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Contact us
Cátedra de Anatomía Patológica, Facultad de Odontología, Universidad de Buenos Aires.
M.T. de Alvear 2142 (C1122AAH) Buenos Aires, Argentina.
http://www.actaodontologicalat.com/contacto.html
actaodontologicalat@gmail.com

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Subgingival biofilm colonization by *Candida albicans* and *Candida dubliniensis* in patients living with HIV from Buenos Aires, Argentina

Verónica Á Dubois[1,2], Pablo A Salgado[1,2,3], Susana L Molgatini[1,2], Laura A Gliosca[1,2]

1. *Universidad de Buenos Aires - Facultad de Odontología - Cátedra de Microbiología y Parasitología, Laboratorio de Diagnóstico Microbiológico y Molecular. Buenos Aires - Argentina.*
2. *Universidad de Buenos Aires - Facultad de Odontología - Instituto de Investigaciones en Salud Pública (IISAP). Buenos Aires - Argentina.*
3. *Universidad de Buenos Aires - Facultad de Odontología - Cátedra de Odontología Preventiva y Comunitaria, Buenos Aires - Argentina.*

ABSTRACT

Oropharyngeal candidiasis (OC) is common among people living with HIV (PLWH). Persistent colonization of oral epithelial surfaces serves as an ecological niche for opportunistic pathogens and is a significant predisposing factor for OC development in PLWH. Mucosal colonization can lead to biofilm formation, directly impacting oral epithelium. ***Aim:*** *To assess Candida albicans and Candida dubliniensis colonization in subgingival biofilms of people living with HIV (PLWH) and undergoing antiretroviral therapy (ART).* ***Materials and Method:*** *A sample of 51 PLWH who were receiving ART was studied, focusing on dental and periodontal parameters. Subgingival biofilm and mucosa samples were collected, and Candida spp. were identified using molecular techniques.* ***Results:*** *Men (average age: 41.11 ± 8.63) predominated. The main cause of HIV was sexual transmission. Fungal-related opportunistic diseases were observed in 18 patients, and LT CD4 counts were evaluated. A total 255 samples were collected, including 204 from gingivo-periodontal sites and 51 from oral mucosa. Candida spp. was detected in 55% of patients, with particular distribution patterns. Positive Candida spp. presence correlated with clinical attachment level and HIV treatments. Microscopic identification revealed the presence of hyphae at the time of microbiological sample collection. Molecular identification confirmed 16 Candida albicans and 36 Candida dubliniensis isolates, challenging their diagnostic importance.* ***Conclusions:*** *The presence of yeast hyphae/pseudohyphae in subgingival biofilms indicates their role in gingivo-periodontal disease dysbiosis. PLWH in this Argentine region face challenges including limited access to healthcare. The study underscores the need for early oral health intervention, emphasizing the diagnostic significance of Candida.*

Key Words: *Candida dubliniensis - Candida albicans - subgingival biofilm - periodontitis - HIV - oral colonization.*

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Corresponding Author:
Laura A. Gliosca
laura.gliosca@odontologia.uba.ar

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Colonización de *biofilm* subgingival por *Candida albicans* y *Candida dubliniensis* en pacientes que conviven con el VIH de Buenos Aires, Argentina

RESUMEN

La candidiasis orofaríngea (CO) es común en pacientes que viven con HIV (PVVS). La colonización persistente de las superficies epiteliales orales sirve como reservorio para patógenos oportunistas. Esta colonización es un factor predisponente para el desarrollo de la CO en PVVS. La colonización mucosa puede llevar a la formación de biofilm, impactando directamente en el epitelio oral. ***Objetivo:*** *Evaluar la colonización por Candida albicans y Candida dubliniensis en biofilm subgingival de pacientes que viven con VIH (PVVS) bajo tratamiento antirretroviral.* ***Materiales y Método:*** *Se estudió una cohorte de 51 PVVS bajo TAR, centrándose en parámetros dentales y periodontales. Se recolectaron muestras de biofilm subgingival y mucosa, identificando Candida spp. mediante técnicas moleculares.* ***Resultados:*** *Predominaron hombres con edad promedio de 41,11 ± 8,63, siendo la transmisión sexual la principal vía. Se observaron enfermedades oportunistas relacionadas con hongos en 18 pacientes, evaluándose los recuentos de CD4 a largo plazo. En total, se recopilaron 255 muestras, incluyendo 204 de sitios gingivo-periodontales y 51 de mucosa oral. Se detectó Candida spp. en el 55% de los pacientes, con patrones de distribución particular. La presencia positiva de Candida spp. se correlacionó con nivel de inserción clínica y TAR. La identificación microscópica reveló la presencia de hifas al momento de la toma de muestras microbiológicas. La identificación molecular confirmó 16 aislamientos de Candida albicans y 36 de Candida dubliniensis, desafiando su importancia diagnóstica.* ***Conclusiones:*** *La presencia de hifas/pseudohifas de levaduras en biofilm subgingival indica su papel en la disbiosis de enfermedades gingivo-periodontales. Los PVVS en esta región argentina enfrentan desafíos debido al acceso médico limitado. El estudio destaca la necesidad de intervenciones tempranas en la salud oral, enfatizando la importancia diagnóstica de Candida spp.*

Palabras Clave: *Candida dubliniensis - Candida albicans - biofilm subgingival - periodontitis - VIH - colonización oral.*

INTRODUCTION

Oropharyngeal candidiasis (OC) is prevalent among people living with HIV (PLWH). Most of these infections arise from *Candida albicans (C. albicans)*, although a global rise in cases attributed to *non-albicans Candida* species in recent years has been reported. OC is an immunosuppression indicator, notably prevalent in patients with a Lymphocyte T CD4 count below 200 cells/ml. OC manifests in approximately 95% of people during stage 1 HIV. With the introduction of antiretroviral therapy (ART), there has been a significant reduction in the incidence of HIV-related oral pathologies, including OC, leading to a decline in systemic disease cases[2]. Nevertheless, the persistent colonization of epithelial surfaces in the oral cavity, which serve as the natural ecological niche for this opportunistic pathogen, remains a robust predisposing factor for the development of OC in PLWH, even if they are under stable treatment with a combination of ART. It is estimated that 90% of infected patients will experience OC at some point during the evolution of HIV infection[1,3,4]. Colonization of mucosal surfaces can give rise to the formation of biofilms, exerting direct impact on the oral epithelium. Biofilm formation induces alterations in cell structure and function, affecting innate immunity and creating an environment conducive to colonization and infection by commensal and pathogenic microorganisms[5]. The virulence of these microorganisms depends on the gene expression associated with their morphotypes. It is important to note that although such colonization does not invariably lead to candidiasis, it is a prerequisite for its onset[4,6-9]. This situation persists despite ART and may be correlated with the residual presence of HIV in mucosal macrophages and dendritic cells[10,11].

There is a broad range of risk factors that predispose individuals to colonization, including smoking, diabetes mellitus, use of oral prostheses, advanced age, use of antibiotics, reduced salivary flow, dietary habits, nutritional status, inadequate oral hygiene, and immunosuppression such as HIV, among others[6,8,12].

Because *Candida dubliniensis (C. dubliniensis)* has become increasingly prevalent in various countries, studies have been conducted to compare its virulence to that of *C. albicans*. *C. dubliniensis* was initially characterized in 1995 by Sullivan et al.[13], and isolated mainly from the oral cavities of PLWH. *C. dubliniensis* was first identified in the subgingival biofilm of periodontal lesions in PLWH in 2001[13,14]. It is known that *C. dubliniensis* can be isolated from individuals with different local or systemic pathologies, and may even colonize the subgingival biofilm of healthy people. In the last five years, *C. dubliniensis* has been associated with the emergence of more recent complications such as meningitis, endocarditis, and recurrent oral and respiratory diseases, particularly in immunocompromised patients[15-17].

The aim of this study was to assess *C. albicans* and *C. dubliniensis* colonization in subgingival biofilm of patients living with HIV (PLWH) undergoing antiretroviral therapy (ART).

MATERIALS AND METHOD

This was an analytical, descriptive, prospective, cross-sectional study. A non-probabilistic sequential sample of PLWH seeking care through spontaneous demand was established at three healthcare facilities in Buenos Aires City (Clínica para la atención de pacientes de alto riesgo (CLAPAR I) University of Buenos Aires, Faculty of Dentistry; the Hospital General de Agudos Dr. Juan A. Fernández, and Hospital de Enfermedades Infecciosas Francisco Javier Muñiz"). The study was approved by the Ethics Committee of University of Buenos Aires, Faculty of Dentistry, with protocol number 002/2019-CETICA-FOUBA). Procedures were carried out after patients were informed about the protocol and voluntarily signed informed consent,

The study included PLWH aged 18 to 60 years with detectable HIV viral load determined by qPCR (Real-Time PCR) using the HIV-1 viral load assay (version 3.0 Abbott), the most recent LT CD4 count, who had been on highly active antiretroviral therapy (HAART) for at least six months before study enrollment. Exclusion criteria were systemic illnesses unrelated to HIV infection, treatment with antibiotics, antifungals, or oral antiseptics within the three months preceding microbiological tests, oropharyngeal or systemic candidiasis lesions, and receiving dental care from other service providers.

Composition of the study population

The study population comprised 59 individuals who underwent a medical history assessment, including the recording of socio-demographic data, prior

opportunistic diseases, and hospitalization history. The patients were evaluated by a calibrated dental professional (Kappa ≥0.80). Dental status and periodontal clinical parameters were documented using Marquis-type periodontal probes at six sites per tooth, considering probing depth (PD) in millimeters, clinical attachment level (CAL) in millimeters, and bleeding on probing (BOP) classified as positive or negative. Radiographic periodontal parameters were also examined[18,19].

Sample collection

Samples were collected according to the following protocol:

1. Mouth rinse with sterile distilled water for one minute.
2. Oral cavity inspection with proper illumination and a dental mirror.
3. Swabbing of all mucosal surfaces using a sterile Dacron swab.
4. Partial isolation of the targeted periodontal site with a sterile cotton roll and suction.
5. Identification of 4 periodontal sites based on clinical-radiographic criteria.
6. Removal of supragingival biofilm (if present) with a Gracey curette (Hu-Friedy) corresponding to the tooth number.
7. Placing sterile paper points (Meta, Biomed) #30-35 into the gingival sulcus or periodontal pocket.
8. Recording periodontal conditions.
9. Collection of subgingival biofilm from the epithelial pocket for smearing on a sterile microscope slide.

Each sample was placed in a tube containing Reduced Transport Fluid (RTF) and transported refrigerated to the laboratory under biosecurity conditions[20] (Fig. 1).

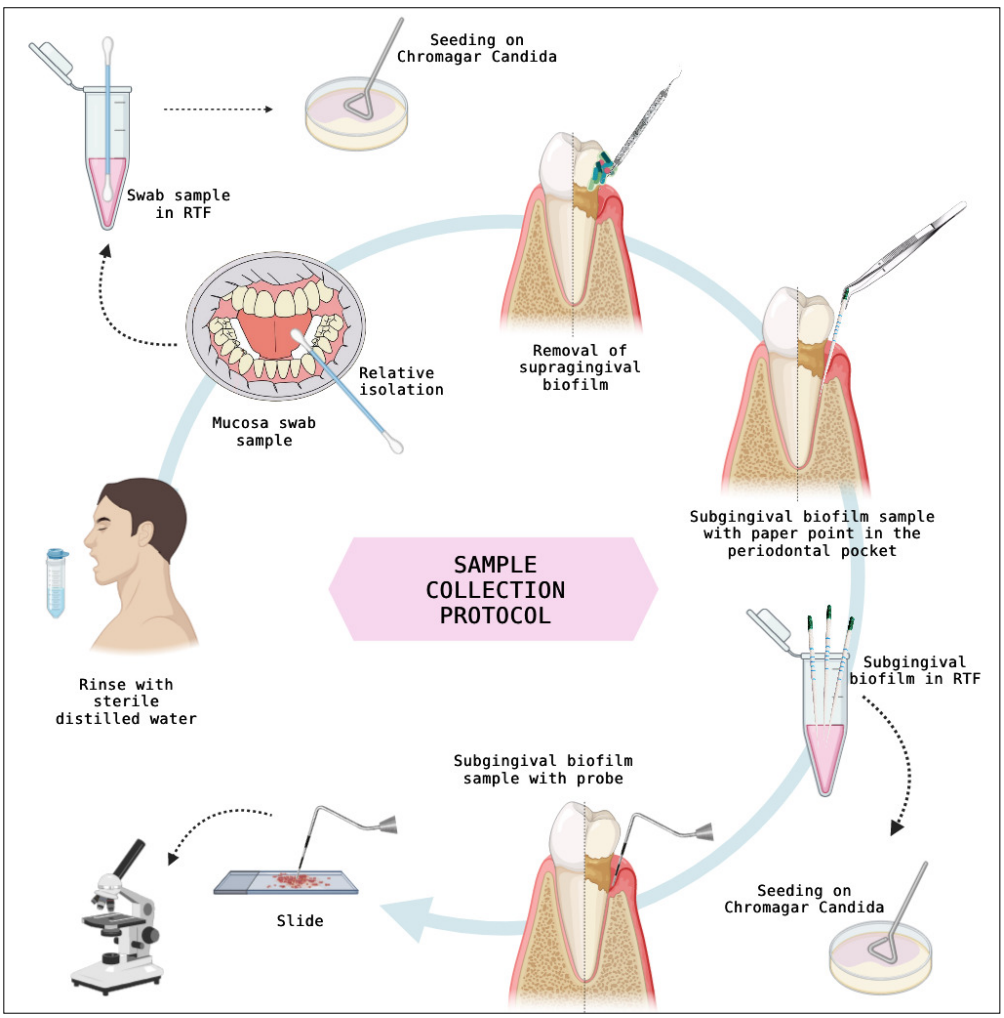

Fig. 1: Sample collection protocol. Created with BioRender.com

Molecular identification of *Candida* species

Each sample was vortexed for one minute. Subsequently, 100 µl of the sample was plated on Chromagar *Candida*® (CHROMagar, France) and incubated at 37 ºC for 48 hours under aerobic conditions, facilitating the presumptive identification of *Candida* spp. The colonies obtained were sub-cultured on Sabouraud Dextrose Agar (SDA) at 37 ºC for 24 hours under aerobic conditions to conduct phenotypic presumptive identification tests[21].

Genomic DNA extraction

Fresh cultures were incubated for 24 hours on Yeast Peptone Dextrose agar (YPD) to extract genomic DNA (gDNA) from a colony of each isolate. Two extraction methods were employed: the commercial Yeast Genomic DNA Kit (Zymo Research, USA) following the manufacturer's instructions, and the commercial Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) with some modifications. The gDNA was quantified in triplicate using the Cytation 3 Cell Imaging multi-mode reader (BioTek, USA)[21].

qPCR Multiplex of ITS regions

Two species-specific primers derived from the internal transcribed spacer (ITS), ITS-1, 5.8S rRNA, and ITS-2 regions of the ribosomal DNA (rDNA) were used with some modifications[7]. The amplification process was assessed using CFX Maestro Software (Bio-Rad Life Science, USA) through melting curve analysis, with a temperature of 86 ºC (±0.5) for C. *albicans* and 82 ºC (±0.5) for C. *dubliniensis*[7].

Multiplex PCR of HWP1 gene

To optimize detection strategies, the HWP1 gene was amplified following the amplification protocol described by Dubois et al. 2023[21], with some modifications, in an Aeris-BG096 thermocycler (Esco Scientific, Singapore). The final amplification products were separated by electrophoresis using 1.3% agarose gel in 1X Tris-acetate-EDTA (TAE) buffer with the addition of 1.5 µl of GelGreen® (Biotium, USA), and visualized using the GelDoc XR+ Gel Documentation System (Bio-Rad Life Science, USA).

The presence of different alleles for the HWP1 gene can be determined[22]. *C. albicans* ATCC 10231 and *C. dubliniensis* CD36 were used as positive reference controls, and *Candida parapsilosis* ATCC 22019 as a negative control[21].

A descriptive statistical analysis was conducted, determining relative frequency expressed in percentages, means/medians, standard deviations and confidence intervals for quantitative variables. Student's t-test was applied to compare quantitative variables. For categorical variables, the chi-square statistic (χ^2) was used. Differences were considered significant when $p<0.05$. The tests were conducted using the software SPSS (v.29.0) and Google Sheets implemented in Google Drive 2023.

RESULTS

In the present study, an initial sample of 59 PLWH undergoing HAART was evaluated. Eight patients were excluded due to unclear medical history regarding the mode of transmission or lack of data. Consequently, the final study population was reduced to 51 patients, representing 86.44% of the initially considered total.

The final study population consisted of 70.6 % males, and average age was 41 ± 8. According to the collected medical history data, the primary mode of HIV transmission was sexual. Concerning the history of HIV-related hospitalizations, most patients had not been hospitalized before. Regarding past opportunistic diseases, 18 patients had had fungal-related opportunistic diseases, of whom 6 had records of antifungal treatment in their medical history, with 1 treated solely with fluconazole, 1 with nystatin and fluconazole, and 1 with fluconazole and voriconazole. The antifungal type was unknown for the remaining cases.

LT CD4 count was evaluated with two cutoff values, 200 cells/ml and 400 cells/ml or higher. Notably, one patient's value was unknown, so the LT CD4 data correspond to the analysis of 50 patients. Details are presented in Table 1.

In relation to HAART treatment, only 3 patients were exclusively taking non-nucleoside analogs. The rest were taking a combination of two or three ART agents. Notably, no patient was exclusively on nucleoside analogs or protease inhibitors (Fig. 2) (Table 2).

Characteristics and composition of the selected sample

A total of 255 samples were collected from the 51 included patients, of which 204 corresponded to

Table 1. Medical and socio-demographic data.

Patients (n=51)		Frequency	%
Gender	Female	10	19.6%
	Male	36	70.6%
	Non-binary	5	9.8%
Nationality	Argentine	46	90.2%
	Foreign	5	9.8%
Residence	CABA	41	80.4%
	AMBA	9	17.6%
	Outside AMBA	1	2%
Employment	Employees	18	35.3%
	Self-employed	17	33.3%
	Students	3	5.9%
	Retirees	1	2%
	Unemployed	10	19.6%
	No response	2	3.9%
Health insurance	Yes	12	23.5%
	No	39	76.5%
HIV transmission	Sexual	46	90.2%
	PDUP	4	7.8%
	Vertical	1	2%
HIV hospitalization history	Yes	17	33.3%
	No	34	66.7%
Opportunistic diseases	Yes	18	35.3%
	No	33	64.7%
Co-infection TB	Yes	1	2%
	No	50	98%
Co-infection HBV	Yes	5	9.8%
	No	46	90.2%
Co-infection HBC	Yes	3	5.9%
	No	48	94.1%
Viral load	≤50	43	84.3%
	> 50	8	15.7%
Patients (n=50)		**Frequency**	**%**
LT CD4	≤200	6	12%
	>200	44	88%
LT CD4	≤400	23	46%
	>400	27	54%

CABA: Buenos Aires Autonomous City, AMBA: Buenos Aires Metropolitan area, PDUP: Parenteral Drug User, TB: Tuberculosis, HBV: Hepatitis B, HCV: Hepatitis C.

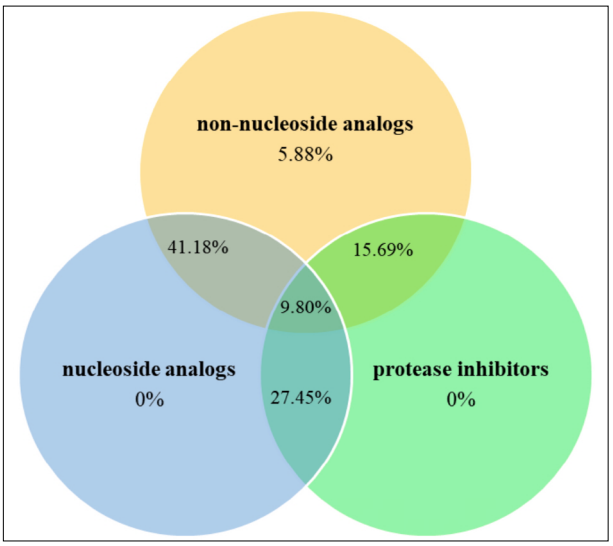

Fig. 2: Percentage and combination of HAART treatments in patients.

Table 2. Number of patients (N) receiving the specified antiretroviral (ART) in the HAART combination.

HAART	Type of ART	N
Nucleoside analogs	ZIDOVUDINE (AZT)	12
	LAMIVUDINE (3TC)	34
	ABACAVIR	14
	DIDANOSINE (ddi)	1
Non-nucleoside analogs	NEVIRAPINE	6
	EFAVIRENZ	17
	EMTRICITABINE (FTC)	9
	TENOFOVIR	18
Protease inhibitors	SAQUINAVIR	1
	LOPINAVIR	10
	RITONAVIR	25
	ATAZANAVIR	7
	FOSAMPRENAVIR	3
	DARUNAVIR	3
	MARAVIROC	1

gingivo-periodontal sites and 51 to oral mucosa samples.

According to the classification of periodontal and peri-implant diseases and conditions, 9 patients (17.65%) were in a state of eubiosis, 5 (9.80%) were diagnosed with gingivitis, and 37 (72.55%) were diagnosed with periodontitis. Among those with periodontitis, 10 (27.03%) were classified as stage I, grade A, 17 (45.95%) as stage II, grade B, and 10 (27.02%) as stage III, grade B.

Regarding the gingivo-periodontal parameters used for subgingival biofilm sampling (n=204), BOP was positive at 121 sites (47.5%), PD > 3 mm at 96 sites

(37.6%), PD ≤ 3 mm at 159 sites (62.4%), CAL > 1 mm at 139 sites (54.5%), and no CAL loss was recorded at 116 sites (45.5%).

Considering *Candida* spp. as the unit of analysis in the 255 collected samples, 28 (55%) patients yielded positive results for the presence of *Candida* spp., representing a total of 82 positive samples collected. Among these, 65/204 (32.4%) were from gingival and periodontal sites, while 17/51 (33.3%) were from mucosal samples.

Different distribution patterns of the samples were observed: yeast was exclusively isolated from the mucosa in one patient (3.6%). In 11 patients (39.3%), it was isolated solely from gingivo-periodontal sites. In 16 (57.1%), presence was detected in both gingivo-periodontal sites and mucosa. In 8 patients (28.6%), *Candida* spp. was isolated from all collected samples, including the 4 gingivo-periodontal sites and the mucosa sample.

Only the presence of *Candida* spp. in gingivo-periodontal sites with negative BOP was statistically significant ($p<0.01$). The association with other parameters is shown in Table 3.

At gingival and periodontal sites, the presence of *Candida* spp. was positive at 34 sites (40.5%) that did not exhibit gingival bleeding on probing (BOP), and this result was statistically significant ($p<0.05$). Regarding probing depth (PD), *Candida* spp. was positive at 26 sites (27.1%) with PD >3 mm and 38 sites (35.2%) with PD ≤ 3 mm. The presence of *Candida* spp. was higher at sites without probing depth. Concerning clinical attachment level (CAL), *Candida* spp. was positive at 39/139 sites (28.1%) where CAL was detected in 25/65 sites (38.5%) without CAL. The finding of *Candida* spp. was higher at sites with CAL >1 mm (Table 3).

Analysis of HIV-related variables, focusing on the HAART treatment in the 28 patients positive for *Candida* spp., showed that 3 (10.71%) were being treated with a combination of nucleoside analogs, non-nucleoside analogs and protease inhibitors, 8 (28.57%) with non-nucleoside and nucleoside analogs, 7 (25.0%) with protease inhibitors and non-nucleoside analogs, and 10 (35.72%) with protease inhibitors and nucleoside analogs.

Regarding viral load, *Candida* spp. was detected in 22 patients (78.58%) with viral load ≤50 copies/ml and in 6 patients (21.42%) with viral load > 50 copies/ml.

Considering the LT CD4 count and excluding the patient with unknown CD4 value, *Candida* spp. was detected in 13 patients (48.15%) with ≤400 cells/ml and 14 patients (51.85%) with >400 cells/ml.

Concerning the CD4 count cutoff at 200 cells/ml, *Candida* spp. was found in 24 patients (88.89%) with values ≥200 cells/ml, and 3 patients with values <200 cells/ml. Among the latter three patients, 2 were in eubiosis, and one had periodontitis. Three other patients in similar conditions were negative for *Candida* spp. On the other hand, *Candida* spp. was detected in 6 patients with viral load >50 copies/ml. Only one of them had CD4 <200 cells/ml, viral load >50 copies/ml and periodontitis. Six additional patients with similar results tested negative for *Candida* gender.

The relationship of viral load and CD4 count with opportunistic infections and co-infections

Table 3. Isolations of *Candida* spp. according to gingivo-periodontal parameters.

Gingivo-periodontal sites (n=204)			***Candida* spp.**			
	Positive		Negative			
	Frequency	%	Frequency	%	p value	
BOP	Positive	30	25.0%	90	75.0%	0.019
	Negative	34	**40.5% ***	50	59.5%	
PD	> 3 mm	26	27.1%	70	72.9%	
	≤ 3 mm	38	35.2%	70	64.8%	
CAL	> 1 mm	39	28.1%	100	71.9%	
	≤ 1 mm	25	38.5%	40	61.5%	

BOP: bleeding on probing, PD: probing depth and CAL clinical attachment level. * **p** <0.05.

parameters in PLWH is shown in Tables 4 and 5.

Table 4. Relationship among CD4 count cutoff 200 cells/ml with opportunistic infections, co-infections and Candida spp.

Patients (n= 50)		Lymphocyte T CD4	
	<200 cells/ml	≥ 200 cells/ml	
	Frequency		
Candida spp	Positive	50%	54.5%
	Negative	50%	45.5%
Fungal diseases	Yes	100%	31.8%
	No	0%	68.2%
Co-infection TB	Yes	0%	2.3%
	No	100.%	97.7%
Co-infection HBV	Yes	16.7%	11.4%
	No	83.3%	88.6%
Co-infection HBC	Yes	16.7%	4.5%
	No	83.3%	95.5%
Viral load	≤ 50	66.7%	86.4%
	> 50	33.3%	13.6%

TB: Tuberculosis, HBV: Hepatitis B, HCV: Hepatitis C. The values correspond to 50 patients due to an unknown count in one of them.

Table 5. Relationship among viral load count cutoff 50 copies/ml with opportunistic infections, co-infections and Candida spp.

Patients (n= 50)		Viral load	
		< 50 copies/ml	≥ 50 copies/ml
		Frequency	
Candida spp	Positive	57.9%	50%
	Negative	42.1%	50%
Fungal diseases	Yes	33.3%	50%
	No	66.7%	50%
Co-infection TB	Yes	2%	50%
	No	98%	50%
Co-infection HBV	Yes	9.3%	14.3%
	No	90.7%	85.7%
Co-infection HBC	Yes	4.8%	12.5%
	No	95.2%	87.5%
LT CD4	< 200	47.6%	25%
	≥ 200	52.4%	75%

TB: Tuberculosis, HBV: Hepatitis B, HCV: Hepatitis C. The values correspond to 50 patients due to an unknown count in one of them.

