%
Musculoskeletal system	2.1%
Antineoplastic and immunomodulating agents	1.1%
Genitourinary system and sexual hormones	0.9%
Various	0.3%
Sensory organs	0.3%
Antiparasitic products, insecticides and repellents	0.1%

Median for DMFT index was 19.9 teeth, consisting of 2.5 decayed, 14.7 missing and 2.7 filled teeth.For CPITN, 57.3% of the sample had CPITN 3 and 4, which implies periodontal pockets deeper than 4 mm (Fig. 2).TNI for dental caries showed that 97.2% of the patients required treatment with prosthesis, endodontics and/or surgery in at least 3 quadrants of the mouth (values 5 to 11 of TNI for dental caries) (Table 6).Only 0.7% of the sample had a healthy mouth but required preventive treatment (1 on the TNI for dental caries) (Table 6).

History of dental care in hospitalized patients

Among the hospitalized patients, 16% reported having visited a dentist within 6 months prior to hospitalization, and 81.3% reported not having been taught oral hygiene technique.

Patients reported the following tooth brushing frequency:

- 1.3% of the patients said they "never" brushed their teeth prior to hospitalization, while 28.7% reported never doing so during hospitalization.
- 40% of the patients said they brushed their teeth "3 times a day" prior to hospitalization, but only 5.3% of patients reported doing so during hospitalization (Fig. 3).

DISCUSSION

The aim of this preliminary study is to trigger discussion on one of the gaps in healthcare, which is comparable to the gap between knowledge production and health policy decision making, which has given rise to increasing development of translational research. The study was conducted at Hospital Italiano in Buenos Aires, which is accredited by the Joint Commission International, and according to the journal "América Economía", is ranked among the five best hospitals in Latin America, and first in Argentina[10]. Current hospital

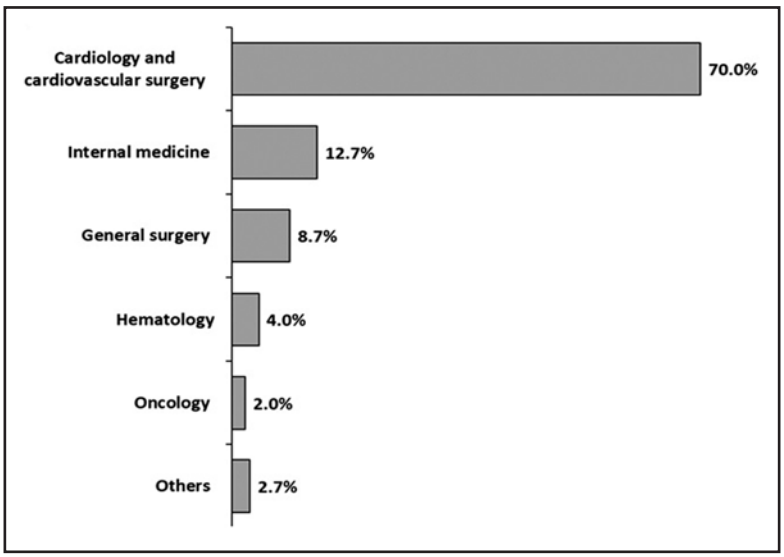

Fig. 1: Services that requested referral to dentistry, in percentages.

Table 4: Reason for referral to dentistry, in percentage.

Reason	Percentage
Presurgical valve	48.00
Pre transplant	39.33
Dental pain	3.33
Evaluation of oral status	3.33
Dental trauma	1.33
Pre bisphosphonate evaluation	1.33
Monitoring dental prosthesis	0.67
Ludwig's angina	0.67
Broken tooth	0.67
Bleeding gums	0.67
Presurgical cardiac tumor	0.67
Total	100.00

Table 5: Description of visible plaque and bleeding on probing.

Percentage	Median	Mean	Maximum	SD
Visible plaque	73.6	8.7	100	22.6
Bleeding on probing	75.4	10.0	100	56.6

Table 6: Percentage of TNI for dental caries.

TNI for dental caries	Description	Percentage
1	Healthy mouth with need for preventive treatment	0.7
2, 3, 4	Dentin-enamel Caries	2.1
5	Pulp problems	1.3
6 a 10	Partially edentulous	85.3
11	Completely edentulous	10.6

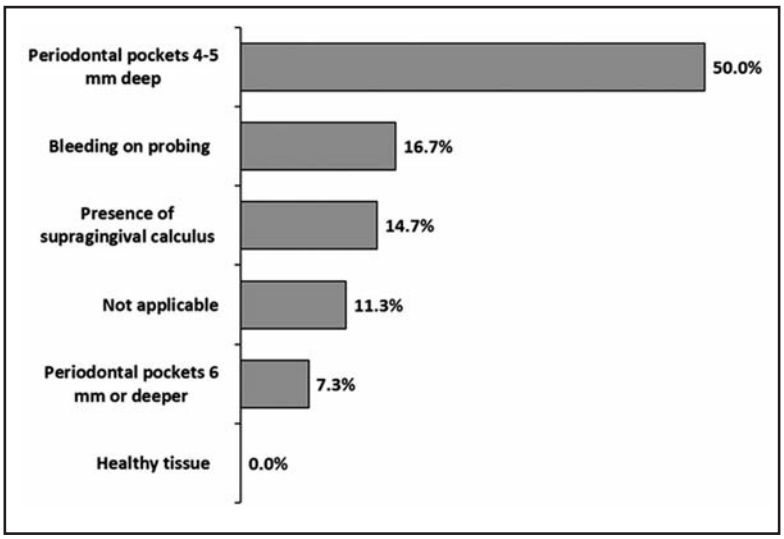

Fig. 2: Percentages of community periodontal index of treatment needs (CPITN).

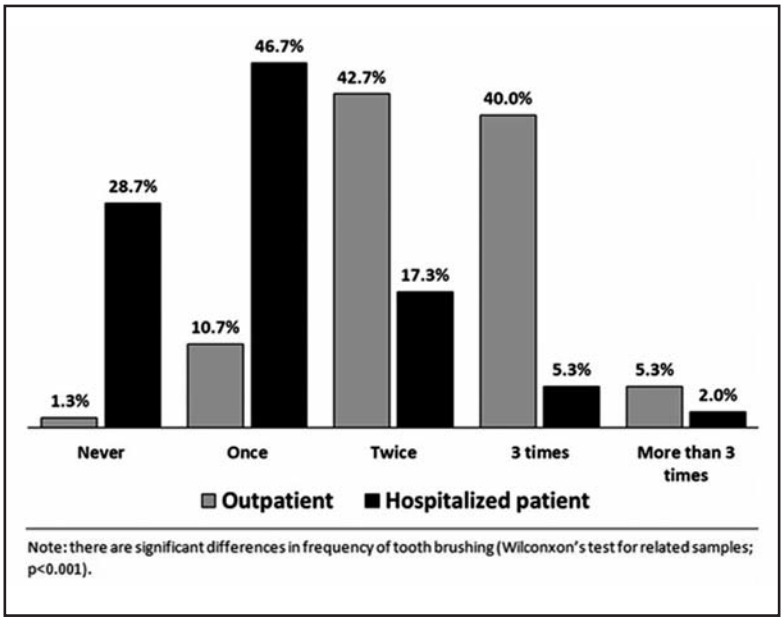

Fig. 3: Comparison of daily hygiene habits before and during hospitalization, in percentages.

policies on initial evaluation of hospitalized patients include assessment of medical, nursing and nutritional areas. The medical area includes referrals to the different services that physicians deem necessary for adequate patient care. Dentistry has been included in the hospital organizational chart with direct dependence on the Surgical Department.The reasons supporting the need for a change in the dynamics of patient healthcare, in the hope of generating a new paradigm in comprehensive healthcare, can be found in scientific evidence. On an individual scale, they include:

1. General diseases or treatments for them with repercussion or manifestations on oral status, and
2. Oral diseases or treatments related to them with repercussion or manifestations on general health.

At the same time, on the healthcare scale, there is a contradiction between the "discourse" and the concrete practice of professional integration. From an epidemiological standpoint, it has been demonstrated that dental caries and periodontal disease are prevalent and could be avoidable[11-12]. Deficient oral hygiene contributes to the development and maturation of dental plaque biofilm, with impact on prevalent diseases. The etiological character of plaque biofilm warrants a re-evaluation of its role in specific prevention. Carrilho N et al. [5] suggest that there may be loss of motivation in maintaining routine hygiene habits such as tooth brushing. Needelman et al. [6] report that as a result of preexisting oral health conditions, problems may become more severe or new problems may arise upon hospitalization, such as infections of the respiratory tract caused by oral microorganisms.

Our results on deficient oral hygiene and consequent presence of biofilm were consistent with those reported in the studies by Carrilho *et al.* [5], Needleman *et al.* [6] and Terezakis *et al.* [7].

This deterioration process can increase as a result of preexisting systemic condition or due to the use of medication administered during hospitalization. Moreover, the impact of emotional conditions that arise during hospitalization is not negligible.

Despite available evidence, no systematic protocol has been implemented to include oral hygiene practice in nursing routines or the routine of caregivers of hospitalized patients.

With regard to established oral diseases, diagnosis performed on the sample of hospitalized patients showed a major sanitary deficiency reflected by indicators of current status as well as need for treatment. It is worth highlighting that 98% of the patients in this study had medical coverage through private systems or "obras sociales" (union-managed health insurance), which included treatment of diseases due to dental plaque biofilm. It is interesting to note that, notwithstanding, only 2.8% had a healthy mouth, while 97.2% had need for oral treatment of caries and gingival-periodontal diseases. The high level of oral disease found in this study is in agreement with the results published by Hanne *et al.* [4], Carrilho *et al.* [5] and Donatsky *et al.* [8].

With regard to the origin of the referrals, there are marked differences between those requested by cardiologists, particularly in pre-surgical situations, and those requested by the rest of the medical specialties.

However, given the chronic inflammatory component of oral diseases, dental referral ought to come from most medical services provided in a hospital, because such diseases may constitute a risk factor for the diseases dealt with by the different specialties.

Dental referral may reflect the degree of interdisciplinarity at a facility and should involve all healthcare professionals in order to optimize the quality of patient healthcare.

Clearly, there is no such thing as "partial health". Oral status is a component of general health. The causes of the "gap" between medicine and dentistry may be found in the corporative autonomies historically developing as from human resource training.

With the aim of proposing changes and improvements in the integration of dentistry in a hospital, we propose further studies to analyze intrahospital medical referrals to the dental department and the existence of protocols or policies of intrahospital oral hygiene, as well as levels of compliance.

Finally, we recommend:

a. Protocolizing the care of hospitalized patients based on a risk approach,
b. Fostering self-care actions to be used to prevent this scenario, and
c. Moving forward in interdisciplinary social practices in healthcare training courses.

CONCLUSIONS

The dental referrals recorded were mainly requested by the cardiology department, particularly for pre-surgical situations.

The oral status of hospitalized patients surveyed in this sample reveals a major gap in care, with no significant difference between sexes.

Patients included in this sample were found to have high levels of need for dental treatment.

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REFERENCES

1. Organización Mundial de la Salud. Documentos básicos 48º Edición. Italia: Ediciones OMS, 2014:1-3.
   URL: https://apps.who.int/gb/bd/PDF/bd48/basic-documents-48th-edition-sp.pdf
2. US Departament of Health and Human Services. A Report of the Surgeon General. Rockville, U.S.: Department of Health and Human Services, National Institute of Dental and Craniofacial Research, National Institutes of Health 2000, 2000:135-159.
   URL: https://profiles.nlm.nih.gov/ps/access/NNBBJT.pdf
3. Alfonsin A. La boca también existe. Buenos Aires, Argentina: Del Hospital Ediciones, 2014:11-16.
4. Hanne K, Ingelise T, Linda C, Ulrich PP. Oral status and the need for oral health care among patients hospitalised with acute medical conditions. J Clin Nurse 2012; 21: 2851-2859.
5. Carrilho NA, De Paula RS, Sant'ana AC, Passanezi E. Oral health statusamong hospitalized patients. Int J Dent Hyg 2011; 9:21-29.
6. Needleman I, Hyun Ryu J, Brealey D, Sachdev M et al. The impact of hospitalization on dental plaque accumulation: an observational study. J Clin Periodontol 2012; 39:1011-1016.
7. Terezakis E, Needleman I, Kumar N, Moles D, Agudo E. The impact of hospitalization on oral health: a systematic review. J Clin Periodontol 2011; 38:628-636.
8. Donatsky O, Ahlgren P, Hansen PF. Oral health status and treatment needs in long-term medicine patients in a Copenhagen hospital department. Community Dent Oral Epidemiol 1980; 8:103-109.

9. Joint Commission International. Accreditation standards for hospitals. 6º Edition Illinois, USA: Department of Publications Joint Commission International, 2017:44-45. URL: https://www.kfshrc.edu.sa/store/media/1oh.pdf
10. Revista América Economía [Disponible en internet] Ranking de Clínicas y Hospitales: Estos son los mejores de América Latina. Publicado 23/11/2017. Accedido el 18-01-2019, desde https://clustersalud.americaeconomia.com/gestion-hospitalaria/ranking-de-clinicas-y-hospitales-estos-son-los-mejores-de-america-latina.
11. US Department of Health and Human Services. A Report of the Surgeon General. Rockville, U.S.: Departament of Health and Human Services, National Institute of Dental and Craniofacial Research, National Institutes of Health 2000, 2000:37-42
URL: https://profiles.nlm.nih.gov/ps/access/NNBBJT.pdf
12. Beaglehole R, Benzian H, Crail J, Mackay J. The Oral Health Atlas: The Challenge of Oral Disease. A Call for Global Action. Second Edition. World Dental Federation; Brighton, UK: Myriad Editions, 2015:16-25.

Implementation of TMD pain screening questionnaire in peruvian dental students

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ABSTRACT
The aim of this study was to screen for painful TMD conditions by implementing the validated Axis I screening instrument from the Diagnostic Criteria for Temporomandibular Disorders. Using the screener as a surrogate, the prevalence of the conditions was estimated among a convenience sample of dental students in Peru. A total 2,562 dental students, 63.7% women, aged 18 to 62 completed the instrument. Prevalence was estimated using both the short and long versions. The prevalence of painful TMD conditions was 19.4% with the short and 16.1% with the long version. The distribution of the conditions according to gender differed significantly between groups (p<.001). Prevalence estimates of painful TMD conditions using the screening instrument seems to be logistically adequate in a field assessment involving multiple geographic and cultural regions in Peru. These estimates seem to be consistent with internationally reported values.
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Keywords: *temporomandibular joint disorders; pain; clinical decision-making.*

Implementacion de un cuestionario de triaje para trastornos temporomandibulares asociados al dolor en estudiantes de odontologia peruanos

RESUMEN
El objetivo de este estudio fue tener una estimación de las condiciones de los trastornos temporomandibulares asociados al dolor, mediante la implementación de un instrumento validado de triaje para la medición del Eje I de los Criterios Diagnósticos para los Trastornos Temporomandibulares (CD/TTM). Usando este instrumento como un sustituto, se estimó la prevalencia de estas condiciones en una muestra por conveniencia de estudiantes de Odontología en el Perú. Un total de 2,562 estudiantes de Odontología, 63.7% mujeres, entre las edades de 18 a 62 años de edad, completaron el instrumento. La prevalencia fue estimada empleando las versiones corta y larga del instrumento. La prevalencia de los TTM asociados al dolor fue de 19.4% con la versión corta y 16.1% con la versión larga. La distribución de estas condiciones fue estadísticamente diferente según el sexo (p<.001). El empleo del instrumento de triaje para estimar la prevalencia de los TTMs asociados al dolor parece ser logísticamente adecuado en un escenario de diversas regiones geográficas y culturales en el Perú. Estas estimaciones parecen ser consistentes con valores internacionalmente reportados.

Palabras clave: *trastornos de la articulación temporomandibular; dolor; toma de decisiones clínicas.*

INTRODUCTION

Comprehensive patient care, both in clinical practice and in academic programs, should include assessment of temporomandibular disorders (TMD). Initial identification of TMD-related conditions should be followed by proper referral to a well-trained professional in the field for evidence-based diagnostic procedures and interventions. This conclusion is based on the incorporation of recent knowledge of the paradigmatic shift to modern concepts supporting TMD etiology, progression and treatment that may deviate from preconceived ideas[1,2].

Historically, TMD diagnostic classifications focused on physical findings, with a lack of consistent, operationalized definitions and criteria. This barrier was overcome by the Research Diagnostic Criteria for TMD (RDC/TMD)[3], which introduced a dual axis system: Axis I for clinical diagnoses and Axis II for pain-related disability and psychological status. More recently, the Diagnostic Criteria for Temporomandibular Disorder (DC/TMD) [4] was published as a result of validation study group recommendations and participation of the international community in this field, presenting validated

criteria for the most common TMDs. This new diagnostic classification system maintains a dual axis and provides sensitivity and specificity values to support its clinical validity for clinical implementation. The DC/TMD recommends the implementation of a standardized examination protocol and utilization of self-report instruments, including a TMD-pain screener [5] to be used by clinicians and researchers. The screening instrument, which shows excellent levels of sensitivity and specificity, can be used routinely for identification of individuals with TMDs in population-based research studies if there is limited infrastructure for including examination protocols, or in clinical settings for early identification prior to proceeding on with appropriate referrals.

The aim of this study was to estimate the prevalence of pain-related TMDs, using as a surrogate the screening instrument presented by the DC/TMD, in a nationwide sample of dental students that included different geographic and cultural regions in Peru.

MATERIALS AND METHODS

A total 4967 dental students enrolled for the 3rd to 10th semesters were invited to participate in this field study, of whom 2562 accepted. They came from different cities, representing 21 geographic locations from regions including the coast, the mountains and the Amazon rainforest. Minimal sample size was estimated using prevalence of 40% [6,7], significance level of 95% and a power of 90%. The proportional sampling procedure was used to determine the sample in each location according to the number of students who enrolled. Exclusion criteria included the presence of systemic joint disease, neurological disease or a previously established diagnosis of TMD.

This instrument was first introduced in 2011 [5] and encompasses the report of pain, jaw stiffness, and factors that may modify pain, such as function and parafunction (Fig 1). The Pain screener is the only screener questionnaire included in the Axis I of the most recently published Diagnostic Criteria for Temporomandibular Disorders [4]. Its inclusion is supported by its reliability and validity estimates, which include Internal reliability ranging from 0.87 to 0.93, in addition to sensitivity and specificity values of 99% and 97%, respectively, for correct classification of true positives and true negatives [5].

The protocol was approved by the Research Ethics Committee of Universidad Alas Peruanas, Lima, Peru. All participants signed consent forms prior to participation.

Triaje para Trastornos Temporomandibulares – Dolorosos

1. En los últimos 30 días, ¿cuánto le ha durado cualquier dolor en su mandíbula o sien, en cualquiera de los lados?
   a. Sin dolor
   b. El dolor va y viene (intermitente)
   c. El dolor siempre está presente (constante)
2. ¿En los últimos 30 días ha sentido dolor o rigidez en su mandíbula al despertar?
   a. No
   b. Si
3. En los últimos 30 días, ¿alguna de las siguientes actividades afectó (es decir el dolor mejoró o empeoró) el dolor en su mandíbula o sien en cualquiera de los lados?
   A. Masticando comidas duras o difíciles de triturar.
      a. No
      b. Si
   B. Abrir la boca o mover la mandíbula hacia los lados o hacia el frente.
      a. No
      b. Si
   C. Hábitos orales tales como mantener los dientes juntos, apretar, rechinar los dientes o masticar goma de mascar.
      a. No
      b. Si
   D. Otras actividades mandibulares tales como hablar, besar o bostezar.
      a. No
      b. Si

Fig. 1: TMD pain questionnaire.

RESULTS

A total 2562 individuals participated in the study. The mean age of the sample was 24.06 (± 5.72 SD) with a range of 18 to 62 years. Due to the broad age range, decade categories were established for data analysis. Gender distribution shows that 63.7% of the participants were female; this finding was consistent among the age categories and screening scores by age and gender.

Based on TMD-pain screening, the percentage of individuals identified with painful TMD using the short-version was 19.4%, more specifically, 22.0% for females and 14.7% for males. Using the long version, the estimates were 16.1%, with 19.0% females and 11.0% males (Table 1).

Gender distribution among the age groups was evaluated using corrected chi square (x^2), and statistically significant differences were found for distribution of men and women ($p<.001$). Nevertheless, there was no significant difference in positive results among the age groups using the distribution screener. ($x^2= 2.932$, $p=.710$ for the short version, and $x^2= 3.344$, $p=.647$ for the long version) (Table 1).

Distribution according to university locations and results of the versions of the screener are shown in Figs. 2 and 3.

The female-to-male ratio among the individuals using the TMD-pain screening questionnaire in both versions was 2.82:1. Distribution of the sample according to sex and age group is shown in Table 2.

All the items in the self-reported screenings have two alternative answers: "no" or "yes", except the first, which has three alternative answers, "a", "b" and "c", the first being the negative response. For

Table 1: Percent of cases identified by gender using the Pain -TMD screener.

		n	3-item version		6-item version	
			Negative	Affirmative	Negative	Affirmative
Sex	Male	931	794	137	829	102
			85.3%	14.7%	89.0%	11.0%
	Female	1631	1272	359	1321	310
			78.0%	22.0%	81.0%	19.0%
Total		2562	2066	496	2150	412
			80.6%	19.4%	83.9%	16.1%

Table 2: Distribution of cases by age groups and gender.

Age group	sex		TMD-pain screener positive		sex		TMD-pain screener positive		Total
	Male	%	3-item	6-item	Female	%	3-item	6-item	
16-20	224	30.2%	17.4%	12.9%	518	69.8%	19.3%	18.1%	742
21-25	362	34.8%	17.1%	11.0%	678	65.2%	21.2%	17.7%	1040
26-30	201	39.7%	11.4%	10.0%	305	60.3%	25.3%	22.0%	506
31-35	78	50.3%	10.3%	12.8%	77	49.7%	27.6%	19.5%	155
36-40	39	56.5%	2.6%	2.6%	30	43.5%	26.7%	20.0%	69
more than 40	27	54.0%	14.8%	7.4%	23	46.0%	34.8%	34.8%	50
Total	**931**	**36.3%**	**14.7%**	**11.0%**	**1631**	**63.7%**	**22.0%**	**19.0%**	**2562**

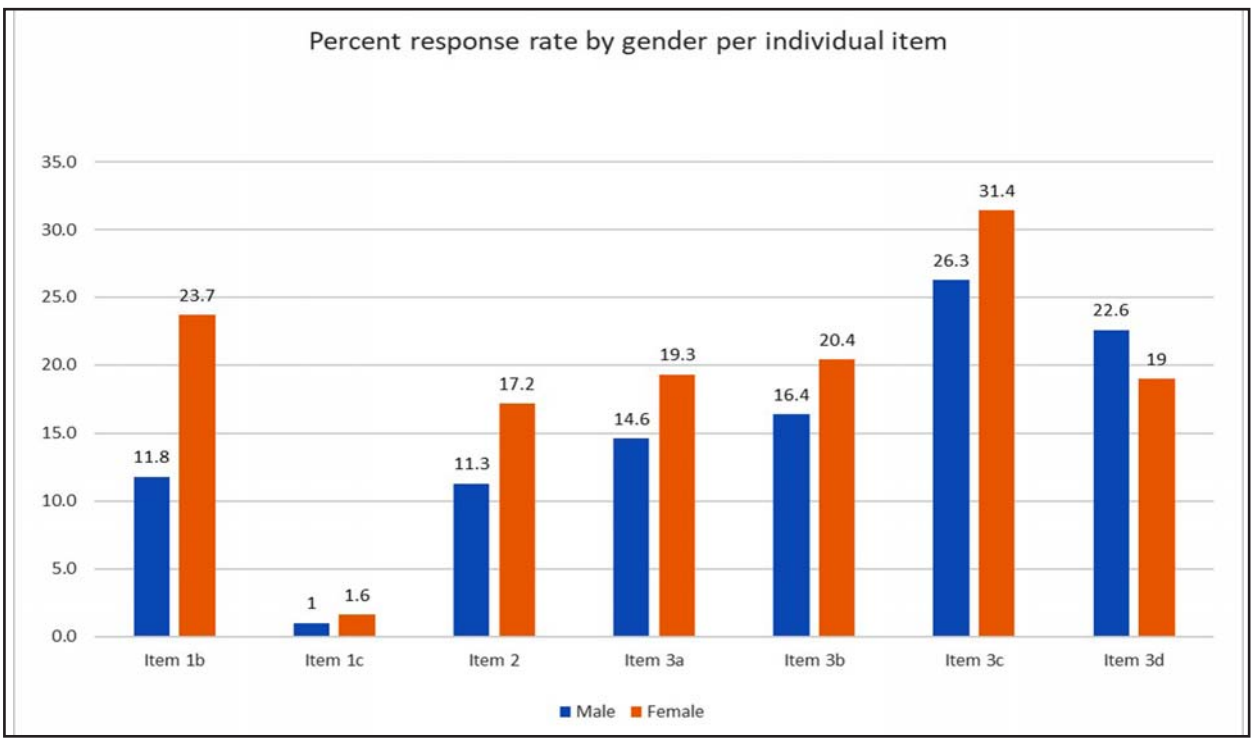

Fig. 2: Percent response rate by gender per individual item.

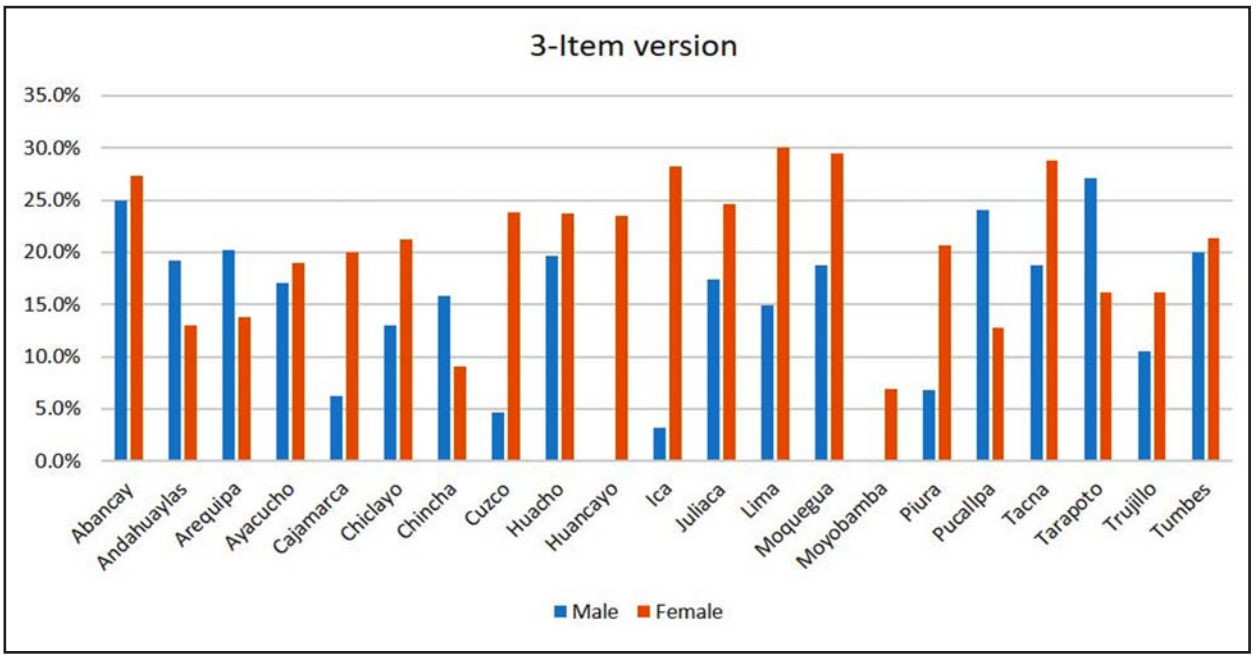

Fig. 3: Percent positive diagnostic allocation by geographic region and gender using the 3-item version of the TMD-Screener.