The collection of subgingival biofilm facilitated the acquisition of samples for a smear, especially at sites with active periodontal disease. The presence of yeast and/or hyphae was observed, along with the periodontopathogenic bacterial microbiota and the inflammatory response. These findings are consistent with the presence of *Candida* spp. infection and other microorganisms associated with periodontal disease. The presence of yeast and hyphae alongside the bacterial microbiota suggests a potential interaction between these microorganisms and their potential contribution to the pathogenesis of gingival and periodontal diseases (Fig. 3).

The molecular identification of the yeasts was previously published by our laboratory[21]. It was determined that 16 isolates corresponded to *C. albicans*, and 36 to *C. dubliniensis*. Four atypical colonies were identified as *C. dubliniensis*. Ten strains tentatively identified could not be recovered. The remaining yeast species tentatively identified, which tested negative in both PCR methods employed, did not belong to either species, and their definitive identification was not conducted. With regard to patients with more than one *Candida*

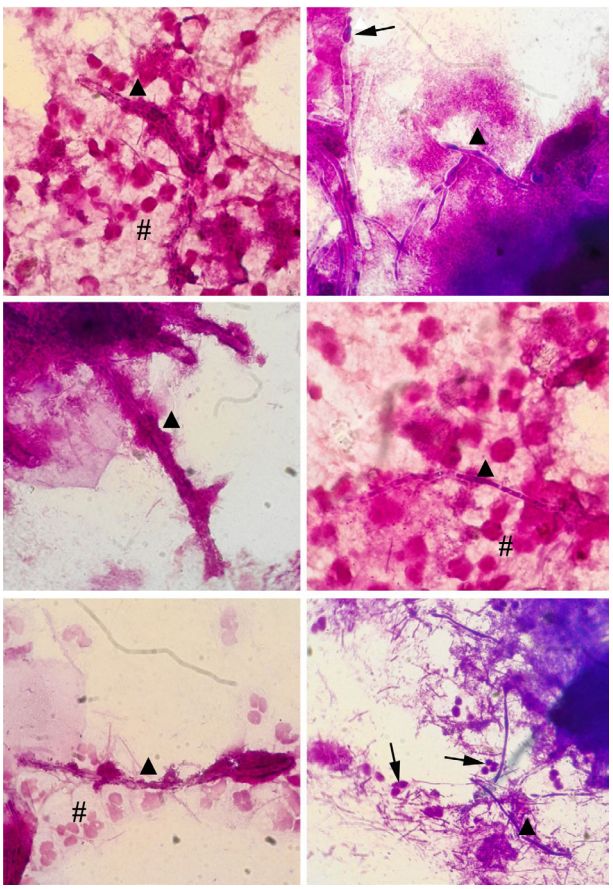

Fig. 3: Micromorphology of subgingival biofilm: Pseudomycelia/hyphae (▲), yeast (↑) and host's inflammatory response cells (#). Gram stain at 1000X

species at the same periodontal or mucosal site, the following findings were observed: In three patients, *C. albicans* and *C. dubliniensis* were found simultaneously at one periodontal site. In one of these patients, three different morphotypes of *C. dubliniensis* with atypical macroscopic and microscopic aspects were present. Two patients exhibited presence of *C. dubliniensis* along with a species compatible with *Nakaseomyces glabrata* (*N. glabrata)* at the same periodontal site. In another patient, *C. albicans* was detected along with a species compatible with *N.glabrata* at one periodontal site. At a mucosal site of another patient, *C dubliniensis* coexisted with a species compatible with *Pichia kudriavzevii* (formerly *Candida krusei*) (Table 6).

Table 6. Candida isolates according to the periodontal status and isolation sites. Only species with molecular confirmation are shown.

Patient	Sample	Diagnosis	*Candida*
01-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
01-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
01-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
01-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
03-0-C	periodontal	Periodontitis, S III, G B	*C.dubliniensis*
03-0-C	periodontal	Periodontitis, S III, G B	*C.albicans*
03-0-C	periodontal	Periodontitis, S III, G B	*C.albicans*
06-0-C	periodontal	Periodontitis, S I, G A	*C.albicans - C.dubliniensis* *
06-0-C	periodontal	Periodontitis, S I, G A	*C.dubliniensis*
07-0-C	periodontal	Eubiosis	*C.dubliniensis* *
08-0-C	periodontal	Eubiosis	*C.albicans - Candida* sp.
10-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
10-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
10-0-C	mucosa	Periodontitis, S II, G B	*C.dubliniensis- Candida* sp.
11-0-C	periodontal	Periodontitis, S III, G B	*C.albicans*
20-0-C	periodontal	Gingivitis	*C.albicans - C.dubliniensis*
20-0-C	periodontal	Gingivitis	*C.dubliniensis*
20-0-C	periodontal	Gingivitis	*C.dubliniensis - Candida* sp.
22-0-C	periodontal	Periodontitis, S I, G A	*C.dubliniensis - Candida* sp.
22-0-C	periodontal	Periodontitis, S I, G A	*C.dubliniensis*
22-0-C	periodontal	Periodontitis, S I, G A	*C.dubliniensis*
23-0-C	periodontal	Periodontitis, S II, G B	*C.dubliniensis*
23-0-C	mucosa	Periodontitis, S II, G B	*C.dubliniensis*
24-0-C	periodontal	Eubiosis	*C.albicans - C.dubliniensis* *
25-0-C	periodontal	Eubiosis	*C.dubliniensis*
25-0-C	mucosa	Eubiosis	*C.dubliniensis* *
25-0-C	periodontal	Eubiosis	*C.dubliniensis*
25-0-C	periodontal	Eubiosis	*C.dubliniensis*
25-0-C	periodontal	Eubiosis	*C.dubliniensis*
27-0-C	periodontal	Eubiosis	*C.albicans*
28-0-C	mucosa	Eubiosis	*C.albicans*
29-0-C	periodontal	Periodontitis, S I, G A	*C.dubliniensis*
33-0-C	periodontal	Periodontitis, S II, G B	*C.albicans*
39-0-C	periodontal	Eubiosis	*C.dubliniensis*
39-0-C	periodontal	Eubiosis	*C.dubliniensis*
39-0-C	periodontal	Eubiosis	*C.dubliniensis*
39-0-C	periodontal	Eubiosis	*C.dubliniensis*
39-0-C	mucosa	Eubiosis	*C.dubliniensis*
50-0-C	periodontal	Periodontitis, S II, G B	*C.dubliniensis*
50-0-C	periodontal	Periodontitis, S II, G B	*C.dubliniensis*
55-0-C	periodontal	Gingivitis	*C.dubliniensis*
57-0-C	mucosa	Periodontitis, S I, G A	*C.dubliniensis*
58-0-C	periodontal	Periodontitis, S II, G B	*C.dubliniensis*

E: stage, G: grade, *C.albicans: Candida albicans, C.dubliniensis: Candida dubliniensis.*
*: sites with presence of *C.dubliniensis* typical and atypical morphology.

DISCUSSION

This study characterized and identified *C. albicans* and *C. dubliniensis* isolated from subgingival biofilm and oral mucosa samples of PLWH, whose compromised immune systems make them notably susceptible to opportunistic infections. The selection of the sample was crucial to explore the potential involvement of these species in the etiology of gingival and periodontal diseases within a specific clinical context.

Males constituted the majority in the population sample. Many of the patients examined encountered obstacles in obtaining healthcare. More than half of them lacked health coverage, and approximately one fifth was unemployed. These variables increase patient susceptibility concerning both health and socioeconomic status in the context of HIV.

According to the latest data from the National Health Surveillance System in Argentina, 164.947 people with HIV have been recorded, of whom 65% receive care in the public health system. The report showed that the infection rate is higher in males than females, and a yearly decrease in mortality rates is observed[22,23]. Only one third of the participants in our study had documented antifungal treatment in their medical history, suggesting a deficiency in regular monitoring and follow-up for these patients within the national health system. Some of the protocolized patients presented co-infections with TBC, HCV and HBV. Unfortunately, we lacked data on the follow-up of these co-infections and HIV controls due to the absence of records in the general medical history of the patients.

A decrease in LT CD4 count below 200 cells/ml and a viral load exceeding 50 copies/ml may indicate disease progression to AIDS[24]. Only a minimal proportion of the patients met these conditions.

Over the past decade, there has been significant progress in comprehending this disease. A direct outcome of this advancement is the steadily increasing life expectancy for PLWH, especially in developing nations. The introduction of HAART has profoundly influenced HIV infection, markedly diminishing the morbidity and mortality linked with AIDS, thereby transforming it into a manageable chronic condition[25,26]. It is recommended to start HAART therapy promptly, irrespective of LT CD4 levels and viral load[27]. All patients included in this study were undergoing antiretroviral treatment. Individuals who contracted HIV after 2010 initiated treatment at the time of diagnosis. However, a significant majority of patients diagnosed before 2010 commenced treatment only when their CD4 cell count decreased and/or viral load increased.

The treatment with ART does not completely restore immunity, which gives rise to various complications associated with inflammation and immunodeficiency[26]. Furthermore, the extended life expectancy of these patients has exposed them to a spectrum of chronic diseases associated with aging, which may manifest prematurely and potentially be exacerbated by HIV and HAART[26,28,29].

ART treatment and immunity modulation suggested that periodontitis is more closely linked to the pathogenesis of gingival and periodontal diseases themselves and the increase in life expectancy than with HIV infection. Therefore, considering periodontitis to be an oral disease associated with HIV remains a topic of controversy and debate[30,31].

In this study, most patients had periodontitis, while a smaller group had gingivitis or eubiosis. Recent studies suggest that gingival tissues might serve as a reservoir for HIV due to the substantial presence of inflammatory lymphocytes in periodontal disease. Observations have indicated that the virus found in the gum does not come from peripheral blood, implying that it might be linked to a reactivation of viral replication within the oral mucosa in the context of chronic periodontitis[29,30,32]. One hypothesis suggests that HIV infection may trigger destructive processes in the oral mucosa, similar to those observed in the gastrointestinal tract, potentially aiding the translocation of microorganisms, and may be influenced by the presence of pro-inflammatory taxa in an environment marked by chronic inflammation associated with early-stage HIV. This could result in changes in the gastrointestinal microbiota, ultimately contributing to an increased likelihood of such translocation[28-30].

Concerning HAART, there is evidence suggesting that non-nucleoside analogs might exacerbate or hasten the onset of chronic diseases, particularly those associated with aging. They are also correlated with decreased bone mass and vitamin D deficiency, directly influencing periodontitis. ART may contribute to oxidative stress in the progression of periodontitis. Furthermore, they can heighten endothelial permeability by suppressing essential binding proteins necessary for the proper functionality of the epithelial barrier. The augmentation of capillary permeability is

particularly linked to inflammatory diseases[26,28-30,33]. Protease inhibitors in combination with nucleoside analogs were associated with a lower diversity of oral microbiota compared to both nucleoside analogs and non-nucleoside analogs[28]. In this research, most patients were under treatment with a combination of nucleoside analogs and non-nucleoside analogs.

According to more recent studies, *C. albicans* is an underlying factor capable of triggering microbial dysbiosis and leaky gut syndrome, emphasizing its significance even in the era of ART. Colonization by commensal strains and the emergence of *non-albicans* species can contribute to oral diseases, disseminated infections, and an increase in antifungal resistance, thereby constituting a public health concern, particularly in resource-limited settings[1,11,34,35].

One study suggesting that colonization at periodontal sites may originate from saliva[36] identified only a few patients in whom *Candida* spp. were isolated from both periodontal sites and mucosa.

Annavajhala et al. observed significantly lower fungal diversity in the subgingival biofilm of teeth with severe attachment loss (≥ 4 mm). Additionally, various taxa of bacteria and fungi were enriched in patients with severe periodontitis[28]. In the current study, the isolation frequency of *Candida* species was lower from periodontal sites with clinical attachment loss.

The heightened viral replication and significant depletion of LT CD4 in the oral mucosa lead to a reduction in the production of interleukin 17 and 22, resulting in systemic immune activation and a potential exacerbation of periodontitis[29,30]. On the other hand, Li et al. have suggested that asymptomatic carriage of oral yeasts was associated with a low LT CD4 cell count (≤200 cells/ml), and this value could serve as a predictive factor for yeast colonization[9]. However, in the current study, it was not possible to identify a pattern that would establish a relationship between the isolation of *Candida* and a decrease in LT CD4 cell count or an increase in HIV viral load in the context of gingivo-periodontal diseases.

It was originally believed that *C. dubliniensis* was not associated with systemic diseases, which suggested that the oral cavity could be its natural ecological niche[37]. Nevertheless, this species has garnered escalating attention in clinical research in recent years. Despite its low incidence in cases of candidiasis, its close phylogenetic relationship to *C. albicans* suggests that *C. dubliniensis* may have long been underestimated in candidiasis diagnosis. While most research has focused on the pathogenicity of *C. albicans,* it is crucial to acknowledge the medical significance of other members of the Candida genus, particularly in regions where their prevalence is unknown. Current medical practices, including the preventive or indiscriminate use of antifungals, seem to be contributing to the rise in the prevalence of alternative species[21,38-41].

While *C. albicans* is the most frequently isolated yeast from the oral cavity, the current research revealed a higher proportion of *C. dubliniensis* isolations, with a very low incidence of co-isolation with these species. In a prior investigation conducted by our laboratory, assessing PLWH with and without HAART, a greater number of *C. dubliniensis* isolations were observed in patients undergoing treatment, while the proportion of *C. albicans* was higher in those not receiving ART[7]. This suggests that ART may act as a regulator of the balance between colonization and infection by *C. dubliniensis*.

The presence of *Candida* spp. filaments in the subgingival microbiota were confirmed in some smears performed on the soft wall of the periodontal pocket. Molecular confirmation of these samples enabled the identification of the strains as *C. albicans* and *C. dubliniensis*. Confirmation of the involvement of these species in the infectious stage of periodontitis emphasizes the importance of conducting smears in microbiological studies to help identify the predominant microbiota composition, understand microbial interactions, and discern their impact on the colonization, infection and persistence of microorganisms in specific environments.

Evidence suggests that the subgingival biofilm microbiota differs taxonomically from microbiota isolated from other niches in the oral cavity[7,42]. To assess the alteration in microbiota composition associated with periodontitis, the subgingival biofilm sample is considered the most representative. DNA sequencing techniques developed in recent decades have revealed that various niches in the oral cavity host significantly different microbial communities with distinct compositions[43].

CONCLUSIONS

C. dubliniensis stood out as the most frequently isolated species in the examined population. This

is a significant discovery because the diagnostic importance of this species has been underestimated, with colonization primarily ascribed to *C. albicans*. The presence of yeast hyphae/pseudohyphae in the subgingival microbiota substantiates the involvement of these species in the dysbiosis of gingival and periodontal diseases.

The HIV population in this region of Argentina is clearly vulnerable, with socioeconomic difficulties and limited access to healthcare. The findings of this study underscore the need to address the oral health of these patients at an early stage, aiming to diagnose gingivo-periodontal diseases and understand their implications in fungal infections.

CONFLICT INTERESTS

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

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Twelve-month healing rates after endodontic therapy with foraminal cleaning using 2% chlorhexidine in mandibular molars diagnosed with apical periodontitis: a prospective clinical study

Cassiano N Almeida[1], Carlos E da Siveira Bueno[2], Alexandre Sigrist De Martin[2], Carlos E Fontana[3], Augusto S Kato[2], Ana Grasiela Limoeiro[4], Wayne M Nascimento[2], Marília FV Marceliano-Alves[5,6,7], Ricardo Ferreira[1], Rina A Pelegrine[2]

1. *Department of Endodontics, Dentistry Course, School of Health Sciences, Vale do Itajaí University (UNIVALI), Itajaí, Santa Catarina, Brazil*
2. *São Leopoldo Mandic, São Leopoldo Mandic Research Institute, Department of Endodontics, Campinas, SP, Brazil.*
3. *PUC Campinas, Center of Life Sciences, Programa de pós-graduação em Ciências da Saúde, Campinas, SP, Brazil.*
4. *Department of Dentistry, Endodontics and Dental Materials, Bauru Dental School, University of Sao Paulo, Bauru, Brazil*
5. *Post-Graduate Program in Dentistry, Iguaçu University, Nova Iguaçu, RJ, Brazil*
6. *Department of Dental Research Cell, Dr. DY Patil Dental College and Hospital, Dr. DY Patil Vidyapeeth, Pune 411018, India.*

ABSTRACT

The success of endodontic treatment depends on the effective disinfection of the root canal system.The literature describes many treatment protocols. ***Aim:*** *The purpose of this prospective in vivo study was to evaluate the healing status observed after endodontic treatment performed with foraminal cleaning in mandibular molars with apical periodontitis.* ***Materials and Method:*** *Fifty mandibular molars were selected and instrumented with ProDesign Logic files. First, mechanized patency was performed with a 25/0.01 instrument; if it was easily achieved, a larger diameter instrument was chosen (30/0.01, 35/0.01, or 40/0.01) that best matched the apical foramen. Then, a respective shaping file corresponding to the patency file was selected for instrumentation. Irrigation was carried out with saline solution and 2% chlorhexidine gel. A 17% EDTA solution was used for the final irrigation and agitated with the EasyClean system. A device was used to standardize the initial, final, and subsequent digital radiographic examinations for the follow-up. A 3-level scoring system was used for evaluation, in which score 1 indicated complete lesion healing, score 2 incomplete healing, and score 3 no healing.* ***Results:*** *There was no significant difference between the frequencies of scores 1 and 2 assigned at the two assessment time points ($p > 0.05$). Complete healing (score 1) was observed in 58%, incomplete healing (score 2) in 42%, and no healing (score 3) in 0% of cases.* ***Conclusion:*** *The endodontic preparation protocol followed by foraminal cleaning favored the healing process in teeth with apical periodontitis.*

Key words: *dental pulp diseases - periapical diseases - root canal therapy - tooth apex - treatment outcome*

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Corresponding Author:
Marilia Marceliano-Alves
mmarceliano@hotmail.com

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A cicatrização em 12 meses após terapia endodôntica com limpeza foraminal após o uso de clorexidina a 2% em molares inferiores com periodontite apical: um estudo clínico prospectivo

RESUMO

O sucesso do tratamento endodôntico depende da desinfecção eficaz do sistema de canais radiculares e há uma abundância de protocolos de tratamento na literatura. ***Objetivo:*** *O propósito deste estudo prospectivo in vivo foi avaliar o estado de cicatrização observado após tratamento endodôntico realizado com limpeza foraminal em molares inferiores com periodontite apical crônica.* ***Material e Métodos:*** *Cinquenta molares inferiores foram selecionados e instrumentados com limas ProDesign Logic. Primeiro, foi realizada a patência mecanizada com instrumento 25/0.01; se foi alcançada facilmente, um instrumento de maior diâmetro foi escolhido (30/0.01, 35/0.01 ou 40/0.01) que melhor correspondesse ao forame apical. Em seguida, uma lima de modelagem respectiva a de patência foi escolhida para a instrumentação. A irrigação foi realizada com solução salina e gel de clorexidina a 2%. Uma solução de EDTA a 17% foi usada para a irrigação final e agitada com o sistema EasyClean. Um dispositivo foi usado para padronizar os exames radiográficos digitais inicial, final e subsequentes para o acompanhamento. Um sistema de pontuação de 3 níveis foi usado para avaliação, no qual a pontuação 1 é a cicatrização completa da lesão; a pontuação 2 é a cicatrização incompleta e a pontuação 3 se refere à ausência de cicatrização.* ***Resultados:*** *Não houve diferença significativa entre as frequências das notas 1 e 2 atribuídas nos dois momentos de avaliação ($p > 0,05$). Cicatrização completa (nota 1) foi observada em 58%, cicatrização incompleta (nota 2) em 42% e nenhuma cicatrização (nota 3) em 0% dos casos.* ***Conclusão:*** *Concluiu-se que o protocolo de preparo endodôntico seguido de limpeza foraminal favoreceu o processo de cicatrização em dentes com periodontite apical crônica.*

Palavras-chave: *doenças da polpa dentária - doenças periapicais - terapia de canal - ápice dentário - resultado do tratamento*

INTRODUCTION

In recent decades, there have been numerous technological advances in the field of endodontics aimed at improving treatment prognosis and thereby helping to preserve teeth[1]. The evaluation of the outcome of endodontic treatment is generally based on the analysis of radiographic findings and the assessment of clinical signs and symptoms associated with the treated tooth. In this context, instrumentation and irrigation play an important role in cleaning and disinfecting the root canal system, thereby contributing to the success of the endodontic treatment[2].

According to Metzger et al.[3], it is not uncommon for failures due to inadequate root canal obturation to result from poor preparation. If the preparation is inadequate, the filling will most likely not be effective, so biomechanical preparation is one of the most important phases in controlling endodontic infection. Cutting and removing tissue with endodontic instruments combined with the flow and antimicrobial action of irrigants can significantly reduce the number of microorganisms in the main root canal and on the dentin walls[4].

The new single-file concept for shaping root canals aims to simplify the preparation technique and reduce surgical time. The ProDesign Logic System (Bassi/Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil) provides a simplified preparation procedure based on the use of a single instrument to shape the root canal[5], providing agility and more conservative preparation. This technical simplification shortens the learning curve and thus streamlines endodontic treatment[6].

Obturation of the root canal system with three-dimensional sealing is another important phase of endodontic treatment. Considering the high prevalence of root canal branching, the complexity of the root canal system should be considered. The continuous wave of condensation technique considers this three-dimensionality and enables the formation of a homogeneous, stable gutta-percha body that penetrates the ramifications[7].

Considering that the success of endodontic treatment depends on a combination of the several factors, it is important to establish an effective treatment protocol to promote and maintain disinfection of the root canal system. The aim of this prospective i*n vivo* study was to evaluate the healing status of 50 mandibular molars with apical periodontitis 12 months after endodontic preparation with foraminal cleaning.

MATERIAL AND METHOD

Sample size was calculated using a formula for sensitivity and specificity for diagnostic studies with the PASS 15 program. A power of 81% was used as the calculation assumption to detect a change in sensitivity from 0.8 to 0.9, and the significance level of the sensitivity test was 0.046. A sample size of 50 teeth was required.

This study was approved by the Institutional Research Ethics Committee of the São Leopoldo Mandic Research Institute (registration number 1.563.724), and conducted in compliance with all ethical standards of the Declaration of Helsinki of the World Medical Association. All patients voluntarily signed an informed consent form before being enrolled in the study. Fifty mandibular molars from patients referred to the Center for Dental Specialties at the Vale do Itajaí University (UNIVALI), Itajaí, Santa Catarina, Brazil, where the study was conducted, were treated and followed up over a 12-month period. All teeth were diagnosed clinically with pulp necrosis and radiographically with apical periodontitis.

Patients were selected according to the following protocol: Medical history, extraoral and intraoral clinical examination, pulp sensitivity testing, and evaluation of a periapical radiography. Patients were excluded if they had advanced periodontal disease or combined endo-periodontal lesions, were taking analgesic, anti-inflammatory, antibiotic or immunosuppressive medications, or had undergone endodontic retreatment.

Initial radiographs of the teeth included in the study were obtained using a digital radiographic sensor (CDR Elite; Dentsply Sirona, York, PA, USA) and an X-ray device (Spectro 70X Eletronic; Dabi Atlante, Ribeirão Preto, SP, Brazil). A silicone mold (Clonage; Nova DFL, Rio de Janeiro, RJ, Brazil) was prepared and adapted to the X-ray positioner to ensure that the sensor was held in the same standardized position during X-ray examinations.

Endodontic treatment

After anesthesia and rubber dam isolation, coronal access was performed using a 1014 drill (KG Sorensen, Barueri, SP, Brazil) and carbide drills

2 and 3 (Injecta, São Bernardo do Campo, SP, Brazil). Initial decontamination of the pulp chamber was performed by completely filling it with 2% chlorhexidine gel (Visnature, Itajaí, SC, Brazil) for 1 minute. The endodontic treatment protocol began with the exploration of the cervical and middle third with #10 K-type hand files (Vdw Gmbh, Munich, Germany) using clockwise/counterclockwise movements (1/4 rotation to the right and ½ rotation to the left) to a point 5 mm short of the radiographic apex to establish a direction for the rotating files. Afterwards, ProDesign Logic System (Bassi/Easy) driven by an endodontic motor (EndoEasy SI; Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil) set to a speed of 350 rpm and a torque of 1 N.cm was used to prepare the canal. In-and-out movements were performed with a maximum amplitude of 3 mm until the working length for exploration was reached, which was determined by subtracting 1 mm from the apparent length of the tooth measured on the first radiograph.

Then, the apical foramen was located with an electronic apex locator (Romiapex A-15; Romibras, Rio de Janeiro, RJ, Brazil) and the working length (WL) was considered in "zero". Hand-use K files #10 and #15 (Vdw GmbH, Munich, Germany) were inserted up to 1 mm beyond the apical foramen to achieve initial foraminal patency. Mechanized patency was then achieved with a 25/0.01 ProDesign Logic file designed to create glide paths. Whenever it reached the foramen effortlessly, a different size file (30/0.01, 35/0.01, or 40/0.01) was used until resistance was encountered.

The final shaping file was selected based on the file used to create the glide path that best matched the apical foramen. The correspondence between glide path and final shaping file is shown in Table 1. The selected final shaping file was coupled to the contra-angle handpiece of the endodontic motor, which was operated at setting #2 of the "Logic" program at a speed of 950 rpm and a torque of 4 N.cm. A brushing motion with light apical pressure was applied against the root canal walls until WL was reached. After three brushing movements, the file was removed, and its blades cleaned with sterile gauze.

During instrumentation, the canals were irrigated with 2% chlorhexidine gel (Visnature, Itajaí, SC, Brazil) using a 3 mL plastic syringe (BD, São Paulo, Brazil) and a 0.20 x 55 mm hypodermic needle (BD, São Paulo, SP, Brazil). Irrigation to remove canal debris was performed with saline (Laborasa, São Paulo, SP, Brazil) using a 5-mL plastic syringe (BD) and a hypodermic needle of the same size (BD).

Table 1. Correspondence between glidepath files and final shaping files (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil) used for the root canal treatments performed in the study.

Glidepath / patency file	Final shaping / preparation file
25/0.01 – red	25/0.06 - red
30/0.01 – blue	30/0.05 - blue
35/0.01 - green	35/0.05 - green
40/0.01 - black	40/0.05 - black

For final cleaning, 5 mL of a 17% EDTA solution (Biodynamic, Ibiporã, PR, Brazil) was added to the canal, and then mechanically agitated with an EasyClean insert (Bassi/Easy). The insert was positioned at WL and reciprocated in three cycles of 20 s. After each agitation cycle, the 17% EDTA solution was removed by rinsing with 5 mL of saline. The canals were then dried with absorbent paper tips (Tanariman Industrial, Manacapuru, AM, Brazil).

The canals were obturated with gutta-percha cones (Odous De Deus, Belo Horizonte, MG, Brazil), the tips of which were placed 2 mm short the WL, and endodontic sealer (Endomethasone; Septodont, Saint-Maur-des-Fossés Cedex, France) was used according to the manufacturer's instructions. The continuous compaction wave technique[7] was applied with the thermoplasticizing tip of the Easy Thermo "FM" device (Bassi/Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil) positioned 5 mm short of the WL. After sealing the apical third of the canal, the rest was filled with heated gutta-percha, using the thermal injector of the same system. Coronal sealing was performed with a temporary sealer (Coltosol; Vigodente, Rio de Janeiro, RJ, Brazil) and composite resin (Z250; 3M, Sumaré, SP, Brazil).

Patients were then referred to a healthcare facility to receive a definitive restoration. A final radiography was obtained and stored in a digital file, using the same standardized procedures as the initial radiography. Patients were re-examined 12 months after the procedure, and new clinical and radiographic examinations were performed to re-evaluate the endodontic treatment performed. The initial, final,

and subsequent radiographs were evaluated by two trained investigators performed the measurements at two different time points, 15 days apart, to verify the agreement of the assessments.

The clinical radiographic assessments were based on the European Society of Endodontology outcome classification criteria, and classified using the following scoring system: score 1, complete healing: no clinical symptoms or presence of lamina dura demonstrated radiographically; score 2, incomplete healing: no clinical symptoms and regression of apical periodontitis demonstrated radiographically; Score 3, no healing: Presence of clinical symptoms and no evidence of regression of apical periodontitis or else progression of apical periodontitis demonstrated radiographically[8]. Figures 1 and 2 show two cases evaluated with scores 1 and 2, respectively.

Statistical analysis

The intraclass correlation test was applied to evaluate the agreement between the investigators. The Shapiro-Wilk normality test, complemented by the nonparametric Mann-Whitney test, was used to compare the scores assigned to the healing status observed in the radiographs. BioEstat v. 5.3 software (Instituto de Desenvolvimento Sustentável Mamirauá, Belém, PA, Brazil) was used to perform the analyses. The significance level applied was 5%.

RESULTS

The correlation test showed that the agreement between the investigators was excellent (Table 2). No significant difference was found between the frequencies of scores 1 and 2 assigned to the radiographs at the two evaluation times ($p = 1.00$, Table 3). Score 3 was not assigned to any of the radiographs.

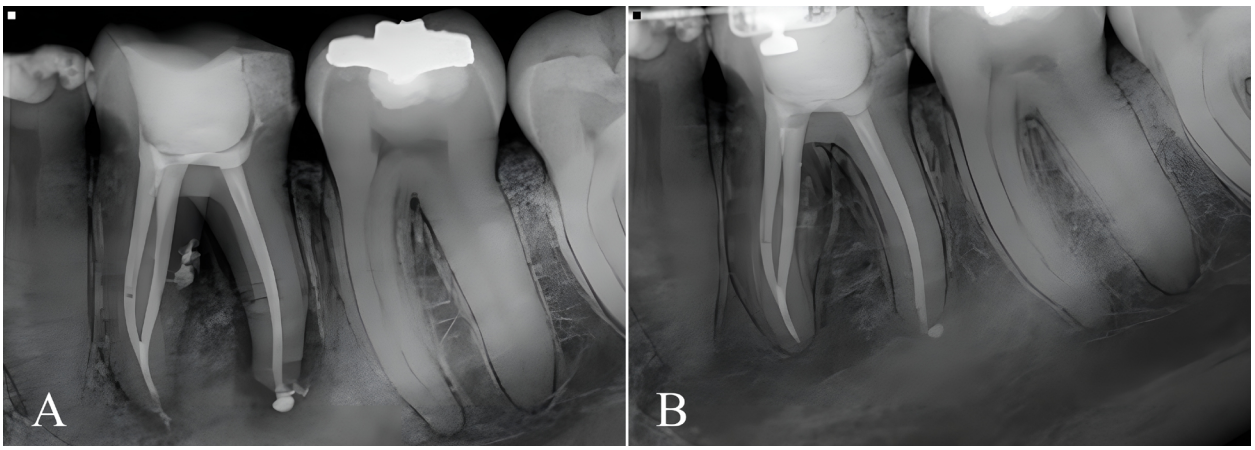

Fig. 1: Representative images of the outcome of endodontic treatment performed with foraminal cleaning. ***A:*** *Radiograph taken immediately after treatment;* ***B:*** *Radiograph taken 12 months post-operatively, classified as Score 1 (complete healing).*

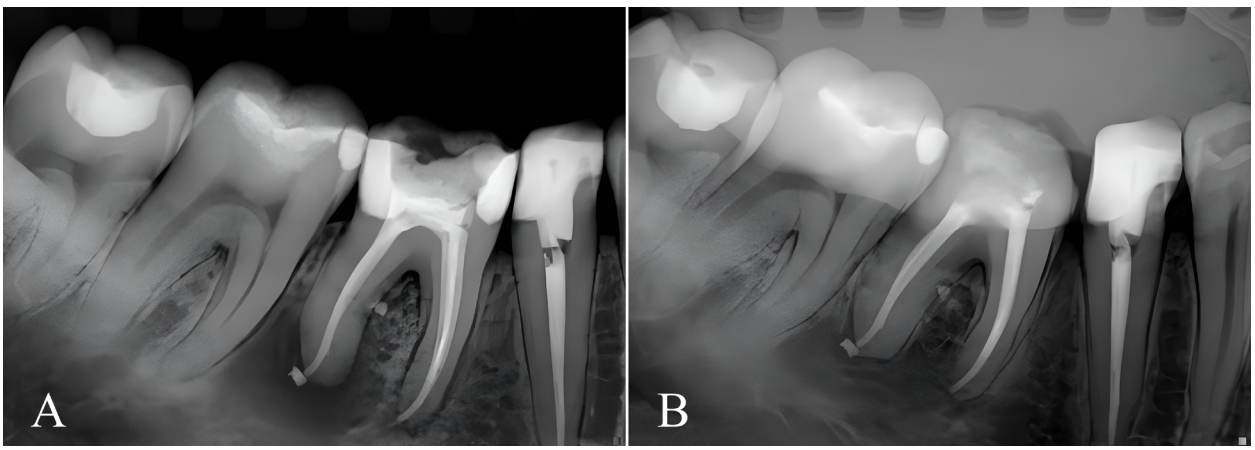

Fig. 2: Representative images of the outcome of endodontic treatment performed with foraminal cleaning. ***A:*** *Radiograph taken immediately after treatment;* ***B:*** *Radiograph taken 12 months post-operatively, classified as Score 2 (incomplete healing).*

Table 2. Results of the intra-class correlation test performed to evaluate inter-examiner agreement in the assessment of the healing status observed in radiographic images taken 12 months after root canal therapy with foraminal cleaning.

Variance between groups	**0.2323**
Experimental error	0.0048
Degree of freedom 1	49
Degree of freedom 2	50
p-value	< 0.0001
Intraclass correlation	0.9595
Replicability	Excellent

Table 3. Median, interquartile deviations and comparison of the scores assigned to the healing status observed in radiographic images taken at two evaluation time points after root canal therapy with foraminal cleaning.

	12 months	12 months and 15 days	p-value
Median (interquartile deviation)	1.00 (1.00) [A]	1.00 (1.00) [A]	1.00
% Score 1	58%	58%	
% Score 2	42%	42%	

Same letters indicate lack of statistically significant differences (Mann Whitney test; p > 0.05).

DISCUSSION

The sample in this study consisted of 50 mandibular molars with pulp necrosis and an initial radiograph suggestive of apical periodontitis, according to a previous study[9]. The overall response rate of patients was 100%, undoubtedly because they were all participants in an oral health program conducted at the University where the study was conducted. Patients were asked to return for follow-up by phone and reminded of the follow-up appointment every 3 months during the 12-month interval.

Initial, final, and follow-up digital radiographs were obtained, the last of which was taken 12 months after completion of endodontic treatment. A silicone mold was made and adapted to the positioner of the radiographic sensor in order to keep it in the same standardized position, thus ensuring higher sensitivity and diagnostic specificity for the assessment of the disease and healing processes[10,11].

The root canals were irrigated with saline and 2% chlorhexidine gel as an auxiliary chemical. A previous study found that both chlorhexidine gluconate and sodium hypochlorite significantly reduced the number of microorganisms in teeth with necrotic pulp or periapical disease, indicating that both solutions can be used successfully as irrigants[12]. These results are in accordance with other studies that showed that chlorhexidine has a broad antimicrobial spectrum, high substantivity, and lower cytotoxicity than sodium hypochlorite[9,13]. In addition, chlorhexidine has shown satisfactory clinical performance, lubricating properties and rheological effect. It inhibits metalloproteinases, is chemically stable, does not stain tissues, and is odorless and water-soluble[14]. Furthermore, because the present study used a technique that promotes targeted cleaning of the apical foramen, the use of a sodium hypochlorite solution could cause accidents[15] or lead to an undesirable inflammatory process[16].

The files used to create the glide path were selected according to the original anatomy of the apical foramen. The criterion for file selection was based on the resistance encountered when attempting to pass them through the apical foramen. This approach was intended to ensure effective cleaning of the apical foramen. A study suggests that foraminal cleaning should be performed to increase the predictability of endodontic treatment and that it is not associated with increased postoperative pain[17].

Final canal irrigation was performed before obturation with a total 5 mL of 17% EDTA solution. After filling each root canal with the EDTA solution to its cervical apical opening, the solution was mechanically agitated with the polymer-based EasyClean instrument, which was positioned at WL and reciprocated in 20-s cycles. A study concluded that irrigation with the EasyClean system was more effective than passive ultrasonic irrigation in removing debris from the more apical regions of the root canal[18].

Root canal orifices were sealed with Coltosol (Vigodente, Rio de Janeiro, RJ, Brazil) and the pulp chamber was sealed with composite resin. Sealing is performed after obturation to prevent bacterial contamination. Teeth with adequate root canal fillings and restorations have a more favorable prognosis considering the basic biological principle of preventing bacterial penetration through the crown[19].

In this study, a clinical and radiographic follow-

up was performed 12 months after treatment, the same follow-up period as used in some other studies[10,11,20], and the results were confirmed after the initial assessment at 12 months. According to a prospective study[21], one of the prerequisites for more favorable periapical healing is the absence of apical periodontitis before endodontic treatment. In the current study, all cases had previous apical periodontitis, which may have contributed to the fact that only 58% of patients achieved complete healing at 12 months. This rate was similar to that reported in a study conducted on teeth with apical periodontitis treated in a single session[11]. In contrast, another study found a radiographic success rate of 80% 12 months after completion of endodontic treatment, although their sample included only 19 cases, which are fewer than the 50 cases followed up in the present study[10].

Another prospective clinical and radiographic study, similar to the current study, but using asymptomatic teeth with pulp necrosis found a 92.9% success rate at the 12-month follow-up, with complete regression of apical periodontitis[20], similar to the present results. However, in that study, success was defined as partial or complete regression of the disease. In contrast, in the present study, partial regression (or incomplete healing) was observed in 42% of cases.

Another study assessed teeth with apical periodontitis and found that at a follow-up period of two years, 96.57% of cases had healed[22]. It has been reported that the maximum expected time for complete radiographic healing of periapical lesions is four to five years[8]. In exceptional cases, teeth have been followed up for up to 17 years. The use of a 12-month follow-up period may be considered a limitation of the present study because longer periods are usually required to allow more reliable follow-up of the healing process.

The incompletely healed apical periodontitis observed in 42% of the cases in the present study showed radiographic signs of regression and no clinical symptoms, but lamina dura formation was not observed in these cases, in contrast to the cases scored 1. This may be attributed to the fact that foraminal preparation was performed in the present study and that compression of the filling material caused a sealer extrusion and it could serve as a tissue irritant or the healing was through fibrous tissue. In order for this treatment outcome can be considered acceptable, it should show a reduction in periapical radiolucency for up to 2 years postoperatively; therefore, longer follow-up periods are recommended for apical periodontitis[23].

Based on the results of this prospective *in vivo* study, it can be concluded that the protocol with foraminal cleaning for the treatment of mandibular molars with a clinical diagnosis of nonvital pulp and a radiograph suggestive of apical periodontitis leads to favorable results in terms of regression of periapical disease. However, further studies with longer observation periods are needed for a more reliable evaluation.

CONFLICT INTERESTS

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

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Treatment of vital teeth involved in the extension of inflammatory radicular cysts: a systematic review

Diego G Calabró[1], Ricardo P Portigliatti[2], Federico Stolbizer[1]

1. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Cirugía y Traumatología Bucomáxilofacial II, Ciudad Autónoma de Buenos Aires, Argentina.
2. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Endodoncia, Ciudad Autónoma de Buenos Aires, Argentina.

ABSTRACT

An inflammatory radicular cyst (IRC) is an odontogenic cyst associated to a non-vital tooth, which may affect sound teeth. There is lack of consensus on whether to perform endodontic treatment on teeth involved in the extension of the lesion prior to surgical treatment. ***Aim:*** *To identify and quantify the therapies most often used on teeth with positive response to pulp tests that are involved in the extension of an IRC, and analyze the criteria upon which different authors based their decisions. Identify and evaluate the reliability of the dental pulp tests used for evaluation and deciding on treatment.* ***Materials and Method:*** *A review was performed of articles indexed in four databases from 2011 to 2023. The review included studies describing diagnosis and treatment of teeth involved in the extension of an inflammatory cyst.* ***Results:*** *Fifteen articles were included in the review: nine case reports, five literature reviews and one comment to the editor. The case reports address 10 cystic lesions, directly associated with a total 21 sound teeth. Three of them report loss of tooth sensitivity, three report preemptive endodontic treatment of teeth with preserved sensitivity, two report recovery of previously lost sensitivity, and one reports preservation of sensitivity after surgical treatment. One of the literature reviews addresses the issue of treating teeth adjacent to a cystic lesion, while the other four, and the comment to the editor, discuss the methods for detecting sensitivity and vitality.* ***Conclusion:*** *Clinical studies with long-term follow-up are needed in order to reach a consensus regarding treatment. Better understanding of pulp status is essential in order to select the treatment best suited to each clinical case.*

Keywords: *radicular cysts - periapical cysts - vitality test - dental pulp test.*

Tratamiento de piezas dentarias vitales involucradas en la extensión de quistes radiculares inflamatorios. Revisión sistemática

RESUMEN

El quiste radicular inflamatorio (QRI) es un quiste odontogénico asociado a una pieza dentaria no vital, que pueden comprometer dientes sanos. Existe una falta de consenso en si se debe o no realizar el tratamiento endodóntico de las piezas dentarias involucradas en la extensión de la lesión, previo al tratamiento quirúrgico. ***Objetivo:*** *Identificar y cuantificar cuáles fueron las conductas terapéuticas más utilizadas en las piezas dentarias con respuesta pulpar positiva involucrada en la extensión de un QRI, y analizar el criterio utilizado por los diferentes autores para la toma de decisión. Identificar y evaluar el grado de confiabilidad de los métodos de respuesta pulpar utilizados para la evaluación y toma de decisión del tratamiento.* ***Materiales y Método:*** *Se realizó una revisión de los artículos indizados en cuatro bases de datos entre los años 2011 y 2023. Se incluyeron estudios que describieran el diagnóstico y tratamiento de las piezas dentarias comprometidas en la extensión de un quiste inflamatorio radicular.* ***Resultados:*** *Se incluyeron 15 artículos en la revisión. En los 9 reportes de casos incluidos, se reportan 10 lesiones quísticas, las cuales tenían una relación directa con un total de 21 dientes sanos. En 3 de los 9 reportes de caso se detecta pérdida de sensibilidad de los dientes, mientras que otros 3 reportes realizan tratamiento endodóntico de dientes con sensibilidad conservada como maniobra preventiva. Otros 2 reportes plantean la recuperación de la sensibilidad que originalmente se encontraba perdida y el caso restante, deja constancia de la conservación de la sensibilidad de las piezas posterior al tratamiento quirúrgico. Se encontraron en esta búsqueda 5 revisiones bibliográficas. Solo una de ellas plantea el tema del tratamiento de los dientes adyacentes a una lesión quística mientras que los otros 4 y el comentario al editor discuten los métodos de sensibilidad y vitalidad.* ***Conclusión:*** *Es necesario el desarrollo de estudios clínicos con seguimiento a largo plazo para lograr un consenso con respecto al tratamiento. Una mejor comprensión del estado pulpar es fundamental para poder optar por el tratamiento que mejor se adapte a cada caso clínico.*

Palabras clave: *quistes radiculares - quistes periapicales - vitalidad pulpar - test pulpar.*

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Corresponding Author:
Diego Calabró
diego.calabro@odontologia.uba.ar

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INTRODUCTION

An inflammatory radicular cyst (IRC) is an inflammatory odontogenic cyst associated to a non-vital tooth. It is the most frequent type of cyst in the jawbone, accounting for 55% of odontogenic cysts[1-4]. Its etiology is necrosis of the pulp tissue that reaches periapical tissues and, via different proinflammatory chemical mediators, stimulates the epithelial rests of Malassez, which give rise to the formation of the cyst membrane. IRCs are slow-growing[2,5] but may compromise sound teeth if they attain considerable size[2-4, 6-8].

There is lack of consensus regarding whether to perform endodontic treatment on adjacent teeth before surgical treatment of an IRC. Some authors consider preemptive endodontic treatment of all teeth involved in the lesion to be "good practice", based on the risk of causing pulp necrosis in originally vital teeth by damaging the neurovascular bundle during enucleation of the cystic membrane[9-11]. Even though the risk is not high, when it does occur, it causes immediate aesthetic damage that requires endodontic treatment and internal whitening. Other authors, in contrast, propose watchful waiting and postponing endodontic treatment, or deciding to perform endodontic treatment according to the degree to which the apices are involved in the lesion, which is very difficult to determine objectively[12-14].

Determining the pulp status of teeth adjacent to an IRC *a priori* is also a controversial issue because there is no consensus on the efficacy of the methods used to evaluate the pulp response to stimuli. In recent years, pulse oximetry using devices adapted to tooth anatomy, and Doppler ultrasound to study blood flow have been considered the most efficient. However, for the time being, these methods are not standardized and are therefore "operator dependent" and difficult to replicate[15-16].

Due to the lack of consensus found in the literature, the aims of this study were:

a) To identify and quantify the most frequently used therapies for teeth with positive response to pulp tests that are involved in the extension of an IRC, and to analyze the criteria upon which different authors based their decisions;
b) To identify and evaluate the reliability of the pulp response tests used for evaluation and deciding on treatment of teeth involved in the IRC extension.

MATERIALS AND METHOD

An exhaustive review was conducted of articles indexed in Pubmed, Medline, Embase and Scielo databases from 2011 to 2023, utilizing the followings MeSH terms, synonyms and free terms**:** "radicular cysts", "periapical cysts", "pulp vitality", "pulp test". Boolean operators (OR and AND) were used for combination of terms. The search key is described in Fig. 1.

Fig. 1: Search strategy for Pubmed, Medline, Embase and Scielo databases

Term= (Cyst) AND Term= (radicular OR periapical).
Term= (Dental pulp Test OR Dental pulp vitality test OR Dental pulp sensitivity test)

The PICO criteria – Population, Intervention, Comparison and Outcome – were used to prepare a structured summary and evaluate the methodological characteristics of each selected article. The criteria were defined in detail as follows: Population: teeth with positive response to pulp test and involved in the extension of an IRC; Intervention: pulp response assessment methods, cold test, electric test, flowmetry, etc.; Comparison: different methods for evaluating pulp response, and Outcome: the outcome of the hypothesis, reported reliability of pulp testing methods, and what decision was made for treatment of the teeth involved in the IRC extension).

Clinical studies, meta-analyses, systematic reviews, control cases, cohort studies, observational and analytical studies describing diagnosis and treatment of teeth involved in the expansion of an inflammatory radicular cyst were included. No randomized control study on the subject was found. Any articles referring to dental trauma history, studies in pediatric patients or patients with temporary dentition, experimental studies and articles without at least the abstract in English were excluded.

RESULTS

The search identified 3420 articles, of which 3346 were excluded because their titles and abstracts revealed that they did not address the treatment of teeth adjacent to an IRC and/or the method used for diagnosing pulp status. The full texts of the 74 remaining articles were analyzed. Of these, 45 were discarded because they did not provide data that would enable evaluation of the procedures. Of the 29 remaining articles, 14 presented one of the

exclusion criteria mentioned above. Thus, only 15 articles were included in the final analysis. Fig. 2 shows the article selection process. Selected articles are listed in Table 1.

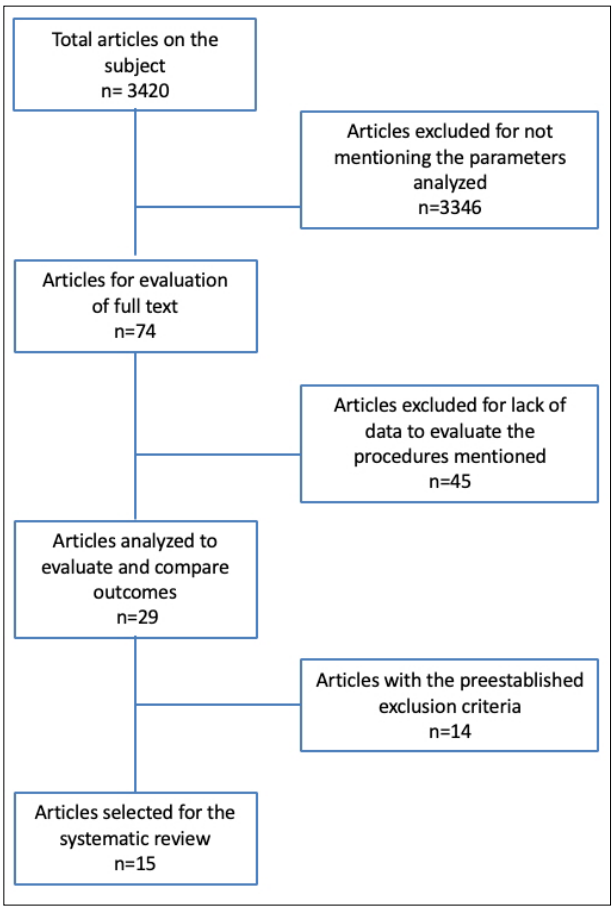

Fig. 2: Flowchart showing article selection process

The articles selected for review consisted of nine case reports, five literature reviews and one comment to the editor.

Altogether, the nine case reports discuss ten cystic lesions that were directly associated with a total of 21 clinically sound teeth (one to three teeth per case, except for one clinical case[10] involving seven teeth). Pulp status in adjacent teeth was evaluated using cold and electric pulp sensitivity tests in seven of the case reports[10-12,14,17-19]. Another case[20] reports an unspecified sensitivity test followed by a cavity test, while the remaining one[9] reports using the two temperature tests (cold and hot) and an unspecified vitality test. Only two of the case reports[10,20] evaluated the cystic lesion by tomographic images, while the rest used periapical or panoramic radiographs. Lesion enucleation is reported in six, but was not the first treatment choice in another two. Regarding treatment of choice and pulp status monitoring, three of the nine cases report loss of sensitivity in teeth that originally had a positive response to the tests used[17-19]. Another three[9-11] report endodontic treatment of the teeth with preserved sensitivity to prevent loss of vitality that was believed would occur during cyst enucleation. Two cases report recovery of the originally lost sensitivity[12,20], and the remaining case reports conservation of tooth sensitivity following surgical treatment of the lesion[14].

Only one of the five literature reviews[13] addresses the subject of treating teeth adjacent to a cystic lesion, highlighting teeth related to odontogenic keratocysts rather than to inflammatory radicular cysts. It notes the lack of established criteria for the therapeutic approach and the clinical importance of preserving teeth with vital pulp, considering that despite the efficacy of the root canal treatment on mature teeth, it is important to consider long-term complications ranging from loss of tooth defensive capacity to potential tooth fracture and extraction. The other four literature reviews[15,21-23] and the comment to the editor[16] discuss methods for testing sensitivity and vitality. Patro et al.[21] considers pulse oximetry to be the most precise method, while Alghaithy et al.[23] concludes that laser doppler flowmetry is closest to the gold standard. Mainkar et al.[15] claims that laser doppler flowmetry and pulse oximetry are the most precise diagnostic methods and do not differ significantly. Balevi[16] states that the cold test is the simplest and most precise pulp sensitivity test available. Donnenmeyer et al.[22] concludes that the effectiveness of pulp tests cannot be determined due to lack of scientific evidence.

DISCUSSION

The studies included in this review propose widely differing treatment criteria, clearly reflecting the

Table 1. Articles selected for review

AUTHOR - REFERENCE	YEAR	TITLE	TYPE
Komabayashi et al.[17]	**2011**	Apical infection spreading to adjacent teeth: a case report	Case Report
Asgary S. et al.[18]	**2013**	Necrosis of intact premolar caused by an adjacent apical infection: a case report	Case Report
Sood N. et al.[9]	**2015**	Treatment of Large Periapical Cyst Like Lesion: A Noninvasive Approach: A Report of Two Cases	Case Report
Martins J. et al.[10]	**2015**	Cystic Lesion with Origin on a Single Long Time Traumatized lower Incisor.	Case Report
Alghaithy et al.[23]	**2016**	Pulp sensibility and vitality tests for diagnosing pulpal health in permanent teeth: a critical review	Review
Mainkar et al.[15]	**2018**	Diagnostic accuracy of 5 dental pulp tests: A systematic review and meta-analysis	Systematic Review and Meta-analysis
Asgary S. et al.[19]	**2018**	Partial Necrosis Consequence of the Infection Spreading from an Adjacent Apical Periodontitis: A Case Report	Case Report
Ben Balevi[16]	**2019**	Cold pulp testing is the simplest and most accurate of all dental pulp sensibility tests	Comment to the editor
Yi Zhao et al.[13]	**2019**	Controversies Regarding the Management of Teeth Associated with Cystic Lesions of the Jaws	Review
Ricucci D. et al.[11]	**2020**	Atypically grown large periradicular cyst affecting adjacent teeth and leading to confounding diagnosis of non-endodontic pathology	Case Report
Elhakim A. et al.[14]	**2021**	Preserving the vitality of teeth adjacent to a large radicular cyst in periapical microsurgery: a case report with 4-year follow-u	Case Report
Patro et al.[21]	**2022**	Diagnostic Accuracy of Pulp Vitality Tests and Pulp Sensibility Tests for Assessing Pulpal Health in Permanent Teeth: A Systematic Review and Meta-Analysis	Systematic Review and Meta-analysis
Asgary S. et al.[20]	**2022**	Pulp Vitality Preservation of an Involved Tooth in a Large Radicular Cyst: A Case Report with 4-Year Recall	Case Report
Donnermeyer et al.[22]	**2022**	Effectiveness of diagnosing pulpitis: A systematic review	Systematic Review
Kim et al.[12]	**2023**	Recovery of Pulp Sensibility After the Surgical Management of a Large Radicular Cyst: A Case Report with a 4.5-Year Follow-up	Case Report

lack of consensus. Ricucci et al., Martins et al. and Sood et al.[9-11] apply endodontic treatment to all teeth involved in the lesion because they believe that cyst enucleation irreversibly damages the neurovascular bundle of tooth apices, leading to subsequent pulp necrosis. Komabayashi et al.[17] report the enucleation of a 20 x 12 mm cyst after finding that root canal treatments alone did not suffice to achieve regression of the pathology. More moderately, Elhakim et al., Kim et al. and Yi Zhao et al.[12-14] propose cyst enucleation while preserving the vitality of adjacent teeth whenever possible.