Table 3: Percent response rate by items.

Item	(n= 2.562)	male (%)	female (%)
1. In the last 30 days, on average, how did long did any pain in your jaw or temple area on either side last?			
a. No pain	2030	40.0	60.5
b. From very brief to more than a week, but it does stop	497	22.1	77.9
c. Continuous	35	25.7	74.3
2. In the last 30 days, have you had pain or stiffness in your jaw upon awakening?			
a. No	2176	38.0	62
b. Yes	386	27.2	72.8
3. In the last 30 days, did the following activities change any pain (that is, make it better or make it worse) in your jaw or temple area on either side?			
A. Chewing hard or tough food			
a. No	2112	37.6	62.4
b. Yes	450	30.2	69.8
B. Opening your mouth or moving your jaw forward or to the side?			
a. No	2077	37.5	62.5
b. Yes	757	32.4	67.6
C. Jaw habits such as holding teeth together, clenching, grinding or chewing gum?			
a. No	1805	38.0	62
b. Yes	757	32.4	67.6
D. Other jaw activities such as talking, kissing or yawning?			
a. No	2042	35.3	10.3
b. Yes	520	40.4	59.62

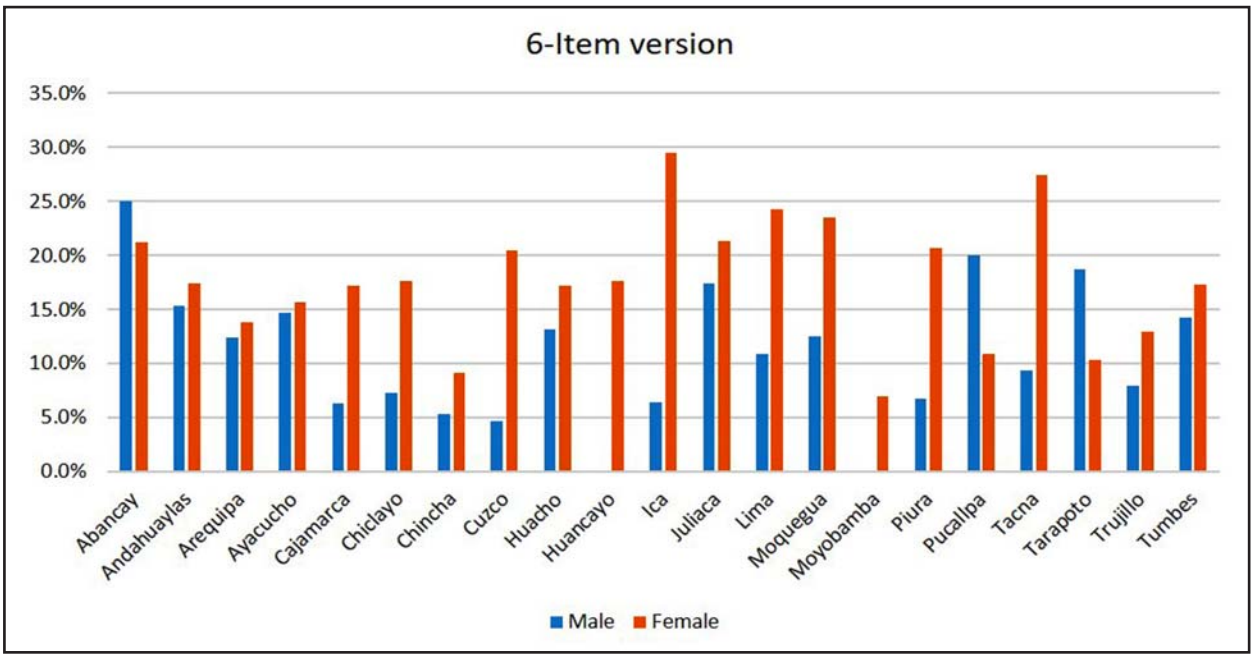

Fig. 4: Percent positive diagnostic allocation by geographic region and gender using the 6-item version of the TMD-Screener.

the first item, 19.4% (497) of all the participants in the study responded affirmatively to alternative "b", and 1.4% (35) responded affirmatively to alternative "c". For item 2, 15.1% (386) responded affirmatively. Of the four conditions included in the third item, 17.6% (450) answered "yes" to the first, and 18.9% (485), 29.5% (757) and 20.3% (520) answered "yes" to numbers 2, 3 and 4, respectively. These results are shown in Table 3.

With regard to the distribution of the responses for the items according to sex, presented in Fig. 2, note that more males than females responded affirmatively only in item 3d.

There was a significant difference in prevalence according to sex in four locations: Abancay, Andahuaylas, Arequipa and Chincha, when comparing the 3-item and 6-item versions (Figs. 3 and 4).

With the 3-item version, male prevalence was noted in Andahuaylas, Arequipa, Chincha, Pucallpa and Tarapoto, but not in Huancayo and Moyobamba. With the 6-item version, male prevalence was found in Abancay, Pucallpa and Tarapoto, but not in Huancayo and Moyobamba. No statistical difference was found in the distribution between TMD and sex with the short version in university locations except in Cuzco, Ica, Lima and Piura. This was also true for the long version, with the addition of Tacna. According to the total results there was a statistical difference ($p<.001$) between TMD and sex in both versions.

Internal consistency of the screener was evaluated in 10% of the sample, and the Cochran alpha Kappa statistic was 0.69.

DISCUSSION

The screener's validity had been previously determined in two versions, with 3 or 6 self-reported items [5]. In the sample evaluated, our main result showed the prevalence of Painful TMD disorders as 19.4% and 16.1% in each version, respectively.

The self-report questions for core symptoms for TMD-pain diagnosis are included in both versions, and address the following: evidence of recent pain and modification of pain by function and parafunction, within the same time frame[5-8].

Using self-report, researchers have implemented field assessment to estimate the prevalence of orofacial pain conditions in representative populations [9],

In the field of TMD, Dworkin, et al.[10] implemented this methodology in USA and reported 12% prevalence of TMD cases, similar to the results obtained in our study. Goncalvez [11], used a five-item TMD symptoms questionnaire in a Brazilian urban population, estimating prevalence of TMD as 25%,

and Bevilaqua [12] reported an estimate of 59% in a convenience sample of dental students in Brazil.

The prevalence of TMD seems to be consistently higher among females [11,13]. However, in our study, this pattern was not maintained across the geographic regions, since males presented higher prevalence in Abancay, Pucallpa and Tarapoto, even though more female students had enrolled. In contrast, in Huancayo and Moyobamba we found no occurrence of TMD pain in males, which may represent a cultural difference.

In conclusion, although two versions of the questionnaire are presented by the screener developers, and in the understanding that the short version may be more efficient for field assessment, we would like to recommend the longer 6-items version because it provides the most conservative and consistent estimates of pain-related TMD prevalence. To the best of our knowledge, our study is the first one to implement the validated instrument for field assessment, since no other publications were found in Index Medicus / Medline journals.

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REFERENCES

1. De Leeuw R, Klasser GD. Orofacial pain: guidelines for assessment, diagnosis and management. Chicago: Quintessence; 2013.
2. Türp JC, Greener CS, Strub JR. Dental occlusion: a critical reflection on past, present and future concepts. J Oral Rehabil 2008; 35446-453.
3. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992; 6:301-355.
4. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/ TMD) for clinical and research applications: recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. J Oral Facial Pain Headache 2014; 28:6-27.
5. Gonzalez YM, Schiffman E, Gordon SM, Seago B, Truelove EL, Slade G, et al. Development of a brief and effective temporomandibular disorder pain screening questionnaire: reliability and validity. J Am Dent Assoc 2011; 142:1183-1191.
6. Campos JA, Gonçalves DA, Camparis CM, Speciali JG. Reliability of a questionnaire for diagnosing the severity of temporomandibular disorder. Rev Bras Fisioter 2009; 13:38-43.
7. Casanova-Rosado JF, Medina-Solís CE, Vallejos-Sánchez AA, Casanova-Rosado AJ, Hernández-Prado B, Avila-Burgos L. Prevalence and associated factors for temporomandibular disorders in a group of Mexican adolescents and youth adults. Clin Oral Investig 2006 Mar; 10:42-49.
8. Ohrbach R, List T, Goulet JP, Svensson P. Recommendations from the International Consensus Workshop: convergence on an orofacial pain taxonomy. J Oral Rehabil 2010; 37:807-812.
9. Lipton JA, Ship JA, Larach-Robinson D. Estimated prevalence and distribution of reported orofacial pain in the United States. J Am Dent Assoc 1993; 124:115-121.
10. Dworkin SF, Huggins KH, LeResche L, Von Korff M, Howard J, Truelove E, et al. Epidemiology of signs and symptoms in temporomandibular disorders: clinical signs in cases in controls. J Am Dent Assoc 1990; 120: 273-281.
11. Gonçalves DA, Dal Fabbro AL, Campos JA, Bigal ME, Speciali JG. Symptoms of temporomandibular disorders in the population: an epidemiological study. J Orofac Pain 2010; 24:270-278.
12. Bevilaqua-Grossi D, Charles TC, de Oliveira As, Monteiro-Pedro V. Anamnestic index severity and signs and symptoms of TMD. Cranio 2006; 24:112-118.
13. Slade GD, Bair E, Greenspan JD, Dubner R, Fillingim RB, Diatchenko L, Maixner W, Knott C, Ohrbach R. Signs and symptoms of first-onset TMD and sociodemographic predictors of it s devolpment: The OPPERA prospective cohort study. J Pain 2013;14l: T20-32 e 1-3.

Coexistence of thyroid disease and oral lichen planus in a Colombian population

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ABSTRACT

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease of unknown etiology. OLP has recently been linked to thyroid disease, mainly hypothyroidism. The aim of this study was to determine the prevalence of thyroid disease in Colombian patients with OLP. A total of 860 clinical records of patients attending the clinics of oral medicine and oral and maxillofacial surgery at IPS CES Sabaneta, Colombia, between 2010 and 2016 were reviewed. Fourteen patients (1.6%) had a diagnosis of OLP. The prevalence of hypothyroidism in patients with OLP was 35.7%, compared to 3.95% in the entire study population (OR 15.92, 95% CI: 5.63-50.09, P = 0.0001). Patients with concomitant hypothyroidism and OLP presented with less severe oral lesions compared to those without thyroid disease. This study supports the notion that patients with OLP should be screened for thyroid disease.

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Keywords: *oral lichen planus; thyroid diseases; hypothyroidism; thyroxine.*

Coexistencia de enfermedad de la tiroides y liquen plano oral en una población colombiana

RESUMEN

El liquen plano oral (LPO) es una enfermedad mucocutánea inflamatoria crónica de etiología desconocida. El LPO ha sido asociado recientemente con la enfermedad de la tiroides, especialmente con hipotiroidismo. El objetivo con este estudio fue determinar la prevalencia de la enfermedad de la tiroides en pacientes colombianos con LPO. Un total de 860 historias clínicas de pacientes que asistieron entre 2010 y 2016 a las clínicas de medicina oral y de cirugía oral y maxilofacial de la IPS CES Sabaneta, Colombia, fueron revisadas. Catorce pacientes (1.6%) habían sido diagnosticados con LPO. La prevalencia de hipotiroidismo en pacientes con LPO fue 35.7%, comparada con 3.95% en toda la población de estudio (RM 15.92, 95% IC: 5.63-50.09, P = 0.0001). Pacientes con hipotiroidismo y LPO concomitante presentaron lesiones orales menos severas comparado con aquellos sin enfermedad de la tiroides. Este estudio respalda la idea de que se debe investigar la presencia de enfermedad de la tiroides en pacientes con LPO.

Palabras clave: *liquen plano oral; enfermedad de la tiroides; hipotiroidismo; tiroxina.*

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease of unknown etiology. In the past decade, a growing body of evidence has accumulated suggesting that OLP is associated with thyroid disease, mainly hypothyroidism.[1-6] Early studies conducted in Finnish and Swedish populations reported a significantly higher prevalence of hypothyroidism (9%-11%) in patients with OLP compared to control subjects (3%-5%).[1,3,4] A more pronounced difference has been noted in Italy and Spain, where 14%-16% of the patients with OLP and 1%-5% of the controls have been diagnosed with hypothyroidism.[2,5,6]

A recent meta-analysis indicated a significantly high prevalence of thyroid disease among patients with OLP compared to controls (OR 2.10; 95% CI: 1.47–3.01).[7] The authors suggested that routine screening for thyroid disease could be beneficial for patients with newly diagnosed OLP. However, more studies are required to elucidate the immunological mechanisms underlying the connection between autoimmune thyroid disease and OLP.

The prevalence of subclinical hypothyroidism in a population of nearly 6000 adults from Medellín, Colombia, has been shown to be 5.9%.[8] However, to the best of our knowledge, no studies investigating the association between thyroid disease and OLP have been conducted in Colombian or Latin American populations. The aims of this cross-sectional study were to 1) determine the prevalence of hypothyroidism or levothyroxine supplementation in Colombian patients with OLP, and 2) describe the clinical characteristics of patients with concomitant OLP and thyroid disease.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of CES University (Act 77, Project Code 370 of 2015).

We reviewed the clinical records of all patients (n=860) attending the clinics of oral medicine, and oral and maxillofacial surgery at Institución Prestadora de Salud (IPS) CES Sabaneta, Colombia, between 2010 and 2016. The WHO clinical and histopathological criteria[9] were used for the diagnosis of OLP. However, biopsies were only performed when the disease did not present with typical clinical manifestations, as has been previously suggested.[10] All lesions had to present with reticular or papular features with or without plaque, erythema or ulcerations. Gingival OLP with erythema but without reticulum or papules, which is sometimes referred to as an oral lichenoid lesion,[11] was also included. Lichenoid contact reactions observed in close contact with amalgam restorations were excluded. Fisher's exact test was used to analyze the difference in the prevalence of hypothyroidism/levothyroxine supplementation in patients with OLP compared to the entire study population.

RESULTS

Fourteen (1.6%; mean age 59.6 years; females n=11) patients were diagnosed with OLP. The lesions in 6 of these patients did not present with typical clinical characteristics and thus the diagnoses were confirmed with histopathologic examination. Only one patient was on systemic steroids for treatment of OLP. Five patients with OLP (35.7%) had been previously diagnosed with hypothyroidism and were taking levothyroxine supplementation. The prevalence of hypothyroidism/levothyroxine supplementation in the study population was 3.9% (34/860). The difference in the prevalence of hypothyroidism/levothyroxine supplementation in patients with OLP compared to the entire study population was found to be statistically significant (OR 15.9, 95% CI: 5.63-50.09, P = 0.0001). In addition, patients with concomitant OLP and hypothyroidism presented with less severe lesions, i.e. reticular and plaque-like, and symptoms compared to those without thyroid disease (Table 1).

DISCUSSION

We found a significantly higher prevalence of thyroid disease, specifically hypothyroidism, in patients with OLP (35.7%) compared to the entire study population (3.9%). This finding is consistent with previous studies conducted in Scandinavia and Mediterranean countries.[1,2,4,5] In addition, the prevalence of hypothyroidism registered in the entire study population corresponds with the results of a recent investigation conducted in Medellín, Colombia.[8]

The biological mechanism underlying the association between hypothyroidism and OLP is unknown. A number of studies have shown that in most cases of concomitant hypothyroidism and OLP, the diagnosis of thyroid disease and the initiation of thyroxine supplementation therapy have preceded the onset of oral lesions.[2,4] Considering that Hashimoto's thyroiditis is the most common cause of hypothyroidism in iodine-replete regions, it has been hypothesized that serum levels of thyroid antibodies may be involved in the pathogenesis of OLP. Chang et al.[12] reported significantly elevated levels of antithyroid-peroxidase (TPOAb) and antithyroglobulin (TgAb) antibodies in Chinese patients with OLP compared to healthy controls. TPOAb have also been associated with an increased risk of erosive OLP in an Iranian population (OR = 4.02, 95% CI 1.21–13.4; P = 0.023).[13] In contrast, Robledo-Sierra et al.[14] did not find an association between OLP and antithyroid

Table 1: Clinical characteristics of patients with oral lichen planus (OLP) (n=14).

Patient	Sex	Age (yr)	Smoking	Allergies	Type of OLP	OLP Symptoms	OLP treatment	Histopathological examination	Thyroid disease	Levothyroxine supplementation
1	F	62	No	None	Reticular	Yes	No	Yes	Hypothyroidism	Yes
2	F	65	No	None	Reticular	No	No	Yes	Hypothyroidism	Yes
3	F	62	No	None	Erythematous	Yes	No	Yes	Hypothyroidism	Yes
4	F	60	No	Penicillin	Reticular	Yes	No	No	Hypothyroidism	Yes
5	F	53	Yes	None	Plaque-like	No	No	No	Hypothyroidism	Yes
6	M	56	No	Penicillin	Erythematous	No	Yes	No	None	No
7	F	52	No	None	Erythematous	Yes	No	No	None	No
8	F	59	No	None	Ulcerative	No	No	No	None	No
9	F	51	Yes	None	Ulcerative	Yes	No	Yes	None	No
10	M	59	No	None	Ulcerative	Yes	No	Yes	None	No
11	M	49	No	None	Ulcerative	Yes	Yes	Yes	None	No
12	F	49	Yes	None	Reticular	No	No	No	None	No
13	M	79	No	None	Reticular	Yes	No	No	None	No
14	F	78	No	None	Reticular	Yes	No	No	None	No

antibodies, i.e. TPOAb, TgAb, and antithyroid-stimulating hormone receptor antibody (TRAb). However, the same study showed a significantly higher expression of thyroid-stimulating hormone receptor in OLP lesions. Previous studies have shown the presence of thyroid-specific antigens, including thyroid-stimulating hormone receptor and thyroglobulin, in the skin of patients with autoimmune thyroid diseases. A similar organ-specific autoimmune response may occur in the oral mucosa and influence the development of OLP in a subgroup of patients, in whom basal keratinocytes expressing thyroid or thyroid-like proteins become a target of cytotoxic T cells.[4,14]

Another aim was to describe the clinical characteristics of patients with concomitant thyroid disease and OLP. Despite the limited number of patients (n=5), it was found that they presented with less severe OLP, namely, more reticular and less erythematous/ ulcerative lesions compared to those without thyroid disease. Similar results were shown in a Swedish study, where OLP patients with thyroid disease presented with more reticular and less erythematous lesions compared to those without thyroid disease. The reason for this is yet to be determined.

CONCLUSION

The prevalence of hypothyroidism in Colombian patients with OLP is remarkably higher than in the general population. This finding is in accordance with what has been reported in other populations, which supports the notion that patients with OLP should be screened for thyroid disease.

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REFERENCES

1. Siponen M, Huuskonen L, Läärä E, Salo T. Association of oral lichen planus with thyroid disease in a Finnish population: a retrospective case-control study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110:319-324.
2. Lo Muzio L, Santarelli A, Campisi G, Lacaita M, Favia G. Possible link between Hashimoto's thyroiditis and oral lichen planus: a novel association found. Clin Oral Investig. 2013;17:333-336.

3. Robledo-Sierra J, Mattsson U, Jontell M. Use of systemic medication in patients with oral lichen planus - a possible association with hypothyroidism. Oral Dis. 2013;19:313-319.
4. Robledo-Sierra J, Landin-Wilhelmsen K, Nystrom HF, Mattsson U, Jontell M. Clinical characteristics of patients with concomitant oral lichen planus and thyroid disease. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;120:602-608.
5. Garcia-Pola MJ, Llorente-Pendas S, Seoane-Romero JM, Berasaluce MJ, Garcia-Martin JM. Thyroid Disease and Oral Lichen Planus as Comorbidity: A Prospective Case-Control Study. Dermatology. 2016;232:214-219.
6. Arduino PG, Karimi D, Tirone F, Sciannameo V, Ricceri F, Cabras M, Gambino A, Conrotto D, et al. Evidence of earlier thyroid dysfunction in newly diagnosed oral lichen planus patients: a hint for endocrinologists. Endocr Connect. 2017;6:726-730.
7. Li D, Li J, Li C, Chen Q, Hua H. The Association of Thyroid Disease and Oral Lichen Planus: A Literature Review and Meta-analysis. Front Endocrinol (Lausanne). 2017;8:310.
8. Carmona Carmona C, Bedoya P, Acevedo J, Cardona Arias J. Prevalence of Thyroid Disorders in an Institution Providing Health Services in Medellin-Colombia. Transl Biomed. 2018;9:1-6.
9. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol. 1978;46:518-539.
10. Al-Hashimi I, Schifter M, Lockhart PB, Wray D, Brennan M, Migliorati CA, Axell T, Bruce AJ, et al. Oral lichen planus and oral lichenoid lesions: diagnostic and therapeutic considerations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103 Suppl:S25 e1-12.
11. van der Waal I. Oral lichen planus and oral lichenoid lesions; a critical appraisal with emphasis on the diagnostic aspects. Med Oral Patol Oral Cir Bucal. 2009;14:E310-14.
12. Chang JY, Chiang CP, Hsiao CK, Sun A. Significantly higher frequencies of presence of serum autoantibodies in Chinese patients with oral lichen planus. J Oral Pathol Med. 2009;38:48-54.
13. Alikhani M, Ghalaiani P, Askariyan E, Khunsaraki ZA, Tavangar A, Naderi A. Association between the clinical severity of oral lichen planus and anti-TPO level in thyroid patients. Braz Oral Res. 2017;31:e10.
14. Robledo-Sierra J, Landin-Wilhelmsen K, Filipsson Nystrom H, Eggertsen R, Larsson L, Dafar A, Warfvinge G, Mattsson U, et al. A mechanistic linkage between oral lichen planus and autoimmune thyroid disease. Oral Dis. 2018;24:1001-1011.

Characteristics and severity of tooth wear in 2 to 5-year-old kindergarten children in Medellin

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ABSTRACT

The aim of the present study was to identify the characteristics and severity of tooth wear in children aged 2 to 5 years attending a kindergarten in the city of Medellin.

The study population comprised 92 children aged 2 to 5 years attending a kindergarten in Medellin; the final sample included 86 children selected using a non-probabilistic sampling method. All assessments were performed using the index devised by Hansson and Nilner (1989).

Prevalence of tooth wear in the studied population was 100%. The observed results confirm previous findings showing that the severity of tooth wear is proportionally related with increase in age, and is mainly due to the physiological wear of teeth. No significant differences in tooth wear facets were observed among the different types of teeth (incisors, canines, molars). It is necessary to develop validated, precise, and comparable indices that allow establishing the etiology and orienting treatment of non-physiological tooth wear.

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Keywords: *tooth wear; dentition permanent; children.*

Facetas de desgaste y características de severidad en niños de 2 a 5 años de un jardín infantil, Medellín

RESUMEN

El objetivo del estudio fue identificar las facetas de desgaste y las características de severidad en niños de 2 a 5 años de un jardín infantil de la ciudad de Medellín.

El estudio incluyó 92 niños entre los 2 y 5 años de un jardín infantil de la ciudad de Medellín, con una muestra final de 86 seleccionados a partir de un muestreo no probabilístico. Se utilizó como instrumento el índice propuesto por Hansson y Nilner (1989).

El 100% (86) de los niños evaluados presentaron facetas de desgaste. Los resultados encontrados confirman datos previos en cuanto a que las severidades de las facetas de desgaste dentario están relacionadas proporcionalmente con el aumento de edad y se deben principalmente al desgaste fisiológico de los dientes. No se encontraron diferencias significativas en la prevalencia o severidad de desgaste entre los grupos anatómicos dentarios (incisivos, caninos y molares).

Es necesario establecer índices validados, precisos y comparables para evaluar la etiología y orientar hacia la solución de las causas no fisiológicas.

Palabras clave: *desgaste dentario; dientes permanentes; niños.*

INTRODUCTION

Among other factors, new eating habits, tooth brushing techniques, and occlusal overloading, have given rise to a new field of oral care within pediatric dentistry: non-carious lesions. In this regard, understanding the difference between normal, or physiological, and pathological patterns of tooth wear contributes to preventing greater damage to the tooth structure at an early stage and to identifying and solving the underlying causes. Hence the importance of early diagnosis and of determining damage severity, which is directly associated with the number of affected teeth and the involvement of the tooth structures (enamel, dentin, and pulp)[1].

Tooth enamel of deciduous teeth contains a lower proportion of mineral salts and is therefore more porous, which can lead to greater tooth wear. Tooth wear facets reveal the mechanical wear of the occlusal surfaces resulting from attrition forces, and are currently an emerging problem within the diseases involving the hard tissues of the teeth.[1-3]

Most studies on tooth wear reported in the literature were performed in adults, whereas tooth wear in deciduous teeth has been less analyzed[4].

In addition to the normal functions of the mouth, other pathological factors such as structural alterations of the teeth, malocclusions, and bruxism, can cause tooth wear of deciduous teeth. The association between tooth wear and emotional stress in children with behavioral disorders is well documented.[5]

Although it has been established that dentin exposure is the best indicator of tooth wear severity[6], it is important in clinical practice to assess other patient-specific factors in order to differentiate between physiological and pathological causes.

In view of the above, the aim of the present study was to identify the characteristics and severity of tooth wear in 2 to 5-year-old children attending a kindergarten in the city of Medellin in 2016.

MATERIALS AND METHODS

A descriptive observational cross-sectional study was conducted. The study population comprised 92 children whose parents agreed to participate in the study and who signed the corresponding informed consent form in keeping with the Declaration of Helsinki. The project was approved by the Research Ethics Committee (CEI) (Resolution N°15. October 26, 2015) of the Autonomous University Foundation of the Americas.

A structured questionnaire eliciting sociodemographic information was filled in by the children's parents, and served as the primary source of information.

The team included three researchers and two assistants, all of whom were trained and calibrated (inter-examiner Kappa index: 0.81)[7]. After tooth brushing, each child was clinically examined by one dentist and one assistant. Dental examination was performed under a direct source of light and using sterile cotton rolls to keep the area dry.

Children aged 2 to 5 years with complete deciduous dentition were included in the study. Teeth presenting caries and/or extensive restorations were not evaluated. Children with behavioral disorders or who refused to undergo oral examination were excluded.

Diagnostic assessment of tooth wear was performed using the following scale devised by Hanson and Nilner, including grade 0 (no wear), as referred by Rendon[5]

Grade 1: Enamel wear facets

Grade 2: Enamel wear facets and islands of dentin measuring up to 1mm in diameter.

Grade 3: Wear of the incisal surface; wear facets are pronounced and extend in a lingual or buccal direction.

Grade 4: Tooth wear facets involving the pulp chamber.

RESULTS

A total of 92 children were evaluated, six of whom were excluded due to multiple active caries (n=3) and behavioral disorders (n=3). Hence, the final study sample comprised 86 children who complied with the inclusion criteria.

Sixty percent of the children were aged 2 to 3 years, and the remaining 40% were aged 4 to 5 years; 56.5% were boys.

The data regarding the sociodemographic factors showed that the majority of children were under the care of women who had finished high school and were home makers. Most families lived in apartments.

One hundred percent of the studied children had tooth wear. Most of the studied children (78%) had moderate, grade 2, tooth wear (Figs. 1 and 2). Grade 3 wear facets were observed in a smaller proportion of children (22%), and were found to increase with age (Table 1). No differences were observed between sexes.

Analysis of the degree of tooth wear according to type of tooth showed the presence of grade 2 wear in 86% of incisors, 88% of canines, and 81% of molars. Thus, the incidence of tooth wear was similar in all anatomical groups of teeth (Table 2).

In the present study, tooth wear grade 0 and 1 were grouped in the same category since the aim was to focus on observable lesions. Because molars had not erupted, or fully erupted, in some of the 2 year-olds, 72 teeth that had not reached occlusion were not included in the final count of the number of studied teeth (Table 2).

DISCUSSION

The present study shows the high prevalence of tooth wear in deciduous teeth in the studied population. Given that the sample included children recruited from a single school, largely homogenous results were expected. Although the causes of tooth wear were not explored in this study, which sought to determine the prevalence and severity of the

Fig. 1: Grade 2 wear facets involving the enamel of the first molar and upper incisors.

Fig. 2: Enamel wear facets and islands of dentin on the upper incisors.

Table 1: Maximum severity of tooth wear in each age group.

Age	Number of studied children	Children showing tooth wear n (%)	
		Grade 2	Grade 3
2-3	53	48 (90.6%)	5 (9.4%)
4-5	33	19 (57.6%)	14 (42.4%)

Table 2: Severity of tooth wear according to age and type of tooth (Number of teeth).

Severity Grade	2 year -olds	3 year -olds	4 year -olds	5 year -olds
	Incisors			
Grade 0-1	13	9	13	2
Grade 2	130	269	191	5
Grade 3	1	2	36	17
	Canines			
Grade 0-1	5	1	2	0
Grade 2	66	131	104	2
Grade 3	1	8	14	10
	Molars			
Grade 0-1	0	19	71	7
Grade 2	71	259	169	0
Grade 3	1	2	0	17

lesions, the homogeneity of the results would indicate that the observed tooth wear was mainly due to physiological processes of mastication, and it is therefore logical that severity should increase as the age of the child increases.

It has been shown that the structure of deciduous teeth is less resistant than that of permanent teeth [8]. This would account for the high prevalence of tooth wear and the similar frequency of tooth wear facets and severity in the different types of teeth and at an early age.

In addition to mastication forces, other factors such as the erosive capacity of certain foods and occlusion disorders can contribute to the development of tooth wear [9,10]. These factors often cause lesions that involve a certain tooth or group of teeth more than other teeth, or that show a greater severity. Recognizing this type of lesion could contribute to assessing increased pulp risk as well as detecting inappropriate eating habits and abnormal occlusion, which in turn may be caused by neurological or psychological disorders that require a multidisciplinary approach [11- 15]. In order to discriminate expected lesions resulting from physiological processes from those caused by other etiopathogenic factors, it is necessary to have objective indicators. The index used in the present study is a semiquantitative tool that involves simple inter-examiner calibration. Nevertheless, it is necessary to reach consensus to establish a validated index with precise quantitative parameters that allows establishing adequate comparisons among studies conducted in different populations.

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REFERENCES

1. Cava, C, Malatto, J, Olivares C, Rodríguez, L, et al. Prevalencia de facetas de desgaste. Kiru 2012; 9:59-64. URL: http://www.usmp.edu.pe/odonto/servicio/2012/Kiruv.9/Kiru_v.9_Art9.pdf
2. Vallejo, E, González, E, Del Castillo, R. El bruxismo infantil. Odontología Pediátrica 2002; 10:135-141. URL:www.odontologiapediatrica.com/wp-content/uploads/2018/ 05/39_9-VALLEJO.pdf
3. Rodríguez, B, Tamayo, A, Tobón, A. Análisis de factores asociados al desgaste dental en niños de cinco años. CES Odontología SD, 1987; 1: 7-13. URL:http://revistas.ces.edu.co/index.php/odontologia/ article/ viewFile/1806/1197
4. Álvarez, E, Arango, M, Arbeláez, D, González, C, et al. Diseño y aplicación de un instrumento para la medición del desgaste dental en pre-escolares de 3 a 5 años del Municipio de Sabaneta. CES Odontología 1998; 11:20-23. URL:http://revistas.ces.edu.co/index.php/odontologia/article/view/839/534
5. Rendón, A. Facetas de desgaste dentario y personalidad: revisión bibliográfica. Kiru 2015; 12: 88-90. URL:http://www.aulavirtualusmp.pe/ojs/index.php/Rev-Kiru0/article/viewFile/466/381
6. Kreulen, CM, Van 't Spijker A, Rodriguez JM, Bronkhorst, EM,et al. Systematic Review of the Prevalence of Tooth Wear in Children and Adolescents. Caries Res 2010; 44:151-159.
7. Cerda JL, Villarroel Del LP. Evaluación de la concordancia inter-observador en investigación pediátrica: Coeficiente de Kappa.URL: https://scielo.conicyt.cl/pdf/rcp/v79n1/art08.pdf
8. Carvalho T, Lussi A, Jaeggi T, Gambon D. Erosive Tooth Wear in Children. Monographs in oral science 2014; 25:262-278. URL:http://www.ncbi.nlm.nih.gov/pubmed/24993274
9. Corica A, Caprioglio, A. Meta-analysis of the prevalence of tooth wear in primary dentition. Eur J Paediatr Dent 2014; 15: 385-388.
10. Pires de Andrade, F, Sales, A, Garcia, P, Abel, Marta, et al. Nutritional status, tooth wear and quality of life in Brazilian schoolchildren. Public Health Nutr 2016; 19:1479-1485.
11. Torlinska, N, Maćkowiak, K, Sowinska, A. Evaluation of deciduous-tooth wear in children living in the middle ages. Oral Health Prev Dent 2015; 13:149-156.
12. Johansson, A, Omar, R, Carlsson, G, Johansson, A. Dental erosion and its growing importance in clinical practice: from past to present. Int J Dent. 2012; 2012:632907. doi: 10.1155/2012/632907.
13. López, F, Castellanos,L, Martín, J, Llamas, J,et al. Clinical measurement of tooth wear: Tooth wear indices. J Clin Exp Dent 2012; 4:e48-53.
14. Ganss, C, Young, A, Lussi, A. Tooth wear and erosion: methodological issues in epidemiological and public health research and the future research agenda. Community Dent Health 2011; 28:191-195.
15. Salas MMS, Nascimento GG, Huysmans MC, Demarco FF. Estimated prevalence of erosive tooth wear in permanent teeth of children and adolescents: An epidemiological systematic review and meta-regression analysis. J Dent 2015; 43:42-50.

Instant and freshness effect of mouth rinses on type 1 (oral) halitosis

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ABSTRACT

Hygiene deficiency causes type 1 (oral) halitosis. There are short and long-term studies on the anti-halitosis effect of mouth rinses but less knowledge on their instant effects. The aim of this study was to compare instant and freshness effects of 8 mouth rinses on type 1 halitosis.

Ninety self-reported halitosis patients (19-58 y.o., median 31) were randomly divided into 9 groups. Cysteine (20 mM) challenge test was applied to obtain maximum halitosis level in the mouth of each patient. Single use of 8 different mouth rinses (R1-R8) and tap water (R0) were tested on each group (n=10). Afterward, patients were requested to score oral freshness effect of the mouth rinse on a 5-point scale (0, bad; 5, fresh). Minimum halitosis level was obtained by rinsing with 20 mMol $ZnCL_2$. In each step, oral gas (organic, NH_3, SO_2, H_2S, H_2) concentrations were quantified by using a portable multi-gas detector (MX6, IndSci, US). The ANOVA or Kruskal Wallis tests were used to compare the quantitative measurements.

R3 (Halitosil Zn) mouth rinse was found to be have the highest instant anti-halitosis effect while the R2 (Colgate plax) had the lowest. The sensation of freshness was highest in R7 (Oxyfresh power mouth rinse lemon-mint) and lowest in R8 (Signal expert protection). The freshness effect was not associated with the anti-halitosis effect (r= 0.185, p=0.608).

Mouth rinses containing $ZnCl_2$ without alcohol are instantly effective on halitosis. Mouth rinses containing ethyl and other alcohols (including glycol, sorbitol, menthol, eucalyptol, thymol, xylitol and eugenol) were found to be less effective on halitosis.

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***Keywords**: halitosis; mouthwashes; breath tests; hydrogen sulfide; ammonia.*

Efecto anti-halitosis instantáneo y efecto de frescura de los enjuagues bucales sobre la halitosis (oral) tipo I

RESUMEN

La deficiencia de higiene causa halitosis tipo 1 (oral). Se han reportado efectos anti-halitosis a corto o largo plazo de los enjuagatorios bucales, pero se desconocen sus efectos instantáneos. El objetivo de este estudio fue comparar el efecto instantáneo y de frescura de 8 enjuagues bucales en la halitosis tipo 1.

Noventa pacientes (19-58 años, mediana 31) que reportaron sufrir halitosis se dividieron aleatoriamente en 9 grupos. Se aplicó la prueba de provocación con cisteína (20 mM) para obtener el máximo nivel de halitosis en la boca de cada paciente. El uso individual de 8 enjuagues bucales diferentes (R1-R8) y agua del grifo (R0) se probó en cada grupo (n = 10). Posteriormente, se pidió a los pacientes que puntuaran el efecto de la frescura oral del enjuague bucal en una escala de 5 puntos (0, malo; 5, fresco). El nivel mínimo de halitosis se obtuvo con 20 mMol de $ZnCL_2$ enjuague. En cada paso, se cuantificaron las concentraciones de gases orales (orgánicos, NH_3, SO_2, H_2S, H_2) mediante el uso de un detector portátil de múltiples gases (MX6, IndSci, EE. UU.)Se encontró que el enjuague bucal R3 (Halitosil Zn) tiene un mayor efecto anti-halitosis instantáneo, mientras que el R2 (Colgate plax) fue el más bajo. El sentido de frescura fue mayor en el enjuague bucal R7 (enjuague bucal Oxyfresh power lemon-mint) mientras que fue bajo en R8 (protección experta de Signal). El efecto de frescura no se asoció con el efecto anti-halitosis (r = 0.185, p=0.608).

Los enjuagues bucales que contienen ZnCl2 sin alcohol son instantáneamente efectivos en la halitosis. Se encontró que los enjuagues bucales que contenían etil y otros alcoholes (incluidos glicol, sorbitol, mentol, eucaliptol, timol, xilitol y eugenol) son menos efectivos para el control de la halitosis.

***Palabras clave:** halitosis; enjuagues bucales; pruebas de aliento; sulfuro de hidrógeno; amoníaco.*

INTRODUCTION

Halitosis is a chronic endogenous malodor, etiologically classified as follows: physiological (type 0), oral (type 1), airway (type 2), gastroesophageal (type 3), bloodborne (type 4) and subjective (type 5).[1]

The dorso-posterior area of the tongue is the most important halitogenic site due to the presence of densely populated biofilm containing anaerobes [2] . The causes of type 1 halitosis are usually poor oral hygiene, plaque stagnation areas, gingivitis and tongue coating.[1-3]

Type 1 halitosis is mostly composed of volatile sulfur compounds (VSCs) but also contains other volatile aromatic compounds including amines (indole, skatole, pyridine, picoline, urea, ammonia, methylated amines, putrescine, cadaverine), short/medium-chain fatty acids or organic acids (propionic, butyric, acetic, valeric acids), alcohols (methanol, ethanol, propanol), volatile aliphatic compounds (cyclopropane, cyclobutane, pentane), aldehydes and ketones (acetaldehyde, acetone, benzophenone, acetophenone).[3] Presence of hydrogen sulfide (H_2S) in the mouth has been accepted as a representative criterium of halitosis. [4] VSCs have been used to compare anti-halitosis effect of mouth rinses tested.[5,6] Other oral gases were assumed to be absent from the mouth cavity of halitosis patients.[7]

Traditional dental or periodontal treatments supported by mouth rinses provide only temporary relief in most patients.[8] Commercially available products, such as mints, toothpaste, mouth rinses, sprays and chewing gums, attempt to mask oral malodor with pleasant flavors and fragrances. [9] According to the literature, good results were reported with chlorhexidine (CHX), while triclosan seems less effective. Essential oils and cetylpyridinium chloride (CPC) are only effective up to 2 -3 hours, and antimicrobial ingredients are only temporarily effective in reducing microorganisms.[9]

In a systematic review of the anti-halitosis effect of mouth rinses; most of the products were found to have beneficial effects in reducing oral malodor. Adequate evidence was provided by both short-term (less than 3 weeks) and long-term studies on the effect of CHX-, CPC- and zinc-containing mouth rinses on oral malodor.[10] The medium-term (less than 2 weeks) efficacy of mouth rinses was also investigated, and CHX, CPC, zinc salts and $ZnCl_2$ were found to be the most effective against oral halitosis.[11]

Patients with halitosis need to use a fast-acting mouth rinse before social approach. Anti-halitosis mouth rinses are usually expected to provide instant effect. There is currently little available knowledge on which are the most fast-acting, instantly effective mouth rinses.

The aim of this study was to quantify instant the anti-halitosis and freshness effects of 8 mouth rinses by comparing different gases using a multi-gas detector.

MATERIALS AND METHODS

Study design and population

This was a single-center, randomized, double-blind, parallel-group clinical trial with a single use of mouth rinses. The study enrolled 90 patients, of whom 52 were female (19-58 y.o., median 31) who self-reported oral malodor. More than 0.7 ppm H_2S in their mouth was confirmed by halitometry. Patients with possible extraoral causes of oral malodor such as upper respiratory infections, as well as those with taste and smell disorders, sinonasal disorders (nasal polyps, chronic rhinosinusitis, allergic rhinitis, septum deviation), any history of asthma, malignancy, head trauma, neurologic and psychiatric disorders (schizophrenia, obsessive-compulsive disorder, social anxiety disorder), metabolic and endocrine disorders (diabetes mellitus, hypogonadism, liver or kidney disease) were excluded. Pregnant or lactating women, patients taking antimicrobials, and smoking or drinking individuals were also excluded.

All subjects who took part in the experiment signed written informed consent, after having received an explanation of the protocol approved by the Ethics Committee of Cumhuriyet University (2016-05-02). This study was conducted in full accordance with the World Medical Association Declaration of Helsinki.

Patients were asked to avoid odorous foods (such as onion, garlic) in their diet for 48 h before their appointment, and to refrain from alcohol intake and smoking 12 h prior to the halitosis examination. Gas measurements were taken blindly between 09:00 and 11:30 a.m.

Baseline gas measurement

Patients were randomly divided into 9 groups. Each participant's baseline volatile organic compound (VOC), NH_3, SO_2, H_2S and H_2 levels in oral air were

measured using a portable multi-gas detector (MX6, IndSci, US) following a previously described procedure.[12] These initial values of the 5 gases were used as individualized control data.

Cysteine challenge test

The cysteine challenge test[13] was carried out as follows: 5 ml of 20 mM (2.43 g/L) aqueous *L*-Cysteine solution (#1.02839.0100, Merck) was placed in the mouth and held in contact with the dorsal part of the tongue for 30 s to generate H_2S in the mouth to challenge oral halitosis.

After 3 min, emerging H_2S in the oral cavity was measured and recorded as maximum halitosis level for each halitosis patient.

After this stage, each group (n=10) gargled for 30s with 10 ml of one of the mouth rinses listed in Table 1.

Oral gas concentrations were read blindly and recorded. Immediately after rinsing, participants were requested to score the oral freshness effect (FE) of the mouth rinse by answering the question "How fresh does your mouth feel?" on a 5-point scale from 0 (bad taste) to 5 (extremely fresh). Answers were recorded.

Finally, to remove the odor of cysteine from the mouth, subjects gargled with 10 ml of 20 mMol $ZnCL_2$ solution (Tekkim, TK800000.01000, Tr) for 30 s, after which oral gases were re-read. This value represented the minimum halitosis level for each halitosis patient.

Statistical analysis

MedCalc v.18.5 software (MedCalc, Belgium) was used for all statistical analyses, and the data were reported as mean ± standard deviation (SD). The *Kolmogorov-Smirnov* test was used to show the normal distribution of quantitative measurements, and the ANOVA or *Kruskal Wallis* tests were used for the comparison of the quantitative measurements between the groups of more than two. A post-hoc test (using *Scheffe* or *Conover*) was performed for pairwise comparison of subgroups when the ANOVA test was positive (p less than the selected significance level). Pearson correlation coefficient was used to analyze the degree of association between two variables. A log transformation was used for the variables that were not normally distributed. The probability of making a Type I error (alpha, significance) is 0.05 in all tests.

Table 1: Mouth rinses used in this study and their content (in alphabetical order).

Number	Mouth rinse	Ingredients
R0	Tap water	Water
R1	Cb12	Water, glycerin, hydrogenated starch hydrolysate, alcohol, zinc acetate dihydrate. Chlorhexidine, diacetate, sodium fluoride, peg-40, hydrogenated castor oil, potassium acesulfame, citric acid, aroma.
R2	Colgate Plax	Water, glycerin, alcohol, propylene glycol, sorbitol, polysorbate 20, sodium benzoate, aroma, menthol, cetylpyridinium chloride, Sodium fluoride (0.025%), experimental sodium saccharin, cl4290.
R3	Halitosil Zn	Zinc chloride, sodium chloride, boric acid, deionized water, glycerin.
R4	Listerine total care zero	Water, sorbitol, propylene glycol, poloxamer 407, sodium lauryl sulfate, aroma, eucalyptol, zinc chloride, benzoic acid, sodium benzoate, methyl salicylate, thymol, sodium saccharin, sodium fluoride, menthol, sucralose, cl16035, cl42090, sodium fluoride (220ppm).
R5	Oderol	Chlorhexidine digluconate (0.025%), zinc lactate, mentha piperita, sucralose, deionized water.
R6	OralB proexpert	Water, glycerine, aroma, cetylpyridinium chloride, poloxamer 407, methylparaben, sodium saccharin, cinnamal, propylparaben, eugenol, cl4290.
R7	Oxyfresh power mouthrinse lemon-mint	Water xylitol, peg-40 hydrogenated castor oil, sodium chloride, Zinc acetate, Natural flavor, Citrus citrus medica limonum (lemon) fruit oil, Citrus grandis (grapefruit), Peel oil, Citrus aurantifolia (Lime) oil, Mentha viridis (spearmint) leaf oil, aloe barbadensis leaf juice sucralose, sodium citrate, citric acid, sodium hydroxide.
R8	Signal expert protection	Water, sorbitol, Peg-40, hydrogenated castor oil, potassium citrate, glycine, sodium benzoate, aroma (flavor), citric acid, zinc sulfate, sodium saccharin, sodium fluoride, propylene glycol, citrus medica limonum juice, Aloe barbadensis leaf extract, limonene, cl 42051, cl 47005.

RESULTS

Initial concentrations of the five gases (VOC, NH_3, SO_2, H_2S, H_2) for each mouth rinse group (n=10) are provided in the left column of Table 2. No significant differences were noted within the baseline readings of the patients. The decrease in baseline concentrations of 5 gases after 1) cysteine rinse, 2) mouth rinse tested and 3) $ZnCl_2$ solution are also shown in Table 2. Table 3 shows the average values of each gas, calculated from the data provided in Table 2.

After the cysteine rinse, H_2S gas increased in the mouth while all other gases decreased. Then,

Table 2: Initial concentrations of gases and their average decrease according to the each step of the study.

Mouth rinse	Gases	Initial concentration (ppm±SD)	Decrease (ppm±SD)		
			Cysteine	Mouth rinse	$ZnCl_2$
R0	VOC	1.27 ± 1,22	0.42 ± 0.40	0.28 ± 0.42	0.28 ± 0.46
	NH_3	5.70 ± 2.66	2.10 ± 1.37	2.8 ± 1.47	0.7 ± 0.48
	SO_2	0-00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00
	H_2S	1.28 ± 0.56	-10.17 ± 4.12	3.4 ± 2.36	7.22 ± 3.29
	H_2	11.20 ± 11.77	6.90 ± 6.22	3.3 ± 5.37	0.60 ± 1.42
R1	VOC	1.43 ± 0.44	0.24 ± 0.26	-36.53 ± 7.55	36.15 ± 7.79
	NH_3	3.7 ± 2.26	1.60 ± 1.57	-8.04 ± 4.1	8.54 ± 3.71
	SO_2	0.00 ± 0.00	0.00 ± 0.00	-0.06 ± 0.13	0.06 ± 0.13
	H_2S	1.29 ± 0.58	-10.02 ± 5.27	0.96 ± 2.16	9.54 ± 5.79
	H^2	16.8 ± 10.12	5.50 ± 5.16	-215.5 ± 94.34	215.4 ± 93.94
R2	VOC	1.52 ± 1.23	0.20 ± 0.35	-53.91 ± 19.08	53.22 ± 19.94
	NH_3	1.80 ± 0.91	0.20 ± 0.78	-1.40 ± 0.96	1.90 ± 0.99
	SO_2	0.00 ± 0.00	0.00 ± 0.00	-0.07 ± 0.11	0.07 ± 0.11
	H_2S	1.54 ± 0.87	-15.11 ± 10.25	1.03 ± 2.05	14.07 ± 11.03
	H_2	12.2 ± 10.62	2.00 ± 2.70	-218.2 ± 95.68	219.1 ± 93.72
R3	VOC	1.75 ± 0.96	0.38 ± 0.40	0.83 ± 0.56	0.4 ± 0.37
	NH_3	3.90 ± 2.21	1.63 ± 1.43	1.54 ± 1.43	0.45 ± 0.68
	SO_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	H_2S	1.42 ± 0.96	-17.10 ± 14.67	17.54 ± 15.25	0.32 ± 0.27
	H_2	15.00 ± 6.76	5.00 ± 3.31	6.63 ± 5.44	2.72 ± 2.61
R4	VOC	1.82 ± 1.11	0.40 ± 0.39	-25.03 ± 17.70	25.18 ± 18.03
	NH_3	3.20 ± 2.60	1.15 ± 1.28	-0.53 ± 0.77	0.38 ± 3.66
	SO_2	0.00 ± 0.00	0.00 ± 0.00	-0.046± 0.11	0.04 ± 0.11
	H_2S	1.52 ± 0.94	-13.88 ± 9.30	3.43 ± 5.20	10.9 ± 9.35
	H_2	6.76 ± 4.95	0.61 ± 3.68	2.84 ± 2.91	1.3 ± 1.31
R5	VOC	1.87 ± 0.61	0.40 ± 0.28	0.24 ± 0.59	0.79± 0.54
	NH_3	4.5 ± 3.54	0.78 ± 0.57	-2.35 ± 1.7	5.0± 3.8
	SO_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	H_2S	1.91± 1.36	-18.37 ± 16.42	10.93 ± 9.79	8.69± 8.15
	H_2	12.71± 10.26	5.07± 3.58	3.71± 5.36	3.42± 4.23
R6	VOC	1.76± 1.00	0.38± 0.56	-2.18± 1.45	2.43± 1.25
	NH_3	2.60±1.95	0.80± 0.63	-1.50± 0.97	2.60± 1.82
	SO_2	0.00± 0.00	0.00 ± 0.00	-0.21± 0.22	0.21± 0.22
	H_2S	1.62± 1.07	-14.94± 12.97	4.42± 4.45	10.98± 9.61
	H_2	12.40± 7.02	6.00± 4.49	-50.9±18.09	52.5±16.56
R7	VOC	2.43 ±1.01	0.40 ± 0.21	-3.75 ± 3.09	4.14± 3.14
	NH_3	2.60 ±1.80	0.72 ± 0.90	-2.90 ± 2.11	3.45± 2.06
	SO_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.000± 0.00
	H_2S	1.62± 1.03	-13.66± 9.15	11.87± 7.39	2.01± 3.26
	H_2	17.54± 9.16	4.45± 3.72	7.09± 4.90	2.27± 4.49
R8	VOC	2.16± 1.24	0.61± 0.45	-27.9± 16.19	27.69± 16.11
	NH_3	3.10± 3.48	1.71± 2.43	-6.07± 4.42	5.21± 5.38
	SO_2	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00
	H_2S	1.32± 0.97	-10.97± 7.61	0.25± 3.53	11.18± 8.75
	H_2	17.42± 29.93	9.21 ± 17.44	3.85 ± 7.72	0 ± 3.13

Negative value means increase

depending on the chemical content of mouth rinse tested, gas concentrations changed. In the final step, after the $ZnCl_2$ solution, all gases decreased sharply, as shown in Table 3.

The average ratios of the inhibitory effect of each mouth rinse on all gases were calculated from Table 2 and sorted as follows (from highest to lowest): 57.88 % (R3), 42.12 % (R0), 11.13 % (R5), -41.39 % (R7), -201.98 % (R6), -344.09 % (R4), -357.93 % (R8), -1070.25 % (R1), -1245.17 % (R2). Negative values represent increase in gas concentrations in the mouth immediately after mouth rinse. R3 was the most effective and R2 the least effective mouth rinse on oral gases, while the others were found to have an intermediate effect.

The freshness effects of the mouth rinses were sorted as follows: R7> R5> R3> R4> R1> R0> R6>R8 (Fig 1). Freshness effect was not correlated with anti-halitosis effect (p=0.608, r= 0.185).

DISCUSSION

This study investigated instant anti-halitosis effects of 8 mouth rinses by comparing different gases using a multi-gas detector in patients with type 1 halitosis. Mouth rinse R3 was the most effective and R2 the least effective against oral gases.

Self-assessment is indeed the primary diagnostic criterium for halitosis because it is the reason why patients request halitosis examination. Self-assessment and/or feedback from other people in the subject's social environment have a prominent place among all diagnostic tests.[14-16] The presence of halitosis in participants was confirmed by halitometry. No self-assessed participant had less than 0.7 ppm H_2S in the oral cavity.

Sulfide detectors are generally used to measure halitosis. The Halimeter (Interscan, Chatsworth, CA) can only detect H_2S gas. Oral Chroma (Abimedical Corporation, Osaka, Jp) is sensitive to only three sulfurous gases. Nevertheless, because there are more than 700[17] or 3481[18] gases in the human breath, most of the gases would have been overlooked if such a sulfide detector had been used in this experiment.

The MX6 multi-gas detector has been used for halitosis examination in previous clinical studies.[7,12] According to the MX6 user manual[19], the hydrogen and photoionization sensors are sufficiently sensitive to more than 72 volatile compounds and 116 organic gases, including major contributors to type 1 halitosis (VSCs, alcohols, aldehydes, ketones, ammonia). A strength of this study was that it measured a wide range of gases, enabling us to compare not only 3 sulfurous gases but also non-sulfurous gases which can be present in the mouth of halitosis patients. On the other hand, there were some limitations, such as the facts that MX6 cannot

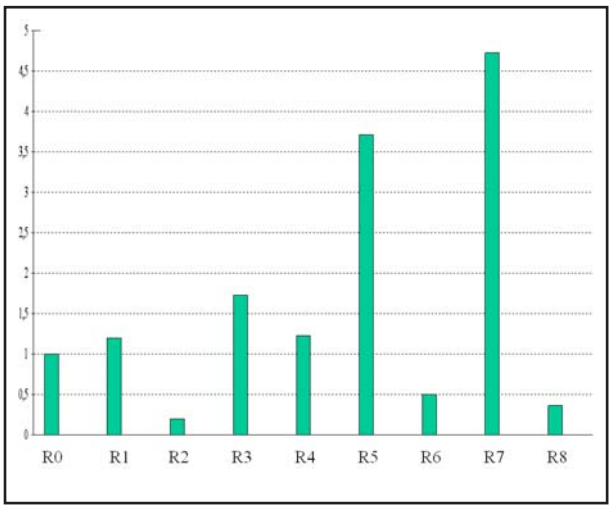

Fig. 1: Freshness effect of the mouth rinses scored by contributors (0 bad taste, 5 extremely fresh). R0-R8 are the mouth rinses listed in the Table 1.