Information gathered so far suggests that each case should be assessed carefully to avoid overtreatment of teeth involved in the extension of an IRC. The greatest challenge is determining pulp soundness using tests to measure sensitivity or vitality. The most frequently used sensitivity tests are thermal (cold or heat), and electric testing, all of which measure tooth nerve fiber response to stimuli, but not tooth vitality. The most frequently used vitality tests are laser doppler flowmetry and pulse oximetry, which ascertain whether there is blood flow in the tooth, and are currently considered to be the most sensitive and precise[15]. Some authors believe that pulse oximetry is the most exact method[21], though there is greater agreement that laser doppler flowmetry is closer to the gold standard[23]. However, their use is largely limited by the need for customized equipment and standardization, and the expense involved.

Other authors therefore believe that the cold test is simplest and most precise method available for testing sensitivity, and it continues to be the main diagnostic tool for evaluating pulp status[16].

Ideally, cone beam computed tomography should be used[19, 24, 25] to determine whether an apex is located within a cyst cavity, and if so, its degree of involvement. However, in daily practice, decisions are usually based on panoramic or periapical radiographs, even though they both provide two-dimensional images which may lead to erroneous conclusions, such as an apex being observed as located within a cyst cavity, when a three-dimensional study might show that it is in fact in front of or behind the cavity, separated by sound bone. In these cases, two-dimensionality may lead to unnecessary treatment a tooth.

Another controversy was identified regarding the cause of loss of sensitivity in teeth adjacent to an IRC. Some authors suggest that the increase in hydrostatic pressure within the cyst lesion may interfere with sensory transmission of the dental pulp, producing a transitory absence of sensitivity to stimuli[7,13,26]. In this case, sound teeth with negative sensory response at the beginning of the treatment might recover sensitivity when pressure is removed from their neurovascular bundles.

Stashenko et al.[27] and Komabayashi et al.[17] claim that suprainfection in an IRC may spread to adjacent teeth and cause loss of vitality, which would be irreversible[17-19]. Establishing whether loss of pulp response is caused by microbes or by an increase in intra-cyst hydrostatic pressure would require microbiological and histopathological studies that would not be possible to implement in a clinical investigation design.

Reestablishment of the blood flow interrupted by damage to the apical neurovascular bundle may be expected in teeth with incomplete root formation, as occurs after reimplantation or autotransplantation, Existing studies do not suffice to draw conclusions about mature teeth. It has been suggested that some autotransplanted teeth with complete root formation and not subjected to root canal treatment may have potential for revascularization[28]. No study was found on revascularization o pulp regeneration following IRC enucleation, but it may be expected that in the near future, knowledge gained regarding treatment of transplanted teeth may be applied to managing teeth adjacent to an IRC. Currently, pulp revascularization treatments in immature permanent teeth with pulp necrosis are considered the first choice. In these cases, it is indicated to clean and disinfect the pulp cavity followed by stimulation of periapical periodontium cells by bleeding into the root canal[29], which promotes the entry of proliferative cells that generate new tissue with different characteristics from pulp and dentin. A vascular network already present in the tooth could reconnect to the newly formed vessels in the healing bone cavity. It is not clear what factors contribute to revascularization: whether patient age, apical diameter of the affected tooth, or type of cyst lesion[13].

It must also be considered whether the degree to which apices are included in the cavity has any influence on loss of tooth vitality. One anatomical study suggested that 98% of the apical ramifications and 93% of lateral canals are located in the last 3 mm of the root[13]. This suggests that vascularization in teeth that are only minimally included in the cyst could be preserved after cyst enucleation by means of irrigation provided by the lateral canals.

CONCLUSION

There is lack of consensus regarding the best way to deal with vital teeth involved in the expansion of an IRC, and on the criteria for decision making.

Widely varying methods have been reported for evaluating pulp status. The use of reliable, replicable methods should be an essential requisite for choosing the treatment best suited to each clinical case.

Clinical studies with long-term follow up are needed in order to reach a consensus on how to deal with vital teeth involved in the extension of an inflammatory radicular cyst.

CONFLICT INTERESTS

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

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ICDA-S-II index improves early-stage diagnosis of carious lesions among schoolchildren in northern Brazil

Davi Lavareda Corrêa[1], Flávia M Flório[2], Vânia Castro Corrêa[1], Suelen Lavareda Corrêa[3], Sue A Lavareda Corrêa Uchôa[3], Cecilia Pedroso Turssi[2]

1. Faculdade de Odontologia, Universidade Federal do Pará (UFPA), Belém-PA.
2. Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Campinas/SP, Brazil.
3. Centro Universitário da Amazônia (UNIESAMAZ), Faculdade de Odontologia, Campinas/SP, Brazil.

ABSTRACT

The Decayed, Missing and Filled Teeth (DMFT) index is widely used for detecting carious lesions, primarily focusing on established cavities, while the International Caries Detection and Assessment System (ICDA-S-II) is designed to identify incipient lesions. ***Aim:*** *The aim of this cross-sectional study was to assess the diagnostic effectiveness of the DMFT index compared to the ICDA-S-II criteria designed for early-stage carious lesion diagnosis in schoolchildren from Belém (Brazil).* ***Materials and Method:*** *A cohort of 107 twelve-year-old schoolchildren from Belém (Brazil) underwent dental examinations by three calibrated examiners using the DMFT and ICDA-S-II indices. The ICDA-S-II assessment involved prophylaxis, relative isolation, and a standardized drying period. Statistical analyses included ANOVA, chi-squared and G tests.* ***Results:*** *No statistically significant differences were observed among examiners for either the DMFT (p = 0.699) or the ICDA-S-II (p = 1.000) indices. Gender did not influence results (DMFT: p = 0.697; ICDA-S-II: p = 0.310). Caries-free prevalence differed significantly, at 32% according to DMFT and 2.8% according to ICDA-S-II (p<0.001).* ***Conclusions:*** *The DMFT index consistently underestimated carious lesions, whereas the ICDA-S-II index enhanced the identification of incipient potentially reversible lesions. DMFT and ICDAS-II indices have demonstrated their efficacy in cavity detection, with the most significant distinction arising in ICDAS-II in the identification of early-stage carious lesions.*

Keywords: *dental caries - oral diagnosis - epidemiology - cross sectional studies.*

O índice ICDA-S-II aumenta diagnóstico de lesões iniciais de cárie em escolares do norte do Brasil

RESUMO

O índice de Dentes Cariados, Perdidos e Obturados (CPOD) é uma ferramenta amplamente utilizada para detectar lesões cariosas, concentrando-se principalmente em cavidades estabelecidas. No entanto, o Sistema Internacional de Detecção e Avaliação de Cárie (ICDA-S-II) é projetado para identificar lesões incipientes. ***Objetivo:*** *Este estudo transversal tem como objetivo avaliar a eficácia diagnóstica do índice CPOD em comparação com os critérios do ICDA-S II projetados para o diagnóstico de lesões cariosas em estágio inicial em crianças escolares de Belém (Brasil).* ***Materiais e Método:*** *Uma coorte de 107 crianças escolares de doze anos de idade, de Belém (Brasil), foi submetida a exames odontológicos usando os índices CPOD e ICDA-S-II por três examinadores calibrados. A avaliação do ICDA-S-II envolveu profilaxia, isolamento relativo e um período de secagem padronizado. As análises estatísticas incluíram ANOVA, qui-quadrado e testes G.* ***Resultados:*** *Não foram observadas diferenças estatisticamente significativas entre os examinadores usando os índices CPOD (p = 0,699) ou ICDA-S-II (p = 1,000). O gênero não influenciou os resultados (CPOD: p = 0,697; ICDA-S-II: p = 0,310). A prevalência de crianças livres de cárie diferiu significativamente, com CPOD em 32% e ICDA-S-II em 2,8% (p < 0,001).* ***Conclusões:*** *Este estudo revela que o índice CPOD subestima consistentemente as lesões cariosas, enquanto o índice ICDA-S-II, projetado para detecção precoce, melhora a identificação de lesões incipientes, potencialmente reversíveis. Os índices DMFT e ICDAS-II demonstraram sua eficácia na detecção de cáries, com a diferença mais significativa surgindo no ICDAS-II na identificação de lesões cariosas em estágio inicial.*

Palavras-chave: *cárie dentária - diagnóstico oral - epidemiologia - estudos transversais.*

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Corresponding Author:
Davi Lavareda Corrêa
davilavareda2@yahoo.com.br

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INTRODUCTION

Dental caries is an important health indicator, and its epidemiology explores the broader aspects of health and disease within a population[1,2]. In addition to providing a comprehensive evaluation of the health status in a population, epidemiological data guide the development of preventive and treatment initiatives in oral healthcare[3,4]. The information gathered can be used to plan effective strategies and track disease trends, ultimately informing the design of appropriate oral healthcare interventions[2,3].

In epidemiological surveys, the Decayed, Missing and Filled Teeth (DMFT) index provides a standardized collective diagnosis of dental caries, and enables swift, straightforward comparisons between studies conducted at different times[4-6]. Recent surveys employing the DMFT index have identified a noteworthy reduction in global prevalence and severity of dental caries in different countries, including Brazil[7,8], where a substantial decline in caries prevalence among 12-year-olds has been reported[8,9]. However, Latin American studies have shown the limitations of the DMFT index for detecting non-cavitated lesions, with research from Colombia, Brazil, Argentina and Venezuela demonstrating that the DMFT index underestimates caries prevalence by failing to account for early-stage lesions, which account for a significant portion of dental caries[10-14].

Previous studies have shown that the decrease in the DMFT index is associated exposure to fluoride from water and toothpaste, focus on oral health education, and improved access to dental services[15,16]. However, this trend should be interpreted with caution because it may not fully reflect the true prevalence of the disease and treatment requirements in the population[17,18]. In Brazil, for instance, dental caries predominantly affects a small segment of the population, leading to disparities among regions, cities, and various demographic groups[8,11,14]. This regional disparity is consistent with findings from other Latin American countries such as Venezuela and Colombia, where socioeconomic factors and access to care contribute to variations in caries prevalence[11,14]. However, with the decline in dental caries prevalence, and progress in caries research, there is a need for an index that can be used to assess pre-cavitated lesions, such as the International Caries Detection and Assessment System (ICDAS-II), the accuracy of which is reported to be comparable to the DMFT[13,19-21].

A limited number of studies compare the DMFT and ICDAS-II indices in Brazil[4,11,13,20,22-27], and because of the importance of identifying lesions in their initial phases, there is a need to evaluate the compatibility between the two indices. This observational cross-sectional investigation was designed to compare caries lesion detection using the DMFT index, which focuses on the decayed component, and the ICDAS-II criteria, in a cohort of schoolchildren from northern Brazil.

MATERIALS AND METHOD

Ethics statement

The study was approved by the Ethics Committee of São Leopoldo Mandic School in Campinas, São Paulo, Brazil (CAAE: 15165913.0.0000.5374). Authorization to conduct the study was granted by the primary schools República de Portugal and Amância Pantoja, affiliated with the Department of Education of Belém (Pará, Brazil). Parents or guardians of the children were provided with detailed information on research aims and procedures and requested to sign an Informed Consent Form. All procedures were carried out in strict accordance with the ethical standards established by the relevant institutional and national committees overseeing human experimentation.

Sample Size

The sample size was determined based on a confidence level of 95%, an absolute sampling error of 10%, a prevalence of caries of 50%, and a non-response rate of 10%. The calculation yielded 107 individuals (BioEstat 5.0 software (Mamirauá Institute in Solimões, Amazonas, Brazil).

Study design

This was an observational cross-sectional study of 107 12-year-olds of both genders, whose DMFT and ICDAS-II indices were evaluated by three examiners. One examiner (DLC) was designated as the "gold standard" examiner due to his specialization in public health, and entrusted with the preliminary screening of participants. The results recorded by the other examiners were subsequently compared to those acquired by the gold standard examiner.

Inclusion and Exclusion Criteria

Eligible participants were children formally enrolled

at municipal schools, whose parents or guardians duly endorsed the consent form. Ineligible were children undergoing orthodontic treatment, or with severe fluorosis, hypoplasia or systemic illness.

Conditions of Examinations and Calibration Training

The examinations and clinical training were conducted in a dental office setup, specially assembled on the school premises exclusively for the research, and equipped with a chair, compressor, cuspidor, saliva ejectors, and an equipment unit with a triple syringe, a stool, and a reflector. Clinical mouth mirrors, clinical tweezers, and a WHO periodontal probe with a 0.5 mm spherical-tipped end were used. The probe was employed for diagnostic purposes without exerting pressure on the dental surface, solely to clarify any uncertainties arising from visual diagnosis. Initial active lesions on smooth surfaces were defined as dental elements presenting as opaque white patches, rough in texture, and lacking luster[28].

Examiner Calibration

The gold standard examiner oversaw the examiner calibration process, which consisted of four stages:

First stage: Theoretical foundation pertaining to DMFT[29,30] and ICDAS-II[20] criteria. Examiners received theoretical materials, instruction on the diagnosis of caries lesions, dental specimens exemplifying incipient and cavitated lesions, and detailed explanations of the codes and criteria for the indices (Tables 1 and 2).

Second Stage: Examination of 10% of the sample

Table 1. DMFT codes and index following WHO classification.

DMFT index		
Code	**Condition**	**Description**
0	Sound	No evidence of caries lesions is present; early disease stages are not considered. The following signs should be categorized as sound: whitish spots, rough spots resistant to CPI probe pressure, grooves and fissures in stained enamel without visible signs of softening, enamel cavities, or softening of walls detectable with the CPI probe, as well as dark, shiny, hard, and fissured areas of enamel on teeth with moderate or severe fluorosis, and lesions resulting from abrasion based on their distribution, history, or tactile/visual examination.
1	Cavitated	A cavitated lesion is when a groove, fissure, or smooth surface exhibits evident cavitation, softened tissue at the base, discoloration of the enamel or wall, or has a temporary restoration (except for glass ionomer). The CPI probe should be used to confirm visual evidence of caries lesions on occlusal, buccal, and lingual surfaces. When in doubt, consider the tooth as sound.
2	Restored with caries	There are one or more restorations, and at the same time, one or more areas have caries. There is no distinction between primary and secondary carious lesions, meaning whether the lesions are or are not in physical association with the restoration(s).
3	Restored and caries-free	There are one or more permanent restorations, and there is no presence of primary or recurrent carious lesions. A tooth with a crown placed due to a carious lesion falls into this category. If the crown results from other causes, such as prosthesis support, it is coded as 7 (H).
4	Lost due to caries	A permanent or deciduous tooth was extracted due to a carious lesion and not for other reasons. This condition is recorded in the box corresponding to the crown. Deciduous teeth: apply only when the individual is in an age group where normal shedding is not a sufficient justification for the absence.
5	Lost for other reasons	Absence is due to orthodontic, periodontal, traumatic, or congenital reasons.
6	Fissure sealant	There is a fissure sealant, or the occlusal fissure has been widened to receive composite. If the tooth has a sealant and is carious, code 1 or B (caries lesion) takes precedence.
7	Bridge or Crown/ Implant Support	Indicates a tooth that is part of a fixed prosthesis. This code is also used for crowns installed for reasons other than carious lesions or for teeth with cosmetic veneers. Extracted teeth replaced by a fixed bridge component are coded in the crown condition box as 4 or 5.
8	Unerupted tooth	When a permanent or deciduous tooth has not yet erupted, consider the eruption timeline. This does not include teeth lost due to congenital problems, trauma, etc.
T	Trauma	Part of the coronal surface has been lost as a result of trauma, and there is no evidence of caries lesion.
9	Excluded tooth	Applied to any permanent tooth that cannot be examined (orthodontic bands, severe hypoplasia, etc.

Table 2. ICDAS-II codes and index following WHO classification.

ICDAS-II index	
Code	**Description**
0	Healthy dental surface: no evidence of caries after prolonged drying (5 s).
1	First visual change in enamel: opacity or discoloration (white or brown) visible in fissures after prolonged drying.
2	Distinct visual change in the enamel when wet; the lesion must be visible when dry.
3	Cavity in enamel (without clinical signs of dentin involvement) visible when wet and after prolonged drying.
4	Shadowing of the underlying dentin.
5	Cavity with visible dentin: visible demineralization with exposed dentin.
6	Extensive cavity (more than half of the surface), with visible dentin exposure.

by all three examiners to address any ambiguous points and reach consensus by discussing clinical findings, diagnostic criteria, codes and how errors were recorded, to achieve an appropriate level of agreement (Kappa > 0.85). After achieving satisfactory performance in the final assessment, clinical training commenced.

Third stage: Examination of 10% of the 107 children by all three examiners. Prior to clinical inspection, the gold standard examiner conducted prophylaxis and used dental floss on each child. During clinical inspection, all teeth were classified using the DMFT and ICDAS-II indices. Following the ICDAS-II criteria manual[31], teeth were initially examined moist, then re-examined after 5 seconds of drying.

Fourth stage: Final discussion of the results, to conclude the exercise. During this stage, emphasis was placed on the need to achieve a high level of agreement before commencing data collection in the field. The clinical data collected were transcribed onto individual forms by a single recorder who had undergone prior training. In the calibration process, inter-examiner agreement among the three examiners was excellent (90.8% to 99.0%), both for DMFT and ICDAS-II (Kappa agreement coefficient 0.80 to 0.96, indicating almost perfect agreement).

Statistical Analysis

The data underwent descriptive statistics, including frequency distributions for categorical variables and calculations of central tendency and variability for quantitative variables. Inter-examiner agreement was assessed using Kappa statistics (k), providing agreement coefficients with a 95% confidence interval. Analysis of Variance (ANOVA) was used to compare DMFT data from different examiners. For categorical variables, the chi-square test was used, with the G-test as an alternative when criteria were not met. Comparisons of observations concerning the presence of caries lesions based on both DMFT and ICDAS-II indices involved the creation of two new variables for each tooth, indicating absence (coded as 0) or presence (coded as 1 or 2 for DMFT and 1 to 6 for ICDAS-II) of caries lesions. This methodology enabled a robust comparison of the two indices using the chi-square test. Median and quartile values of teeth with caries lesions diagnosed using DMFT and ICDAS-II (codes 1 to 6) were subjected to the Wilcoxon test. The statistical analyses were conducted using BioEstat 5.0 software (Mamirauá Institute in Solimões, Amazonas, Brazil) at 5% significance level (α).

RESULTS

Among the 107 participants, 67 (62.6%) were female and 40 (37.4%) were male.

Inter-examiner agreement was excellent (Table 3). Agreement was higher for examinations employing the DMFT index than the ICDAS-II index (Table 3). The component denoting dental decay had the highest prevalence, accounting for 89.4% of cases, followed by filled teeth at 8.4%, and missing teeth at 2.2%. The DMFT index, as observed by the gold standard examiner (#1), was determined to be 2.11 teeth. Examiners 2 and 3 reported DMFT indices of 1.88 and 1.91, respectively (Table 4). There was no statistically significant variance among the observations made by the different examiners (p = 0.69).

DMFT indicated absence of caries in 32.7% of

Table 3. Agreement between the examiners and the gold standard examiner, and Kappa statistics by index used for dental caries detection.

		Examiner 1		Examiner 2		Examiner 3	
Index	**N**	**%**	**k (CI95%)**	**%**	**k (CI95%)**	**%**	**k (CI95%)**
DMFT	107	99.6	0.97 (0.96-0.97)	98.9	0.89 (0.88-0.90)	99.4	0.94 (0.93-0.95)
ICDAS-II	107	97.8	0.94 (0.94-0.95)	93.5	0.85 (0.83-0.86)	95.4	0.89 (0.88-0.90)

Legends: N: sample size; %: agreement; k: Kappa coefficient 95% CI: 95% confidence interval.

Table 4. Mean and proportions relative to DMFT.

		Sound	Decayed		Filled		Missing		DMFT		ANOVA
Examiner	**N**	$\bar{x}$	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	***S***	***p-value***
1	107	23.78	1.88	89.4	0.18	8.4	0.05	2.2	2.11	2.22	
2	107	24.11	1.69	90.0	0.14	7.5	0.05	2.5	1.88	1.94	0.69
3	107	24.02	1.73	90.6	0.13	6.9	0.05	2.5	1.91	2.09	

Legends: N: sample size; $\bar{x}$: mean; %: percentage composition; S: standard deviation.

Table 5. Proportion of individuals with (DMFT ≥ 1) and without caries lesion (DMFT = 0).

	DMFT = 0		DMFT ≥ 1		chi-square
Examiner	**AF**	**%**	**AF**	**%**	**p-value**
1	35	32.7	72	67.3	
2	36	33.6	71	66.4	0.98
3	36	33.6	71	66.4	

Legends: AF: absolute frequency; %: Percentage.

the children when assessed by the gold standard examiner, and 33.6% when assessed by the other examiners (Table 5). The chi-square test showed no statistically significant disparities among the observations recorded by the various examiners (p = 0.98).

For the ICDAS-II index, the predominant condition observed was denoted by code 1, which, when cumulatively considered alongside code 2, accounted for 77.0% of the cases scrutinized by the gold standard examiner. Statistical analysis employing the G-test indicated a consistent level of observation among all three examiners, with a p-value of 1.00 (Table 6).

The gold standard examiner identified 2.8% of children as caries-free according to the ICDAS-II index, while examiner 2 recorded a slightly higher rate at 3.7%. All three examiners observed that a considerable portion of participants (29.9%) had at least one tooth with an incipient lesion lacking cavitation. Cavitated carious lesions were found in at least one tooth in 67.3% of the children examined by the gold standard examiner and examiner 3, while examiner 2 documented a slightly lower percentage of 66.4%. There was no statistically significant discrepancy among the three examiners, as indicated by a p-value of 0.99 (Table 7).

Table 6. Mean and proportion of the components of the ICDAS-II index and of teeth with caries.

			ICDAS-II														
			Code 1		Code 2		Code 3		Code 4		Code 5		Code 6		Σ Code 3-6		G-test
		N	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	**%**	$\bar{x}$	**S**	**P-value**
Examiner	1	107	3.57	40.9	3.15	36.1	1.41	16.2	0.05	0.5	0.37	4.3	0.17	1.9	2.00	2.19	
	2	107	3.15	41.0	2.69	35.0	1.29	16.8	0.05	0.6	0.34	4.4	0.17	2.2	1.85	1.94	1.00
	3	107	3.49	42.4	2.87	34.9	1.29	15.7	0.05	0.6	0.36	4.4	0.17	2.0	1.87	2.05	

Legend: N: sample size. Σ Code 3-6: Sum of teeth coded 3 to 6 according to ICDAS-II. $\bar{x}$: mean. %: percentage. S: standard deviation.

Table 7. Patients according to caries severity using the ICDAS-II index.

		ICDAS-II						
		Without cavity or initial lesion (code 0)		Without cavity and with initial lesion (codes 1 and 2)		With cavity (codes 3, 4, 5, and 6)		G-test
Examiner	N	AF	%	AF	%	AF	%	P-value
1	107	3	2,8	32	29,9	72	67,3	
2	107	4	3,7	32	29,9	71	66,4	0.99
3	107	3	2,8	32	29,9	72	67,3	

Legend: AF: absolute frequency; %: percentage.

The relatively infrequent occurrence (2.8%) of individuals without caries lesions detected by the ICDAS-II index differed significantly ($p < 0.001$) from observations using the DMFT index, where 34.6% were found to be free of caries lesions (Fig. 1A). When the DMFT and ICDAS-II indices were evaluated within the context of caries cavity detection, no significant difference was found ($p = 0.88$) (Fig. 1B).

Median DMFT was 2, with the 1st and 3rd quartiles reported as 0 and 3 teeth, respectively. For the ICDAS-II index (codes 1 to 6), the median number of teeth exhibiting visible lesions was 8, with the 1st and 3rd quartiles reported as 5 and 12 teeth, respectively. Evaluation of these observations showed a statistically significant contrast between ICDAS-II index (codes 1 to 6) and DMFT ($p < 0.001$) (Fig. 1C).

Similarly, for the ICDAS-II index (codes 3 to 6), the median number of teeth with caries cavities was 1, with the 1st and 3rd quartiles reported as 0 and 3 teeth with cavities, respectively. Interestingly, this specific aspect of cavity observations did not yield any significant differences ($p = 0.76$) (Fig. 1D).

DISCUSSION

Dental caries has long been recognized as a global public health concern[1-3], though its prevalence and severity have been reduced by scientific development, increased awareness of the significance of oral health, and improved access to treatment[20,21]. As a result, dental caries indicators have been adapted and made more stringent for epidemiological surveys[20,31]. For instance, a study in Venezuela found that DMFT significantly underestimated the prevalence of non-cavitated lesions, whereas including initial caries lesions in the index increased the DMFT score by 33% in a Venezuelan population[14]. Research from Argentina and Colombia also highlights the importance of detecting non-cavitated lesions, which account for a significant portion of total carious lesions[10,12]. In Brazil, according to the latest census data from 2022, 12-year-old children present a DMFT index of 1.6, which is lower than the value found in our current study[9]. Dental caries is a substantial concern in many countries, some of which report prevalence rates exceeding 50% among 12-year-olds[32-34], and disproportionate impact on socioeconomically disadvantaged populations. In these countries, division, polarization, and significant economic and social disparities exert direct influence on the overall health of the population[35].

According to DMFT, 32.7% of participants were caries-free. The prevalence of dental lesions was higher than in the previous survey conducted in Brazil, which reported that 43.5% of 12-year-old children were free from dental caries[31]. This difference could be attributed to social factors correlated to the causes of the disease, such as family dynamics, workplace environment and economic policies[36]. The DMFT results were compared to ICDAS-II, given its international relevance and widespread utilization in other countries. The current study found similar results using the DMFT and the ICDAS-II indices.