Table 3: Average values of each gas at baseline, after cysteine, mouth rinse and $ZnCl_2$, respectively.

	Initial (ppm±SD)	Gas concentrations (ppm±SD) after:		
		Cystein	Mouth rinse	$ZnCl_2$
VOC	1.80 ±1.03	1.41 ± 0.87	17.60 ± 21.10 *	1.13 ±1.06*
NH_3	3.48 ± 2.70	2.29 ±1.90	4.44 ± 4.00	1.20 ± 2.10
SO_2	0.00 ±0.00	0.00 ± 0.00	0.03 ± 0.11	0.00 ± 0.00
H_2S	1.51 ± 0.96	15.4 ± 11.7	9.32 ± 8.6 *	0.98 ± 0.57
H_2	13.53 ±13.80	8.58 ± 8.30	52.3 ± 96.3*	3.89 ± 5.92

* statistically significant

distinguish which particular organic gas is present in a gas sample, and that some unpredictable cross-reactions can occur between gas sensors.

However, the effectiveness of oral healthcare products depends on active ingredients and their concentrations.[9] In the current study, the dose-dependent effects of ingredients could not be investigated because, due to business considerations, mouth rinse manufacturers are unwilling to disclose information regarding the ratio of active ingredients.

Some clinical parameters, such as oral hygiene, periodontal health, carious lesions, etc., were not considered because the aim of the study was not to detect causes of halitosis. Patients with periodontitis were allowed to enroll in the study because they are the most representative halitosis patients. The study does not cover the long-term effect of mouth rinses, which may differ from their instant effect.

Minimum halitosis level

Zinc at concentrations of 1% seems to be a safe, effective metal for inhibiting halitosis.[9] The zinc ion inhibition on VSC production has been largely attributed to its affinity for sulfur, although a restricted antimicrobial effect of zinc on plaque bacteria has been reported.[20]

$ZnCl_2$ is the most effective zinc salt against oral malodor due to its water-soluble structure. Zinc citrate has been found to be less effective because it is difficult to ionize, while other zinc salts show intermediate levels of effectiveness on oral halitosis.[21] $ZnCl_2$ has already been used to achieve a minimum halitosis level in the mouth.[7,21,22] Unexpectedly, $ZnCl_2$ not only reduced H_2S but also reduced other gases. After $ZnCl_2$ rinsing, the VOC, NH_3, SO_2, H_2S and H_2 decreased 93.65%, 72.45%, 0%, 89.27%, 93.06%, respectively (data calculated from Table 2).

Maximum halitosis level (cysteine challenge test)

To challenge halitosis, using cysteine rinse in healthy individuals is often used and widely accepted way of designing such studies. [4,5,21,23,24] can be useful to distinguish type 1 halitosis from other types of halitosis.

Degradation of cysteine by oral bacteria is the biochemical process in the formation of artificial oral malodor. The magnitude of the H_2S (peak) response provides an idea of the tendency of the oral ecology to produce halitosis.[21] The H_2S peak value depends on the proteolytic capacity of the microbiota of an individual's mouth, independently of age and sex and momentary alterations of type 1 halitosis level. [7,12,21,22]

Usually, 8.23 mM L-cysteine rinse for 1 min, or 10 mM,[23] 6mM (pH 7.2)[5] or 6 mM (pH 7.1) for 20 s, [24] or 0.06 mM (pH 7.2) for 30 s have been used in the cysteine challenge test.

In this study, oral H_2S increased sharply after cysteine (20 mM) rinse, and oral bacteria produced H_2S by using cysteine, rather than carbohydrates, as an energy source. Other oral gases (H_2 or VOC) decreased, since carbohydrates were not used by bacteria during cysteine challenge test (Table 3).

Previous black and white approach to diagnosis of oral halitosis by one-time measurement with a halitometer may be an oversimplification.[1] It is assumed that halitosis fluctuates every 2 minutes throughout the day.[25] Single instant gas readings, or comparison of the reading to a predefined mathematical cut-off value, can lead to misdiagnosing halitosis. It might be more accurate to consider a scale between maximum and minimum levels for each individual. Oral gas readings can be compared with reference to these two points.[1,12] For this reason, this study determined maximum and minimum halitosis levels by using cysteine and $ZnCl_2$ solution, respectively. This procedure, which was used to diagnose halitosis in this study, can be called "scalar approach".

Effect of water on halitosis

Water rinse has been found not to reduce halitosis.[13] However, some evidence on this matter is conflicting, since rinsing with 15 ml of water for 30 s has been found to reduce oral H_2S by 30% - 50%.[26] In the present study, oral gas concentrations of VOC, NH_3, SO_2, H_2S and H_2 decreased 32.94, 77.70, 0.00, 29.69 and 23.25%, respectively, by rinsing with tap water (R0) (data calculated from Table 2). The anti-halitosis effect of water rinse probably simply consisted of a dilution effect on oral gases, which returned to initial values immediately after the water rinse. NH_3 was the most affected gas to be suppressed by water rinse (data not shown).

Mouth rinses

Good short-term results on halitosis were reported with CHX. However, CHX-containing mouth rinses

are not suitable for long periods of use because they can destabilize oral microbiota or cause tooth staining, calculus formation, transient taste disturbance and harmful effects on the oral mucosa.[27-29] Rinsing with 0.2% alcohol-free CHX for 1 week caused irritation of oral mucosa and burning sensation, and disturbed taste perception compared to the placebo rinse.[29] In this mouth rinse series, R1, R2, R5 and R6 cannot be used for more than one week due to the possible adverse effect of their antiseptic content.

The present study found that CB12 mouth rinse reduced oral H_2S but increased other gases in the oral cavity. This was confirmed by an additional test; unfavorably halitosis treatment , this mouth rinse was found to spontaneously release very high concentration of VOC (52.96±8.20 ppm, n=10), NH_3 (3.6±1.1 ppm, n=10) and H2 (2057.2±154.7 ppm) in the headspace of its bottle at room temperature (data not shown).

Triclosan, and metal ions such as stannous and zinc, appear to be effective in controlling oral malodor. The effects of Triclosan (34.5 mmol/L) and Zinc Citrate (39.8 mmol/L), alone or in combination, have been tested in vitro for oral bacteria grown. Inhibitory effects were observed on oral bacteria except for *S. gordonii* and *S. oralis*.[30] However, the ability to kill bacteria may not truly reflect the success of the anti-halitosis effect. Antimicrobial mouth rinses merely suppress bacterial activity in the mouth for a limited time.[9]

Essential oils and CPC were found to be more effective than triclosan up to 2 or 3 h. Metal ions and oxidizing agents, such as hydrogen peroxide and chlorine dioxide, are active in neutralizing VSCs. Thymol, Eucalyptol, Menthol, CPC, ClO_2 + Zn-acetate containing mouthwashes reduce oral malodor within 4 h of a single usage. CPC was found to be the most effective ingredient.[31] In this study, R2 contained CPC, but was found to be the least effective, while another CPC containing R6 was found to have an intermediate level of effect on oral gases.

Silwood CJ et al,[6] tested six products on 6 volunteers according to 7 treatment regimens. Twenty ml of 5 of products were examined. VSC concentrations were recorded at 30 minutes intervals after administration. All products reduced VSC within 20 minutes of treatment. VSC concentrations returned to their baseline values within 5 h. The most effective oral health care products were found to be chlorite anion and chlorine dioxide. In accordance with the literature, the present study found that R3 and R7, which contain chlorite anion (Sodium chlorite), were both reasonably effective on oral gases.

Mouth rinses containing a combination of sodium chlorite and $ZnCl_2$ have been found to be more effective than those containing $ZnCl_2$ alone.[20] In the present study, R3 contained this combination that was found to be the most effective rinse.

Alcohol(s)

The term "alcohol" traditionally refers to ethanol (ethyl alcohol), although glycol, sorbitol, menthol, eucalyptol, thymol, xylitol and eugenol are also alcohols which may present in mouth rinses. All alcohols are mild skin irritants.[32] It has been reported that a high content of alcohol, an acidic pH and other ingredients constitute potential irritants individually or synergistically with the other components in the mouth rinse.[33]

The widespread use of alcohol-containing mouth rinses has also resulted in a number of reports of mouth rinse-associated adverse effects, including potential association with oral cancer.[34,35] There are controversial reports showing no causal relationship between the use of alcohol-containing mouth rinses and the development of oral cancer.[36] However, a hydroalcoholic vehicle containing 21.6 to 26.9 percent of alcohol in a mouth rinse meets the requirements of the FDA policy regarding fixed combinations of over-the-counter active ingredients with antigingivitis/antiplaque action.

Alcohol is not required in anti-halitosis mouth rinses since it exacerbates halitosis by drying the oral mucosa.[2,37-40]. Most manufacturers do not need to disclose alcohol content of rinses as ingredient but Listerine, Plax, Scope, Signal, ACT, Viadent and CB12 contain 26.9%, 7.5%, 18.9%, 14.5%, 6%, 10% and 1.7% of alcohol, respectively.[39] In the current study, R1 and R2 were clearly labeled as containing alcohol. In agreement with the literature, R1 and R2 were found to be the least effective on oral gases, possibly due to their alcohol content. There is not enough documentation to distinguish which alcohol contributes to oral malodor. Nevertheless, alcohols should not be preferred in the content of an anti-halitosis mouth rinse.

In 1978, Bernstein ML reported two cases of oral mucosa white lesions associated with Listerine

mouth rinse, and subsequently reported the experimental induction of hyperkeratotic white lesions in hamster cheek pouches with the same mouth rinse.[33] The present study found that Listerine Total (R4) was not an effective mouth rinse on type 1 halitosis.

Instead of ethanol, R4, R6, R7 and R8 contained other alcohols such as sorbitol, glycol, thymol, eucalyptol, menthol and xylitol. These were found to have intermediate effectiveness on oral gases. All alcohol-containing mouth rinses (R1, R2, R4, R6, R8) released high concentrations of H_2 and VOC to the headspace of their bottle (data not shown). The reason for some gases increasing in the oral cavity immediately after rinsing may be the alcohol content of the mouth rinse tested.

On the other hand, alcohol-free mouth rinses (R3, R5) were found to be the most effective against oral gases. H_2 gas in the breath can be recognized by the MX6 and is also known as an indicator for inflammation. [12] It may be a good idea to monitor oral inflammation by detecting oral H_2 gas in the mouth cavity. However, SO_2 did not significantly alter within the study. Even though SO_2 is sulfurous, it might not be a halitosis gas. Further studies on oral halitosis gases are needed in the future.

Freshness effect

Even although the freshness effect of mouth rinses has been found to be independent of anti-halitosis action, it provides a feeling of psychological relief for patients. This is why it is important to assess the instant success of anti-halitosis mouth rinses. The three freshest mouth rinses (R7, R5, R3) are alcohol free, except R7, which contains xylitol, and all contained zinc salts.

CONCLUSIONS

1. Mouth rinses containing Zn salts, especially $ZnCl_2$, were instantly effective on halitosis.
2. Mouth rinses containing ethyl and other alcohols (glycol, sorbitol, menthol, eucalyptol, thymol, xylitol and eugenol) were less effective on halitosis than the alcohol-free mouth rinses.
3. Freshness effect was not associated with anti-halitosis effect.

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REFERENCES

1. Aydin M, Harvey-Woodworth CN. Halitosis: a new definition and classification. Br Dent J. 2014; 217: E1
2. Quirynen M, Zhao H, Soers C, Dekeyser C, Pauwels M, Coucke W, Steenberghe D. The impact of periodontal therapy and the adjunctive effect of antiseptics on breath odor-related outcome variables: a double-blind randomized study. J Periodontol 2005; 76:705-712.
3. Campisi G, Musciotto A, Di Fede O, Di Marco V, Craxi A. Halitosis: Could it be more than mere bad breath? Intern Emerg Med 2011; 6:315-319.
4. Thrane PS, Young A, Jonski G, Rölla G. A new mouthrinse combining zinc and chlorhexidine in low concentrations provides superior efficacy against halitosis compared to existing formulations: a double-blind clinical study. J Clin Dent 2007; 18:82-6.
5. Young A, Jonski G, Rölla G. Combined effect of zinc ions and cationic antibacterial agents on intraoral volatile sulphur compounds (VSC). Int Dent J 2003; 53:237-242.
6. Silwood CJ, Grootveld MC, Lynch E. A multifactorial investigation of the ability of oral health care products (OHCPs) to alleviate oral malodour. J Clin Periodontol 2001; 28:634-641.
7. Aydin M, Derici MC, Unal Y, Yeler D, Demir YI. [Type 1 halitosis is not related with oral Candida colonisation] Mikrobiyol Bul 2019; 53: 192-203.
8. Krespi YP, Shrime MG, Kacker A. The relationship between oral malodor and volatile sulfur compound–producing bacteria. Otolaryngology–Head and Neck Surgery 2006; 135: 671-676.
9. Broek AM, Feenstra L, Baat C. A review of the current literature on management of halitosis. Oral Dis 2008;14:30-39.
10. Blom T, Slot DE, Quirynen M, Van der Weijden GA. The effect of mouthrinses on oral malodor: a systematic review. Int J Dent Hygiene 2012; 10: 209-222.
11. Slot DE, De Geest S, van der Weijden FA, Quirynen M. Treatment of oral malodour. Medium-term efficacy of

mechanical and/or chemical agents: -a systematic review-. J Clin Periodontol 2015; 42(: 303–316. 12. Aydin M. Özen ME. Kirbiyik U, Evlice B, Ferguson M, Uzel I. A new measurement protocol to differentiate sources of halitosis. Acta Odontol Scand 2016;11:1-5.
13. Kleinberg I, Codipilly DM. Cystein challenge testing: a powerful tool for examining oral malodour processes and treatments in vivo. Int Dent J 2002; 52:221-228.
14. Aydin M, Bollen CM, Özen ME. Diagnostic Value of Halitosis Examination Methods. Compend Contin Educ Dent 2016; 37:174-178.
15. Pham TA, Ueno M, Shinada K, Kawaguchi Y. Comparison between self-perceived and clinical oral malodor. Oral Surg Oral Med Oral Pathol Oral Radiol 2012; 113:70-80.
16. Grzegorek TE, Michalik J, Kepa J, Wierzbicka M, Aleksinski M, Pierzynowska E. Subjective patients' opinion and evaluation of halitosis using halimeter and organoleptic scores. Oral Dis 2005; 11:86-88.
17. Velde VS, van Steenberghe D, Van Hee P, Quirynen M. Detection of odorous compounds in breath. J Dent Res 2009; 88:285-289.
18. Phillips M, Herrera J, Krishnan S, Zain M, Greenberg J, Cataneo RN. Variation in volatile organic compounds in the breath of normal humans. J Chromatogr B Biomed Sci Appl 1999; 729:75-88.
19. Ind Sci. MX6 User manual of MX6. PID response factors, 2010. https://www.indsci.com/WorkArea/DownloadAsset.aspx?id=308
20. Codipilly DP, Kaufman HW, Kleinberg I. Use of a novel group of oral malodor measurements to evaluate an anti-oral malodor mouthrinse (TriOralTM) in humans. J Clin Dent 2004; 15: 98.104.
21. Kleinberg I, Codipilly D. H(2)S generation and E(h) reduction in cysteine challenge testing as a means of determining the potential of test products and treatments for inhibiting oral malodor J. Breath Res 2008; 2:017018.
22. Waler SM. On the transformation of sulfur-containing amino acids and peptides to volatile sulfur compounds (VSC) in the human mouth. Eur J Oral Sci 1997;105:534-537.
23. Lopes RG, Mota ACC, Soares C, Tarzia O, Deana AM, Prates RA, França CM, Fernandes KPS, Ferrari RAM, Bussadori SK. Immediate results of photodynamic therapy for the treatment of halitosis in adolescents: a randomized, controlled, clinical trial. Lasers Med Sci 2016; 31:41-47.
24. Young A, Jonski G, Rölla G. Effects of metal salts on the oral production of volatile sulphur containing compounds (VSC). J Clin Periodontol 2001; 28:776-781.
25. Springfield J, Suarez FL, Majerus GJ, Lenton PA, Furne JK, Levitt MD. Spontaneous fluctuations in the concentrations of oral sulfur-containing gases. J Dent Res 2001; 80: 1441-1444.
26. Van der Sluijs, Slot DE, Bakker EWP, Weijden GA. The effect of water on morning bad breath: a randomized clinical trial. Int J Dent Hyg 2016; 14:124-134.
27. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, Whelton H, Riley P. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev 2017; 31;3: 8676.
28. Helms JA, Della-Fera MA, Mott AE, Frank ME. Effects of chlorhexidine on human taste perception. Arch Oral Biol 1995; 40:913-920.
29. Gurgan CA, Zaim E, Bakirsoy I, Soykan E. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as an adjunct to non-surgical periodontal treatment: A double-blind clinical study. J Periodontol 2006; 77:370-384.
30. Bradshaw DJ, Marh PD, Watson GK, Cummins D. The Effects of Triclosan and Zinc Citrate, Alone and In Combination, on a Community of Oral Bacteria Grown In vitro. J Dent Res 1993; 72:25-30.
31. Borden LC, Chaves ES, Bowman JP, Fath BM, Hollar GL. The effect of four mouthrinses on oral malodor. Compend Contin Educ Dent 2002; 23: 531-536.
32. Jürgen F, Helmut B, Wolfgang L, Dieter M. Alcohols, Aliphatic, Ullmann's Encyclopedia of Industrial Chemistry, Weinheim: Wiley-VCH, 2000.
33. Bernstein ML. Carlish R. The induction of hyperkeratotic white lesions in hamster cheek pouches with mouthwash. Oral Surg Oral Med Oral Pthol 1979; 48:517-522.
34. Reidy JT, McHugh EE, Stassen LF. A review of the role of alcohol in the pathogenesis of oral cancer and the link between alcohol-containing mouthrinses and oral cancer. J Ir Dent Assoc 2011; 57:200-202.
35. McCullough MJ, Farah CS. The role of alcohol in oral carcinogenesis with particular reference to alcohol-containing mouthwashes. Aust Dent J. 2008; 53:302-305.
36. Vecchia CL. Mouthwash and oral cancer risk: An update. Oral Oncology 2009; 45:198-200.
37. Ayers KM, Colquhoun AN. Halitosis: causes, diagnosis, and treatment. N Z Dent J 1998; 94:156-160.
38. Ben-Aryeh H, Horowitz G, Nir D, Laufer D. Halitosis: an interdisciplinary approach. Am J Otolaryngol 1998; 19:8-11.
39. Gagari E, Kabani S. Adverse effects of a mouthwash use. Oral Surg Oral Med Oral Pathol Radiol Endod 1995; 80: 432-439.
40. Young A, Jonski G, Rölla G. The oral anti-volatile sulphur compound effects of zinc salts and their stability constants. Eur J Oral Sci 2002; 110:31-34.

Arch parameters and dental discrepancy (crowding and spacing) in a sample of an Afro-Colombian population

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ABSTRACT

The aim of this study was to determine the differences in arch length, inter-canine distance, inter-premolar distance, inter-molar distance and arch shape between dental discrepancies (crowding and spacing) in a sample of dental casts from the Afro-Colombian population of San Basilio de Palenque. An analytical, cross-sectional study was conducted on a convenience sample of 63 subjects aged 11 to 57 years, of Afro-Colombian origin, with full dentition from first molar to first molar, without extensive caries or restorations, and excluding casts with defects due to loss. The differences between arch (upper and lower) variables were analyzed according to dental discrepancies. Plaster models digitalized with a TRIOS3 Mono scanner with exactitude (6.9 ± 0.9 µm) and precision (4.5 ± 0.9 µm) were analyzed with Orthonalyzer software. Statistical analyses were done on SPSS software (Version 20 for Windows) and Real Statistics. Spacing discrepancy of 68.25% was found for upper arch and 66.66% for lower arch; crowding discrepancy of 19.04% for upper arch and 20.63% for lower arch, and an adequate ratio of 12.69% for both arches. No statistically significant difference (p>0.05) was found between arch parameters except for inter-premolar distance on the lower arch. The most frequent arch shape in the population was oval for both upper arch, with 76.19%, and lower arch, with 71.42%. Tooth size was larger in males than females but the difference was not statistically significant.

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Parámetros de arco y discrepancia dental (apiñamiento y espaciamiento) en una muestra de población Afrocolombiana

RESUMEN

El objetivo de este estudio fue determinar las diferencias en longitud de arco, distancia intercanina, interpremolar, intermolar y la forma de arco entre discrepancias dentales (apiñamiento y espaciamiento), en una muestra de modelos dentales de la población afrocolombiana de San Basilio de Palenque. Se realizó un estudio analítico transversal, en una muestra por conveniencia de 63 sujetos con un rango de edad entre 11 y 57 años, de origen afrocolombiano, quienes tuvieron dentición completa de primer molar a primer molar, sin caries extensas, ni restauraciones; se excluyeron los modelos con defectos por el vaciado. Se analizaron las diferencias entre las variables de los maxilares (superior e inferior) con las discrepancias dentales. Se utilizaron modelos de yeso que fueron digitalizados con el escánerTRIOS3 Mono con una exactitud de (6.9 ± 0.9 µm) y una precisión de (4.5 ± 0.9 µm)y analizados con el software Orthonalyzer. Los análisis estadísticos se llevaron a cabo utilizando el software SPSS (Versión 20 para Windows) y Real Statistics. Se encontró una discrepancia de espaciamiento de un 68,25% para el arco superior y 66,66% en el arco inferior; y una discrepancia de apiñamiento en el arco superior de 19,04% e inferior de 20,63% y una relación adecuada de 12,69% para los dos arcos. No se encontraron diferencias estadísticamente significativas (p>0.05) en los parámetros de arco a excepción de la distancia interpremolar del arco inferior. La forma de arco más frecuente en la población fue ovalada tanto en el arco superior con un 76,19% como en el arco inferior con un 71,42%. En cuanto al tamaño dental, se presentó mayor tamaño en los hombres que en las mujeres, pero este no fue estadísticamente significativo.

Palabras clave: *arcada dental, maloclusión, distema; corona dental; maxilar superior.*

INTRODUCTION

Jaws undergo a number of changes during growth and development, beginning with the embryo stage and continuing through the development of deciduous and permanent dentition[1]. In 2015, Mauad et al.[2] reported that the greatest changes in size and shape of the jaws occur at two important times during growth and development: the first

during the deciduous dentition period (approximately 3-6 years of age) and the second during the eruption of permanent dentition and until it is functional. These changes involve the size and shape of dental arches and continue to occur until the growth stage has been completed[3]. Jaws change in size and shape as a result of multiple factors, including bone remodeling, position of teeth, sutural expansion of the upper jaw, nutrition, environment and genetics, among others[4-6].

Adequate growth should lead to proper occlusion between jaws, although in this regard, tooth size should also be taken into account[7]. Tooth size should correlate appropriately with the size of the bony bases, since any discrepancy between them could cause crowding or spacing[8], with impacted, rotated, displaced or excessively spaced teeth[9]. The causes of discrepancy are not yet absolutely clear. They have been related to different etiologies, such as reduction in arch size, tooth size, and relationship between tooth size and number of teeth[10].

The association between dental crowding and tooth and arch size have been studied previously. Howe et al.[11] reported that crowding was associated to smaller dental arches rather than to larger teeth. Mills[12] reported a significant correlation between crowding and arch width. Radznic[13] established that arch length is highly correlated to crowding. Tooth-size discrepancy can also cause crowding or spacing, and tooth mesiodistal width is considered a primary etiological factor in spacing anomalies causing malocclusion[14]. Barrett[15] showed that tooth position in the dental arch can be determined considering tooth size and amount of space available for teeth in the dental arch. Fastlicht[16] reported two factors responsible for dental crowding: larger tooth mesiodistal dimensions and smaller dental arch sizes. In 2018, Haidi et al.[17] conducted a study on a Saudi population, finding that tapered dental arches were prevalent, with males having larger arches than females, and discrepancy in tooth size in the anterior segment. In 2000, Burris[18] reported that American blacks had square dental arches, and that they were larger than in whites. There is little information available on the shape and size of dental arches in Colombian populations. Different facial features may influence dental arch shape and be related to the bony bases[19]. In 2016, Rodríguez et al.[20] conducted a study on Colombians of three ethnic descents, including a sample of Afro-Colombian population from Puerto Tejada, Cauca, on dental arch shape and size, finding that the oval is the most frequent in all ethnic groups for both jaws. Inter-canine distance varied among arch shapes, with significant difference in the upper arch, particularly in triangular arches. No significant difference was found between upper and lower arch shape.

Populational studies have shown that there is a wide range of tooth sizes[17]; e.g., American blacks have significantly larger dental crown size than American whites[21], though they have been reported to have more spacing on the arches[22]. Other studies have shown variations in crowding and spacing in different ethnic groups. Spacing prevalence ranges from 6% to 50%, and crowding ranges from 5% to 80% in different populations[23-25].

The population in Colombia is considered mestizo, as a result of a mixture of three large populations: indigenous, African and European, which began at the time of the conquest with the arrival of Europeans, mostly Spaniards, who brought with them African slaves[26]. Currently there are relatively isolated Afro-Colombian populations, such as the people of San Basilio de Palenque, who have kept alive many of their customs, as well as maintaining their genetics. Genetic studies on the population of San Basilio de Palenque show a high African component, with haplogroup E1b1a-M2 from eastern Africa, indicating that these people probably have unaltered African heritage[26-28]. The aim of the current study was to determine differences in arch length; inter-canine, inter-premolar and inter-molar distances, and arch shape between dental discrepancies (crowding and spacing) in a sample of dental casts from the Afro-Colombian population of San Basilio de Palenque. Although Afro-descendant communities have been reported to have larger teeth than other populational groups, they also have a high frequency of diastemas, so it is assumed that this tooth size-arch length discrepancy may be due to greater arch length and inter-canine, inter-premolar and inter-molar distances.

MATERIALS AND METHODS

An analytical, cross-sectional study was performed on a sample of 63 casts from 32 males and 30 females, aged 11 to 57 years, of Afro-Colombian origin, from the town of San Basilio de Palenque, Mahates Bolívar district, Colombia, who signed

informed assent and consent. Casts were taken on subjects who had complete permanent dentition from first molar to first molar, upper and lower, without previous orthodontic treatment, without extensive caries, restorations or rehabilitations on the mesial and distal surfaces. Casts with impression defects, loss or fracturing, which prevented variables from being recorded, were excluded. This study was approved by the University ethics committee.

A TRIOS 3 Mono scanner was used to digitalize the plaster casts with exactness 6.9 ± 0.9 µm and precision 4.5 ± 0.9 µm. Orthonalyzer software was used to view the casts in 3D for analysis. First, the casts were formatted and canine tips, vestibular cusps for first and second molars, and mesiovestibular cusp of first upper and lower molars were located in order to measure the following distances: maxillary and mandibular inter-canine distance, distance between maxillary and mandibular first premolars, distance between maxillary and mandibular second premolars, maxillary and mandibular intermolar distance (Fig. 1). Maxillary and mandibular arch length was evaluated by measuring the distance between the vestibular surfaces of upper central incisors and another tangent line connecting the most distal points of the second premolars (Fig. 2). Arch shape was determined by superimposing square, oval and triangular arch shapes in occlusal view on the 3D cast, both upper and lower, and considering the shape most similar to the cast analyzed (Fig. 3).

Dental arch discrepancy (crowding and spacing) was considered as the difference between arch perimeter and the sum of mesiodistal diameters from distal face of the second premolar to the distal face of the contralateral tooth, on both upper and lower arches. Arch perimeter (basal region available in the arch) was determined by adding the length segments of maxillary and mandibular arch (A1 B1 C1 D1) where A1 is the distance between the distal surface of the second right premolar to the mesial surface of the canine on the same side, B1 is the distance between the mesial surface of the right canine to the midline, C1 is the distance from the midline to the mesial surface of the left canine, D1 is the distance from the mesial surface of the left canine to the distal surface of the second premolar on the same side. The sample was classified into 3 groups: crowding (values less than -1 mm), spacing (values higher than 1 mm) and normal (values from -0.9 mm to 0.9 mm).

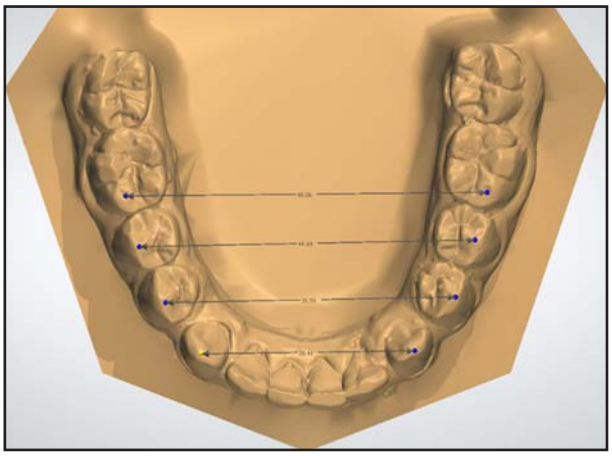

Fig. 1: Measurement of transversal distance between homologous teeth on digital maxillary cast.

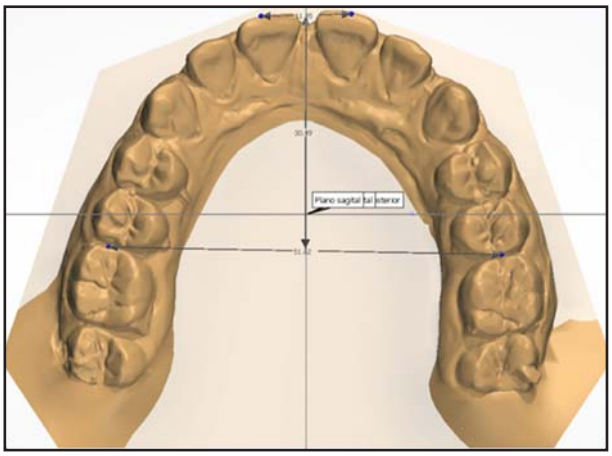

Fig. 2: Measurement of arch length on digital mandibular cast.

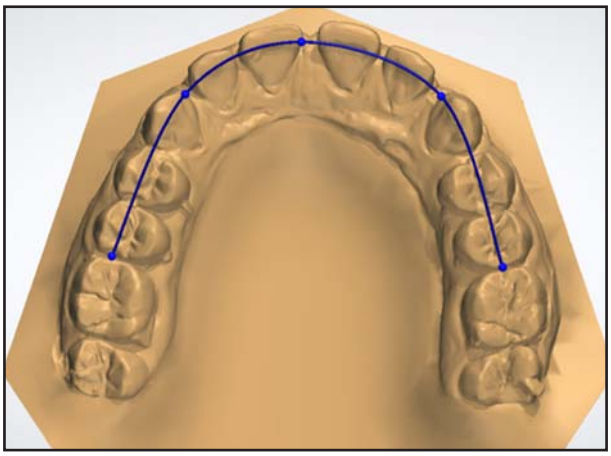

Fig. 3: Determination of arch shape on maxillary digital cast.

Statistical analysis was performed using SPSS software (Version 20 for Windows) and RealStatistics. All measurements were taken by a single observer, trained in taking measurements. To determine intra-rater error, measurements were taken 15 days apart for 19% of the sample, and Bland Altmand graphs[29] and Dahlberg and Houston statistics[30] were applied.

RESULTS

The results of the paired T-test for each variable show that there was no difference in the average of the measurements taken by a single rater ($p>0.05$), accepting H0 for equal means. The Dalhberg values for each variable (0.30 mm, 0.40 mm, 0.28 mm, 0.40 mm, 0.39 mm, 0.30 mm, 0.30 mm) show control of the systematic random error for one rater.

In the upper arch, mean distances between canines, between premolars (first and second), between molars and arch length were greater in males than females; nevertheless, there was no significant difference ($p<0.05$) between any of the variables in upper and lower arches (Tables 1 and 2).

No significant difference was found between sexes for upper and lower tooth size ($p>0.05$) although measurements were greater for males than females ($p<0.05$) (Tables 3 and 4).

In the upper arch, spacing discrepancy was present in 68.25% of the sample, crowding in 19.04%, and normal ratio in 12.69%. For lower arch, there was spacing in 66.66%, crowding in 20.63% and normal ratio in 12.69%.

For upper arches, in subjects with spacing, 74.41% of the arches were oval, 18.59% were square and 6.97% were triangular. In subjects with crowding, 75.0% of the arches were oval, 25.0% were triangular and none square. In subjects with normal arch ratio, 87.54% of the arches were oval and 12.45% triangular (Table 5).

For lower arches, in subjects with spacing, 73.80% of the arches were oval, 14.28% square and 11.89% triangular. In subjects with crowding, 61.51% of the arches were oval, 30.73% triangular and 7.65% square. In subjects with normal ratio, 75.01% of the arches were oval and 24.98% were triangular (Table 6).

Table 1: Comparison of upper arch variables for both sexes.

	UPPER ARCH								
	Female				Male				
	Mean	SD	Min	Max	Mean	SD	Min	Min	p
Inter-canine distance	36.53	3.37	30.07	44.01	35.66	2.64	29.25	38.15	0.27
Distance between 1st premolars	44.86	3.24	37.94	52.11	44.00	2.70	36.36	48	0.25
Distance between 2nd premolars	49.79	3.53	42.63	57.15	49.01	2.52	42.02	53.67	0.31
Distance between 1st Molars	54.69	3.37	46.91	62.09	53.81	3.34	42.1	59.01	0.30
Arch length	27.42	2.95	21.19	32.61	27.18	**2.24**	23.09	31.7	0.72

*significance p<0.05

Table 2: Comparison of lower arch variables for both sexes.

	LOWER ARCH								
	Female				Male				
	Mean	SD	Min	Max	Mean	SD	Min	Min	p
Inter-canine distance	28.53	2.69	23.92	38.15	27.65	2.7	21.19	32.96	0.20
Distance between 1st premolars	36.35	2.32	31.66	41.99	36.36	0.29	32.26	41.26	0.98
Distance between 2nd premolars	42.44	3.08	36.54	49.51	41.41	3.5	28.62	47.94	0.22
Distance between 1st Molars	48.24	3.64	42.89	61.89	47.23	3.2	41.33	53.81	0.25
Arch length	24.01	2.99	30.97	17.16	23.94	2.48	18.82	30.22	0.92

*significance p<0.05

Tables 7 and 8 show means and SD for the different variables in the three groups (spacing, crowding and normal ratio) in upper and lower arches. The means for all variables were higher in the spacing group, except upper arch length, although there was no significant difference in the variables between groups (p>0.05).

To explore the groups internally, the categories spacing and crowding were divided. Crowding was considered moderate-to-severe in subjects with a difference greater than -4 mm in available space / required space ratio; and spacing was considered moderate-to-severe in subjects with a positive difference of + 4 mm for this ratio. Comparison of the moderate-to-severe crowding and moderate-to-severe spacing groups showed significant differences (p<0.05) in the distances between first and second premolar for both arches (Tables 9 and 10).

Table 3: Comparison of upper tooth sizes per sex.

	1st Molar		2nd Premolar		1st Premolar		Canine		Lateral		Central	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MALE	10.25	0.76	7.20	0.92	7.26	0.65	7.65	0.78	6.54	0.81	8.41	0.71
FEMALE	10.35	0.82	6.77	0.67	7.13	0.65	7.50	0.56	6.33	0.85	8.30	0.71
P	**0.62**		**0.25**		**0.41**		**0.34**		**0.30**		**0.55**	

*significance p<0.05

Table 4: Comparison of lower tooth sizes per sex.

	1st Molar		2nd Premolar		1st Premolar		Canine		Lateral		Central	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MALE	11.02	0.83	7.41	1.08	7.16	0.69	6.69	0.69	5.77	0.65	5.18	0.51
FEMALE	11.06	0.97	7.28	0.66	7.18	0.62	6.51	0.65	5.67	0.61	5.07	0.59
P	**0.88**		**0.58**		**0.96**		**0.42**		**0.60**		**0.59**	

*significance p<0.05

Table 5: Discrepancy ratio and arch shape in upper arch.

Discrepancy	Arch Shape					
	Oval		Square		Triangular	
Spacing	32	74.41%	8	18.59%	3	6.97%
Crowding	9	75.0%	0	0%	3	25.0%
Normal	7	87.54%	0	0%	1	12.45%

Table 6: Discrepancy ratio and arch shape in lower arch.

Discrepancy	Arch Shape					
	Oval		Square		Triangular	
Spacing	31	73.80%	6	14.28%	5	11.89%
Crowding	8	61.51%	1	7.65%	4	30.73%
Normal	6	75.01%	0	0%	2	24.98%

Table 7: Descriptive analysis of Spacing, Crowding and Normal in upper arch.

	Spacing		Crowding		Normal		
	Mean	SD	Mean	SD	Mean	SD	P
Inter-canine distance	36.45	0.41	35.28	0.87	35.35	1.55	0.12
Distance between 1st premolars	44.85	0.39	43.30	0.97	43.69	1.46	0.07
Distance between 2nd premolars	49.79	0.41	48.59	0.88	48.41	1.59	0.11
Distance between molars	54.76	0.40	54.02	0.89	53.05	1.53	0.23
Arch length	27.05	0.42	27.82	0.47	27.8	0.89	0.11

*significance p<0.05

Table 8: Descriptive analysis of Spacing, Crowding and Normal in lower arch.

	Spacing		Crowding		Normal	
	Mean	SD	Mean	SD	Mean	SD
Inter-canine distance	27.92	0.38	27.49	0.58	28.77	0.83
Distance between 1st premolars	36.42	0.37	35.45	0.49	37.51	0.73
Distance between 2nd premolars	42.29	0.44	40.39	0.66	43.84	0.79
Distance between molars	47.65	0.45	45.95	0.73	49.11	0.96
Arch length	24.07	0.45	23.99	0.41	23.43	1.09

Source: Author

Table 9: Difference between severe crowding and severe spacing in upper arch.

	Severe Crowding		Severe Spacing			
	Mean	SD	Mean	SD	p (T<=t) one tailed	p (T<=t) two tailed
Inter-canine distance	35.05	5.09	36.93	6.63	0.10	0.20
Distance between 1st premolars	43.10	11.89	45.55	6.96	0.12	0.24
Distance between 2nd premolars	48.27	18.28	50.21	7.25	0.22	0.44
Distance between molars	51.82	43.70	55.11	7.23	0.19	0.39
Arch length	27.71	6.19	27.69	8.08	0.49	0.98

*significance p<0.05

Table 10: Difference between severe crowding and severe spacing in lower arch.

	Severe Crowding		Severe Spacing			
	Mean	SD	Mean	SD	p (T<=t) one tailed	p (T<=t) two tailed
Inter-canine distance	26.46	1.93	28.08	2.71	0.13	0.27
Distance between 1st premolars	34.75	1.04	36.98	2.21	0.019*	0.03*
Distance between 2nd premolars	40.52	1.05	42.69	2.58	0.019*	0.03*
Distance between molars	44.97	2.65	48.19	3.93	0.07	0.15
Arch length	23.05	1.42	24.03	3.14	0.18	0.37

*significance p<0.05

DISCUSSION

The aim of this study was to determine differences in arch length; inter-canine, inter-premolar and inter-molar distances, and arch shape among subjects with crowding and spacing dental discrepancies, in an Afro-descendant population, in which there is usually larger tooth-size and frequent presence of diastemas.

In the Afro-Colombian population study sample, we found a greater frequency of spacing discrepancy (68.25% for upper arch and 66.66% for lower arch). These results are similar to those reported by Burris[18] for a black American population, in whom greater presence of spacing was found.

In this population, the frequency of spacing was high, but no significant difference was found in arch parameters between groups (crowding and spacing). However, because orthodontics traditionally classifies discrepancies as severe, moderate and mild, the sample was divided accordingly. Signi-

ficant differences between inter-premolar distances were found between the extremes – severe crowding compared to severe spacing. This is in agreement with a study on a Pakistani population by Faruqui[31]. However, our results differ from other studies in which the difference was greater for inter-canine and inter-molar distances[17,20]. These results should be considered with caution, due to the small sample size.

The most frequent arch shape in the study population was "oval", both for upper arch (78.98%) and lower arch (70.10%). These results are in agreement with Bedoya-Rodríguez et al.[20], who found a frequency of 78.6% oval for upper arch and 74.3% for lower arch in an Afro-descendant population in the Cauca Department, Colombia. However, our results differ from those reported by Burris[18] in American blacks, in whom the most frequent arch shape was square. These results may be a consequence of allometries in the growth and development processes in North American and Latin American populations.

Comparison between sexes showed no significant difference in study variables. Therefore, no distinction is made according to sex, and the sample is taken as a single group, in contrast to other studies that found significantly larger measurements in males than in females for all variables[6,18].

Dental crowding and spacing are known to be the outcome of several etiological factors in permanent dentition. However, discrepancy in the mesiodistal size of upper and lower teeth is considered to be a determining factor[12,14]. Although there is no statistically significant difference in tooth size between sexes, teeth were slightly larger in males than in females, whereas transverse arch measurements were greater in females. For orthodontic diagnosis, the amount of space deficiency or excess in the arch is usually established according to the mesiodistal size of teeth and the perimeter of the arches [32-34]. Some studies suggest that other factors, such as tooth shape, bony base shape, arch length and arch transversal width play an important part in special discrepancies and have implications in the diagnosis and treatment plan[5,6,17,18].

Crowding is also caused by dental compensation to problems of arch-length discrepancy. The current study did not consider this variable because profile radiographs were not available. It is therefore recommended to use craniofacial radiographs in future studies in order to relate malocclusions to dental discrepancies, considering that this population tends to have biprotrusion[35].

Inclination of tooth long axes may be one of the reasons for spacing. Maxillary teeth, particularly incisors, tend to be more inclined over the basal arch, as mentioned by Moorrees[8] in a study finding that the teeth in the mandibular arch had considerable inclination with respect to the occlusal plane and therefore the maxillary dental arch presented a larger circumference than the bony base arch.

A possible cause of dental discrepancy (spacing and crowding) in the study sample may be alterations in tooth size, considering that diagnostic exploration found low Bolton's index in 34.9% of this population[14].

One of the limitations in this study is the lack of standardization in transverse arch measurements. Some authors use palatine and lingual cusps as reference points[8], others measure from the distal fossae in premolars to the central fossa in molars[29,36] and still others consider vestibular cusps, as we did in the current study[17,18]. Nevertheless, the study sample is not consistent with the representation of the Afro-Colombian population, in addition to which oral status made it difficult to meet the exclusion criteria because the population is highly susceptible to dental caries and edentulism[37].

One of the problems in odontometric studies is the low reproducibility of measurements. In this study, error was controlled by ensuring that the rater was sufficiently trained to achieve consistent results.

Analyses for orthodontic diagnosis should take into account that if standards from other populations are used, tooth size may vary as a result of sexual dimorphism, greater ethnic influence or specific kinds of malocclusion[33]. It is therefore essential to evaluate the patient individually rather than according to measurements or analyses performed on other populations.

The current study found no significant differences for arch parameters (arch length, arch shape, inter-canine distance, inter-premolar distance, inter-molar distance, arch perimeter, tooth size) of dental discrepancies (crowding and spacing). Greater spacing discrepancy was found for both arches. Moreover, no significant differences were found for the variables (arch length, arch shape, inter-canine distance, inter-premolar distance, inter-molar distance, arch perimeter, tooth size) between males and females. The most frequent arch shape was

oval. For subjects with spacing, the upper arch was oval in 74.41% and the lower arch was oval in 73.80%. For subjects with crowding, the upper arch was oval in 75.0% and the lower arch was oval in 61.51%. There were significant differences for inter-premolar distance in the lower arch when severe crowding and severe spacing were compared. Diagnosis of dental discrepancies should consider not only arch size and tooth size, but also tooth inclination and the individual skeletal component.

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REFERENCES

1. Sillman JH. Dimensional changes of the dental arches: Longitudinal study from birth to 25 years. Am J Orthod 1964; 50:824-842.
2. Mauad BA, Silva RC, Aragón MLS de C, Pontes LF, Silva Júnior NG da, Normando D. Changes in lower dental arch dimensions and tooth alignment in young adults without orthodontic treatment. Dental Press J Orthod 2015;20:64–8.
3. Lazenby RA. Continuing periosteal apposition II: The significance of peak bone mass, strain equilibrium, and age-related activity differentials for mechanical compensation in human tubular bones. Am J Phys Anthropol 1990; 82:473-84.
4. Harris EF. A longitudinal study of arch size and form in untreated adults. Am J Orthod Dentofacial Orthop 1997; 111:419-27.
5. Correia GDC, Habib FAL, Vogel CJ. Tooth-size discrepancy: A comparison between manual and digital methods. Dental Press J Orthod 2014; 19:107-113.
6. Cassidy KM, Harris EF, Tolley EA, Keim RG. Genetic influence on dental arch form in orthodontic patients. Angle Orthod 1998; 68:445-454.
7. Arbeláez CJ. Discrepancias de tamaño dental (índice de Bolton) en una muestra de modelos digitales tridimensionales de pacientes con oclusión ideal; validación del método y descripción de hallazgos métricos (tesis Posgrado de Ortodoncia. Universidad Nacional de Colombia, Bogotá, Colombia. 2015.
URL:http://bdigital.unal.edu.co/50061/1/Discrepancias%20de%20tama%C3%B1o%20dental%20%28%C3%ADndice%20de%20bolton%29%20en%20una%20muestra%20de%20modelos%20digitales%20tridimensionales%20de%20pacientes%20con%20oclusi%C3%B3n%20ideal%3B%20validaci%C3%B3n%20del%20m%C3%A9todo%20y%20descripci%C3%B3n%20de%20hallazgos%20m%C3%A9tricos-Catalina%20Jaramillo%20Arbel%C3%A1ez-2015.pdf
8. Moorrees CFA, Reed RB. Biometrics of crowding and spacing of the teeth in the mandible. Am J Phys Anthropol 1954; 12:77-88.
9. Merz ML, Isaacson RJ, Germane N, Rubenstein LK. Tooth diameters and arch perimeters in a black and a white population. Am J Orthod Dentofac Orthop 1991; 100:53-58.
10. Bolton W. Disharmony in tooth size and it relation to the analysis and treatment of malocclusion. The Angle orthodontist 1958; 28:113-130.
11. Howe RP, McNamara JA, O'Connor KA. An examination of dental crowding and its relationship to tooth size and arch dimension. Am J Orthod 1983; 83:363-73.
12. Mills LF, Hamilton PM. Epidemilogical studies of malalignment of Malalignent, A Methodfor computing Dentalarch Circumference. Angle Orthod 1965; 35:244-248.
13. Radnzic D. Dental crowding and its relationship to mesiodistal crown diameters and arch dimensions. Am J Orthod Dentofac Orthop 1988; 94:50-6.
14. Bolton WA. The clinical application of a tooth-size analysis. Am J Orthod 1962; 48:504-529 .
15. Barrett MJ, Brown T, Macdonald MR. Dental observations on Australian aborigines: mesiodistal crown diameters of permanent teeth. Aust Dent J 1963; 8:150-6.
16. Fastlicht J. Crowding of mandibular incisors. Am J Orthod Dentofacial Orthop 1970; 58:156-63
17. Haidi, O. Alhajrasi M, Felemban N, Hassan A. Dental arch dimensions, form and tooth size ratio among a Saudi sample. Saudi Med J 2018; 39:86-91.
18. Burris BG, Harris EF. Maxillary Arch Size and Shape in American Blacks and Whites. Angle Orthod 2000; 70:297-302.
19. Bravo YL, Burbano PE, Bedoya A,Osorio JC, Tamayo J, Martinez CH. Variabilidad de las medidas de los arcos dentales y su relacion con la diferenciacion poblacional - Revision sistematica.Rev. Colomb Investig en Odontol 2015; 5:157-175.
URL:https://pdfs.semanticscholar.org/b3cd/64ac6d2953e9660cb801cf9ca5c6af31f418.pdf
20. Bedoya-Rodrígez A, Montoya-Gómez J, González-Benavidez V, Tamayo-Cardona J, Martínez-Cajas H. Forma y tamaño del arco dental en poblaciones de tres ascendencias étnicas colomianas. Ces Odontol 2016; 29:20-32.
21. Richardson ER, Malhotra SK. Mesiodistal crown dimension of the permanent dentition of American Negroes. Am J Orthod 1975; 68:157-64.
22. Kelly JE, Harvey CR. An assessment of the occlusion of the teeth of youths 12-17 years. Vital and health statistics.

Series 11, Data from the national health survey. 1977; 162:1-65. URL: https://www.cdc.gov/nchs/data/series/sr_11/sr11_162.pdf
23. Gábris K, Márton S, Madléna M. Prevalence of malocclusions in Hungarian adolescents. Eur J Orthod 2006; 28:467-70.
24. Mugonzibwa EA, Eskeli R, Laine-Alava MT, Kuijpers-Jagtman AM, Katsaros C. Spacing and crowding among African and Caucasian children. Orthod Craniofacial Res 2008; 11:82-89.
25. Hack GD, Patzelt SBM. Evaluation of the Accuracy of Six Intraoral Scanning evices: An in-vitro Investigation. ADA Prof Prod Rev 2015; 10:1-5. URL:https://www.beldental.cz/upload/uploads/files/ke_stazeni/evaluation_of_the_accuracy_of_six_intraoral_scanning_devices.pdf
26. Noguera MC. Estudio molecular de estructura Genética en poblaciones indígenas y afrocolombianas mediante marcadores nucleares y mitocondriales. 2011; 1-271. URL: https://repository.javeriana.edu.co/handle/10554/16657.
27. Padilla M, Tello L, Moreno F, Osorio JC, Bedoya A. Analysis of Dental Arch Dimensions in Three Colombian Ethnic Groups. Int J Morphol 2013; 31:100-106.
28. Mesa NR, Mondragon MC, Soto ID, Parra M V., Duque C, Ortiz-Barrientos D, et al. Autosomal, mtDNA, and Y-chromosome diversity in Amerinds: Pre- and Post-Columbian patterns of gene flow in South America. Am J Hum Genet 2000; 67:1277-1286.
29. Houston, WJ. The analysis of errors in orthodontic measurements. Am J Orthod 1983; 83: 382-390.
30. Dahlberg G. Statistical methods for medical and biological students. Br Med J 1940; 2(4158): 358-359. URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2179091/pdf/brmedj04110-0016c.pdf No se encontró en PubMed.
31. Faruqui S, Fida M, Shaikh A. Comparison of tooth and arch dimensions in dental crowding and spacing. POJ 2012; 4:48-55. URL:http://applications.emro.who.int/imemrf/Pak_Orthod_J/Pak_Orthod_J_2012_4_2_48_55.pdf
32. Kundi IU, Bashir U, Zahid S, Shaheed S, Usman K. Bolton tooth size analysis of pakistanis of 13 to 20 years in islamabad city. Pakistan Oral Dent J 2012;32:421-6. URL: http://podj.com.pk/archive/Dec_2012/p-14.pdf
33. Lacerda dos Santos R, Melo M. Discrepancia Dentaria de Bolton y Finalización de Ortodoncia: Consideraciones Clínicas. Int. J. Odontostomat 2010;4:93-100. URL: http://dx.doi.org/10.4067/S0718-381X2010000100014
34. Cuanalo LO, González de la Fuente MV, Nácar MS, Santillán NP, Sánchez CL, Moreno W. Forma de los arcos dentales en pacientes atendidos en la clínica multidisciplinaria Zaragoza. Vertientes 2011;14:82-87. URL: https://www.medigraphic.com/pdfs/vertientes/vre-2011/vre112d.pdf
35. McKoy-White J, Evans CA, Viana G, Anderson NK, Giddon DB. Facial profile preferences of black women before and after orthodontic treatment. Am J Orthod Dentofac Orthop 2006;1:17-23.
36. Alvaran N, Roldan SI, Buschang PH. Maxillary and mandibular arch widths of Colombians. Am J Orthod Dentofac Orthop 2009; 135:649-656.
37. IV Estudio Nacional De Salud Bucal ENSAB IV. MINSALUD. 2014. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/ENSAB-IV-Situacion-Bucal-Actual.pdf

Validation of an adherence assay to detect group mutans streptococci in saliva samples

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ABSTRACT

The aim of the present study was to validate and establish a cut off point and the predictive value of an adhesion test (AA-MSMG), as a microbiological method for evaluating cariogenic risk. The study is based on a variant (20% sucrose) of a selective medium descripted by Gold et al. (MSMG). This method differentiates mutans group streptococci (MGS) by exacerbating the production of insoluble extracellular polysaccharide which gives adhesion to surfaces such as glass, plastic and dental enamel. Caries assessment according to ICDAS was conducted in 154 patients (aged > 21 years) who were attended at Preventive and Community Dentistry Department, School of Dentistry, University of Buenos Aires, Argentina, between August 2017 to August 2018. The study population was assigned to groups according to the presence/ absence of caries lesions: Group A: ICDAS lesion code = 0 (L=0) on all dental surfaces (n=23); and Group B: L>1 (n=131). After mouth-rinsing with distilled water, saliva samples were collected with fasting and hygiene protocol, and sent immediately to the Microbiological Diagnosis Laboratory, Microbiology Department, School of Dentistry, University of Buenos Aires. Samples were homogenized and serially diluted to the tenth. 100 µl of the dilutions were cultured in 25 cm^2 sterile plastic flasks containing 9.9 ml of modified selective medium described by Gold (MSMG-selective and differential medium). Cultures were incubated in an anaerobic atmosphere at 36 ± 1ºC for 48 hours. The supernatants were eluted and the samples washed with sterile distilled water. Colony forming unit counts were performed by calibrated researchers (Kappa ≥0.75) using a stereoscopic microscope at 50X.

Mutans group streptococci (MGS) counts ranged from 1x10^4 to 1x10^5 CFU/ml in group A, and were higher than 1x10^6 CFU/ml in Group B.

Statically analysis of results (ROC) showed that the AAMSMG has a satisfactory predictive value (91%) and established a cutoff point in 1.68x10^5 UFC / ml. This would indicate that individuals whose MGS saliva counts are higher than the cutoff value would be 5 times more likely to develop dental caries. Adherence assay could be a useful microbiological predictor of caries risk.

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Keywords: *mutans group streptococci; saliva; carious lesions.*

Validación del Test de adherencia para el recuento de estreptococcos del grupo mutans en muestras de saliva

RESUMEN

El objetivo del presente estudio fue validar, establecer el punto de corte y valor predictivo de una técnica microbiológica para evaluar el nivel de estreptococos del grupo mutans en saliva. La técnica consiste en un test de adherencia que emplea un medio selectivo modificado (20% sacarosa) descripto por Gold et al. (TA-MSMG). Este método permite diferenciar a los estreptococos del grupo mutans (SGM) exacerbando la producción del polisacárido extracelular insoluble que le confiere adhesión a superficies como vidrio, plástico y esmalte dental. De acuerdo con los criterios de ICDAS se sembraron 154 salivas de pacientes mayores de edad, que asistieron al Servicio de Odontología Preventiva y Comunitaria de la Facultad de Odontología de la Universidad de Buenos Aires entre los meses de agosto de los años 2017 y 2018.