The ICDAS-II index for dental caries lesions (codes 1 to 6) showed that codes 1 and 2 accounted for 77.0% of the cases, highlighting incipient lesions. This is supported by findings by Melgar et al.[23], who reported that non-cavitated lesions accounted for 60% of the total caries burden in young children in Brazil, demonstrating the need for tools like ICDAS to capture the full spectrum of caries stages. This

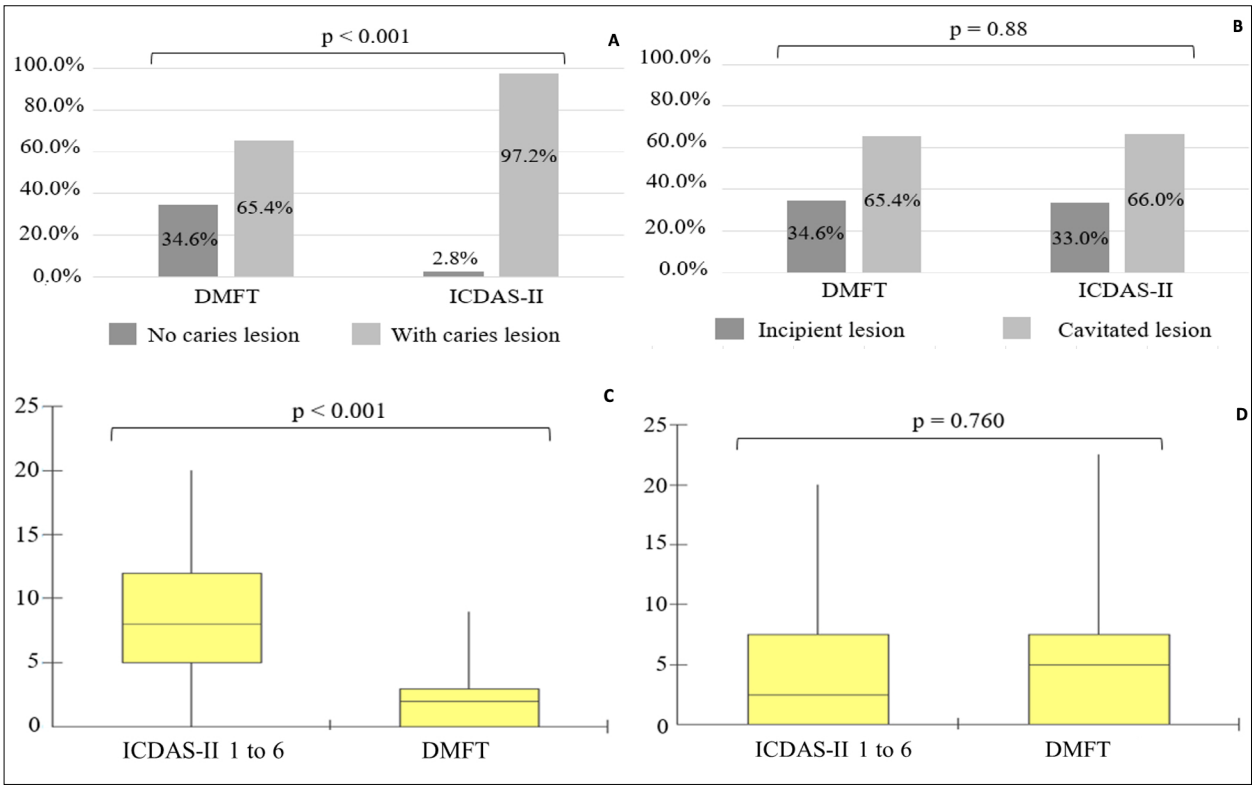

Fig. 1: Graphic results of the statistical analysis. A) Patients with or without dental caries lesions considering DMFT and ICDAS-II indices. B) Patients with or without dental cavities due to caries based on DMFT and ICDAS-II indices. C) Median and quartile values of teeth with caries lesions diagnosed using DMFT and ICDAS-II (codes 1 to 6) in Wilcoxon test. D) Median and quartile values of teeth with cavities due to caries observed using the DMFT and ICDAS-II in Wilcoxon test (codes 3 to 6).

underscores the importance of paying attention to non-cavitated white spot lesions, which are potentially reversible[22,31], but if not diagnosed in a timely manner, can progress to cavities, making oral hygiene and disease control more challenging, and often requiring invasive treatment.

The prevalence of dental caries, as assessed by the ICDAS-II index in this study, is considered high, as 97.2% of the examined children had at least one tooth classified with codes 1 to 6. This agrees with previous studies by Guido *et al*.[37] and Soto-Rojas *et al*.[38], which also employed this index. Furthermore, 67.3% of the children presented caries lesions in the cavitated stage (codes 3 to 6), indicating the need for restorative treatment. The importance of early diagnosis is thus emphasized, particularly when caries lesions are still in stages 1 and 2 of the ICDAS-II scale.

A consensus has yet to be established regarding the cutoff point for defining cavitated dental caries lesions using the ICDAS-II scoring system[39,40]. Some studies designate lesions as cavitated when classified with code 4 or higher[41,42]. Similar issues regarding the classification of cavitated versus non-cavitated lesions have been reported in Venezuela and Colombia, where researchers emphasize the importance of defining appropriate cutoff points to ensure consistent reporting in epidemiological studies[11,14]. In the current study, lesions scoring 3 or higher were considered cavitated. Score 3 is often used as the threshold for comparison with the DMFT [2,20] because it closely resembles the DMFT criteria, given that the World Health Organization criteria only recognize dental caries when lesions penetrate into the dentin[7,29,30].

It is important to highlight that in the current study, the number of individuals identified with no dental caries differed significantly depending on whether the DMFT or ICDAS-II indices were used. According to DMFT, 34.6% of children were considered free from caries lesions (DMFT = 0), whereas according to ICDAS-II, only 2.8% showed no signs of dental caries (code 0). This discrepancy arises because DMFT code 0 includes ICDAS-II codes 1 and 2. These findings align with the conclusions reported in other studies that DMFT

underestimates the presence of dental caries because it does not account for the early stages of carious lesions[43,44]. They also agree with other studies from Latin America, such as de Souza et al.[11] and Acevedo et al.[14], highlighting the advantages of ICDAS in capturing early-stage lesions, which DMFT typically overlooks. As a result,, children identified as "caries-free" by the DMFT index who have non-cavitated lesions do not receive personalized treatment, despite collective preventive measures being in place[45,46], and these lesions may progress to irreversible stages. Interestingly, the findings in the current study regarding the absence of caries in permanent teeth using the ICDAS-II differ from Kuhnisch et al.[46], who reported that 70% of children were free from dental caries in their permanent dentition. This discrepancy may be attributed to the fact that Kuhnisch et al. collected data from Europeans.

ICDAS-II is still relatively unfamiliar to dentists, and despite its advantage of detecting incipient caries lesions, it has limitations. Training for ICDAS-II calibration takes approximately twice as long as for DMFT[43]. While the DMFT is faster and easier to administer, it tends to underestimate the presence of caries lesions, potentially leading public health programs overlooking the true extent of dental caries in the population. Including ICDAS in public health programs has proven beneficial in countries like Brazil, where research shows, it can help target preventive measures more effectively by identifying lesions in the early stages of development[11,23].

The results of the current study underscore the strategic importance of including early-stage lesions in epidemiological surveys, and show that the use of resources such as artificial light and professional prophylaxis prior to clinical examination would be helpful to improve efficiency[29]. Examiner calibration is crucial to identify incipient lesions that may be underestimated by traditional criteria, with the subject being classified as healthy. Diagnosing lesions in early stages is fundamental for applying minimally invasive interventions and adopting a more conservative approach with disease control measures[30,41]. This change in diagnosis would contribute to achieving more precise allocation of public health services, improving diagnostic techniques, and providing appropriate guidance for preventive and therapeutic measures in oral health programs[41,42,46]. In Latin American countries such as Venezuela and Brazil, adopting ICDAS in public health surveys has already demonstrated its potential to provide more precise data on caries prevalence, particularly in underserved populations where non-cavitated lesions are often missed[14,23].

In conclusion, our study shows that the DMFT index is a valuable tool in dental caries diagnosis, but may tend to underestimate its extent. It also underscores the effectiveness of the ICDAS-II index in identifying incipient lesions, which, in turn, provides a critical contribution to early-stage treatment. This insight emphasizes the importance of enhancing the accuracy of caries detection and enabling timely interventions, ultimately promoting better oral health outcomes. In Latin America, ICDAS can improve caries detection and prevention efforts significantly by capturing early-stage lesions, which can halt the progression of the disease and reduce the need for invasive treatments in the future.

CONFLICT INTERESTS

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

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Exploring furcation involvement diagnosis and treatment practices: a cross-sectional survey among general dentists in southern Santa Fe Province, Argentina

Juan C Llaudet[1], Enrique Pussetto[1], Martin Carrion[2], Carlos D De la Vega Elena[3,4], Fabio Herrero[1,2,4]

1. Instituto Universitario Italiano de Rosario, Facultad de Odontología, Especialidad en Periodoncia, Rosario, Argentina.
2. Instituto Universitario Italiano de Rosario, Facultad de Odontología, Cátedra de Periodoncia, Rosario, Argentina.
3. Instituto Universitario Italiano de Rosario, Facultad de Odontología, Cátedra de Bioinformática V, Rosario, Argentina.
4. Instituto de Investigaciones Instituto Universitario Italiano de Rosario, Rosario, Argentina

ABSTRACT

The risk of losing a tooth with furcation involvement is twice as high as that of losing a multirooted tooth with good periodontal status. Early diagnosis of furcation involvement increases the likelihood of retaining the tooth in the oral cavity. ***Aim:*** *To explore the behavior and limitations of general dentists in the southern region of Santa Fe Province in the detection and clinical management of furcation lesions.* ***Materials and Method:*** *A cross-sectional study was conducted using an anonymous online questionnaire consisting of 32 questions to assess general dentists' experience in diagnosis and management of furcation lesions (instrumental, classification and treatment), how likely they were to refer patients, and their self-perception regarding certain topics. Invitations to participate were extended twice between November 2022 and March 2023 via email sent by the Dental Association of the 2nd District of Santa Fe Province. The invitation emails contained a link to the questionnaire on the Google Forms platform, which participants accessed after reading the information sheet and providing informed consent to participate. Once the survey was completed, the responses were exported as a matrix from the Google Forms platform and anonymized.* ***Results:*** *Most of the 121 surveyed dental professionals reported difficulties with diagnosis, limitations in handling instruments, and challenges in selecting appropriate treatments.* ***Conclusions:*** *As reported by similar studies in other countries, there is a clear need for further training and development of continuous education programs for general dentists in the region.*

Key words: *diagnosis - furcation involvement - periodontitis - treatment - dentists - professional training*

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Corresponding Author:
Fabio Herrero
fmherrero65@gmail.com

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Diagnóstico y tratamiento de lesiones de furcación: una encuesta entre odontólogos generales del sur de la provincia de Santa Fe, Argentina

RESUMEN

Una pieza dentaria con lesión de furcación duplicaría el riesgo de pérdida en comparación con otro diente multirradicular con un buen estado periodontal. El diagnóstico temprano de una lesión de furcación aumenta la permanencia de la pieza dentaria en la cavidad oral. ***Objetivo:*** *Explorar el comportamiento y las limitaciones de los odontólogos generales del sur de la Provincia de Santa Fe en la detección y manejo clínico de las lesiones de furcación.* ***Materiales y Método:*** *Se realizó un estudio transversal utilizando un cuestionario anónimo en línea compuesto por 32 preguntas para investigar la experiencia de los profesionales odontólogos en cuanto al diagnóstico y tratamiento de las lesiones de furcación. También se relevó las posibilidades de derivación y la autopercepción de los profesionales respecto a los problemas planteados. Los profesionales fueron invitados a participar dos veces entre noviembre de 2022 y marzo de 2023 a través de correo electrónico enviado por el Colegio de Odontólogos de la 2da. Circunscripción de la Provincia de Santa Fe. Los correos de invitación contenían un enlace al cuestionario en la plataforma Google Forms, al que los participantes accedían después de leer la hoja informativa y proporcionar su consentimiento explícito para participar (consentimiento informado). Una vez completado el cuestionario, las respuestas se exportaron como matriz desde la plataforma Google Forms y se anonimizaron.* ***Resultados:*** *La mayoría de los 121 profesionales odontólogos encuestados mostraron dificultades diagnósticas, deficiencias en el manejo de instrumentos y desafíos en la elección del tratamiento.* ***Conclusiones:*** *Como se ha informado en estudios similares en otros países, queda evidente la necesidad de mayor capacitación y desarrollo de programas de educación continua para los odontólogos generales en la región.*

Palabras clave: *diagnóstico - lesión de furcación - periodontitis - tratamiento - odontólogos - formación profesional*

INTRODUCTION

Furcation involvement occurs when alveolar bone resorption extends to the bifurcation or trifurcation areas of a multirooted tooth due to periodontal disease[1]. Current classifications commonly used for furcation lesions include the measurement of horizontal extension[2], vertical measurement[3], and categorization based on clinical and radiographic parameters[4].

The presence of furcation involvement is associated with an increased risk of tooth loss, both in patients under supportive periodontal therapy[5-10] and in patients without periodontal therapy[11]. As a complexity factor, furcation lesions class II and III determine the stage of periodontitis according to the 2018 classification of periodontal diseases[12]. Therefore, adequate diagnosis is unlikely without complete probing of furcation lesions.

Although there is sufficient evidence regarding the best treatment options for furcation involvement at all stages, the best prognosis is achieved through prevention and early detection. An untreated grade I furcation lesion will progress and require more complex treatment[11], but adequate diagnosis and treatment will reduce the risk of tooth loss[10,13]. A grade II-III furcation lesion requires more complex treatment to minimize the risk of tooth loss[14-16].

Currently, furcation lesions are significantly underdiagnosed in primary care[17]. In Argentina there is no information available on the detection and clinical management of furcation involvement by general dentists, nor on the potential need to improve education and continuous professional development.

The aim of this study was to explore the behavior and limitations of general dentists in the southern region of Santa Fe Province in the detection and clinical management of furcation lesions.

MATERIALS AND METHOD

Study Design

This was an observational, online survey-based, cross-sectional study with prospective data collection.

Population

The study population consisted of general dentists practicing in the southern region of Santa Fe Province. Inclusion criteria were dentists registered with the Dental Association of the 2nd District of Santa Fe Province at the time of the study, and practicing in one of the following departments in the province: Rosario, Caseros, Constitución, Iriondo, San Lorenzo, General López or Belgrano. Dentists specialized in periodontics were excluded from the study.

Recruitment

Invitations to participate were e-mailed twice between November 2022 and March 2023 by the Dental Association of the 2nd District of Santa Fe Province. They contained a link to the Google Forms questionnaire, which participants accessed after reading the information sheet and providing informed consent.

Questionnaire

The questionnaire comprised 32 questions (Table 1) covering: a) Clinical diagnosis of furcation involvement, b) Radiographic diagnosis of furcation involvement, c) Perception of prognosis of these lesions, d) Treatment management of these lesions, and e) Need to update knowledge on the topic. It was based on a questionnaire published previously by Nibali et al.[17] with modifications and additional questions. Once the established data collection period concluded, the questionnaires were exported as a matrix from Google Forms and anonymized.

Statistical Analysis: Descriptive statistics were performed using IBM SPSS Statistics software (version 25).

Ethical Considerations

This study was approved by the Institutional Committee of Ethics and Bioethics (Res. CEB IUNIR No. 44/22 dated November 29, 2022). Participation was voluntary and anonymous. Precise information was provided about the research and its objectives, and prior consent was obtained before the respondents accessed the online Google Forms questionnaire. Project members signed a confidentiality agreement.

The study followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist for cross-sectional studies.

Table 1. Self-administered questionnaire provided to general dentists in the south of the Province of Santa Fe, regarding their behavior and limitations in the diagnosis and management of furcation lesions.

A. Demographics and General Information of Survey Participants	
Question	Options
A1. Gender	( ) Female ( ) Male ( ) Other
A2. Age	( )
A3. Department of the 2nd Circumscription of the Province of Santa Fe where you work	( ) Belgrano ( ) Caseros ( ) Constitución ( ) General López ( ) Iriondo ( ) Rosario ( ) San Lorenzo
A4. Professional setting	( ) Works in the private sector ( ) Works for the Public Service ( ) Works for both the Public Service and in the private sector
A5. University of Graduation (Undergraduate)	( )
A6. Year of Graduation as a Dentist	( )
A7. Do you have postgraduate degree(s)?	( ) No ( ) Yes. If yes, please specify: ( )
B. Routine Practices	
B1. How many periodontal patients do you estimate you treat per week?	( ) <5 ( ) 5 to 10 ( ) 11 to 20 ( ) >20
B2. How many patients do you refer to a specialist per week?	( ) <5 ( ) 5 to 10 ( ) 11 to 20 ( ) >20
B3. In your daily practice, do you have access to the Nabers Probe?	( ) Yes ( ) No ( ) Maybe
B4. How often do you use the Nabers probe?	( ) On every patient ( ) On most patients ( ) Only on patients with advanced periodontitis ( ) Never
B5. In your daily practice, do you have access to periodontal probes?	( ) Yes ( ) No ( ) Not sure
B6. How often do you use periodontal probes?	( ) On every patient ( ) On most patients ( ) Only on patients with advanced periodontitis ( ) Never
B7. When performing a periodontal probing, how many sites do you evaluate per tooth?	( ) 2 ( ) 4 ( ) 6 ( ) I never perform probing
B8. How many cases of furcation lesions have you treated with periodontal surgery throughout your professional life?	( ) None ( ) 1 to 10 ( ) 11 to 50 ( ) > 50
C. Experience Report	
C1. Flap access periodontal surgery	( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5
C2. Regenerative periodontal surgery	( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5
C3. Root resection surgery	( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5
C4. Implant surgery	( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5
D. Knowledge and Self-perception	
D1. Do you think there is furcation involvement in this case? (image)	( ) Yes ( ) No ( ) Not sure
D2. Do you think there is furcation involvement in these first molars? (image)	( ) Yes ( ) No ( ) Not sure
D3. Do you think there is furcation involvement in these first molars? (image)	( ) Yes ( ) No ( ) Not sure
D4. Do you think there is furcation involvement in these first molars? (image)	( ) Yes ( ) No ( ) Not sure
D5. Do you believe the furcation lesion affects dental prognosis? (image)	( ) Yes ( ) No ( ) Not sure
D6. How would you manage a case of advanced furcation as shown in the following radiograph (both affected molars)?	( ) Would treat ( ) Would refer to a specialist ( ) Would not treat
D7. How confident are you that this tooth can be maintained functional for at least 5 years? (image)	(1) No confidence (2) Low confidence (3) Medium confidence (4) High confidence (5) Very high confidence
D8. How confident are you in detecting furcation lesions?	(1) No confidence (2) Low confidence (3) Medium confidence (4) High confidence (5) Very high confidence
D9. How confident are you in knowing how to manage furcation lesions?	(1) No confidence (2) Low confidence (3) Medium confidence (4) High confidence (5) Very high confidence

continue on next page

Table 1. (cont.)	
D11. Do you think an implant would have a higher survival rate than a tooth with furcation involvement in a periodontally stable patient?	( ) Yes ( ) No ( ) Not sure
D12. What are the main challenges you face when treating a furcation lesion? Check all that apply.	( ) Lack of time ( ) Lack of adequate instruments ( ) Lack of experience in detection and classification of furcation lesions ( ) Lack of knowledge about referral and management protocols ( ) Other
E. Interest in Continuous Education	
E1. Are you interested in learning more about detection, classification, and management of furcation lesions?	( ) Yes ( ) No ( ) Not sure
E2. How would you like to learn more about furcation lesions? Check all that apply.	( ) Theoretical course with in-person mode ( ) Theoretical course with virtual mode ( ) Theoretical-Practical course (Workshop) ( ) Theoretical-Practical course (Clinical)

RESULTS

A total of 121 complete surveys that met the selection criteria were analyzed.

Table 2. Demographics and general information of survey participants		
Sex	**N**	**%**
Female	64	52.9
Male	57	47.1
Total	121	100.0
Age		
21-30 years	6	5.0
31-40 years	28	23.1
41-50 years	47	38.8
51-60 years	31	25.6
>60 years	9	7.4
Total	121	100.0
Department in Santa Fe Province		
Belgrano	3	2.5
Caseros	4	3.3
Constitución	10	8.3
General López	6	5.0
Iriondo	1	0.8
Rosario	83	68.6
San Lorenzo	14	11.6
Total	121	100.0
Professional setting		
General Dentist in Private Sector	78	64.5
General Dentist in Public Service	2	1.7
General Dentist in Public Service and Private Sector	36	29.8
Missing Data	5	4.1
Total	121	100.0
University of Graduation		
Instituto Universitario Italiano de Rosario	5	4.1
Universidad de Buenos Aires	1	0.8
Universidad Nacional de Córdoba	2	1.7
Universidad Nacional del Litoral	1	0.8
Universidad Nacional de Rosario	111	91.7
Universidad Nacional de Tucumán	1	0.8
Total	121	100.0
Year of Graduation (Dentist)		
1966-1981	6	5.0
1981-1996	30	24.8
1996-2011	60	49.5
2011-2024	25	20.7
Total	121	100.0
Postgraduate Degree/s		
No	71	58.7
Yes	50	41.3
Total	121	100.0

Surveyed Population

Most of the surveyed dentists reported practicing in the Departments of Rosario and San Lorenzo. Of the surveyed professionals, 52.9% were female and 47.1% were male, with ages ranging from 26 to 73 years. Regarding professional development, 64.5% worked in the private sector, 29.8% worked in both the public and private sectors, and 1.7% worked exclusively for the public sector. Nearly all respondents graduated from the School of Dentistry

of Universidad Nacional de Rosario (UNR), with a smaller percentage graduating from the School of Dentistry of Instituto Universitario Italiano de Rosario (IUNIR) and Universidad Nacional de Córdoba (UNC), mostly between 1999 and 2010. Only 41.3% reported having a postgraduate degree (Table 2).

Experience in Periodontal Treatment

Among the respondents, 46.3% reported treating fewer than 5 periodontal patients per week, while 41.3% treated 5 to 10 patients per week. A smaller proportion treated 11 to 20 patients, and even fewer treated more than 20 patients weekly. Regarding periodontal probes, only 51.2% reported having access to a Nabers probe, with 85% using it only on periodontal patients (Tables 3 and 4).

Table 3. Routine Practices

B1. How many periodontal patients do you estimate you treat per week?	N	%
<5	56	46.3
5 to 10	50	41.3
11 to 20	12	9.9
>20	3	2.5
Total	121	100.0
B2. How many patients do you refer to a specialist per week?		
<5	100	82.6
5 to 10	19	15.7
11 to 20	1	0.8
>20	1	0.8
Total	121	100.0
B3. In your daily practice, do you have access to a Nabers probe?		
Yes	62	51.2
No	53	43.8
Maybe	6	5.0
Total	121	100.0
B4. How often do you use a Nabers probe?		
On every patient	2	1.7
On most patients	9	7.4
Only on patients with advanced periodontitis	50	41.3
Never	60	49.6
Total	121	100.0
B5. In your daily practice, do you have access to periodontal probes?		
Yes	115	95.0
No	6	5.0
Total	121	100.0
B6. How often do you use periodontal probes?		
On every patient	14	11.6
On most patients	48	39.7
Only on patients with advanced periodontitis	45	37.2
Never	14	11.6
Total	121	100.0
B7. When performing a periodontal probing, how many sites do you evaluate per tooth?		
0	9	7.4
2	29	24.0
4	63	52.1
6	20	16.5
Total	121	100.0
B8. How many cases of furcation lesions have you treated with periodontal surgery throughout your professional life?		
None	80	66.1
1 to 10	26	21.5
11 to 50	12	9.9
> 50	3	2.5
Total	121	100.0

Periodontal probes were available to 95%, but only 11.6% used them on all patients.

Concerning the number of sites evaluated per tooth, only 16.5% evaluated them correctly (at 6 sites), while the rest had deficiencies.

Two-dimensional radiographic images were used to assess ability to detect presence or absence of furcation involvement in molars. Three different cases were presented: in Case 1 (Fig. 1), only 9.9% expressed uncertainty regarding the presence of furcation involvement in the molar; in Case 2 (Fig. 2), 23.1%; and in Case 3 (Fig. 3), 15%. The remaining participants provided either positive or negative responses. The divergent answers suggest that diagnosis based solely on two-dimensional radiographic images does not provide certainty regarding the presence or absence of furcation lesions.

Table 4. Experience Report

Procedure / Experience Level	N	%
C1. Periodontal flap surgery		
No experience	70	57.9%
Low experience	19	15.7%
Medium experience	15	12.4%
High experience	12	9.9%
Very high experience	5	4.1%
Total	121	100.0%
C2. Regenerative periodontal surgery		
No experience	80	66.1%
Low experience	16	13.2%
Medium experience	14	11.6%
High experience	11	9.1%
Very high experience	0	0%
Total	121	100.0%
C3. Root resection surgery		
No experience	72	59.5%
Low experience	20	16.5%
Medium experience	15	12.4%
High experience	11	9.1%
Very high experience	3	2.5%
Total	121	100.0%
C4. Implant surgery		
No experience	58	47.9%
Low experience	12	9.9%
Medium experience	17	14.1%
High experience	24	19.8%
Very high experience	10	8.3%
Total	121	100.0%

Table 5. Knowledge and Self-perception

Question	N	%
D1. Do you think there is furcation involvement in this case? (image)		
No	9	7.4%
Not sure	30	24.8%
Yes	82	67.8%
Total	121	100.0%
D2. Do you think there is furcation involvement in these first molars? (image) (Case 1)		
No	6	5.0%
Not sure	12	9.9%
Yes	103	85.1%
Total	121	100.0%
D3. Do you think there is furcation involvement in these first molars? (image) (Case 2)		
No	76	62.8%
Not sure	28	23.1%
Yes	17	14.0%
Total	121	100.0%
D4. Do you think there is furcation involvement in these first molars? (image) (Case 3)		
No	33	27.3%
Not sure	18	14.9%
Yes	70	57.9%
Total	121	100.0%
D5. Do you think furcation involvement affects dental prognosis? (image)		
No	1	0.8%
Not sure	3	2.5%
Yes	117	96.7%
Total	121	100.0%
D6. How would you manage a case of advanced furcation as in in the following X-ray (both affected molars)?		
Would refer to a specialist	87	71.9%
Would treat	34	28.1%
Total	121	100.0%
D7. How confident are you that this tooth can remain functional for at least 5 years? (image)		
No confidence	23	19.0%
Low confidence	36	29.8%
Medium confidence	42	34.7%
High confidence	17	14.0%
Very high confidence	3	2.5%
Total	121	100.0%
D8. How confident do you feel when detecting furcation lesions?		
No confidence	5	4.1%
Low confidence	23	19.0%
Medium confidence	55	45.5%
High confidence	30	24.8%
Very high confidence	8	6.6%
Total	121	100.0%
D9. How confident do you feel about knowing how to manage furcation lesions?		
No confidence	28	23.1%
Low confidence	44	36.4%
Medium confidence	34	28.1%
High confidence	14	11.6%
Very high confidence	1	0.8%
Total	121	100.0%

Table 5. (cont.)

D10. Do you think an implant would have higher survival rate than a tooth with furcation involvement in a periodontally stable patient?		
No	52	43.0%
Not sure	44	36.4%
Yes	25	20.7%
Total	121	100.0%
D11. What do you believe are the major challenges you face when dealing with a furcation involvement? Check all that apply.		
Lack of experience in detection and classification of furcation lesions	67	55.4%
Unfamiliarity with referral and management protocols	42	34.7%
Lack of appropriate instruments	33	27.3%
Lack of time	14	11.6%
Poor patient response to oral care techniques	6	5.0%
Not a periodontal specialist	2	1.7%
Patient›s time and ability to cover costs	1	0.8%
Root anatomy	1	0.8%
Total	121	

Perception of Prognosis in Teeth with Furcation Lesions

A high percentage (96.7%) agreed that furcation lesions affect the survival of multirooted teeth.

Confidence in Detection and Management of Furcation Lesions

When presented with a case of furcation lesion in two molars, only 28.1% of respondents stated they would treat them, while 71.9% would refer them to a periodontal specialist (Table 5). These percentages are consistent with the answers regarding the difficulty general dentists encounter in managing this type of lesions (Fig. 4).

However, when asked whether a molar with a furcation lesion, treated and preserved in the mouth, would have a higher survival rate than if it were extracted and replaced with an implant, 43% answered in favor of treating the molar versus 20.7% who opted for replacement with an implant. These responses indicate that although dentists consider these lesions clinically challenging to manage, they prioritize treatment over extraction and implant placement.

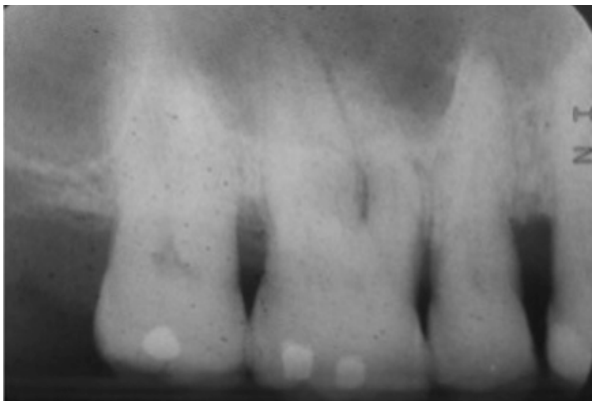

Fig. 1: Case 1. Teeth 16 and 17.

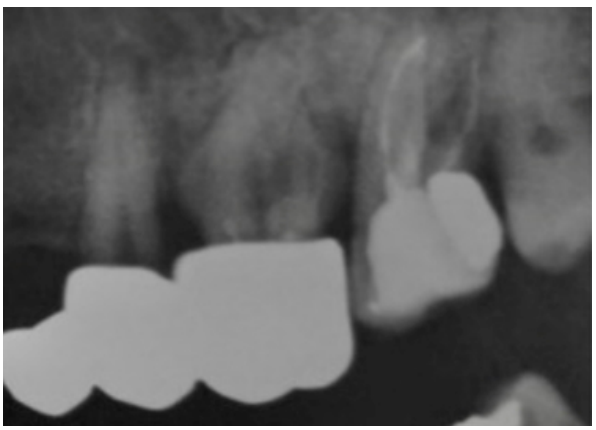

Fig. 2: Case 2. Teeth 26 and 27.

Fig. 3: Case 3. Tooth 36.

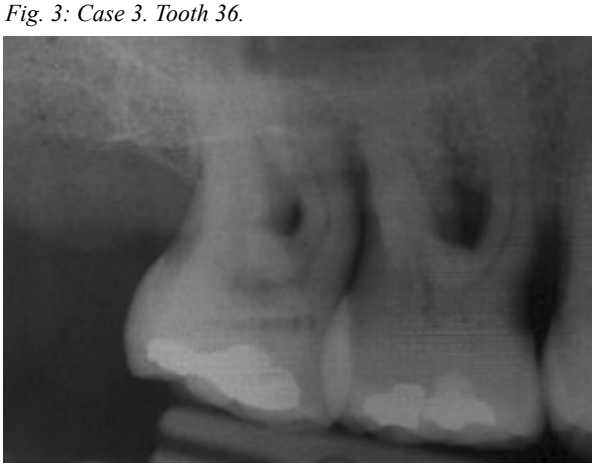

Fig. 4: Case 4. Teeth 16 and 17.

Barriers to Dealing with Furcation Lesions and Ways to Improve Them

A significant proportion (55.4%) of respondents said they had difficulties in diagnosing and treating furcation lesions. Nonetheless, 72.7% expressed interest in learning more about the detection, classification, and management of furcation lesions (Table 6).

Table 6. Interest: Continuous Education

E1. Are you interested in learning more about detection, classification, and management of furcation involvement?	N	%
No	17	14.0%
Not sure	16	13.2%
Yes	88	72.7%
Total	121	100.0%
E2. How would you like to learn more about furcation lesions? Check all that apply.		
Theoretical course with in-person mode	15	12.4%
Theoretical course with virtual mode	38	31.4%
Theoretical-Practical course (Clinical)	36	29.8%
Theoretical-Practical course (Workshop)	32	26.4%
Total	121	100.0%

DISCUSSION

This is the first study in Argentina using a survey format to analyze general dentists' knowledge of diagnosis and management of furcation involvement. The analysis considered the new Classification of Periodontal Diseases presented in 2018, which was defined in the 2017 Workshop by Tonetti et al.[12], and the current classifications for furcation involvement, including those by Hamp et al.[2] and Tarnow et al.[3]. A correct understanding of terminology is essential to be applied to each unit of analysis[1].

Clinical diagnosis of periodontal pathologies requires the use of a probe (Marquis/North Carolina), and specifically, Nabers probes are used for furcation lesions[8,10]. In this study, most dentists were aware of them, but only a low proportion used them on all patients. In contrast, only half had access to Nabers probes and used them in their daily practice. Not knowing about and/or not using Nabers probes make it difficult to diagnose furcation lesions. The percentages recorded in the current study were similar to those found recently by Nibali et al.[17] among dentists from different countries.

Only a few dentists reported correct use of periodontal probes, i.e., on six sites per tooth[18]. Incorrect use makes it difficult to establish a definitive diagnosis, as periodontal lesions cannot be identified if the probe is not used at all sites of each tooth. Similarly, furcation lesions cannot be diagnosed if the probe is not placed in each furcal entrance in premolars and molars. Appropriate treatment can only be planned based on suitable diagnostic practice[2,3].

Most respondents said they treated a significant number of patients (though not more than 10) with periodontal pathologies per week.

These results show that general dentists encounter a high degree of difficulty in identifying and making a definitive diagnosis of periodontal lesions, especially in patients with severe periodontitis (stages III and IV). When furcation lesions remain undiagnosed, treatment is neither planned nor executed correctly.

Furthermore, current criteria propose that different periodontal pathologies (gingivitis and periodontitis) should be diagnosed by general dentists, not only by specialists[12], which was not found to be the case in this study. Sanz et al.[14], Dommisch et al.[15] and Jepsen et al.[16], believe that 70% of these pathologies can be treated by general dentists.

Respondents' answers regarding the diagnosis of upper and lower molars using two-dimensional periapical radiographs revealed difficulties in determining the presence of furcation lesions in all three clinical cases analyzed. This reflects (a) the limitation of periapical radiography as a single diagnostic method, which creates difficulty not only in identifying the furcation lesion but also in categorizing the type of furcation lesion based on current classifications, and (b) the difficulty encountered by general dentists in making a correct diagnosis through imaging alone, without clinical examination. The same limitation applies to specialists[14].

However, nearly all respondents considered the impact of furcation lesions on the prognosis of affected teeth to be significant, demonstrating an awareness of the challenges associated with treating these lesions, and the risk they pose to tooth retention[5]. These results are similar to those reported for a group of specialists in a European study on general dentists and specialists[10].

Another significant finding of the current study was that most professionals lacked confidence in effectively managing furcation lesions, and preferred

to refer cases of advanced furcation lesions (Hamp type 2/3) to periodontal specialists. However, when asked about whether an implant would offer better survival than a tooth affected by furcation involvement in a periodontally stable mouth, responses indicated preference for preserving the affected tooth rather than placing an implant.

A furcation lesion is not an absolute indication for extraction and despite the reserved prognosis, tooth survival with appropriate preventive protocols can range from 10 to 20 years or more[6,7]. The findings in the current study undoubtedly show lack of clarity in the clinical management of furcation lesions, and in the knowledge of treatment outcomes and prognosis, compared to an implant as a replacement for a tooth. It should be noted that replacing a tooth with an implant is more costly than treating a furcation lesion[19].

Limitations in the acquisition of theoretical knowledge in undergraduate dental education significantly influence practice. As evidence-based knowledge constitutes the foundation of professional diagnosis, treatment and preventive strategy planning, not only for furcation lesions, but also for oral health in general, the importance of updating it should be emphasized.

Another aim of this study was to assess general practitioners' need for updates in this field: a high proportion responded affirmatively regarding their need to update their knowledge of classification, detection and clinical management of furcation. There is a need to reinforce knowledge of current instruments, diagnosis, and management of periodontal diseases in general and furcation involvement in particular.

CONCLUSION

The findings suggest the need for further education in the diagnosis and clinical management of periodontal diseases, as well as the implementation of continuous training programs for general dentists.

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CONFLICT INTERESTS

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

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Mandibular size as a predictor of vertical dimension of occlusion based on cephalometric analysis

Omar E Gárate-Silva

Facultad de Odontología, Universidad de Chile.

ABSTRACT

There are multiple methods for determining Vertical Dimension of Occlusion (VDO), but most of them require scientific validation. ***Aim:*** *To study the correlation between mandibular cephalometric measurements and VDO in young Chilean adults with complete dentition and known inclusion criteria, by using modified Knebelman's technique.* ***Materials and Method:*** *The study population consisted of 96 young Chilean adults aged 18 to 35 years. Inclusion criteria were complete natural dentition, bilateral molar support, skeletal class I or mild class II, presence of anterior coupling, and asymptomatic temporomandibular joints. Exclusion criteria were prior or ongoing orthodontic treatment, having undergone orthognathic or other facial surgery, poor oral habits (mouth breathing, or lingual, labial or object interposition), severe dental crowding (IOTN score > 2), too much beard and/or soft tissue under the chin. Anthropometric measurements were taken with a modified digital vernier caliper. Mandibular cephalometric measurements were taken with the QuickCeph 2000 software on digital lateral cephalometric x-rays. All anthropometric and cephalometric measurements were taken by one operator. Based on the mandibular cephalometric measurements with the highest correlation, a mathematical model was proposed to predict the VDO [VDO' = (XAEO-STF)*0.3 + (R3R4 dist.)0.5 + (Go-Ar dist.)-0.3 + (Ar-Po Mand.Depth.)*0.4 – 8], whose predictive capacity will be tested.* ***Results:*** *The three cephalometric measurements with highest correlation with VDO were selected. The resulting predictive model correlated significantly with actual VDO (r= 0.77), in addition to having significant correlation values according to the Björk-Jarabak facial biotypes.* ***Conclusions:*** *The proposed mathematical model demonstrated a strong correlation with the Vertical Dimension of Occlusion. It is a reliable method, uninfluenced by the patient's sex or biotype, and is useful for restoring the VDO within a physiological range close to its original state.*

Keywords: *vertical dimension of occlusion - cephalometry - mandibular size - modified Knebelman technique - predictive model.*

Tamaño mandibular como variable predictora de la dimensión vertical oclusal a partir del análisis cefalométrico

RESUMEN

Existen múltiples métodos para determinar la Dimensión Vertical de Oclusión (DVO), pero la mayoría requiere de validación científica. ***Objetivo:*** *Estudiar la correlación entre mediciones cefalométricas mandibulares y la DVO en adultos jóvenes chilenos con dentición completa y criterios de inclusión conocidos, utilizando la Técnica de Knebelman modificada.* ***Materiales y Método:*** *La población de estudio es de 96 jóvenes chilenos de 18 a 35 años. Criterios de inclusión: dentición natural completa, soporte molar bilateral, clase esqueletal I o clase II suave, presencia de acople anterior y articulaciones temporomandibulares asintomáticas. Criterios de exclusión: aquellos con tratamiento de ortodoncia previa o en curso, sometidos a cirugía ortognática u otra cirugía facial, con malos hábitos orales (respiración bucal, interposición lingual, labial y de objetos), presencia de apiñamiento dentario severo (índice IOTN> 2), cantidad excesiva de barba y/o tejido blando bajo el mentón. Se realizaron mediciones antropométricas con un pié de metro digital modificado y cefalométricas mandibulares mediante el software QuickCeph 2000 en telerradiografías de perfil digitales. Tanto las mediciones antropométricas como cefalométricas fueron realizadas por un mismo operador. A partir de las mediciones cefalométricas mandibulares con mayor correlación, se plantea un modelo matemático para predecir la DVO [DVO'= (XAEO-STF)*0.3 + (dist.R3R4)*0.5 + (dist.Go-Ar)*-0.3 + (Depth.Mand. Ar-Po)*0.4 – 8], cuya capacidad predictiva será puesta a prueba.* ***Resultados:*** *Se seleccionaron las tres medidas cefalométricas con mayor correlación con la DVO. El modelo predictivo resultante correlacionó significativamente con la DVO real (r= 0.77) y además obteniendo valores de correlación significativos según los biotipos faciales de Björk-Jarabak.* ***Conclusiones:*** *El modelo matemático planteado demostró una buena correlación con la Dimensión Vertical de Oclusión. Es un método fiable, no influenciado por el sexo o biotipo del paciente y útil para restaurar la DVO dentro de un rango fisiológico cercano al original.*

Palabras clave: *dimensión vertical de oclusión - cefalometría - tamaño mandibular - técnica de Knebelman modificada - modelo predictivo.*

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Corresponding Author:
Omar E Gárate-Silva
omarenrique.gs@me.com

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CLINICAL IMPLICATIONS

Enables simple, reliable determination of VDO, independently of biotype, sex or edentulousness, by means of a mathematical equation with 4 variables: one clinical measurement and 3 measurements taken on a lateral cephalometric x-ray.

INTRODUCTION

Determining the Vertical Dimension of Occlusion (VDO) is a basic step in planning prosthetic rehabilitation for an edentulous patient without stable occlusal references. Clinicians should be aware of their skills and limitations upon choosing a method. Goldstein et al. claim that there is a difference, philosophically speaking, between restoring and increasing VDO[1]. There is currently vast evidence supporting the need to restore VDO in patients who have lost occlusal landmarks or have severe dental wear, and many successful outcomes have been achieved[2]. Calamita et al. (2019) concluded that VDO can be considered a dimension that is not immutable over time, and can therefore be found within a range of physiological tolerance[3]. Lassman et al. note that there is no scientific evidence to support any direct association between altering the VDO and the development of temporomandibular disorders[4].

Batra says that although there are multiple tools and/or methods for determining VDO, most of them lack solid supporting scientific evidence[5]. Moreover, most current methods for determining VDO share a characteristic, since they all use the Postural Vertical Dimension (PVD) variable in some reference. Although there is no solid evidence showing that PVD changes over a person's lifetime, it has been established that it may be compromised due to loss of muscle tone as a result of aging (also related to sarcopenia and loss of muscle function in edentulous patients). As PVD is 3-dimensional, it can be affected by numerous factors, making its use unreliable for determining VDO[3].

A study by Silva et al. uses a craniometric method based on lateral craniometric x-rays, like the current study. Silva et al. propose a VDO predictive model based on cranial height and diameter (glabella-opisthocranion distance), in which a value of 0.702 was found for Pearson's correlation[6].

A well-known method for determining VDO is Knebelman's clinical craniometric method, which determines VDO directly, without the need to consider interocclusal distance or VDP. Knebelman S. Craneometric method for establishing occlusal vertical dimension. 1987. U.S. Patent number No. 4718850. Many studies have analyzed the different variables involved in Knebelman's method with the aim of testing and validating it scientifically. Chou et al. found that Knebelman's method had acceptable reproducibility, but that there were significant differences among the groups studied, and that it had not clearly defined participant exclusion and inclusion criteria[7]. In another study, Morata et al. determined that VDO is variable according to sex and facial biotype; that the most reliable facial measurement on the skin is the left side of the face, where average Pearson's correlation was found to be 0.56, and that when segmented according to biotype (classified using Facial Morphological Index), the highest correlation was found in the mesoprosopic group (r=0.60)[8].

Considering the simplicity of Knebelman's craniometric method, the aim of the current study is to reformulate it methodologically with a few adjustments, in hope of achieving better results for Pearson's correlation, which is an important parameter for comparison with other similar prior studies. Instead of using Knebelman's craniometer, we propose to use a digital vernier caliper, which is more readily available and easier to use. In addition to the measurements proposed by Knebelman, we propose some cephalometric references known to be stable and independent of edentulism. Finally, we evaluate the correlation between these measurements and the original VDO of the study subjects, with stable occlusal references and known inclusion criteria. The research hypothesis is that the variables associated to mandibular size (determined by facial biotype) could improve the predictive capacity of Knebelman's craniometric method, expressed as a function of the Pearson's correlation obtained, thereby enabling prediction of VDO by means of a mathematical equation[9].

MATERIALS AND METHOD

Sample selection

The sample consisted of 96 healthy young Chilean adults aged 18 to 35 years (49 male and 47 female), recruited over a period of six months, who were dentistry students at the University of Chile. Inclusion criteria were having complete natural dentition, bilateral molar support, skeletal class I or mild class II (without need for treatment), presence of anterior

coupling, temporomandibular joint with normal movement range and no associated symptoms. Exclusion criteria during screening (clinical examination phase) were prior or ongoing orthodontic treatment, prior orthognathic surgery or any other surgery altering facial morphology, poor oral habits (mouth breathing or lingual, labial or objects interposition), severe dental crowding (IOTN score > 2), excessive beard and/or soft tissue under the chin[10].

Informed consent and ethical considerations

Each participant signed and informed consent which had been approved by the Ethics Committee of the School of Dentistry of the University of Chile. Digital lateral cephalometric radiographs followed rigorous standards for protection against radiation.

Facial dimension measurements

An ordinary vernier caliper modified with a fixed metal extension on one of the arms (Fig. 1) was used to measure the distance in millimeters between the anthropometric points equivalent to those in Knebelman's Craniometric Method described in the method proposed by Gaete et al.[11], as follows: Subnasale (Sn') to Mentum (Me'). cutaneous, at Maximal Intercuspation (MIC); and between the landmarks Outer Canthus of the Eye (AEO) – right and left sides with eyes closed – and the Facial Tragus Sulcus (STF). All these measurements were taken on the skin with minimum compression (Fig. 2).

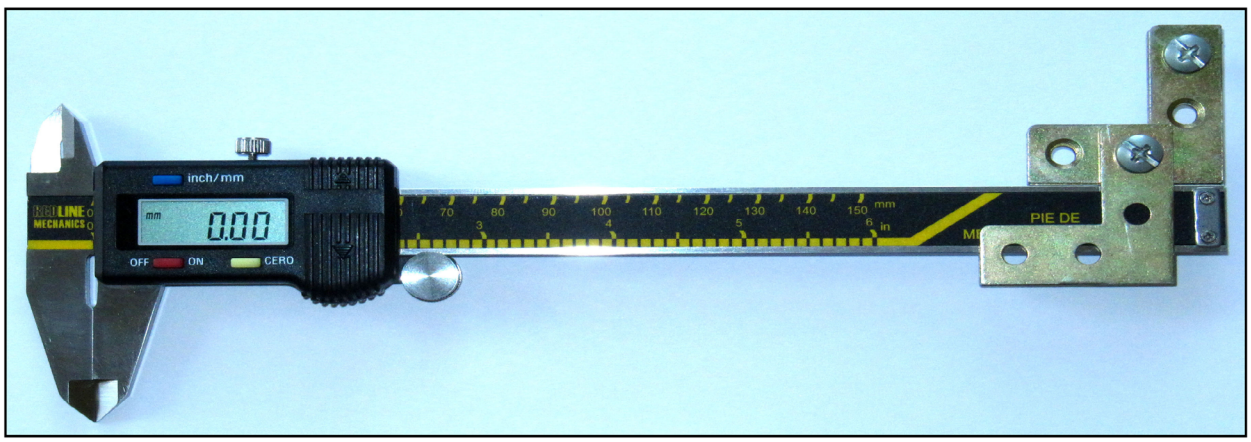

Fig. 1: Modified digital vernier caliper

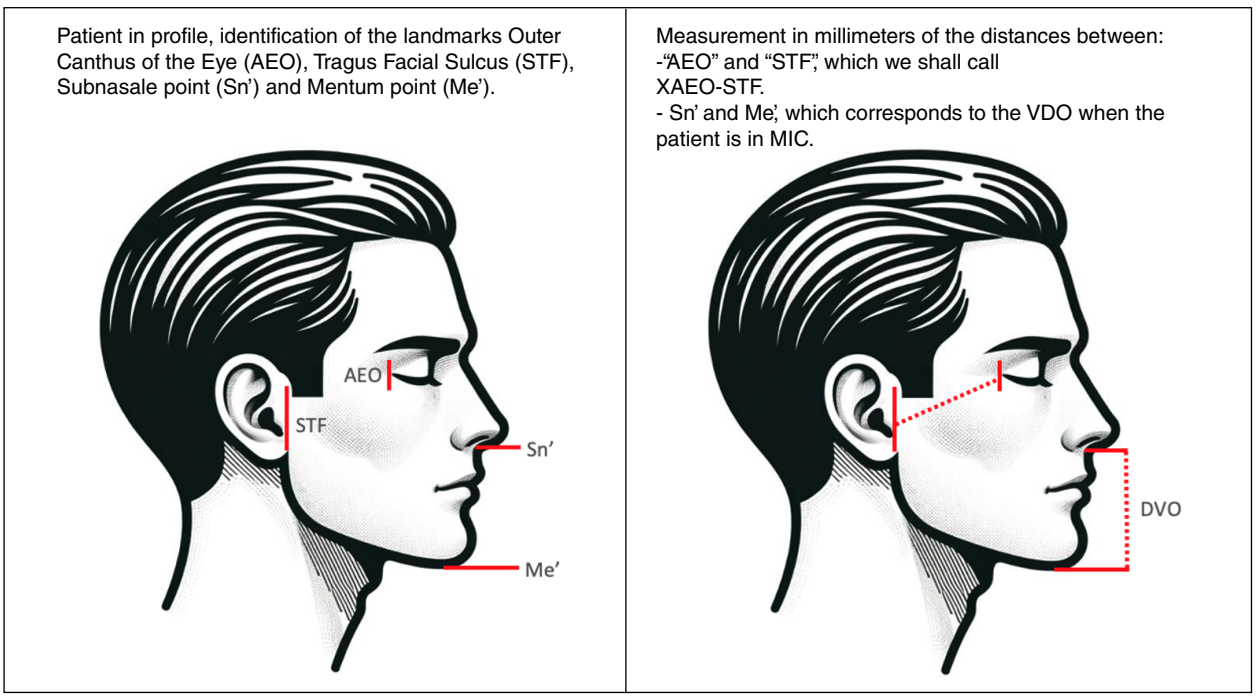

Fig. 2: Modified Knebelman method, references for measurement

All measurements were taken by one operator, with the digital vernier caliper placed at 0.0 each time. To avoid errors in measurements, the procedure was repeated up to three times per measurement so that the values recorded for a measurement would not vary by more than 1mm.

Measurements to assess mandible size

Out of all the types of cephalometric analyses described by different authors, the eight mandibular cephalometric measurements that can be made on a lateral cephalometric x-ray were selected. One operator reproduced the known cephalometric landmarks on each digital cephalometric x-ray using QuickCeph 2000 software.

The following measurements were proposed at the beginning of the study: Gonial Angle (part of Ricketts and Steiner's cephalometric analysis), Mandibular Arc Angle (part of Björk-Jarabak's cephalometric analysis), Gonion-Mentum Distance (part of Ricketts' cephalometric analysis), Distance R1-R2 and Distance R3-R4 (part of VTO analysis), Sigmoid Notch Depth, Articular Distance to Pogonion (part of Ricketts' cephalometric analysis) and Articular Distance to Gonion projected -Go'- (part of Björk-Jarabak's cephalometric analysis) (Fig. 3).

The eight cephalometric measurements taken on the lateral cephalometric radiography were analyzed using Stata 10® software and subjected to Pearson's analysis of correlation and statistical significance ($p < 0.05$ in the T-test) in relation to the VDO variable found for study participants.

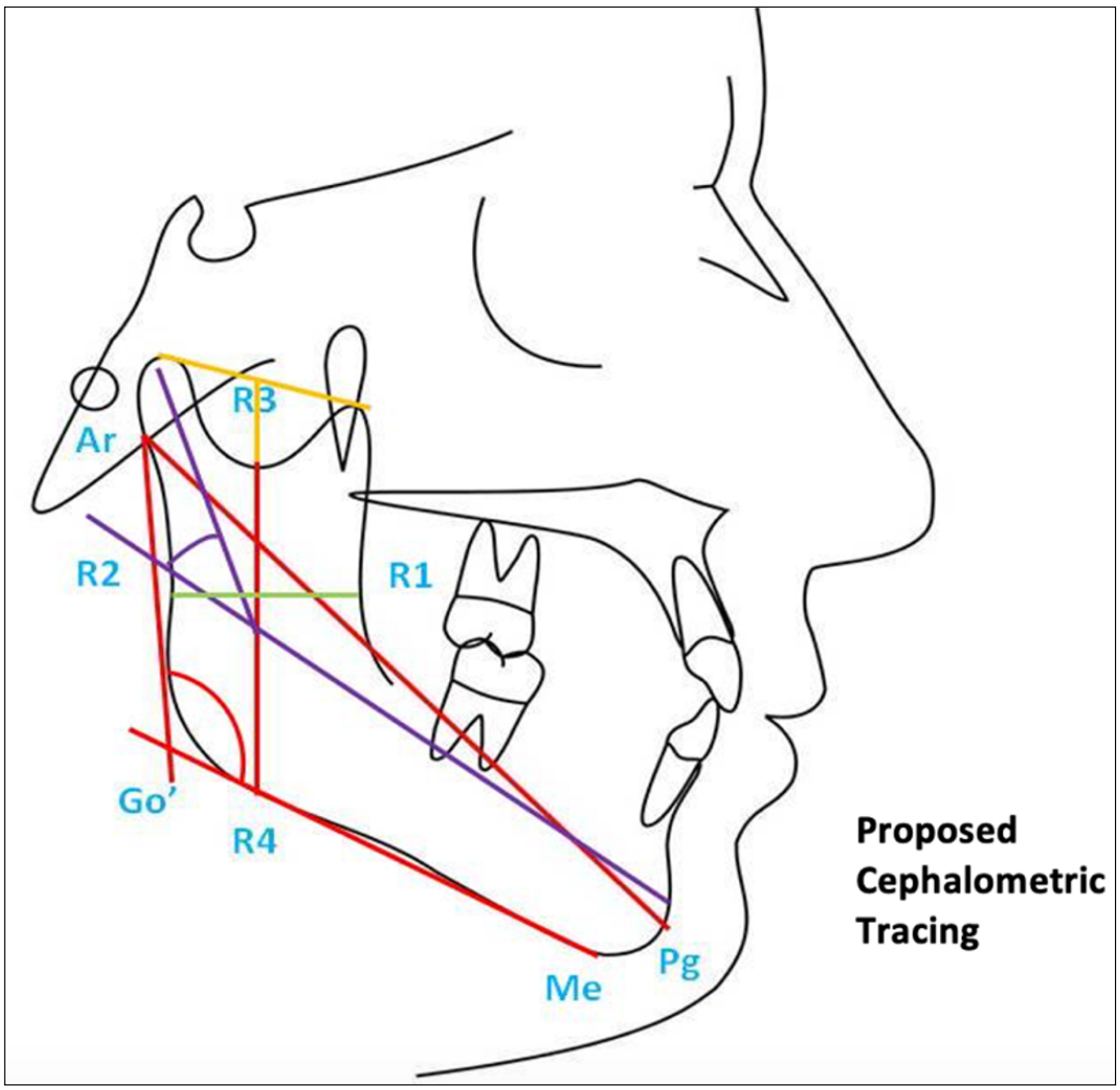

Fig. 3: Initial cephalometric tracing proposed with the landmarks for measuring the suggested variables.