La población estudiada fue asignada a dos grupos según la presencia / ausencia de lesiones de caries: Grupo A: código de lesión ICDAS = 0 (L = 0) en todas las superficies dentales (n = 23); y Grupo B: L> 1 (n = 131). Después de realizar un enjuague bucal con agua destilada, las muestras de saliva se recogieron según protocolo (ayuno de 4 horas y suspensión de higiene dental de 12 hs). Las muestras se remitieron de inmediato al Laboratorio de Diagnóstico Microbiológico, Departamento de Microbiología de la Facultad de Odontología, Universidad de Buenos Aires. Para su procesamiento, las muestras fueron homogeneizadas y diluidas al décimo. Se cultivaron 100 µl de las diluciones en botellas de plástico estériles de 25 cm^2 que contenían 9,9 ml de medio de Gold modificado (MSMG-20% sacarosa). Los cultivos se incubaron en atmósfera anaeróbica a 36 ± 1ºC durante 48 horas. El

sobrenadante se eluyó y las muestras se lavaron con agua destilada estéril. Los recuentos de unidades formadoras de colonias SGM fueron realizados por investigadores calibrados (Kappa ≥0.75) utilizando un microscopio estereoscópico a 50X. Los recuentos de SGM presentaron una variación entre $1x10^4$ y $1x10^5$ UFC/ml en el grupo A, mientras que en el Grupo B fueron superiores a $1x10^6$ UFC/ml.
El análisis estadístico de los resultados determinó una curva ROC que establece para el TA-MSMG un valor predictivo del 91% y un punto de corte en $1.68x10^5$ UFC SGM / ml. Esto indicaría que los individuos cuyos recuentos en saliva de SGM sean superiores al valor de corte, tendrían 5 veces más posibilidades de desarrollar caries (5:1). Este método podría ser un instrumento útil al momento de evaluar (indicador microbiológico) el riesgo cariogénico del paciente.

Palabras clave: *Estreptococos del grupo mutans; saliva; caries.*

INTRODUCTION

Mutans group streptococci - MGS (*S. mutans*–*S. sobrinus*) belong to the indigenous microbiota of the oral cavity. However, given their metabolic characteristics, they are closely associated with caries lesions in humans[1]. Caries, a multifactorial ecological infectious disease, is the result of environmental changes triggered by the interaction of many contributing factors including the environment, bioavailability of substrates (sucrose), tooth surface, and the presence of cariogenic biofilm[2]. The persistence of such particular conditions has been found to result in an increase in the concentration and distribution of mutans group streptococci. The ecological change is evidenced by qualitative and quantitative remodeling of the supragingival biofilm, which favors the production of large amounts of soluble and insoluble extracellular polysaccharides and a marked pH decrease at the saliva-plaque interface. Production of insoluble, mutans polysaccharides on the smooth surfaces of teeth is an undisputed characteristic of sucrose-dependent biofilm, and is a relevant cultural feature of MGS[3].

Supragingival biofilm, which remodels and consolidates through time, is able to withstand environmental changes due to its ability to reorganize and become metabolically autonomous. This is typical of mature biofilm (*Quorum sensing*)[4]. The strategy of preventive dentistry is based on identification and establishment of the elements that will facilitate diagnosis and foreseeing possible short and mid-term changes in the oral environment. To this end, indicators have been developed to determine the likelihood of a patient having the conditions needed for the occurrence of microbiological, ionic, and chemical imbalances that promote demineralization of dental tissues (cariogenic risk – CR). Such indicators include mutans streptococci and *Lactobacillus* spp (LB) counts in saliva[5].

Making clinical decisions is an extremely complex process, and must ultimately rely on the usefulness of a diagnostic test for patient management. It is therefore essential to have detailed knowledge and a thorough understanding of the precision of different available diagnostic tools; in other words, to understand the accuracy of each test in order to classify patients in categories or according to status with regard to the disease. For this purpose, statistical parameters such as sensitivity and specificity of the diagnostic tests are assessed, and the positive and negative predictive values are calculated based on the prevalence of the disease that is to be diagnosed, and are used to determine the usefulness of the tool.

Efforts to obtain a reliable indicator for enumerating salivary mutans streptococci have resulted in the development of different isolation and identification methods using selective and/or differential media[6]. Some commercial kits are used as reference tables (*Cariesscreen*®[7] *Dentocult*SM®[8]), whereas others are diagnostic tests with a set cut- off point.

Clinical microbiology laboratories seek to develop different selective and differential media that allow recovering the largest amount of MGS serotypes occurring in the samples, and significantly diminish the indigenous background microbiota. [9-11] The broth used at our laboratory is a modification of a selective medium designed by Gold for mutans group streptococci [12-15] (20%P/V sucrose SIGMA®) Selectivity and specificity are adjusted to enhance the production of insoluble, or mutant polysaccharides, which are specific to MGS.

The aim of the present study was to validate and establish a cut-off point and the predictive value of an adhesion test (AA-MSMG), as a microbiological method for evaluating cariogenic risk.

MATERIALS AND METHODS

Study Population

The study population comprised 154 male and female patients aged more than 21 years (25.27± 7.25), who attended the Department of Preventive and Community Dentistry, School of Dentistry, University of Buenos Aires between August 2017 and August 2018.

All enrolled patients gave their written informed consent. They were interviewed to obtain information regarding on their diet, oral hygiene habits, frequency of dental visits, and recent medical-dental treatments, and were clinically examined and scored according to the ICDAS-II criteria.[16,17] The patients were then assigned to one of the following groups : Group A (n=23): patients without carious lesions (*ICDAS II, code 0*) (L=0); Group B (n=131): patients with carious lesions (L >= 1) [18].

METHOD

Patients who had received antibiotic therapy or dental treatment within one month prior to sample collection were excluded from the study. The participants were instructed to come fasting and without brushing their teeth on the day of sample collection. Unstimulated saliva was collected by spitting into a falcon tube for 1 minute. The obtained sample was sent immediately to the Microbiological Diagnosis Laboratory, Microbiology Department of the School of Dentistry, University of Buenos Aires and processed in keeping with the biosafety protocols of the Institution. (Resol CD 287/07).

Saliva samples were homogenized by vortexing for 30 seconds and then were diluted to the tenth in phosphate buffer solution (0.01M PBS pH 7). 100µl of the pure and diluted samples were cultured in sterile plastic flasks (25cm^2 contact surface area), containing 9.9 ml of modified selective media described by Gold (MSMG-selective and differential medium with 20% sucrose). The inoculated flasks were placed horizontally and incubated in anaerobic conditions at 36 ±1ºC for 48 hours. Following the incubation period, the flasks were gently inverted several times in order to eliminate the colonies not adhered to the plastic surface. The supernatants were discarded, and the flasks were rinsed twice with sterile distilled water.Colonies were counted by calibrated observers (index Kappa≥ 0,75) in three 1cm^2 fields , randomly distributed on the contact surface of the flasks, using a stereoscopic microscope at 50X magnification. Total counts were corrected taking into account the total contact surface area (25 cm^2), the 100 µl culture aliquot and the dilution factor applied to the sample. Total salivary SGM CFU /ml counts of saliva were thus calculated for each patient.

An MI strain of *Streptococcus mutans (Sm*MI) (Arginine Dihydrolase, Mannitol, Sorbitol, Esculin, Voges Proskauer – ROSCO® tablets and 6.5% sodium chloride – hypertonic broth Britania S.A) from the collection of microbial cultures of the Microbiology Department was used as intra-assay control. The inoculum was adjusted to Nº 0.5 on McFarland's scale, and the culture was processed under the same conditions as the experimental samples. Sensitivity was determined by limiting dilution. In addition, the viability of different strains of indigenous microbiota was tested using a modification of the selective medium developed by Gold *et al* (MSMG). The studied strains were: *Staphylococcus aureus* (*Sa*) ATCC 25923, *Candida albicans* (*Ca*), *Enterococcus faecalis* (*Ef)* ATCC 29212, *Escherichia coli* (*Ec*) ATCC 25922, *S mutans* (*Sm*) ATCC 25175, mutans group streptococci (MGS SmMI) and *Streptococcus sanguinis* (SsMI), wild strains included in our microbial culture collection.

Statistical Analysis

In order to determine the diagnostic value of the AA-MSMG test (sensitivity, specificity, and adequate overall accuracy), the data were statistically analyzed by plotting the Receiver Operating Characteristic curve (ROC curve) using SPSS 25[19].

RESULTS

Although the cultures of the studied strains (*Sa, Ca, Ss*MI, *Ef* and *Ec*) were found to grow in MSMG, none of them adhered to the plastic surface of the flasks as a result of insoluble extracellular polysaccharide production. Total counts, macro and microscopic morphologic features of the *Sm*MI and *Sm* ATCC 25175 strains were consistent with reference values for growth in this medium (Figs 1, 2 and 3). 1 x 10^3 CFU/mL of saliva was the lowest number of GMS that can be detected using this technique. For validation of the AA-MSMG as a diagnostic tool, the data was processed by ROC (Receiver Operating Characteristic)[20] (Figs.4 and 5). Data analysis established 78.63% sensitivity, 56.52% specificity, 91.15% positive predictive

Fig. 1: Polystyrene flask with mutans streptococci adhered colonies.

Fig. 2: Colonies adhered with insoluble extracellular polysaccharide.

Fig. 3: Mutans group streptococci colony with insoluble extracellular polysaccharide.

value (PPV), 31.71% negative predictive value (NPV) (Table 1), 1.68x10^5 UFC/ml cut off point (Table 3), 0.722 areas below the curve (Fig 4), and 4.782 risk to develop caries (Table 2). This method used in a population of 154 people showed that levels of MGS counts higher than the cut-off point (1.68 x 10^5 CFU/ml), increase the microbiological risk of developing caries up to 5 times.

DISCUSSION

The caries process differs according to the genotype of each individual and the environmental stimulus to which the individual is subjected. Nevertheless,

Table 1: Analysis of ROC curve parameters.

		95% C.I.	
		Lower limit	Upper limit
Prevalence of the disease	85.06%	79.44%	90.69%
Correctly diagnosed patients	75.32%	68.52%	82.13%
Sensitivity	78.63%	71.61%	85.65%
Specificity	56.52%	36.26%	76.78%
Positive predictive value	91.15%	85.91%	96.39%
Negative predictive value	31.71%	17.46%	45.95%
Likelihood Ratio +	1.808	1.125	2.906
Likelihood Ratio -	0.378	0.233	0.615

The positive predictive value of the AA-MSMG makes it a reliable method to be used as a population-screening tool

Table 2: ROC curve. Risk estimation parameters.

		95% Confidence interval	
	Value	Low	High
Reason for the advantages for Sm Count (High/Low)	4.782	1.898	12.050
For cohort Presence of caries= Yes	1.335	1.075	1.657
For cohort Presence of caries= No	0.279	0.133	0.587
Number of valid cases	154		

AA-MSMG results in a population of 154 people shown that levels of MGS counts higher than the cutoff point (1.68 x 105 CFU/ml), increase the microbiological risk of developing caries up to 5 times

Table 3: Group mutans streptococci counts obtained using available methods.

Method	Reference Values (CFU/ml saliva)
AA-MMG	**$1.68x10^5$**
Cariesscreen	$1x10^4$-$1x10^5$= low risk $2.5x10^5$-$5x10^5$= moderate risk **>$5x10^5$= high risk**
Dentocult SM	Class 0-1: <$1x10^5$ Class 2: between 0-1 and 3 **Class 3: >$1x10^6$**

Cariesscreen® and DentocultSM® are commercial kits behave as reference tables, whereas the AA-MSMG is a diagnostic test with a lowest set cutoff point.

in view of the multifactorial etiology, world-wide distribution, and high prevalence of dental caries, it is paramount to develop tools that allow measuring the level of infection by microbial agents that are indicators of the disease (MGS – LB)[21], and which can be applied in populations with high social vulnerability in developing countries. The method used in the AA-MSMG involves collecting unstimulated saliva and counting colonies adhered by mutans after serial rinsing, using a stereoscopic microscope.

The method (AA-MSMG -contact surface area) promotes an increase in the microbial specificity in the sample. It allows and enhances insoluble exopolysaccharide (IEPS) production by mutans streptococci and provides the latter with stable and insoluble adherence and cultural features that are specific to colonies producing IEPS. Using serial dilutions facilitates microbial counts by decreasing operator error. Obtaining negative cultures for MSG could mean that ecological balance has been reestablished in healthy proportions.

Commercially available kits show ranges of unspecific cariogenic microbial counts according to tables provided by the manufacturer, and establish a direct correlation with different levels of caries risk. Although the usefulness of the AA-MSMG to assess caries risk *per se* has not been studied to date, the results of the present study suggest that AA-MSMG can be used as a microbiological indicator.

The analysis of the obtained results showed that sensitivity of the AA-MSMG was 78.63% and PPV was 91%. Kigmanet al[22], however, obtained a sensitivity of barely 31% using a similar culture technique in MSB (Mitis Salivarius Bacitracin).[6] The variations in sensitivity could be associated

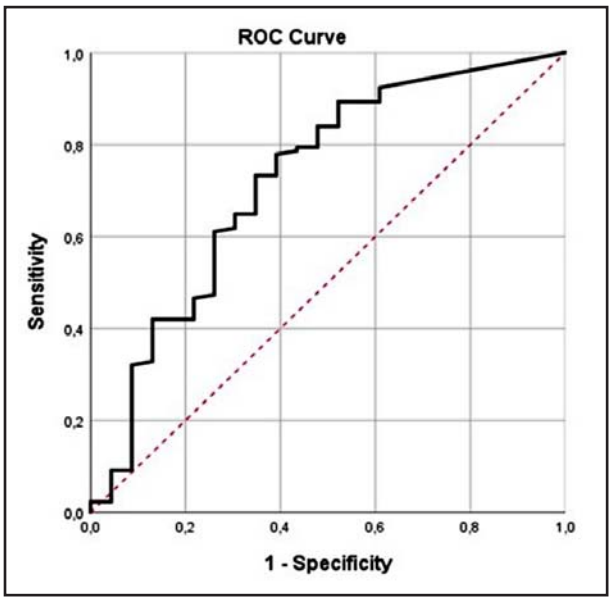

Fig. 4: ROC Curve. Area under the curve:

			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
0.722	0.063	0.001	0.598	0.846

the test result variable(s): RTO *Sm* has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the non parametric as sumption
b. Null hypothesis: true area = 0.5

with the methodological characteristics of the test and the use of magnification to perform microbial counts. In comparison with other commercial methods, such as CARIESCREEN®[9], their results are consistent with the cutoff point obtained in the present study using the AA-MSMG.

Studies conducted by Buischi *et al.*[23] in Brazil and by Klock *et al.*[24] in Sweden showed that MGS counts in saliva above 1.10^6 CFU/ml correlated positively with the presence of dental caries. Their results are above the cut-off point obtained in the present study.

Other researchers, such as Sullivan *et al.*[25], have challenged the true predictive value of microbiological indicators (MGS-LB) in comparison with other indicators of caries risk. As is often observed in the literature, microbiological indicators are thought to have poor validity *per se*, and are considered reliable only when they present simultaneously with high microbial counts (MGS) and clinical evidence of caries. In view of their PPV, microbiological

indicator must be considered as an additional parameter that provide information about the level of infection in a patient, and which could possibly modified to contribute to restore the homeostasis of the oral environment. This method has few limitations; it needs trained observers to perform the counts of specific and characteristic adhered colonies, and basic laboratory equipment. Microbiological diagnostic laboratory can employ it because the method no poses severe operative difficulties.

In view of the sensitivity, PPV, ABC and risk values, AA-MSMG would seem to be an adequate cariogenic-screening test. It could be considered as a valid diagnostic test to measure the infection level of a cariogenic microorganisms group such as mutans streptococci. This test will safely estimate the likelihood of an individual developing caries when salivary MGS counts are above the cut-off point. It will reliably measure biological risk factor and contribute to establishing cariogenic risk with greater certainty.

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REFERENCES

1. Biral RR. Correlation between streptococci of human dental plaques and dental caries. Aust Dent J 1976; 21: 143-146.
2. Takahashi N, Washio J, Mayanagi G. Metabolomics of supragingival plaque and oral bacteria. J Dent Res 2010; 89: 1383-1388.
3. Kleinberg I. A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to Streptococcus mutans and the specific-plaque hypothesis. Crit Rev Oral Biol Med 2002; 13: 108-125.
4. Li YH. A quorum-sensing signaling system essential for genetic competence in Streptococcus mutans is involved in biofilm formation. J Bacteriol 2002; 184: 2699-2708.
5. Quivey RG, Kuhnert WL, Hahn K. Genetics of acid adaptation in oral streptococci. Crit Rev Oral Biol Med 2001; 12: 301-314.
6. Preliasco A, Sabelli C, Preliasco de Davison M, Muscia H, et al. Adherence test in relation to caries activity. Rev Ateneo Argent Odontol. 1989; 24:5-9.
7. Soderholm G, Birkhed D. Caries predicting factors in adult patients participating in a dental health program. Community Dent Oral Epidemiol 1988; 16: 374-377.
8. Bentley CD, Broderius CA, Crawford JJ. Evaluation of the Cariescreen SM method for enumeration of salivary mutans streptococci. Gen Dent 1991; 39: 188-191
9. Connelly J, Tanyan C, Webster C. Mutans streptococci monitoring with Cariescreen: a component of dental examinations. Oral Health 1994; 84: 19-21.
10. Little WA. Comparative recovery of *Streptococcus mutans* on ten isolation media. J Clin Microbiol 1977; 5: 578-583.
11. Syed SA, Loesche WJ. Efficiency of various growth media in recovering oral bacterial flora from human dental plaque. Appl Microbiol 1973; 26: 459-465.
12. Saravia ME. Recovery of mutans streptococci on MSB, SB-20 and SB-20M agar media. Arch Oral Biol 2013; 58: 311-316.
13. Tanzer JM. Glucose-sucrose-potassium tellurite-bacitracin agar, an alternative to mitis salivarius-bacitracin agar for enumeration of Streptococcus mutans. J Clin Microbiol 1984; 20: 653-659.
14. Sabelli CA. Is *Streptococcus mutans* adherence saliva test predictive for caries? Acta Odontol Pediatr 1987; 8: 53-56.
15. Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973; 18: 1357-1364.
16. Pitts N. "ICDAS"—an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dent Health 2004; 21: 193-198.
17. Pitts NB. Modern concepts of caries measurement. J Dent Res 2004. 83 Spec No C: C43-7.
18. Pitts NB. Modern perspectives on caries activity and control. J Am Dent Assoc 2011; 142: 790-792.
19. IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.
20. Albeck MJ, Borgesen SE. ROC-curve analysis. A statistical method for the evaluation of diagnostic tests. Ugeskr Laeger 1990; 152: 1650-1653.
21. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Community Dent Oral Epidemiol 2005; 33: 248-255.
22. Kingman A. Salivary levels of *Streptococcus mutans* and lactobacilli and dental caries experiences in a US adolescent population. Community Dent Oral Epidemiol 1988;16: 98-103.
23. Buischi YA. Salivary Streptococcus mutans and caries prevalence in Brazilian schoolchildren. Community Dent Oral Epidemiol 1989; 17: 28-30.
24. Klock B, Svanberg M, Petersson LG. Dental caries, mutans streptococci, lactobacilli, and saliva secretion rate in adults. Community Dent Oral Epidemiol 1990; 18: 249-252.
25. Sullivan A. Number of mutans streptococci or lactobacilli in a total dental plaque sample does not explain the variation in caries better than the numbers in stimulated whole saliva. Community Dent Oral Epidemiol 1996; 24: 159-163.

Effect of periapical inflammation on calcium binding proteins and ERK in the trigeminal nucleus

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ABSTRACT

Peripheral inflammation induces plastic changes in neurons and glia which are regulated by free calcium and calcium binding proteins (CaBP). One of the mechanisms associated with the regulation of intracellular calcium is linked to ERK (Extracellular Signal-Regulated Kinase) and its phosphorylated condition (pERK). ERK phosphorylation is important for intracellular signal transduction and participates in regulating neuroplasticity and inflammatory responses. The aim of this study is to analyse the expression of two CaBPs and pERK in astrocytes and neurons in rat trigeminal subnucleus caudalis (Vc) after experimental periapical inflammation on the left mandibular first molar. At seven days post-treatment, the periapical inflammatory stimulus induces an increase in pERK expression both in S100b positive astrocytes and Calbindin D28k positive neurons, in the ipsilateral Vc with respect to the contralateral side and control group. pERK was observed co-expressing with S100b in astrocytes and in fusiform Calbindin D28k neurons in lamina I. These results could indicate that neural plasticity and pain sensitization could be maintained by ERK activation in projection neurons at 7 days after the periapical inflammation.

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Key words: *neuroplasticity; pERK kinase; S100b protein; Calbindin D28k; apical periodontitis; trigeminal nucleus.*

Efecto de la inflamación periapical sobre las proteínas fijadoras de calcio y ERK en el núcleo trigeminal

RESUMEN

La inflamación periférica induce cambios plásticos en las neuronas y en la glía, los cuales están regulados por el calcio libre y las proteínas fijadoras calcio (CaBP). Uno de los mecanismos asociados con la regulación del calcio intracelular está vinculado con la fosforilación de la proteína quinasa ERK. Asimismo, ERK fosforilado es importante para la transducción de señales intracelulares y participa en la regulación de la neuroplasticidad y las respuestas inflamatorias. El objetivo de este estudio es analizar la expresión de dos CaBPs y pERK en astrocitos y neuronas del subnúcleo caudal del trigémino (Vc) después de una inflamación periapical experimental en el primer molar inferior izquierdo en ratas. A los siete días posteriores al tratamiento, el estímulo inflamatorio periapical induce un aumento en la expresión de pERK, en el número de astrocitos positivos para la proteína marcadora astroglial S100b y en neuronas positivas para Calbindina D28k, en el Vc ipsilateral respecto del lado contralateral y el grupo de control. Además, se observó coexpresión de pERK tanto en astrocitos S100b positivos, como en neuronas fusiformes Calbindin D28k positivas, de la lámina I. Estas observaciones podrían indicar que la neuroplasticidad y la sensibilización al dolor podrían mantenerse mediante la activación de ERK en las neuronas de proyección a los 7 días de la inflamación periapical.

Palabras clave: *neuroplasticidad; quinasa pERK; proteína S100b; Calbindina D28k; periodontitis apical; núcleo trigeminal.*

INTRODUCTION

Periodontal sensory information is transmitted by the maxillary and mandibular branches of the trigeminal nerve. The principal relay for orofacial sensory information (A delta and C fibers) are the laminae I, II and V of the trigeminal subnucleus caudalis (Vc), which is the first center involved in orofacial pain modulation[1,-4]. After peripheral

inflammatory reaction, the primary afferent neuron at the dorsal horn of the Vc releases glutamate onto the ipsilateral fusiform second-order neurons, which in turn increases the influx of calcium[2]. These fusiform neurons localized in lamina I project the nociceptive information to other higher centers of the central nervous system[4]. At the same time, glia and interneurons of Vc participate in the local modulation of the nociceptive information[1,5,6] and may induce morphological modifications as well as biochemical, molecular, and functional synaptic changes. Some of these plastic changes are regulated by free calcium and Calcium Binding Proteins (CaBP)[7,-9].

The calcium binding protein S100b is expressed only in mature astrocytes[10,11], and Calbindin D28k, calretinin, parvalbumin are present in neurons[12,13]. One of the mechanisms associated with the regulation of intracellular calcium is linked to the mitogen-activated protein kinase (MAPK) pathways. Some of these pathways are important for intracellular signal transduction and participate in regulating neural plasticity and inflammatory responses[9,14,15]. There are four well-characterized subfamilies of MAPKs: Extracellular signal-Regulated Kinase (ERK) 1/2, ERK5, c-Jun N-terminal Kinase (JNKs) and p38[16,17]. Some authors have shown a sequential activation of ERK immediately after a peripheral injury. Initially, ERK is transiently phosphorylated (pERK) in dorsal horn neurons, but only for a period of few hours after injury, then in the microglia and some weeks later in astrocytes[14,18,19]. It is assumed that activation of ERK in glial cells is important for the maintenance of pain states[15,20].

However, there are no studies linking astroglia and neuron activation of trigeminal nociceptive pathway after apical periodontitis. The aim of this study is to analyze the expression of calcium binding proteins and ERK activation in astrocytes and neurons in the trigeminal subnucleus caudalis (Vc) at seven days after experimental periapical inflammation in rats.

MATERIALS AND METHODS

This study was carried out on 16 female Wistar rats, 50 days old (weight about 140-170 gr). The animals in these experiments were used according to the Guide for the Care and Use of Laboratory Animals, National Academy of Science, USA and the experimental protocol was authorized by the Ethics Committee of the School of Dentistry (8/1 1/2012-40) and Medicine (CD nº 2057/14) of Buenos Aires, Argentina. They were housed in a room at about 24 °C, 12 h light/dark cycle and free access to water and standard laboratory rat food. The animals were divided into an experimental and a control group, (n= 8 rats per group), in order to perform immuno-histochemistry, immunofluorescence and Western blot analysis. All rats were deeply anaesthetized by intraperitoneal injection of ketamine (100 mg/kg) plus xylazine (14 mg/kg). The rats in the experimental group were operated on the left mandibular first molar to remove the occlusal enamel and dentin, in order to locate the pulp chambers and root canal orifices. The working length of root canal (occlusal-apical) was previously standardized by our laboratory at 4 mm. Later, apical preparation of mesial and distal root canals was done by instrumentation up to a 25 K-file (Dentsply-Maillefer, Ballaigues, Switzerland). After drying with sterile paper points (Sure Endo - Excel Dental Supplies Ltd, mesial and distal root canals were filled with gutta-percha cones (Sure Endo- Excel Dental Supplies Ltd.) and ZOE-based sealers (Grossman´s sealer- Farmadental) by the lateral compaction technique. Grossman´s sealer was manipulated following the manufacturer´s recommendations. Each cavity was then sealed with glass ionomer restorative cement (Fuji II LC). After recovery from anesthesia, animals were monitored for signs of discomfort or pain, and body weight was monitored until they were euthanized. After the first day post-treatment, all operated animals returned to normal behavior, including feeding. Animals in the control group were not exposed to the experimental treatment and received only the anesthetic protocol. All animals in both groups were euthanized at 7 days. Experimental (n=5) and control (n=5) animals were anesthetized with ketamine/xylazine and fixed transcardiacally using a modification[6] of the original perfusion method described by Gonzalez Aguilar and De Robertis[21]. After euthanasia, brains were removed and post-fixed for 4 hours at 4-5º C. Subsequently, the brains were washed 3 times in phosphate buffer saline (PBS) (1 hour) and then stored at 4 °C in PBS containing 0.01% sodium azide. Cryoprotection was performed by immersion in 10% and 30% buffered sucrose (120 min. and overnight respectively). After that, brainstems were frozen at -20ºC. Cryostat brainstem sections (30 µm) were sectioned using a slide microtome and collected in phosphate

buffer saline (PBS) to obtain floating sections for immunohistochemistry. In this study, we selected coronal sections from spinal cord cervical levels C4 to -5.6 interaural levels (6 – 1.5 mm caudal to obex)[22].

The right (unstimulated, contralateral) side of each brainstem was marked on the ventral face with a blade for identification of the paired control side (Fig 1A). Immunostaining was done by our immunohistochemical technique protocol in free floating sections with a procedure using an enzyme method with chromogen; avidin- biotin complex (ABC) or immunofluorescence[6]. The primary antibodies used were: anti astroglial calcium binding protein S100b (rabbit polyclonal, Sigma-Aldrich Cat# HPA015768, RRID:AB_1856538, 1:800), Calbindin D28-K (rabbit polyclonal, CB38, Swant Immunochemicals, Cat# CB38, RRID:AB_2721225, 1:5000) and phosphorylated ERK (Santa Cruz Biotechnology Cat# sc-7383, RRID:AB_627545, 1:1000). Negative controls were performed by replacing primary antibody with goat serum. Briefly, for the ABC method, sections were washed in TS for 40 minutes and then incubated with appropriate biotin conjugated secondary antibody 1:2000 (Jackson Immuno Research). When immunofluorescence labeling was performed, anti S100b or Calbindin D28-K was combined with pERK**.** After washing for 40 minutes with TS, rhodamine red or fluorescein isothiocynanate (FITC) conjugated secondary antibodies were used.

Western blot analyses were performed on brainstem extracts from control (n=3) and operated rats (n=3). Brainstems were cut along in the sagittal plane to obtain the ipsilateral and contralateral side of Vc of each group. Tissues were homogenized in a Potter-Elvehjem homogenizer, in 5 vol of TS containing 1 mM EDTA, 0.5% Triton X-100, 1 mM PMSF, and protease inhibition cocktail (Sigma-Aldrich). The homogenate was centrifuged at 12000 g, 4ºC for 20 min and the supernatant was assayed for protein concentration with the BCA Protein Assay Kit (Thermo Scientific Pierce), according to the manufacturer's instructions. Aliquots of supernatant corresponding to 30 µg of total protein were loaded onto SDS-12% polyacrylamide gels. The proteins were electrotransferred to a PVDF membrane in Tris-glycine-methanol buffer and examined with different antibodies.

Membranes were blocked for 1 h at room temperature in a blocking solution containing 5% normal goat serum, 0.1 %, Tween-20 in PBS (pH 7.4). The membrane was then incubated overnight at 4°C with primary antibodies in the blocking solution. The following primary antibodies were used in the western blot experiments: rabbit polyclonal S100b (1:1000), rabbit polyclonal Calbindin D28-K (1:1000), mouse monoclonal pERK (1:1000) and mouse monoclonal GAPDH (Glyceraldehyde 3-phosphate dehydrogenase, Santa Cruz Biotechnology Cat# sc-166574, RRID:AB_2107296; 1:5000) as loading control.

Membranes were rinsed three times with 0.1% Tween-20 in PBS for 10 min each, followed by incubation for 1 h at room temperature with HRP donkey anti-mouse IgG or anti-rabbit IgG, 1:2000 (Millipore). The blots were washed three times for 20 min each and then processed for DAB/Ni immunochemistry. Development of peroxidase activity was carried out with 0.04% w/v 3,3' diaminobenzidine (Sigma-Aldrich) plus 2.5% w/v nickel ammonium sulphate (Baker) and 0.01% H_2O_2. The reaction was stopped by adding tap water.

The hemimandibles were dissected, post-fixed overnight and radiographed. Then they were decalcified in 10% EDTA/PBS for about 5 weeks, dehydrated, clarified in toluene and embedded in Paraplast. They were cut at 10 µm longitudinal sections, collected on glass slides and stained with hematoxylin-eosin to analyze the degree of periapical injury and extent of inflammatory tissue after canal instrumentation and filled up to the apex.

All stained sections were photographed in a Zeiss Axiophot light microscope equipped with epifluorescence by switching between FITC and rhodamine filter sets. Photomicrographs were captured at 24 bits per pixel resolution (8 bpp x 3 colors) using an Olympus Q-color 5 camera, and identical light exposure parameters. The morphometric analysis was performed using Image Pro 6 (Media Cybernetics, Silver Springs, MD, USA). Based on normal morphological criteria, on each side and two levels of Vc per animal, the immunoreactive cells were quantified per dorsal area of 0.01 mm^2.

Results are shown as mean and SEM. Data were statistically compared by two-way analysis of variance (ANOVA) followed by the post-hoc Bonferroni test. Statistical difference was considered significant for p-values less than 0.05. All statistical analyses were performed using "Graph Pad Prism" (version 5.03; Graph Pad Software).

RESULTS

Histological analysis of periapical tissues

Conventional histological stain was used to evaluate the periapical inflammatory reaction. The histological analysis in the experimental group showed the extent of damage and the inflammatory reaction on the left mandibular first molar at 7 days post treatment. The connective tissue of the periodontal ligament showed an acute inflammatory infiltrate (arrow in Fig. 1B) after preparation of root canal and was filled up to the apex. This reaction was usually accompanied by widening of the periodontal ligament at the expense of resorption of the cortical alveolar bone. At 7 days, the histopathological reaction would be compatible with a diagnosis of apical periodontitis and probably sensory receptor stimulation (Fig. 1B). In the control group and the right hemimandible of the experimental group, the molars showed normal histological features (Fig. 1C). None of them showed any histological signs of an inflammatory process in the periodontal ligament.

Astrocytes and neurons activation in the subnucleus caudalis

To determine whether the periapical inflammation can induce activation of astrocytes and neurons on the subnucleus caudalis, we analyzed the expression of S100b in mature astrocytes, Calbindin D28k in neurons and pERK in astrocytes and neurons present in the subnucleus caudalis (Vc). In the

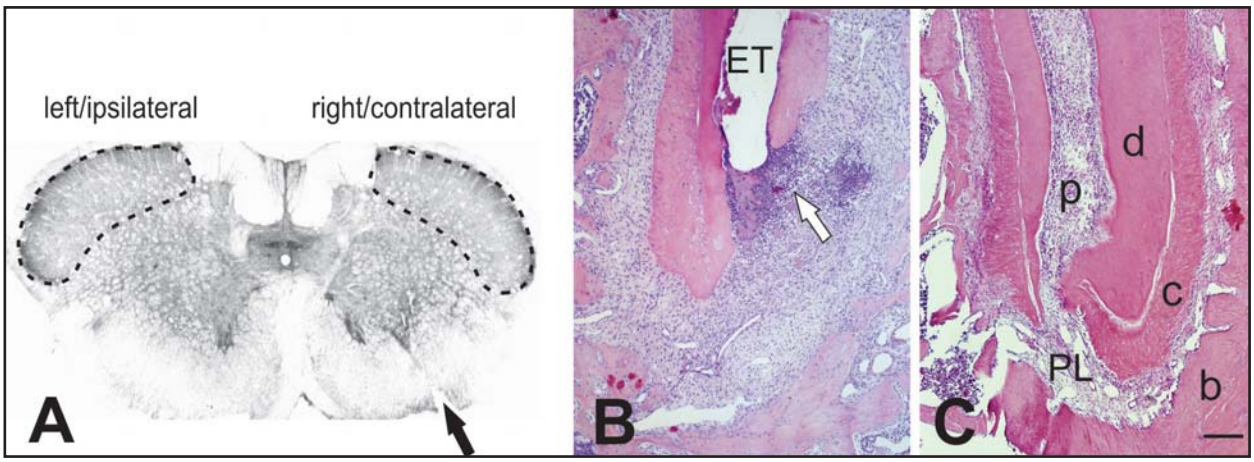

Fig. 1: A: coronal sections of paired ipsilateral and contralateral subnucleus caudalis (Vc). The right (contralateral) side of each brainstem was marked on the ventral face with a blade (arrow) for identification of the paired control side. B-C: Sections of apical zone on the left (B) and right (C) mandibular first molar of experimental group and stained with hematoxilin and eosin. B: Image showing an inflammatory reaction in the periodontal ligament (white arrow) seven days after root canals instrumentation and filled with gutta-percha cones and ZOE-based sealers (Grossman´s sealer- Farmadental). p= pulp; d= dentin; c= cement; b= alveolar bone; PL= periodontal ligament; ET= endodontic treatment. Scale bar= 100um.

Table 1: Astrocytes and neurons activation in the subnucleus caudalis after periapical inflammation.

	S100b astrocytes	Calbindin D28K neurons	Calbindin D28K fusiform neurons	pERK Glia /neurons
CONTROL GROUP				
Ipsilateral side	10.57 ± 0.67	11.94 ± 1.57	0.63 ± 0.09	28.43 ± 3.52
Contralateral side	10.66 ± 1.00	13.94 ± 0.72	0.68 ± 0.08	31.19 ± 2.48
EXPERIMENTAL GROUP	#	#	#	#
Ipsilateral side	**16.23 ± 1.76** *	**16.34 ± 0.88** *	**1.18 ± 0.14** *	**35.35 ± 4.09** *
Contralateral side	13.76 ± 2.32	14.46 ± 1.01	0.83 ± 0.14	31.71 ± 4.42

The results in the table are expressed as mean and SEM. Data (control Vs experimental group) were statistically compared by two-way analyses of variance (ANOVA) followed by the post-hoc Bonferroni test. Note the significant increase in the ipsilateral Vc in the experimental group with respect to their paired contralateral Vc (*) and to control group (#). $p<0.05$.

experimental group, the number of S100b positive astrocytes per area was significantly increased on the ipsilateral side (16.23 ± 1.76) as compared to the paired contralateral side (13.76 ± 2.32) and to the control group (10.57 ± 0.67) (Table 1 and Fig. 2 A-B).

The expression pattern of Calbindin D28K, at 7 days after periapical inflammation, demonstrates that the number of the CB positive neurons in the superficial lamina of the ipsilateral Vc (16.34 ± 0.88) was significantly greater than in the contralateral side (14.46 ± 1.01) and the control group (11.94 ± 1.57). Additionally, in the lamina I, we analyzed the number of fusiform neurons showing morphological features with an elongated soma and two long primary extensions emerging from each end of the soma. We quantified (0.63 ± 0.09 cells/area) in the control group and (1.18 ± 0.14 cells/area) in the ipsilateral side of the experimental group showed statistically significant differences (Table 1 and Fig. 2 C-D).

Finally, we quantified the pERK expression in different cell populations of Vc at 7 days of peripheral inflammation. The number of positive cells per area was found to increase significantly on the ipsilateral side (35.35 ± 4.09) compared to the paired contralateral side (31.71 ± 4.42) (Table 1 and Fig. 2 E-F).

Western blot analysis also revealed an increase in the amount of S100b, CB D28K and pERK proteins on the ipsilateral side of the Vc in the experimental group (Fig. 3).

Moreover, we analyzed ERK activation linked calcium binding proteins in different cell populations related to plasticity. To identify pERK in astrocytes or neurons, double immunofluorescence labeling with S100b or Calbindin D28K was performed, respectively. We observed that pERK and S100b colocalize in the cell body and processes of astrocytes. In addition, pERK expression was observed in some cells with different morphology from astrocytes, and which did not

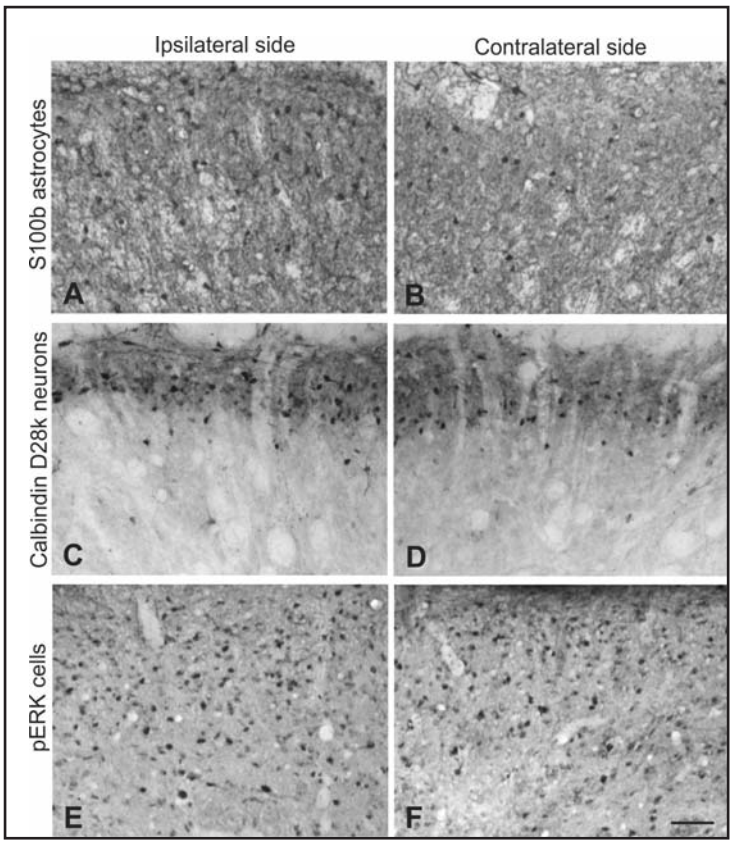

Fig. 2: Coronal sections of brainstems showing the superficial (I and II) and deeper laminae (III) on both sides of Vc of experimental group. (A-B): Images showing S100 b positive astrocytes. (C-D): Experimental group, showing Calbindin D28k positive neurons. (E-F): Experimental group, showing pERK positive cells. The ipsilateral side (A-C-E) showed a significant increase in the number of immunoreactive cells relative to their paired contralateral Vc (B-D-F) for the three study proteins. Moreover, the ipsilateral side of Calbindin D28K (C) showed an increase in the number of fusiform neurons in superficial laminae relative to the contralateral side.

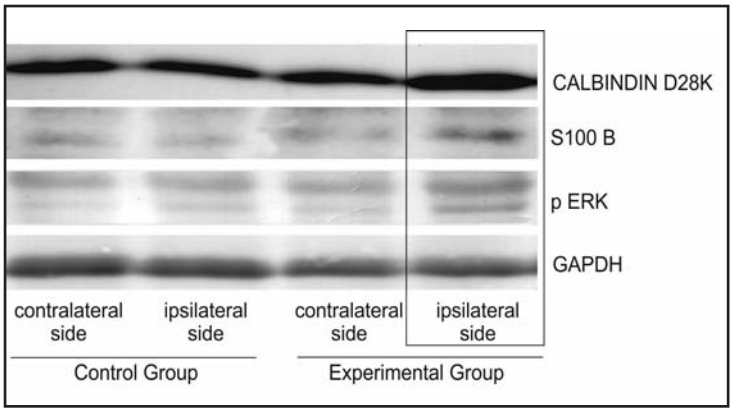

Fig. 3: Western Blot analysis showed an increase in the amount of Calbindin D28K, S100b and pERK proteins on the ipsilateral side of the experimental group 7 days after periapical inflammation. GAPDH was used as a loading control.

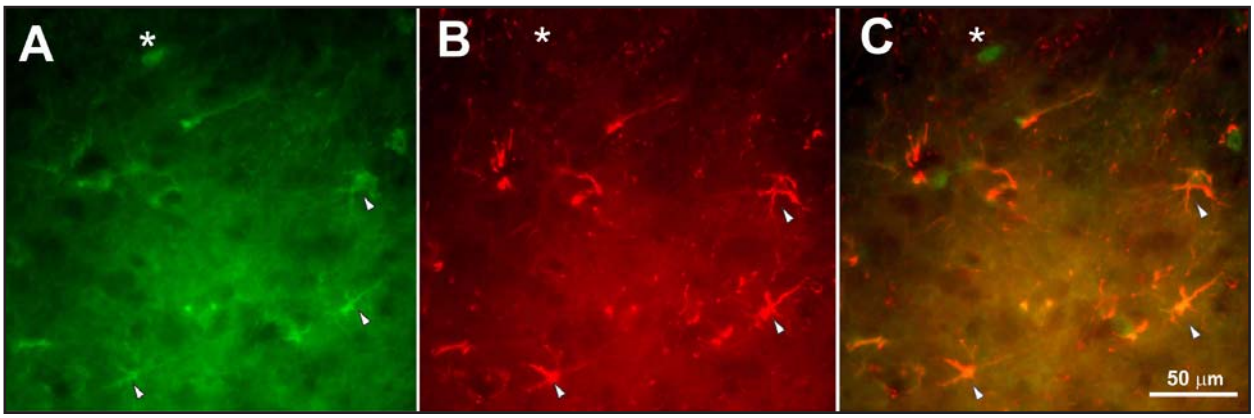

Fig. 4: Double immunofluorescence of S100b and pERK. A: pERK expression in cells of the superficial laminae (arrowheads). B: S100b expression in the cell body and processes of astrocytes (arrowheads). C: double labeled for pERK (green) and S100b (red). Note the presence of cells with different astrocyte morphology in laminae I of ipsilateral Vc. It presents pERK expression but it is not S100b IR cell (asterisk). Scale bar= 50um.

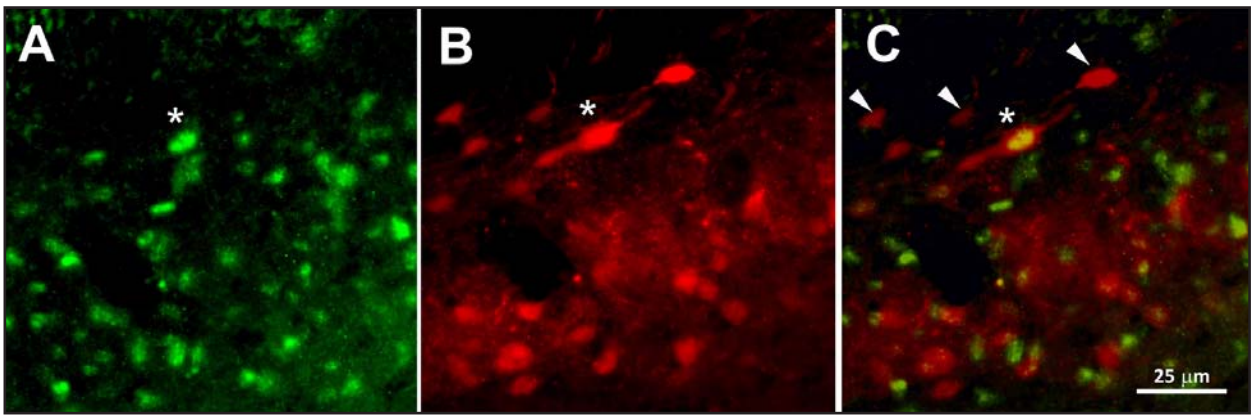

Fig. 5: Double immunofluorescence of pERK and Calbindin D28K in the ipsilateral side of the Vc in the experimental group. A: pERK expression in cells of the lamina I B: Calbindin D28K expression in neurons of superficial laminae. C: Double labeling with pERK (green) and Calbindin D28k (red). Note that CB D28k and pERK colocalize in fusiform neurons in laminae I of ipsilateral Vc at 7 days. Other neurons only express Calbindin D28k (arrowheads) Scale bar=25um.

express S100b (Fig. 4 C asterisk). Double immunofluorescence labeling with pERK and Calbindin D28K showed that these proteins colocalize in fusiform neurons presents in lamina I of the ipsilateral Vc at seven days after the peripheral injury. (Fig. 5 C asterisk).

DISCUSSION

Periapical inflammation is a common problem in dental practice, especially related to endodontic treatment, because canal instrumentation or root canal sealers frequently induce an inflammatory reaction in the periodontal ligament (apical periodontitis)[23,24]. It has been demonstrated that ZOE-based sealers are direct activators of periodontal sensory neurons[23,25]. There is usually greater incidence of post-endodontic pain following treatment of teeth with vital pulp[23]. One possibility is that periapical vital tissue promotes abnormal accumulation of fluid in the interstitium (edema) with consequent compression and activation of mechanoreceptors or free nerve endings[24]. Another possibility is that peripheral inflammatory injuries induce central sensitization, keeping the circuit in a pathological state related to the development of hyperalgesia/allodynia[26,27]. Previous studies observed that rats have responses that are similar to human periapical inflammatory reaction[28,29]. In this study, we considered that the apical inflammation induced by root canal treatment is a good experimental model for studying plasticity in the trigeminal subnucleus caudalis (Vc). However,

there are few studies linking the activation of nociceptive pathway after apical periodontitis.

After peripheral inflammatory reaction, the glia and interneurons of Vc participate in the local modulation of the nociceptive information[1,5,6]. According to this idea, in a previous study, we demonstrated that a pulpal inflammatory process in adult rats generated plastic changes in S100b positive astrocytes, in the ipsilateral side of Vc. On this side, the expression of protein was significantly greater than in the control animals, which displayed low expression of S100b, suggesting its role in pulpal nociceptive pathway[26]. The calcium binding protein S100b is synthesized and released by activated astrocytes and is involved in the regulation of intracellular free calcium or calcium homeostasis and in the activation of some kinases[10,12].

Moreover, Calbindin D28K (CB), another member of the calcium-binding protein family, is expressed in neurons of medulla and Vc[13]. Most CB positive neurons in the dorsal horn are involved in transmission and modulation of the nociceptive pathway[30]. We have reported different subtypes of Calbindin D28k immunohistochemistry positive neurons in the trigeminal subnucleus caudalis, and the morphology and distribution on the dorsal laminae I of the Vc[31].

In addition, our results show that the experimental model of periapical inflammation induces plastic changes in calcium-binding proteins S100b and calbindin D28K expressed in astrocytes and neurons of ipsilateral subnucleus caudalis, respectively. Moreover, we observed an increase in the number of fusiform cells present in ipsilateral lamina I, considered excitatory projection neurons that would transmit nociceptive information to the thalamus.

On the other hand, pERK is actually involved in the response to potentially harmful, abiotic stress stimuli and considered a fast molecular marker in injury or nociception. pERK has been shown to localize to cytoplasm, nucleus and projections[32]. There is currently evidence that MAPKs expression in neurons or glial cells plays an essential role in the development and maintenance of persistent pain[33,20]. According to these concepts, here we demonstrate that pERK-IR cells were observed mostly in the ipsilateral dorsal horn after experimental apical periodontitis.

It is noteworthy that S100b and Calbindin D28K colocalize with pERK in astrocytes or neuron of Vc, respectively. We did not quantify co-expression of CaBPs and pERK in cells of Vc. Studies reported in the literature found that ERK is phosphorylated in dorsal horn neurons transiently for a period of few hours after stimulus and some weeks later in astrocytes[18,19,14.] However, we demonstrated that Calbindin D28K and pERK colocalize in some fusiform neurons at lamina I of ipsilateral Vc, at a later time-point (7 days) than was described previously. This indicates that some Vc projection neurons presents in lamina I, show long-term pERK activity at 7 days, and could maintain the nociceptive information to thalamus and cortex. This result could be interpreted through the increase of S100b and pERK proteins in astrocytes at the ipsilateral Vc, indicative of glial activation. Astrocytes that respond to alterations in neuronal environments and nanomolar levels of extracellular S100b could be released by activated astrocytes[10] and act on neurons, possibly inducing ERK pathway activation. However, more results are required to confirm this hypothesis.

CONCLUSION

Prevention and management of acute periapical pain is an integral part of endodontic treatment in patients. We find that apical periodontitis stimulus increases S100b, Calbindin D28k and pERK protein expression in astrocytes and neurons of the ipsilateral Vc and suggest that ERK activation in fusiform calbindin neurons in lamina I (nociceptive projection neurons), could prolong neural plasticity and pain sensitization for 7 days.

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REFERENCES

1. Capra NF, Dessem D. Central connections of trigeminal primary afferent neurons: topographical and functional considerations. Crit Rev Oral Biol Med 1992; 4:1-52.
2. Sessle BJ. Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates. Crit Rev Oral Biol Med 2000; 11:57-91.
3. Craig AD. Pain mechanisms: labeled lines versus convergence in central processing. Annu Rev Neurosci 2003; 26:1-30.
4. Guy N, Chalus M, Dallel R, Voisin DL. Both oral and caudal parts of the spinal trigeminal nucleus project to the somatosensory thalamus in the rat. Eur J Neurosci 2005; 21:741-754.
5. Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. Trends Neurosci 2001; 24:450-455.
6. Canzobre MC, Rios H. Nicotinamide adenine dinucleotide phosphate/neuronal nitric oxide synthase-positive neurons in the trigeminal subnucleus caudalis involved in tooth pulp nociception. J Neurosci Res 2011; 89:1478-1488.
7. Miyamoto E. Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. J PharmacolSci 2006; 100:433-442.
8. Nelson MT, Todorovic SM. Is there a role for T-type calcium channels in peripheral and central pain sensitization? Mol Neurobiol 2006; 34:243-248.
9. Latremoliere A, Woolf C.J. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009; 10:895-926.
10. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta 2009; 1793:1008-1022.
11. Brunne B, Zhao S, Derouiche A, Herz J, May P, Frotscher M, Bock,HH. Origin, maturation, and astroglial transformation of secondary radial glial cells in the developing dentate gyrus. Glia 2010; 58:1553-1569.
12. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 2003; 4:517-529.
13. Ichikawa H, Jin HW, Terayama R, Yamaai T, Jacobowitz DM, SugimotoT. Calretinin-containing neurons which co-express parvalbumin and calbindin D-28k in the rat spinal and cranial sensory ganglia; triple immunofluorescence study. Brain Res 2005 Nov 9;1061:118-123.
14. Fukui T, Dai Y, Iwata K, Kamo H, et al. Frequency-dependent ERK phosphorylation in spinal neurons by electric stimulation of the sciatic nerve and the role in electrophysiological activity. Mol Pain. 2007 Jul 16;3:18.
15. Ji RR, Gereau RW 4th, Malcangio M, Strichartz GR. MAP kinase and pain. Brain Res Rev 2009; 60:135-148
16. Cruz CD, Cruz F. The ERK 1 and 2 pathway in the nervous system: from basic aspects to possible clinical applications in pain and visceral dysfunction. Curr Neuropharmacol 2007; 5:244-252.
17. Hasegawa M, Kondo M, Suzuki I, Shimizu N, Sessle BJ, Iwata K. ERK is involved in tooth-pressure-induced Fos expression in Vc neurons. J Dent Res 2012; 91:1141-1146.
18. Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain 2005; 114:149-159.
19. Shimizu K, Asano M, Kitagawa J, Ogiso B, Ren K, Oki H, Matsumoto M, Iwata K. Phosphorylation of Extracellular Signal-Regulated Kinase in medullary and upper cervical cord neurons following noxious tooth pulp stimulation. Brain Res 2006; 1072:99-109.
20. Worsley MA, Allen CE, Billinton A, King AE, Boissonade FM. Chronic tooth pulp inflammation induces persistent expression of phosphorylated ERK (pERK) and phosphorylated p38 (pp38) in trigeminal subnucleus caudalis. Neuroscience 2014; 269:318-330.
21. Gonzalez Aguilar F, De Robertis E. A formalin-perfusion meted for histophysiological study of the central nervous system with the electron microscope. Neurology 1963; 13:758-771.
22. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. Academic Press 2007.
23. Marshall JG, Liesinger AW. Factors associated with endodontic posttreatment pain. J Endod 1993; 19:573-575.
24. Gotler M, Bar-Gil B, Ashkenazi M. Postoperative pain after root canal treatment: a prospective cohort study. Int J Dent 2012; 2012:310467.
25. Ruparel NB, Ruparel SB, Chen PB, Ishikawa B, Diogenes A. Direct effect of endodontic sealers on trigeminal neuronal activity. J Endod 2014; 40:683-687.
26. Canzobre MC, Rios H. Pulpar tooth injury induces plastic changes in S100B positive astroglial cells in the trigeminal subnucleus caudalis. Neurosci Lett 2010;470:71-75.
27. Cao Y, Li K, Fu KY, Xie QF, Chiang CY, Sessle BJ. Central sensitization and MAPKs are involved in occlusal interference-induced facial pain in rats. J Pain 2013; 14:793-807.
28. Murazabal M, Erausquin J, Devoto FH. A study of periapical overfilling root canal treatment in the molar of the rat. Arch.Oral Biol 1966; 11:373-383.
29. Yu SM, Stashenko P. Identification of inflammatory cells in developing rat periapical lesions. J Endod 1987; 13:535-540.
30. Gamboa-Esteves FO, Kaye JC, McWilliam PN, Lima D, Batten TF. Immunohistochemical profiles of spinal lamina I neurones retrogradely labelled from the nucleus tractus solitarii in rat suggest excitatory projections. Neuroscience 2001; 104:523-538.
31. Canzobre MC, Rios H. Experimental endodontics: expression of calbindin in neurons of the trigeminal subnucleus caudalis. Abstract, J Dent Res 2012; 91, 55.
32. Gao YJ, Ji RR. c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury? Open Pain J 2009; 2:11-17.
33. Piao ZG, Cho IH, Park CK, Hong JP, et al. Activation of glia and microglial p38 MAPK in medullary dorsal horn contributes to tactile hypersensitivity following trigeminal sensory nerve injury. Pain 2006; 121:219-231.