RESULTS

Comparison of the average values in the study group (49 males and 47 females) between VDO and the AEO-STF measurement (average between right and left sides) showed that AEO-STF varied by up to 5.56 mm more than VDO in the study subjects (Table 1).

Table 1: Averages, standard deviation, minimum and maximum values of clinical measurements

Values for total sample

Variable	Average	Standard Deviation	Minimum Value	Maximum Value
VDO (Sn-Me)	69.70	5.88	59.22	84.04
right AEO-STF	75.45	4.88	60.01	86.32
left AEO-STF	75.07	4.72	59.40	86.06

Values of the sample segregated according to sex (Male: M; Female: F)

Variable	Average		Standard Deviation		Minimum Value		Maximum Value	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
VDO (Sn-Me)	73.15	66.39	5.38	4.23	59.38	59.22	84.04	74.53
right AEO-STF	77.67	73.33	4.37	4.41	60.33	60.01	86.32	81.60
left AEO-STF	77.13	73.09	4.20	4.36	59.40	63.28	86.06	81.37

The eight cephalometric measurements recorded initially were subject to statistical analysis on Stata 10® software, and any variables with low Pearson's correlation or low statistical significance ($p < 0.05$ in the T-test) in relation to the VDO variable were discarded. Thus, three cephalometric measurements remained for consideration in the final predictive model: Dist.R3-R4, Dist.Ar-Pog, and Dist.Go'-Ar (Fig. 4 and Table 2).

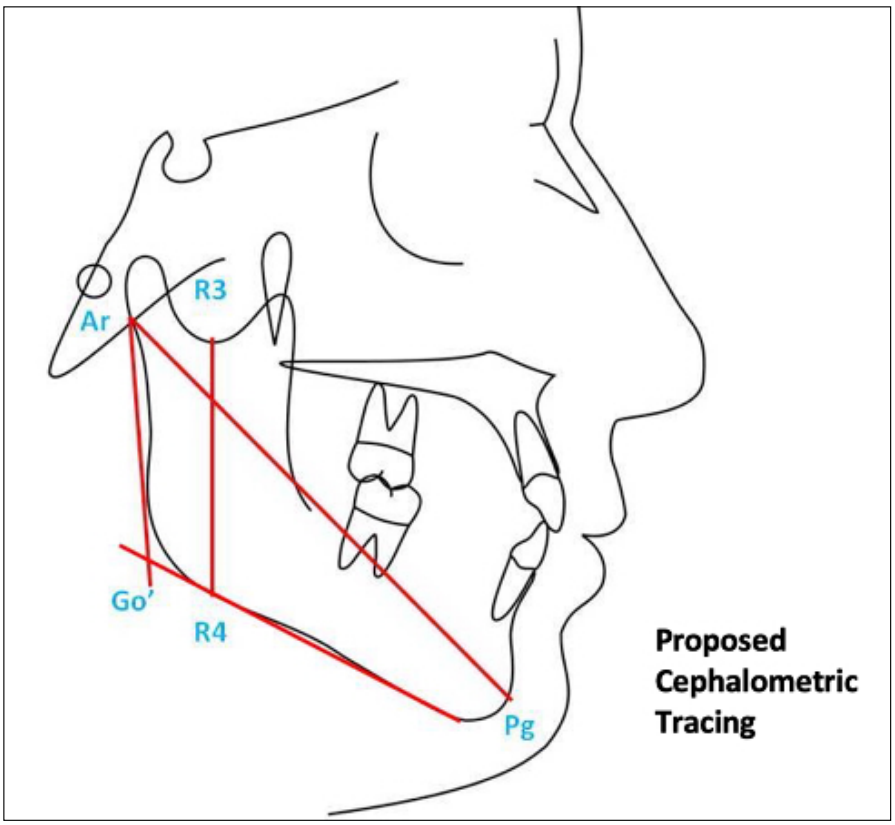

Fig. 4: Final cephalometric tracing with the landmarks for measuring the variables proposed

Table 2: Averages, standard deviation, minimum and maximum values of the selected cephalometric measurements

Values for total sample

Variable	Average	Standard Deviation	Minimum Value	Maximum Value
R3-R4	49.40	4.74	39.68	60.16
Depth. Ar-Pog	105.32	7.18	89.20	123.30
Go'-Ar	48.75	6.31	35.00	71.50

Values of the sample segregated according to sex (Male: M; Female: F)

Variable	Average		Standard Deviation		Minimum Value		Maximum Value	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
R3-R4	53.36	46.56	3.77	3.73	44.48	39.68	60.16	54.43
Depth.Ar Pog	110.22	100.63	5.64	5.07	95.60	89.20	123.30	111.9
Go'-Ar	52.18	45.47	6.12	4.52	37.70	35.00	71.50	53.50

After the preliminary analyses, the clinical measurements (XAEO-STF) and cephalometric measurements (Dist.R3-R4, Dist.Ar-Pog, Dist. Go'-Ar) together were subjected to Multiple Linear Regression Analysis with the aim of finding a mathematical model to predict VDO. The following equation was found: [VDO'= (XAEO-STF)*0.3 + (dist.R3R4)*0.5 + (dist.Go-Ar)*-0.3 + (Depth. Mand.Ar-Po)*0.4 – 8].

Pearson's Correlation for the total study population was 0.77 (interpreted as follows: 0.1 to 0.3 low correlation, 0.3 to 0.5 medium correlation, and 0.5 to 1 high Correlation:)[12]. A subsequent step investigated the correlation between the VDO found using the predictive model and the original VDO (Sn-Me), but segregating the subjects according to Björk-Jarabak, biotypes, finding the following: Hyperdivergent Biotype: R-squared 0.8473 and Pearson's Correlation 0.92 (Fig. 5), Normodivergent Biotype: R-squared 0.8917 and Pearson's Correlation 0.94 (Fig. 6), Hypodivergent Biotype: R-squared 0.5560 and Pearson's Correlation 0.74 (Fig. 7).

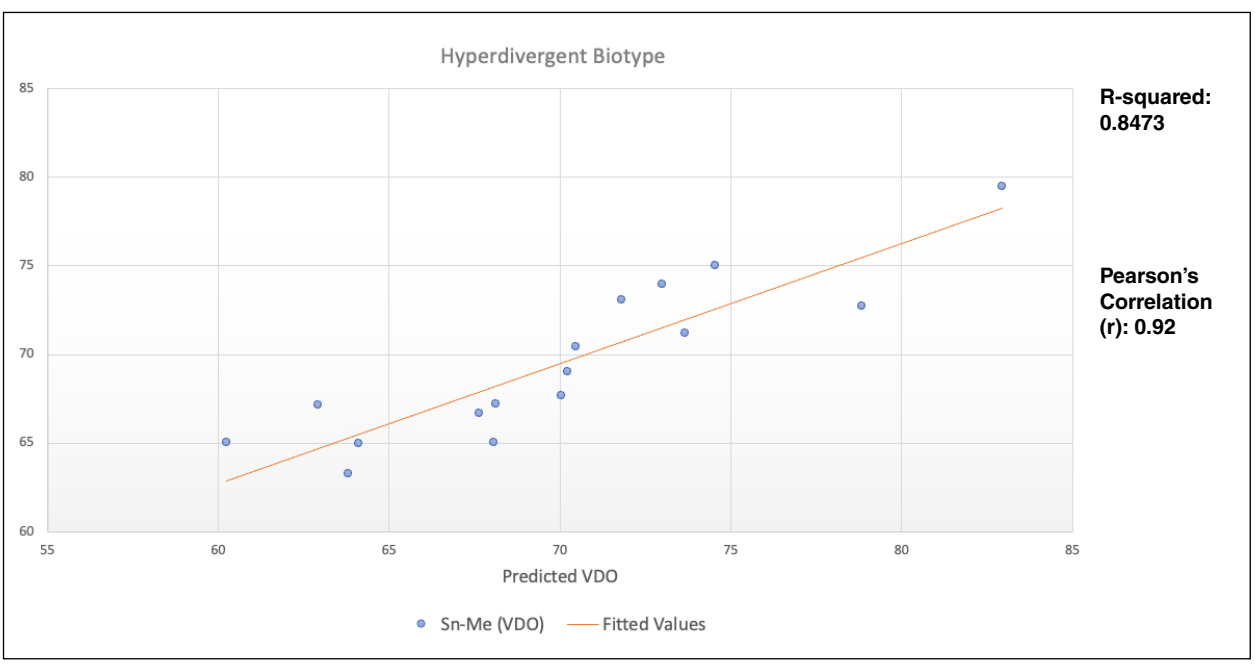

Fig. 5: Dispersion of the variable predicted Vertical Dimension of Occlusion (Predicted VDO) in relation to the variable original VDO in Hyperdivergent biotype

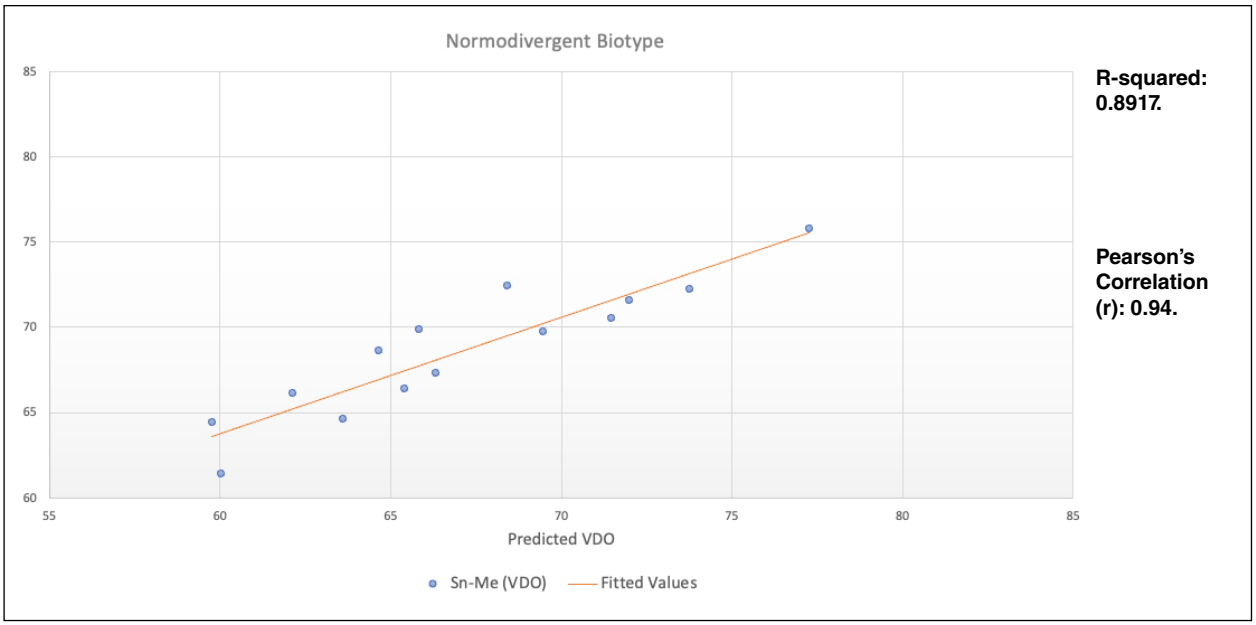

*Fig. 6: Dispersion of the variable **predicted Vertical Dimension of Occlusion** (Predicted VDO) in relation to the variable **original VDO** in Normodivergent biotype*

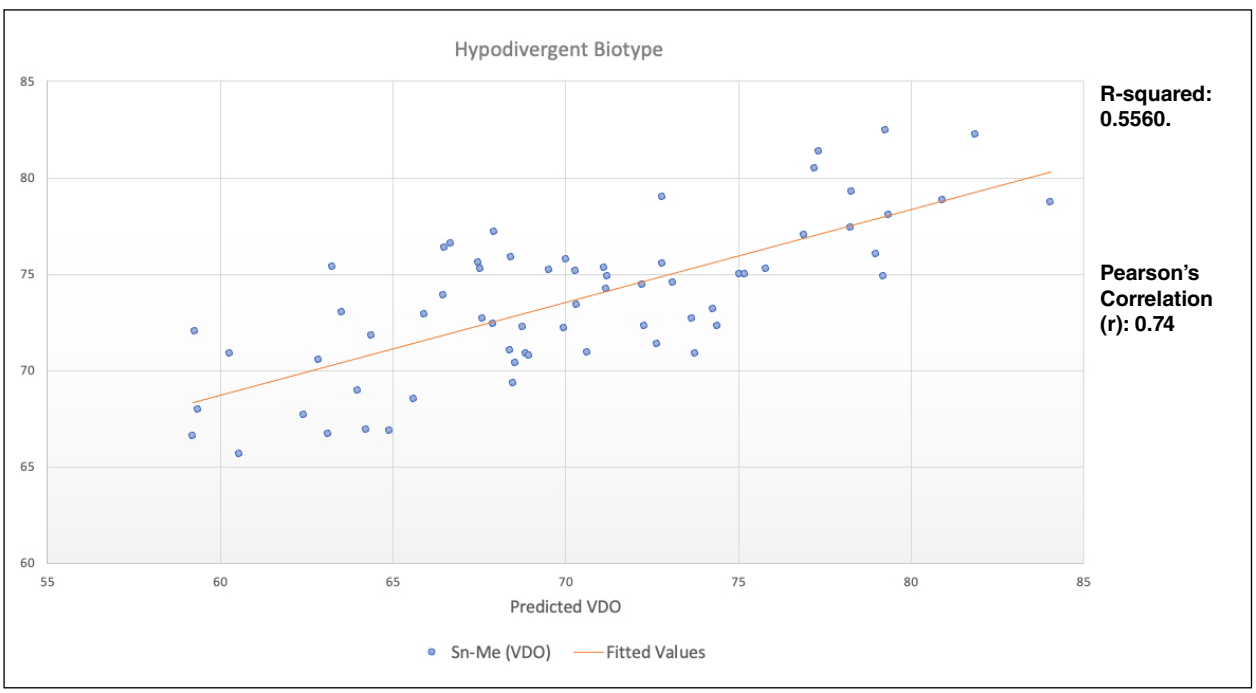

Fig. 7: Dispersion of the variable predicted Vertical Dimension of Occlusion (Predicted VDO) in relation to the variable original VDO in Hypodivergent biotype

DISCUSSION

Our initial parameter for comparison corresponds to Knebelman's Craniometric Method, whose practicality and simplicity make it attractive to use. However, determining Vertical Dimension of Occlusion involves more than simply subtracting an arbitrary predetermined value from the distance between the eye and the ear (AEO-STF), as originally proposed by Knebelman. The current study proposes an integrated method for determining VDO which includes both clinical and cephalometric information, and would be applicable to all patients, regardless of sex, biotype and edentulousness.

Based on the results of the current study, Knebelman's

proposition can be contrasted empirically. Knebelman's method states that the distance from cutaneous Subnasale (Sn') to cutaneous Mentum (Me'), which would correspond to the Vertical Dimension of Occlusion, is 3 to 5 mm shorter than the distance from the anterior wall of the external auditory canal to the lateral edge of the orbit cavity (equivalent to the distance from Outer Canthus of the Eye to Tragus Facial Sulcus in our method) because the difference in the average values found is 5.56 mm (Table 1). Moreover, analysis of the data in the report by Morata et al., and comparison of its average anthropometric values to the VDO recorded for its participants shows a difference of 2.18 mm, which is outside the range proposed by Knebelman. The study by Chou et al. on Knebelman's method says that determining the Vertical Dimension of Occlusion requires consideration of other factors that influence its final value[7]. It was this statement that led us to seek other variables, beyond the eye-ear distance preliminarily proposed by Knebelman. The statistical analysis in the article by Chou et al. does not provide the average values found, but only the correlation coefficients for each group analyzed, so it is not possible to compare it to the original Knebelman method as done in our study. In general terms, the correlation values in our study (R-squared and Pearson's r) are higher than those reported by Chou et al. (in which the highest value was only in the group "White Woman", with R-squared 0.76, while the in the others, the values were 0.56, 0.41 and 0.36), and than those reported by Morata et al.[8] (in which average Pearson correlation value was 0.56 and the highest correlation value [0.60] corresponded to the mesoprosopic group).

With the aim of predicting VDO based on cephalometric landmarks, Silva et al. proposed a model based on cranial height and diameter (Dist. Glabella-Opisthocranion), which informs a value of 0.702 for Pearson's correlation[6]. In this case too, our study found higher values for Pearson's correlation, and is therefore a better predictor in mathematical terms[11].

Since in the current study, the values of the clinical measurements for right and left AEO-STF are statistically very similar (Table 1), and considering future clinical use of this method, it is concluded that the measurement of either the right side or the left side can be used equally.

In a subsequent step, all the parameters in this study were subjected to a multivariate analysis with the aim of determining the degree of influence of the variables on the determination of the Subnasale-Mentum measurement (Sn-Me or VDO). In this case, Multiple Linear Regression analysis was used on the 4 variables: Average (right and left sides) of the Distance Outer Canthus of the Eye to Tragus Facial Sulcus (XAEO-STF), Distance R3-R4, Distance Go-Ar, and Distance Ar-Pog (Depth. Mand.) as a function of the measurement Sn'-Me' (or VDO). The results of the analysis show that all the proposed predictive factors explain 58.6 % of the variable Sn-Mn (R-squared 0.5860), with Pearson's correlation coefficient (r) 0.77. This implies a higher value than those described in previous studies (cutaneous distance Sn-Me) (Fig. 5).

Based on the data obtained, we propose a method to determine a reliable VDO, individualized for each patient, with a higher degree of certainty than its predecessors. Although there are significant differences in the magnitude of the measurements according to sex and for each study variable (Tables 1 and 2), we do not propose to calculate VDO differently for each group. The mathematical model is regulated implicitly and through the measurements themselves, because the cephalometric and facial variables implicitly include these variations, with the measurements in each individual corresponding to one another proportionally. Thus, calculated VDO will be higher for males than for females, because in this study population, the measurements in males are greater in than in females.

Analysis of the model as a predictor of Vertical Dimension of Occlusion upon segregating subjects according to biotype shows high correlation values, particularly for the hyperdivergent (Fig. 6) and normodivergent (Fig. 7) biotypes. Although the hypodivergent biotype (Fig. 8) has a lower Pearson's coefficient than the other biotypes, it is still high. This shows that the proposed model has considerable predictive capacity for Björk-Jarabak facial biotypes.

Finally, our initial hypothesis is proved: there is a high correlation between VDO *predicted* by mandibular size and *actual* VDO, with Pearson's correlation (r) 0.77. In addition, the method has advantages, such as being independent of biotype, being simpler and more reliable than other methods, being easily performed, using an easily available digital vernier caliper, having better predictive

values than other methods proposed, and most importantly, being independent of edentulousness (a condition that affects the facial index calculation used by Morata et al. as one of the variables for their VDO predictive model). All this makes the method proposed herein more reliable and advantageous than those described previously in the literature.

CONCLUSIONS

Mandibular size, represented by the cephalometric variables Dist.R3-R4, Dist.Go'-Ar and Depth. Mand.Ar-Pog, correlates well to the variable VDO. These variables are important adjustment factors for Knebelman's method, and improve its precision as a predictor for determining VDO.

Statistical analysis of the predictive model created [DVO'= (XAEO-STF)*0.3 + (dist.R3R4)*0.5 + (dist.Go-Ar)*-0.3 + (Depth.Mand.Ar-Po)*0.4 – 8] found a value of 0.77 for Pearson's correlation (r), which is considered significant and is one of the highest reported in the literature.

It is worth highlighting that this method is independent of patient sex and biotype, and most importantly, that the cephalometric landmarks and measurements of the mandible and the facial reference magnitudes for this mathematical VDO predictor model do not vary with edentulism, providing reliability to both the method and the result that can be obtained with the equation. Thus, with a high degree of certainty, this method could be used to predict VDO in completely edentulous patients or patients with unstable occlusal references undergoing oral rehabilitation, enabling restitution of the VDO within the range of physiological tolerance and even very close to the original.

CONFLICT INTERESTS

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

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Non-pathological facial asymmetry in adult women: an approach to bite force, occlusal contact distribution and masticatory muscle thickness

Anna L Alves Fernandes[1], Lilian Mendes Andrade[1], Laís Valencise Magri[2], Jardel F Mazzi-Chaves[2], Sergio Rodrigues[1], Isabela Hallak Regalo[1], Paulo B de Vasconcelos[1], Selma Siéssere[1,3], Simone C Hallak Regalo[1,3], Marcelo Palinkas[1,3]

1. Departamento de Biologia Básica e Oral, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Brasil.
2. Departamento de Odontologia Restauradora, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, São Paulo (SP) Brasil.
3. National Science and Technology Institute for Translational Medicine (INCT-TM), Brazil.

ABSTRACT

Facial asymmetries, often subtle, can indicate imbalances that go beyond aesthetic concerns. ***Aim:*** *This cross-sectional study analyzed molar bite force, occlusal contact distribution, and masseter and temporal muscle thickness in adult women with facial symmetry and slight non-pathological facial asymmetry, which generally goes unnoticed and is often considered a variation of normality.* ***Materials and Method:*** *Forty-two women aged 18 to 30 were evaluated for slight non-pathological facial asymmetry using the VECTRA M3. Two groups were established: facial symmetry (n=21) and slight non-pathological facial asymmetry (n=21). Molar bite force was measured with a dynamometer. T-Scan was used to evaluate occlusal contact distribution. Masseter and temporal muscles thickness was assessed using ultrasound. Analyses used a 5% significance level (Student's t-test).* ***Results:*** *No significant difference was found in maximum molar bite force between the groups, though the slight asymmetry group showed lower force in clinical observation. No significant difference was observed in masseter and temporal muscle thickness during rest and maximum voluntary contraction, but clinically, the masseter was thicker than the temporal in both groups, being thicker in the slight non-pathological facial asymmetry group than in the facial symmetry group. Occlusal contact distribution did not differ significantly between groups, but clinically, the slight non-pathological facial asymmetry group showed less distribution in the occlusal contacts of the first permanent molars and more pronounced distribution in the left hemiarch compared to facial symmetry group.* ***Conclusion:*** *Although no significant difference was observed between groups in this study, the numerical analysis of the results highlights the importance of evaluating the stomatognathic system in dental procedures, particularly with regard to non-pathological facial asymmetry.*

Keywords: *facial asymmetry - masticatory muscles - three dimensional imaging - bite force - occlusion - ultrasonography*

Assimetria facial não patológica de mulheres adultas: uma abordagem da força de mordida, distribuição de contatos oclusais e espessura dos músculos mastigatórios

RESUMO

Assimetrias faciais, muitas vezes discretas, podem indicar desequilíbrios que ultrapassam questões estéticas. ***Objetivo:*** *Este estudo transversal analisou força de mordida molar, distribuição do contato oclusal e espessura dos músculos masseter e temporal em mulheres adultas com simetria facial e assimetria facial leve não patológica, que geralmente passa despercebida e muitas vezes é considerada variação da normalidade.* ***Materiais e Método:*** *Quarenta e duas mulheres com idades entre 18 e 30 anos foram avaliadas quanto à assimetria facial leve não patológica usando o VECTRA M3. Foram estabelecidos dois grupos: simetria facial (n=21) e assimetria facial leve não patológica (n=21). Força de mordida molar foi medida com dinamômetro. T-Scan avaliou a distribuição do contato oclusal. A espessura dos músculos masseter e temporal foi avaliada por meio de ultrassonografia. As análises utilizaram nível de significância de 5% (teste t de Student).* ***Resultados:*** *Não foram encontradas diferenças significativas na força máxima de mordida molar entre os grupos, embora o grupo com leve assimetria tenha apresentado menor força na observação clínica. Não foram observadas diferenças significativas na espessura dos músculos masseter e temporal durante o repouso e contração voluntária máxima, mas clinicamente o masseter foi mais espesso que o temporal em ambos os grupos, sendo mais espesso no grupo com assimetria facial leve não patológica em comparação ao grupo com simetria facial. Distribuição dos contatos oclusais não apresentou diferenças significativas entre os grupos, mas clinicamente, o grupo com assimetria facial leve não patológica apresentou menor distribuição nos contatos oclusais dos primeiros molares permanentes e distribuição mais pronunciada na hemiarcada esquerda em comparação ao grupo com simetria facial.* ***Conclusão:*** *Embora não tenham sido observadas diferenças significativas entre os grupos neste estudo, a análise numérica dos resultados ressalta a importância da avaliação do sistema estomatognático em procedimentos odontológicos, principalmente no que se refere à assimetria facial não patológica.*

Palavras-Chave: *assimetria facial - músculos mastigatórios - imagem tridimensional - força de mordida - oclusão - ultrassonografia*

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Corresponding Author:
Marcelo Palinkas
palinkas@usp.br

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INTRODUCTION

Symmetry is important for aesthetic sense and beauty standards, suggesting order, proportion and elegance in architecture and arts, and health, strength and favorable reproduction in animals. In humans, it affects relationships, quality of life, and aesthetic well-being[1]. The importance attributed to symmetry contrasts with the fact that human faces may exhibit slight asymmetry that is often unnoticed[2,3].

Although highly prevalent across the population, facial asymmetry is rarely addressed in dental literature. There is a lack of epidemiological information, studies, and histological and genetic research to determine the real etiology and factors associated with such disharmony, which affects patients' aesthetics and is significantly challenging to correct by means of clinical interventions[2].

Evaluation of facial asymmetry by dental and medical professionals can determine the intensity of asymmetries and be used to plan effective treatment. There are not usually any major structural impairments or functional problems associated with non-pathological facial asymmetry, but patients often seek treatment due to the impact on their facial attractiveness[4].

Dynamic modifications in facial structures can have implications on the complex stomatognathic system, which consists of interdependent dynamic and static structures, including the temporomandibular joint, bones, muscles, teeth, tongue, lips, cheeks, glands, blood vessels and nerves[5]. These structures work together, and any anatomical or functional alteration can result in functional imbalance, affecting proprioceptive information and thus, chewing, swallowing and speech[6-8].

It is essential to understand the origin and clinical aspects of facial asymmetry in order to comprehend the effects of the function of masticatory and facial muscles on the craniomandibular system, as well as the variations in development and how they relate to functional and aesthetic outcomes[9,10]. Evaluation methods can provide information about the cause and consequences of facial asymmetries, especially non-pathological ones, and such information is essential for planning corrective procedures and controlling functional imbalances[11].