Student learning strategies according to years spent studying for a degree in dentistry

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ABSTRACT

Learning strategies are a set of organized, conscious, intentional tasks performed by a student to achieve a learning objective effectively in a given social context. The aim of the present study was to determine the type and frequency of use of different learning strategies among students taking the subject "Comprehensive Clinic II ", which corresponds to the 3rd year of the 6-year general syllabus of the undergraduate course at the School of Dentistry, Buenos Aires University, and to analyze the use of these learning strategies according to the number of years elapsed between each student's admission to dental school and the time he/she took that subject.

Dental students (n=189) filled in the Learning and Study Strategies Inventory (LASSI). Seventy-five percent were female. The tool includes 10 dimensions, organized in 77 items. Responses to each question were recorded using a Likert type scale (5 choices). Total scores were obtained by assigning values to the responses. The students were grouped according to time elapsed from year of admission to dental school to the year in which they took the subject (Institutional Persistence, TI). Statistical analysis included mean and confidence intervals for scores (total and for each domain) and comparisons among TI groups using one-way ANOVA and Tukey's post hoc test. Total score for the sample was 275.3 (71.5% of maximum possible score). There were differences in the use of learning strategies reported by dental students in the tool. Students with shorter institutional persistence times scored higher in the following dimensions: attitude and interest, motivation, self-discipline, willingness, self-testing and reviewing.

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Key words: *dental education; learning strategies; dental students.*

Relación entre estrategias de aprendizaje y permanencia en el ciclo curricular en estudiantes de Odontología

RESUMEN

Las estrategias de aprendizaje son un conjunto organizado, consciente e intencional de lo que hace el estudiante para lograr con eficacia un objetivo de aprendizaje en un contexto social dado. El objetivo de este trabajo fue determinar el tipo y frecuencia de uso de estrategias de aprendizaje en estudiantes del curso de Clínica Integrada II, que corresponde al 3er año del programa general de 6 años de la Carrera de Odontología, y analizar su utilización en función del tiempo de permanencia en la Facultad. Los alumnos (n=189) respondieron el cuestionario "Inventario de Estrategias de Aprendizaje y Estudio" (LASSI). Este instrumento consta de 10 dimensiones organizadas en 77 reactivos. Las respuestas a cada reactivo fueron registradas mediante una escala de Likert (5 opciones). El puntaje total se obtuvo mediante asignación de valores a las respuestas elegidas. Los estudiantes fueron agrupados de acuerdo con el tiempo transcurrido entre el ingreso a la carrera y el la fecha en que cursaron la asignatura elegida. (Permanencia institucional, TI). Procesamiento estadístico: cálculo de media e intervalo de confianza para puntajes (total y por dominio) y comparación entre grupos (PI) mediante ANOVA de un factor y post hoc Tukey. El componente femenino fue 75%. El puntaje total para la muestra fue 275,3 (71,5% del máximo posible). Se comprobaron diferencias en el uso de estrategias de aprendizaje. Los alumnos con menor tiempo de permanencia institucional mostraron puntajes mas altos en las siguientes dimensiones: actitud e interés, motivación, autodisciplina, disponibilidad y autoevaluación / repaso.

Palabras Clave: *educación odontológica; estudiantes de odontología; estrategias de enseñanza.*

INTRODUCTION

Current educational research focuses on students achieving academic performance that will enable them to attain significant learning. This includes cognitive, linguistic, motor and social skills, which can take many forms, expressed in attitudes and behaviors[1].

According to Cabrera et al.[2], it is critical to determine the variables associated with academic performance, since low performance levels in

university students are associated with high drop-out and low persistence-to-degree rates.

Learning strategies are academic procedures applied in a controlled fashion, following a pre-established plan, to attain a set target[3]. They include any thoughts, behaviors and emotions that facilitate the acquisition, understanding, and subsequent transfer of new knowledge and skills. Underlying these strategies are components associated with skill and self-regulation. With regard to affective and motivational states, if there is no interest in learning, learning just does not happen.

The Learning and Study Strategies Inventory (LASSI) is a tool to diagnose students' learning strategies and study methods. It is based on the assessment of thoughts and implicit and explicit behaviors that lead to learning. It can be used to evaluate the intellectual profile of a student, and ultimately, to plan actions aimed at improving student achievement[4].

This diagnostic process enables the identification of weaknesses. Once a diagnosis has been established, the information can be used by the students to re-orient their strategies, and by the teacher to implement actions aimed at improving learning. A timely re-assessment enables outcomes to be measured.

Mayor et al.[5] and Beltrán[6] emphasize the importance of studying the relationship between what is taught and how students learn it, i.e., the content compared to the process. Hence, it is important to identify the predominant learning strategies used by young and adult university students.[7-9]

Table 1: LASSI domains.

ANX	Anxiety and worry about school performance
ATT	Attitude and interest
CON	Concentration and attention to academic tasks
INP	Information processing, acquiring knowledge and reasoning
MOT	Motivation, diligence, self-discipline and willingness to work
SFT	Self-testing, reviewing and preparing for classes
SMI	Selecting main ideas and recognizing important information
STA	Use of support techniques and materials aids
TMT	Use of time management principles for academic tasks
TST	Test strategies and preparing for tests

The aim of this study was to determine the type and frequency of use of different learning strategies among students taking the subject "Comprehensive Clinic II ", which corresponds to the 3rd year of the 6-year general syllabus of the undergraduate course at the School of Dentistry, Buenos Aires University, and to analyze the use of these learning strategies according to the number of years elapsed between each student's admission to dental school and the time he/she took that subject.

MATERIAL AND METHODS

The study was conducted on a selected sample of 3rd year dentistry students at Buenos Aires University. The "Learning and Study Strategies Inventory" (LASSI) was applied. The LASSI consists of a validated inventory of 77 items distributed among 10 domains (Table 1)[10]. Responses were recorded using a Likert-type scale with the following five choices: not at all typical of me, not very typical of me, somewhat typical of me, fairly typical of me, very much typical of me. All participants agreed to participate in the study, and signed a written consent, keeping their anonymity.

The sample (189 students) was divided into 5 groups according to year of admission (from 2013 to 2017). The sample size selected provides 90% statistical power to detect relevant differences between groups.[11]

The socio-demographic profile of the students in each group was determined (Table 2).

Statistical analysis involved:

a) Calculating the score for each response per domain.
b) Calculating scores corresponding to items and domains answered by the students.
c) Comparing mean age between sexes using Student Test for independent samples.
d) Comparing scores corresponding to the 10 dimensions and total score according to year of admission using Analysis of Variance (ANOVA) and Tukey's HSD post hoc test for independent samples.

The null hypothesis was rejected at a significance level of $p < 0.05$.

RESULTS

Statistically significant differences ($p=0.015$) in the ATT (attitude and interest) domain were observed among the students who began their professional

Table 2: Sex per Group and Age.

Year of admission		Sex		Total
		Female	Male	
2013	N %	20 80.0%	5 20.0%	**25** **13.2%**
2014	N %	19 79.2%	5 20.8%	**24** **12.7%**
2015	N %	30 76.9%	9 23.1%	**21** **20.6%**
2016	N %	46 80.7%	11 19.3%	**57** **30.2%**
2017	N %	34 77.3%	10 22.7%	**44** **23.3%**
Total	**N** **%**	**149** **78.8%**	**40** **21.2%**	**189** **100.0%**

Pearson Chi-Square: p=0.990

Age (years)	N	%	Mean	Standard Deviation	Minimum	Maximun
Female	149	78.8%	23.7	3.7	20	56
Male	40	21.2%	24.7	3.9	20	38
Total	**189**	**100.0%**	**23.9**	**3.8**	**20**	**56**

Student Test for independent samples: p=0.163

training in the years 2013, 2016 and 2017.

Statistically significant differences (p=0.006) in the MOT domain (motivation, self-discipline, willingness) were observed among the 2013, 2014, and 2017 groups (Table 3).

Analysis of the TST domain (test strategies) showed statistically significant differences at a value of p=0.001 between groups with longer (2013, 2014 and 2015 groups) and shorter (2016 and 2017 groups) institutional persistence times (Table 3).

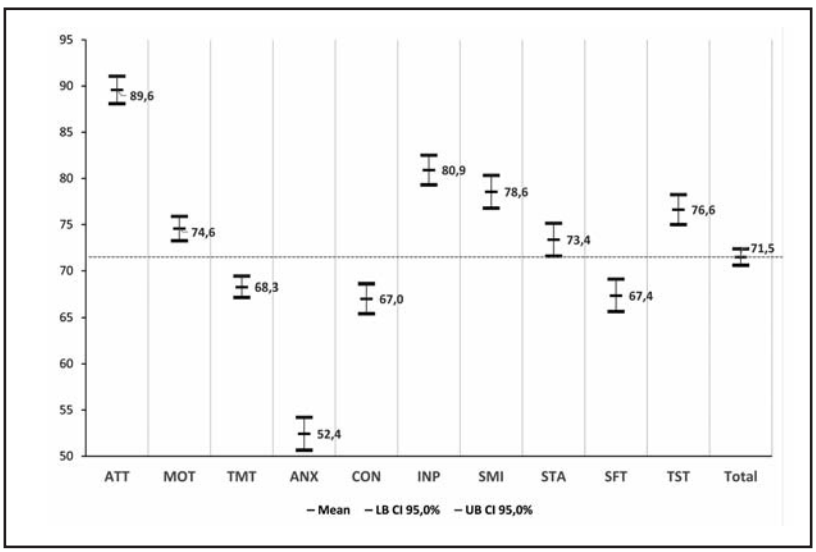

Fig. 1: Domains: mean percentage of the maximum possible score.

The results showed differences in the use of learning strategies among dentistry students, with students with fewer years elapsed from year of admission to time of the subject showing higher scores.

A mean cut-off was established for the mean percentage of the maximum possible score for each domain; mean value was 71.5% (Fig. 1).

In students with fewer years elapsed between admission and taking the subject, the values corresponding to domains ATT (Attitude and Interest), MOT (motivation), INP (information processing), SMI (selecting main ideas), STA (study aids) and TST (test strategies) were higher than the mean percentage of the maximum possible score; whereas scores corresponding to domains TMT (time management), ANX (anxiety and worry about academic performance), CON (concentration

Table 3: Scores for domains per group.

Domains	Year of admission	N	Mean	Std. Dev.	95% CI for mean		% of the maximum score of the mean	p-value
					Lower Bound	Upper Bound		
ATT	2013	25	29.2	3.8	28.2	30.2	83.5	0.015
	2014	24	31.3	3.6	29.7	32.8	89.3	
	2015	39	31.1	4.5	29.6	32.5	88.8	
	2016	57	32.0	2.6	31.3	32.7	91.3	
	2017	44	32.1	2.7	31.3	32.9	91.6	
	Total	**189**	**31.4**	**3.6**	**30.8**	**31.9**	**89.6**	
MOT	2013	25	27.6	3.2	26.9	28.3	68.9	0.006
	2014	24	30.0	3.5	28.5	31.5	75.0	
	2015	39	29.3	3.1	28.3	30.3	73.3	
	2016	57	30.6	3.2	29.8	31.5	76.5	
	2017	44	30.5	3.6	29.4	31.6	76.2	
	Total	**189**	**29.8**	**3.7**	**29.3**	**30.4**	**74.6**	
TST	2013	25	28.0	5.1	25.9	30.1	70.0	0.001
	2014	24	29.5	5.8	27.0	31.9	73.6	
	2015	39	29.4	4.4	28.0	30.8	73.5	
	2016	57	32.0	3.7	31.0	33.0	80.0	
	2017	44	32.2	3.4	31.1	33.2	80.4	
	Total	**189**	**30.7**	**4.5**	**30.0**	**31.3**	**76.6**	

and attention), and SFT (self-testing and reviewing) were lower than the mean percentage of the maximum possible score (Fig. 1)

The highest value corresponded to the ATT domain (attitude and interest), with a mean percentage of the maximum possible score of 89.6. The lowest value, 52.4, corresponded to the ANX domain (anxiety and worry about academic performance).

DISCUSSION

Analysis according to demographic variables

In agreement with previous studies[12] in a population with similar socio-demographic characteristics, our results showed significant differences between male and female participants.

At present, strategic learning presents as a need of the community for information and knowledge, and has a significant impact on academic performance.[13-16]

Modern educational research increasingly assigns importance to academic success and failure at universities. There is consensus on their multifactorial causes, including inadequate learning strategies. Reliable tools are therefore needed to evaluate learning strategies. In dental degree courses, it is important to analyze the teaching-learning process.

Many different learning problems can impact students at a given time. There is evidence showing that one of these is not knowing how to learn, which implies that most students do not use adequate strategies to achieve significant learning.[17]

Association between learning strategies and LASSI domains

- ***Attitude and Interest:*** Students' overall attitudes to studying and their motivation to achieve academic success have an impact on their diligence when studying, particularly in autonomous situations when they must study alone. Our results showed significant differences among groups according to year of admission, and scores were lower among students with longer times elapsed between admission and taking the subject, which could be explained by a decrease in interest in achieving the expected goal (graduation).

- ***Motivation and Self-discipline:*** This domain measures students' overall motivation to achieve academic success. However, although the level of general motivation is important, so is the student's motivation to perform specific tasks related to achievement. Students who score low on this measure need to work on goal-setting, perhaps at the more global levels on the attitude scale, but certainly at the more specific level of individual tasks and assignments. The highest score in this domain corresponded to the group with the shortest length of time elapsed between admission and taking the subject, and was significantly higher than in the remaining groups. This would partly explain the delay in taking a 3rd year course among students who began their studies in 2013. In agreement with a 2006 study conducted by Escurra[18] in students of psychology, we found no significant differences in the remaining domains. Huber et al.[19] highlight the importance of creating a conceptual framework that is in line with the cultural group on which the study is to be conducted.

- ***Time Management:*** Managing time effectively is an important strategic factor in learning. Most students have demands on their time and can only advance in their studies if they prepare and follow realistic schedules. Time management was best in the group of students who began studying for the degree in 2017, though the difference compared to the remaining groups was not statistically significant.

- ***Anxiety and Worry about Academic Performance:*** Current conceptions of anxiety emphasize the effects of thought processes and how they affect academic performance. Cognitive worry, an important component of anxiety, is manifested in negative self-referent statements. Our results, below the mean cut-off, involve more cognitive-affective components than learning strategies, which would explain the low level of worry found. Indeed, this was the domain with the lowest value in all study groups.

- ***Concentration:*** The score on this scale measures students' ability to concentrate and direct their attention to their study tasks. Students who score high on this measure are effective at focusing their attention and maintaining a high level of concentration. Students who score low are less successful at avoiding interfering thoughts, emotions, feelings and situations. Techniques for focusing and maintaining concentration help students to implement effective learning strategies and to make learning and studying more effective and efficient. Students with shorter times since beginning their studies had better scores. This was the group of students who were on-schedule with intended times in the general syllabus.

- ***Information Processing:*** Learning is enhanced by the use of elaboration and organization strategies. These strategies help build bridges between what a student knows and what he/she is endeavoring to learn and remember. The results for this domain, above the cut-off line, are probably due to the fact that the curriculum contains a program for reinforcement of previous information, a priority condition that enables the student to organize new knowledge.

- ***Selecting Main Ideas***: Effective and efficient studying requires students to be able to select the important material for in-depth processing. Most lectures, discussions and textbooks contain redundant material and extra examples of what is being taught or presented. Separating the more relevant from the less relevant is a major task. If a student cannot identify the important information, learning will become more complicated due to the large quantity of material to be learned. The lack of this skill will also increase the student's likelihood of not having sufficient time to study all the material to be covered. Student scores on this scale measure their skills at selecting the important information which they must focus on for further study in the classroom or autonomously. Students who had spent fewer years studying for the degree scored high in this domain. This may be due to better recall of study methods acquired in high school for selecting information.

- ***Study Aids***: Students need to know how to use study aids created by other people and how to create their own. Authors usually use headings, special fonts, blank spaces, special marks and summaries to help students learn from written

materials. However, these elements and aids will only be useful to students if they know how to recognize and use them. It is also important for students to know how to create their own study aids using methods such as diagrams, text underlining, graphs and summaries. Other supplementary activities that support and enhance learning include activities such as participating in revision groups or comparing notes with other students to confirm that their own are complete and accurate.

The result obtained for this item would be explained by one of the weaknesses of the LASSI, which is that it does not reflect new technologies used by undergraduate students.

- ***Self-testing***: Reviewing and testing level of understanding is important for acquiring knowledge and monitoring comprehension. Both these strategies support and contribute to meaningful learning and effective performance. Without them, learning could be incomplete, or errors might go undetected. Reviewing and self-testing contribute to knowledge consolidation and integration across topics. Using mental reviews, reviewing class notes and texts, thinking up questions to guide reading or help prepare for a test are all important methods for checking understanding, consolidating new knowledge, and determining the need for additional studying. Our study found low values in both study groups. This may be because the students complying with intended timing for the degree and who have good scores in other domains have little available time for reviewing. A more profound analysis of this item may lead to a change in the general number of hours in the degree course.
- ***Test Strategies*** (Preparing for tests): Preparing for tests includes knowledge on the type of test, such as whether it will be a short-answer or a multiple-choice test, or whether the student will be required to provide a description, or apply concepts, principles and ideas. Test preparation also includes knowing methods for studying and learning the material in a way that will facilitate memorization and use of the material. In other words, test-taking strategies include knowing the characteristics of the test and how to create an effective test-taking plan. The results obtained in the present study are in keeping with findings reported by Olaussen et al.[20] in a study conducted in Norway in 2012.

In this line of research, it would be interesting to include studies on teaching methodology applied to the different times of the degree course analyzed herein.

CONCLUSION

This study found differences in the use of learning strategies reported by students of dentistry through the LASSI. Students with fewer years elapsed between admission and taking the subject scored higher in the following dimensions: attitude and interest, motivation, self-discipline and willingness, self-testing and reviewing.

Further studies are needed to confirm these findings before the tool can be adopted or rejected for reliable use in the broader educational context in the dentistry degree at Buenos Aires University.

Some of the most important aspects of education are student intent, planning and effort. These aspects have not received sufficient attention, and members of the faculty could modify their pedagogic practices and use resources that adjust to student profiles.

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REFERENCES

1. Schunk D, Learning theories and Educational Perspective. North Carolina, USA. Pearson 6º Ed. 2012.
2. Cabrera A, Deil-Amen R, Prabhu R, Terenzini PT, Lee C. Franklin RE Jr. Increasing the college preparedness of at risk students. Journal of Latinos and Education 2006; 5: 79- 97. URL: https://www.tandfonline.com/doi/abs/10.1207/s1532771xjle0502_2
3. Dugan R. Examining the construct validity of academic self-regulation (SASR) using the survey of academic self-regulation. Abstr. Humanities and Social Sciences 2008; 68 (8-A), 3277. URL: https://books.google.com.ar/books/about/Examining_the_Construct_Validity_of_Acad.html?id=y12qwgEACAAJ&redir_esc=y
4. Weinstein C, Palmer D, Acee T. User's Manual Learning and Study Strategies Inventory. Third Edition. FL, USA. H&H Publishing Company 2016; 1-55.
5. Mayor J, Suengas A, González J. Estrategias metacognitivas. Aprender a aprender y aprender a pensar. Madrid, España. Síntesis 1993.
6. Beltran J. Estrategias de Aprendizaje. En C Jenovard y J Beltran Psicología de la Instrucción. Variable y procesos básicos. Madrid, España. Eudema 1996.
7. Quesada R. Como planear la enseñanza estratégica. Mexico. Mexico Limusa.2001
8. Zabalza MA. La enseñanza universitaria, el escenario y sus protagonistas. Madrid, España. Narcea 2002.
9. Zabalza MA. Competencias docentes del profesorado universitario. Madrid, España. Narcea 2003.
10. Stover JB, Uriel F, Fernández Liporace M. Inventario de Estrategias de Aprendizaje y Estudio: Análisis Psicométricos de una Versión Abreviada. Rev. Argentina Ciencias del Comportamiento (RACC) 2012, 4: 4-12. URL: https://revistas.unc.edu.ar/index.php/racc/article/ view/5162
11. Faul F, Erdfelder E, Lang AG, Buchner A. A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 2007; 39, 175-191. URL: https://link.springer.com/article/10.3758/BF03193146
12. Argentieri A. Basterrechea L. Salgado P. Gomez Diz W. Bordoni N. Estrategias de aprendizaje en estudiantes universitarios. Sociedad Argentina de Investigación Odontológica. Res 4 2016 Abstr. URL: http://www.saio.org.ar/new/descargas/Libro_XLIX_Reunion_Cientifica_Anual.pdf
13. Hernández J, Pozo C. El fracaso académico en la Universidad: Diseño de un sistema de evaluación y detección temprana. Psicología Educativa 1999; 5:27-40. URL: http://web.ebscohost.com/ehost/results?vid=3&hid=2&sid=587899c8-abb4-450b-b739-b8c4e88709e1%40sessionmgr107
14. Massone A, González G. Análisis del uso de estrategias cognitivas de aprendizaje, en estudiantes de noveno año de educación general básica. Revista Iberoamericana de Educación 2003; 33. URL: https://www.researchgate.net/publication/237607379_
15. Rosário P, Mourão R, Núñez JC, González Pienda J, Solano P, Valle A. Eficacia de un programa instruccional para la mejora de procesos y estrategias de aprendizaje en la enseñanza superior. Psicothema 2007; 19: 422-427.
16. Yip MC, Chung OL. Relation of study strategies to the academic performance of Hong Kong. University students. Psychological Reports 2002; 90: 338-340. URL: https://journals.sagepub.com/doi/10.2466/pr0.2002.90.1.338
17. Nisbet J. Schucksmith J. Estrategias de aprendizaje. Madrid, España. Ed Santillan, 1986.
18. Escurra Mayaute LM. Análisis psicométrico del Inventario de Estrategias de Aprendizaje y Estudio en estudiantes universitarios de psicología de Lima metropolitana. Persona 2006; 127-170. URL: http://repositorio.ulima.edu.pe/handle/ulima/2303
19. Huber S. Bassen A. Towards a sustainability reporting guideline in higher education. Int J Sustainability Higher Education 2018; 19: 2. 218-232. DOI: 10.1108/IJSHE-06-2016-0108
20. Olaussen B. Braten I. Students use of strategies for self-regulated learning: Cross cultural perspectives. Scand J Educal Res, 43:4, 409-432, DOI: 10.1080/0031383990430405

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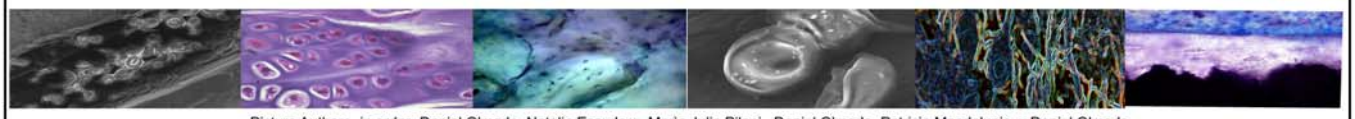

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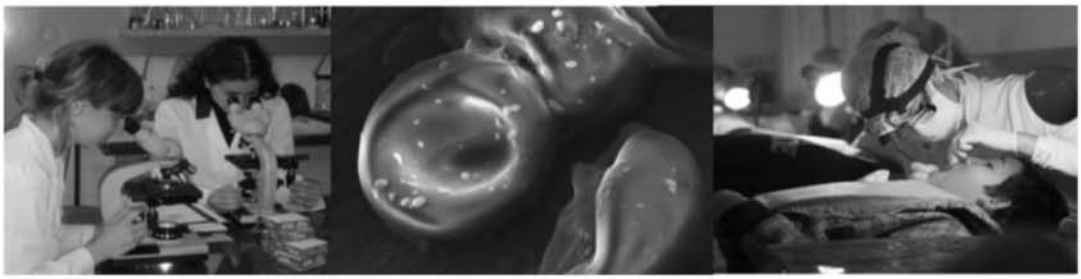

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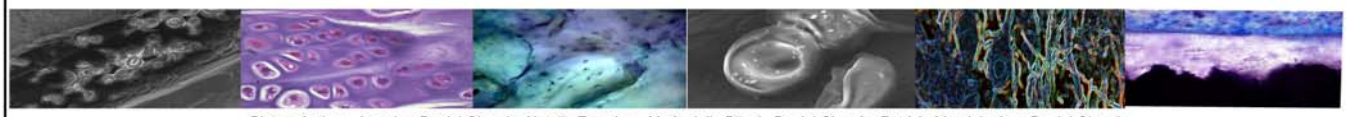

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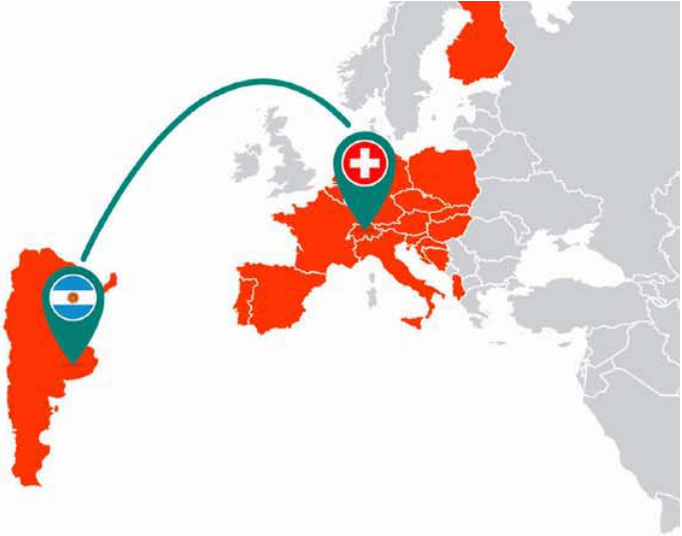

Con más de 50 años de tradición y liderazgo en Suiza, **elmex** trae a Argentina una nueva línea de protección y fortalecimiento del esmalte dental, con beneficios científicamente comprobados.

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

A la luz de esto, podemos hacer una comparación entre el uso de la línea elmex y el uso del protector solar en la rutina diaria: elmex es para los dientes como el protector solar para la piel, un aliado preventivo a los impactos del medio ambiente.

Conozca la línea completa:

elmex®

Previene la caries dental y el desgaste temprano de los dientes[1]

elmex® SENSITIVE

Alivio inmediato y protección prolongada[2] contra la sensibilidad.

elmex® ULTRA SOFT

Delicado, preciso y eficiente

+5500 PUNTAS ULTRAFINAS

1. Contra caries y con el uso continuo del régimen completo.
2. Cuando es usado según lo indicado en la caja y con el uso continuo del régimen completo.

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