The aim of this cross-sectional study was therefore to evaluate molar bite force, occlusal contact distribution, and the thickness of the masseter and temporal muscles in adult women with facial symmetry or slight non-pathological facial asymmetry. The null hypothesis assumes the absence of significant differences in molar bite force, occlusal contact distribution and masseter and temporal muscles thickness between the symmetric and slight non-pathological asymmetry groups.

MATERIALS AND METHOD

Sample

This cross-sectional study was approved by the ethics committee at the Ribeirão Preto School of Dentistry, University of São Paulo, Brazil (protocol # 59833522.0.0000.5419). All subjects were informed of the objectives and procedures, and signed an informed consent form.

The G* Power software (Franz Faul, Kiel University, Kiel, Germany) was used to calculate the sample size via a priori test, considering $\alpha = 0.05$, effect size of 1.31, and 95% power with the variable of left masseter muscle thickness in maximum voluntary contraction based on the pilot project with 5 subjects. The minimum sample size was determined as n=18 for each group.

Recruitment was conducted through an open invitation sent via email to the population. The invitation contained a link to information about the project and the informed consent form. Interested subjects consented to participate in the study through the Research Electronic Data Capture (REDCap) system[12]. The demographic questionnaires and baseline data, including personal information, anthropometrics, parafunctional habits, and history of pre-existing conditions were completed after the subjects had expressed their agreement to participate in the study.

The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) questionnaire, sent via email as an online survey, was used to assess the presence of temporomandibular dysfunction. Researchers received notifications upon completion of the responses. A total 77 subjects responded to the questionnaires provided in REDCap, of whom 6 did not fully complete the forms, and 2 did not attend the scheduled date for the initial examination. After completing the distributed questionnaires, the 69 subjects who met the online criteria received email invitations to attend the research laboratory for a clinical examination to confirm inclusion or exclusion criteria, resulting in the non-selection of 27 subjects for the study.

The eligibility criteria ensured sample homogeneity and the validity of the results. Inclusion criteria were having a Class I standard profile, natural dentition, all teeth present except third molars, body mass index between 18.5 and 24.9, and age 18 to 30 years. Exclusion criteria were symptoms of temporomandibular dysfunction, ulcerations, skin hypersensitivity, uncompensated neurological and systemic pathologies, periodontal disease, parafunctional habits, being on continuous use of muscle relaxants that could interfere with neuromuscular physiology or having undergone prior orofacial harmonization procedures.

After applying the inclusion and exclusion criteria, 42 adult women (mean age ± SD: 23.14 ± 4.14 years) were selected to compose two groups matched by age and body mass index: symmetric (n=21) and with slight non-pathological asymmetry (n=21) (Table 1). The variable in this study was slight non-pathological facial asymmetry, defined as absent or present. The measure was obtained by classifying the subjects based on the root mean square (RMS) values through stereophotogrammetry.

Table 1. Data on the characteristics of the group with facial symmetry (FSG) and the group with mild non-pathological facial asymmetry (FAG). Significant difference, Student's t-test (p < 0.05).

Groups	Age	Body Mass Index
FSG	22.3 ± 4.1	22.1 ± 2.4
FAG	23.1 ± 3.6	21.4 ± 2.1
p value	0.52	0.35

Facial asymmetry analysis

The classification method for subjects as symmetric or asymmetric was based on clinical examination criteria established. During the initial assessment, trained calibrated examiners evaluated facial symmetry using predefined parameters, considering anatomical landmarks, proportions, and any visible deviations. The classification was further supported by objective measurements derived from stereophotogrammetry methods, which generated RMS values to enhance consistency and reliability in distinguishing between symmetric and asymmetric subjects.

The 3D imaging system VECTRA M3 (Canfield Scientific, Inc., Parsippany, NJ, USA) was used for image acquisition[3,13,14]. The Vectra M3 software enabled the creation of a symmetry plane that aligned two surfaces in three-dimensional space through manual alignment using reference points, and subsequently automatically through the integrated closest-point algorithm.

During the examination, the subjects remained seated in an upright position, with the torso straight, shoulders relaxed, hands on the lap, and feet fully supported on the ground. The face was cleaned with hypoallergenic wet wipes to remove any oiliness and makeup residue. Instructions were given on positioning in front of the 3D camera according to the manufacturer's guidelines. Subjects were asked to keep their face at rest and expressionless while focusing on the front mirror during image capture.

Images were prepared for quantitative comparison between the original facial surface and the mirrored image according to the following protocol: the first point was marked at the Trichion, on the hairline, followed by points from left to right. Starting from the Frontotemporale, the image was rotated to expose the left side of the face, marking until the Cheilion, and then the image was rotated to expose the Gonion. The selection continued until the Menton, returning to the frontal view to complete the contour of the mandibular body. The image was rotated laterally to expose the right Gonion and moved up from the Gonion to the Trichion, bordering the muscle insertion. After marking the points, the excess area was delineated and removed. Subsequently, the facial area of interest was delimited, gridlines were applied, and positioning was adjusted along the y-axis . Then, the entire face was selected, and the option to find symmetry was applied. The image was viewed in both frontal and lateral views. The gridlines were removed, and the original face was mirrored, preparing it for comparative quantitative analysis.

The quantitative comparison between the original facial surface and its mirrored image calculated the distance between them, enabling quantification of non-pathological asymmetry. The root mean square (RMS) of the means of the distances between corresponding points in the overlaid images was calculated. To enhance visualization, the regions contributing most significantly to the asymmetry were analyzed using a color map generated by the software.

Criteria for classifying non-pathological facial asymmetry

The Receiver Operating Characteristic (ROC) curve analysis was used to compare the clinical

examination - considered the gold standard for facial asymmetry classification- to subjects' RMS scores. This analysis determined the point of greatest agreement between the two assessments, which was defined as the cutoff point for group classification.
The Jamovi software (The Jamovi Project, 2023, version 2.3.17) was used for this analysis. The cutoff point that optimized both sensitivity and specificity was 0.68, with a sensitivity of 94.44% and a specificity of 83.33%. Subjects with RMS of up to 0.68 were classified as symmetrical, while those with values above 0.68 were considered to have mild, non-pathological facial asymmetry.
The classification into symmetric and asymmetric was used exclusively for methodological purposes in this study, aiming to identify the potential impacts that visible asymmetry could have on morpho-functional balance. Non-pathological facial asymmetry was defined as a subtle asymmetry, likely resulting from self-compensatory mechanisms, with no readily observable effects on function, though subtle effects might still have been present.

Maximum molar bite force analysis
Maximum molar bite force was analyzed with a digital dynamometer (IDDK, Kratos - Equipamentos Industriais Ltda, Cotia, São Paulo, Brazil) with a capacity of 1000 N. The dynamometer was initially placed in the region of the first upper and lower permanent molars on the right side and then on the left side. Probe tips were protected with disposable latex finger cots (Waripaer, São Paulo, Brazil) as a biosafety measure. Three measurements were recorded at 2-minute intervals, alternating between sides[7,15,16].

Occlusal contact distribution analysis
The T-Scan® III occlusal analysis system (Tekscan Inc., Ann Arbor, MI, USA) evaluated occlusion in terms of the sequence of occlusal contact distribution on the first upper and lower permanent molars, as well as the right and left hemiarches. Subjects were instructed to occlude their teeth on the recording sensor several times, until a repetitive contact pattern was captured. Then, three closures were recorded, during which subjects were instructed to bite down as hard as possible, maintaining the pressure for 5 seconds. The equipment recorded the relative force as a percentage of the individual maximum occlusal force[17-19].

Masseter and temporal muscle thickness analysis
The Sonosite® Nano Maxx ultrasound (SonoSite, Inc., Bothell, Washington, USA) with a 13 MHz linear transducer was used to determine masseter and temporal muscle thickness in centimeters. The location of the muscle belly was confirmed by digital palpation and movement of the linear transducer[20]. For the masseter muscle, the linear transducer was positioned transversely to the muscle fibers, approximately 1.5 to 2.0 cm above the angle of the mandible towards the zygomatic arch. For the temporal muscle, the linear transducer was positioned transversely over the anterior belly of the muscle, located in the region of the temporal fossa, about 1.0 to 1.5 cm backward and upward from the lateral canthus of the eyelids, on both sides[7,21]. Ultrasound images were acquired during the mandibular rest task and during maximum voluntary clenching. Ultrasound images were acquired three times by one appropriately trained examiner.

Statistical analysis
The data were subjected to the Shapiro-Wilk test for normality, which showed normal distribution. All analyses were performed with a significance level of 5% (Student's t-test) using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS
Table 2 shows the results of molar bite force, distribution of occlusal contacts, and masseter and temporal muscle thickness in adult women with facial symmetry and mild non-pathological facial asymmetry. There was no significant difference in maximum molar bite force between groups, although the group with mild non-pathological facial asymmetry showed lower force in clinical observation. The distribution of occlusal contacts did not differ significantly between the groups, but clinically, the group with mild non-pathological facial asymmetry showed less distribution in occlusal contacts of the first permanent molars and more pronounced distribution in the left hemiarch compared to the symmetrical group. In terms of masseter and temporal muscle thickness, there was no significant difference during rest and maximum voluntary contraction, but clinically, the masseter was thicker than the temporal in both groups, being thicker in the group with mild non-pathological facial asymmetry than in the symmetrical group.

Table 2. Mean ± standard error and p value of molar bite force, distribution of occlusal contacts and thickness of the masseter and temporal muscles of the group with facial symmetry (FSG) and the group with mild non-pathological facial asymmetry (FAG). Significant difference, Student's t-test (p < 0.05).

Variables	FSG	FAG	p value
Bite Force (N)			
Right	387.75 ± 38.73	382.45 ± 30.98	0.82
Left	400.01 ± 40.89	388.63 ± 30.01	0.96
Muscle thickness (cm)			
Rest			
Right masseter	0.64 ± 0.01	0.67 ± 0.01	0.20
Left masseter	0.65 ± 0.01	0.66 ± 0.01	0.54
Right temporal	0.28 ± 0.007	0.28 ± 0.007	0.95
Left temporal	0.27 ± 0.007	0.27 ± 0.007	0.84
Maximum voluntary contraction			
Right masseter	1.20 ± 0.03	1.22 ± 0.02	0.60
Left masseter	1.22 ± 0.04	1.25 ± 0.02	0.61
Right temporal	0.44 ± 0.01	0.42 ± 0.01	0.97
Left temporal	0.40 ± 0.01	0.39 ± 0.001	0.84
Distribution of occlusal contacts (%)			
Upper first molar	16.20 ± 1.15	15.25 ± 1.78	0.65
Upper second molar	15.67 ± 1.37	15.39 ± 1.57	0.89
Lower first molar	15.80 ± 2.11	15.55 ± 1.62	0.64
Lower second molar	15.00 ± 1.60	14.23 ± 1.59	0.73
Right hemiarch	49.00 ± 1.30	46.36 ± 1.72	0.15
Left hemiarch	50.95 ± 1.31	54.20 ± 1.83	0.73

DISCUSSION

The null hypothesis was accepted since there was no significant difference in molar bite force, distribution of occlusal contacts, and masseter and temporal muscle thickness between women in the symmetrical and mild non-pathological asymmetry groups. Notwithstanding, clinical analysis showed differences between groups, highlighting the importance of the clinical approach in observing subtle anatomical differences that may affect the functions of the stomatognathic system.

Although the results revealed no significant difference between the groups for maximum molar bite force, during clinical analysis, lower maximum molar bite force was observed in the group with mild non-pathological asymmetry. The muscles involved in facial kinetics function in synchrony, responding to contractile forces, which may result in morphological modifications in asymmetric subjects[22].

In response to asymmetry, the masticatory and facial muscles can be activated compensatorily on both sides, regardless of the degree of asymmetry promoting neuromuscular adaptations[23]. This occurs at motor level, and is an important aspect to consider in the analysis of the stomatognathic system, as it shows the nervous system's capacity to regulate muscle function and strength[24].

Facial asymmetry can induce adaptations in the pattern of muscle force, resulting in uneven distribution, and consequently, a decrease in the overall strength of the stomatognathic system. Even

mild facial discrepancies can affect the distribution of occlusal force, leading to a decrease, especially during attempts at functional compensation[25].

No significant difference was found in the distribution of occlusal contacts on the upper and lower first permanent molars or on the right and left hemiarches between the symmetrical and mild non-pathological asymmetry groups. However, clinical analysis revealed a less uniform distribution of occlusal contacts on the upper and lower first permanent molars in the group with mild non-pathological asymmetry. Specifically, the asymmetrical group showed more pronounced distribution on the left hemiarch.

Facial asymmetry, when not considered pathological, and often imperceptible visually, can also influence the functionality of dental occlusion and the distribution of occlusal contacts, resulting in potential occlusal misalignments[26]. One of the manifestations of occlusal misalignment is the presence of abnormal occlusal contact points, which can arise from poorly adjusted dental restorations or irregular tooth wear, thereby altering the craniomandibular cervical system[27].

These contact points can interfere with the normal distribution of occlusal loading force during the chewing process, promoting inadequate distribution of occlusal contacts on the posterior teeth[28]. It is relevant to highlight that, although abnormal occlusal contact points on the first permanent molars were not evaluated in this study, they are important in interpreting the results. These abnormal contact points on the teeth can affect the proper distribution of occlusal contacts, and should be considered when analyzing occlusion and masticatory function in subjects with facial asymmetry.

Differences in muscle strength between the left and right sides can also lead to an asymmetric distribution of load during the chewing process. Stronger or more active muscles on one side of the face may promote greater force and, consequently, a more pronounced distribution of occlusal contacts on that side[29]. A preference for chewing predominantly on one side of the mouth provides an asymmetric distribution of occlusal contacts[30], and asymmetries in the contraction patterns of the masticatory muscles, resulting in an uneven distribution of load in the dental hemiarch during chewing. These factors may account for the more pronounced distribution in the left hemiarch in the group with mild non-pathological facial asymmetry. However, this study did not identify the preferred chewing side.

This study investigated potential differences in masseter and temporal muscle thickness between groups with symmetry and mild non-pathological facial asymmetry, during resting and maximum voluntary dental clenching, finding no significant difference. However, in clinical analysis, it was observed that the masseter muscle was thicker than the temporal muscle in both groups, being even thicker in the group with mild non-pathological asymmetry. These findings are consistent with data from the literature, which report thicker masseter muscles than temporal muscles[16,31].

Thicker masseter muscles than temporal muscles, both at rest and during maximum voluntary dental clenching, can be explained by anatomy. Firstly, the masseter muscle plays a crucial role in chewing, elevating the mandible during mouth closure and applying the necessary force to crush food[32]. This predominant function in chewing naturally leads to greater development and thickness of the masseter than the temporal muscle, which has a secondary – though important – function: stabilizing and positioning the jaw during chewing and other mandibular movements[33].

In addition to the anatomical and morphological differences between the masseter and temporal muscles that contribute to this discrepancy in thickness, it is important to highlight their distinct functional characteristics. The masseter muscle, considered a thick muscle with synergistic action with the medial pterygoid, is inserted in the mandible, giving it a mechanical advantage in applying force during chewing. It also has a higher proportion of fast-twitch muscle fibers, enabling it to generate more substantial and sustained force during chewing than the temporal muscle, which has a fan-shaped structure originating from an extensive area of the lateral aspect of the skull and is designed more for movement than force[33,34].

Mild non-pathological facial asymmetry may lead to a greater thickness in the masseter muscle, suggesting a morphological compensation in the stomatognathic system, which could result in an imbalance in force distribution and muscular activity during chewing to maintain mandibular functional stability[35]. This demonstrates the importance of considering not only the external facial appearance but also the

internal adaptations of the stomatognathic system when analyzing subjects with facial asymmetries.

There are several limitations to this study. Firstly, only a few of the numerous available tests were used to assess masticatory function; future studies should incorporate a variety of tests and consider different age groups. Another limitation is the absence of comparisons with the underlying bone structure. Ethical constraints prevented the use of imaging examinations with ionizing radiation for this purpose. Moreover, preferred chewing side and abnormal occlusal contact points on the first permanent molars were not investigated.

The pursuit of balance transcends all areas of dentistry, from maintaining oral health to developing innovative techniques and continuous professional improvement. The findings of this study emphasize the relevance of clinical observation of non-pathological facial asymmetries, highlighting that even mild discrepancies can affect the functionality of the stomatognathic system without apparent significant difference. Therefore, when planning rehabilitation and aesthetic procedures, it is important to consider not only facial appearance but also the function of the anatomical structures involved in the masticatory process. This approach can contribute to more satisfactory, long-lasting results.

CONCLUSIONS

The authors of this study highlight that, although no significant difference was observed in maximum molar bite force, masseter and temporal muscle thickness, or distribution of occlusal contacts between the groups of women with facial symmetry and those with slight non-pathological facial asymmetry, clinical analysis revealed noteworthy subtleties. The group with slight non-pathological facial asymmetry had lower bite force, less uniform distribution of occlusal contacts – particularly in the left hemiarch – and greater masseter muscle thickness compared to the symmetrical group. The results emphasize the need to assess the stomatognathic system in dental procedures, especially concerning non-pathological facial asymmetry

CONFLICT INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this manuscript.

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Apical extrusion following different glide path instrumentation in curved canals of mandibular molars

Marlene P Torres[1], Ana GS Limoeiro[2], Wayne M Nascimento[1], Virgílio MB Braga[1], Ana Raquel LS Miranda[3], Thais MC Coutinho[4], Marilia FV Marceliano-Alves[4,5,6], Marcos Frozoni[1]

1. *Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Departament of Endodontics, Campinas, SP, Brazil*
2. *Departamento de Odontologia, Endodontia e Materiais Dentários, Faculdade de Odontologia de Bauru, Universidade de São Paulo, Bauru, Brasil*
3. *Departamento de Odontologia, Centro Universitário do Pará, Belém, Brasil*
4. *Programa de Pós-Graduação em Odontologia, Universidade Iguaçu, Nova Iguaçu, Brasil*
5. *KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT - Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, Leuven, Belgium.*
6. *Laboratoire des Pathologies Orofaciales, Imagerie et Biothérapies, École de Chirurgie Dentaire, Laboratoire d'Excellence INFLAMEX, Université Paris Cité, URP 2496, Montrouge, France*

ABSTRACT

Endodontic failures are usually the result of technical errors compromising the proper endodontic procedures required to control and prevent periradicular infections. Among these errors, the extrusion of materials through the apical foramen has been associated with periapical inflammation, postoperative pain, tissue necrosis, delayed periapical healing and long-term failure. ***Aim:*** *To compare the debris extruded through the apical foramen when different glide path files are used prior to endodontic instrumentation with the WaveOne Gold Primary file.* ***Materials and Method:*** *Twenty-four mesial roots of extracted human mandibular first molars were divided into three groups (n=8): C-Pilot hand file (CPH) #15/.02; R-Pilot (RPL); WaveOne Gold Glider (WGG). The roots were placed in Eppendorf tubes containing 1.5% agar gel and weighed before and after instrumentation to calculate the weight of debris and irrigant solution extruded. Apical extrusion of debris was compared using one-way analysis of variance. The Games-Howell test was used for multiple comparisons due to heteroscedasticity, with a significance level of 5%.* ***Results:*** *Debris extrusion was significantly lower with the WaveOne Gold Glider file than with the R-Pilot file, which in turn produced less extrusion than the C-Pilot file.* ***Conclusion:*** *All glide path files caused apical extrusion, with the WaveOne Gold Glider causing the least.*

Keywords: *apical extrusion - automated instruments - debris - glide path*

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Corresponding Author:
Ana Grasiela Limoeiro
grasielalimoeiro@gmail.com

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Extrusão de debris após diferentes protocolos de glide path em canais curvos de molares inferiores

RESUMO

O fracasso do tratamento geralmente é resultado de erros técnicos que comprometem os procedimentos endodônticos adequados necessários para controlar e prevenir infecções perirradiculares. Entre esses erros, a extrusão de material obturador através do forame tem sido associada à inflamação periapical, dor pós-operatória, necrose tecidual, atraso na cicatrização periapical e falhas a longo prazo. ***Objetivo:*** *Comparar os detritos extruídos através do forame apical quando diferentes instrumentos para exploração do canal radicular são usados antes da instrumentação com o instrumento WaveOne Gold Primary.* ***Material e Métodos:*** *Vinte e quatro raízes mesiais de primeiros molares inferiores humanos extraídos foram divididas em três grupos (n=8): Lima manual C-Pilot (CPH) #15/.02; R-Pilot (RPL); WaveOne Gold Glider (WGG). As raízes foram colocadas em tubos Eppendorf contendo gel de ágar a 1,5% e pesadas antes e depois da instrumentação para calcular o peso dos detritos e da solução irrigante extruídos. A extrusão apical de detritos foi comparada usando análise de variância unidirecional. O teste de Games-Howell foi usado para múltiplas comparações devido à heterocedasticidade, com um nível de significância de 5%.* ***Resultados:*** *A extrusão de detritos foi significativamente menor com o instrumento WaveOne Gold Glider do que com o R-Pilot, que por sua vez produziu menos extrusão do que o C-Pilot.* ***Conclusão:*** *Todos os instrumentos de caminho de deslizamento causaram extrusão apical, sendo o WaveOne Gold Glider o que causou a menor extrusão.*

Palavras-chave: *extrusão apical - instrumentos automatizados - detritos - exploração do canal radicular*

INTRODUCTION

Endodontic failures are usually the result of technical errors compromising the proper endodontic procedures required to control and prevent periradicular infections. Among these errors, the extrusion of materials through the apical foramen has been associated with periapical inflammation, postoperative pain[1], tissue necrosis, delayed periapical healing and long-term failure[2].

All instruments and techniques used in root canal preparation may transfer varying amounts of debris into the periradicular area. The debris may consist of hard tissue (dentin), pulp tissue, microorganisms and/or irrigants[3].

Rotary NiTi instruments have advantages over hand files in terms of faster preparation and better preservation of canal geometry[4], but they still pose a challenge in terms of debris extrusion[5]. The amount of extruded content can be influenced by endodontic file design and kinematics[6], technique (hand, reciprocating or rotary)[7,8], number of instruments used to prepare the canal, and file taper, cross-section and cutting capacity[9].

There is controversy in the literature about which instruments cause the most apical debris extrusion, though some studies have found that reciprocating instruments may produce more than rotary instruments do[10-13].

There are also contradictory results regarding the amount of debris extrusion caused by different types of irrigants[11]. One study found that 5.25% hypochlorite caused more debris extrusion than 2.5% hypochlorite or 2% chlorhexidine[12]. In another study, more debris was extruded with chlorhexidine gel than with 2.5% NaOCl[11]. EDTA gel, citric acid and peracetic acid in the root canals during preparation produced less apically extruded debris than other solutions did[13]. Irrigation, though essential to the success of endodontic treatment, may facilitate the extrusion of debris into the periapical region[14], causing inflammatory reactions such as pain, edema, and the possibility of delayed tissue repair[15].

Creating a path from the canal opening to the apical foramen, referred to as a "glide path," prior to canal preparation helps to improve file performance and may reduce the amount of apically extruded debris[15]. A glide path can be created using hand, rotary or reciprocating instruments[6].

The following are two of the reciprocating files available on the market that can be used to prepare glide paths:

- WaveOne Gold Glider (Dentsply Sirona, Charlotte, USA), which has thermomechanical treatment, parallelogram-shaped cross-section, tip diameter 15, and variable taper of 2-6%[1],
- R-Pilot (VDW, Munich, Germany), which has M-wire heat treatment, S-shaped cross-section, tip diameter 0.125 mm and constant taper of 4%[16].

Both these files have been previously evaluated for debris extrusion prior to instrumentation with the Reciproc Blue R25 reciprocating system[1].

The aim of this *ex vivo* study was to evaluate the apical extrusion of debris using different files when preparing the glide path prior to endodontic instrumentation with the WaveOne Gold file. The null hypothesis was that there would be no difference between the systems in terms of the amount of debris extruded.

MATERIALS AND METHOD

This study was approved by the local research ethics committee (approval number R002/2023).

Required sample size was calculated with the program G*Power 3.1.9.4, using the analysis of variance model. With an effect size of 0.734, which was determined in the study by Gunes[17], a significance level of 5 % and a power of 90 %, the calculation showed that a total sample of 24 mesial roots would be required to detect significant differences among groups.

Selection and preparation of the specimens

Forty-eight mesial canals from 24 type IV mesial roots from human mandibular first molars, extracted for reasons unrelated to this study, were selected. Teeth with previous endodontic treatment, incomplete rhizogenesis, root fractures, internal or external resorption and foramina larger than 0.10 mm were excluded. Mesial roots with moderate curvature (10º to 20º) according to Schneider's Classification[18] were included. To confirm the inclusion criteria, periapical radiographs were taken in the mesiobuccal and distolingual directions with a K #08/.02 hand file (Dentsply Maillefer, Ballaigues, Switzerland) in the canal, during the working length measurement, as explained below. The teeth were immersed in 5% sodium hypochlorite for 30

minutes, their outer surface was cleaned with a periodontal curette, and they were stored in saline solution and refrigerated at 4°C until use.

For sample selection, the teeth were fixed with utility wax (Lysanda, São Paulo, SP, Brazil) to the platform of a micro-CT scanner (SkyScan 1273; Bruker micro-CT, Kontich, Belgium) and scanned at 70 kV, 114 mA, 14 µm pixel size, 360° around the vertical axis, rotation step of 0.5, and 2 average frames using a 1.0-mm-thick aluminum filter. Images were reconstructed using N. Recon v.1.6.9.16 software (Bruker micro-CT), with ring artifact correction of 5, beam hardening correction of 50%, and smoothing of 8 to create axial slices of the internal structure of all root canals. Next, the 3-dimensional quantitative analysis (volume, major and minor apical diameter) was performed using CTAn v1.14.4.1 software (Bruker micro-CT), to pair samples and select groups considering similar morphological parameters ($p>0.05$) assessed by micro-CT. Finally, a total 24 teeth (48 mesial canals) were included in the study (Fig. 1).

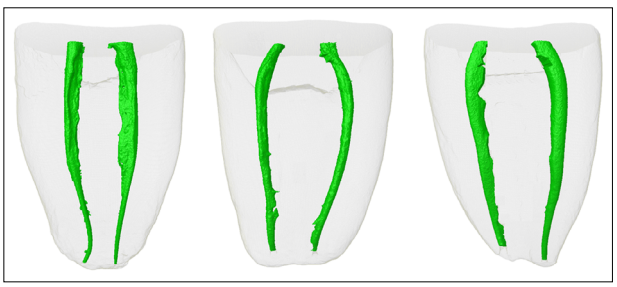

Fig. 1: Representative CT images of the Vertucci´s type IV mandibular molars used in the present study.

CTAn v1.14.4.1 software v.1.5.2 (Bruker microCT) was used to measure, the minor diameter of each canal at the apical foramen on the axial plane. Mean minor root canal diameter measured on the micro-CT scans was 0.26 ± 0.05 mm (median = 0.26 and range = 0.14-0.34).

Sample preparation

The teeth were prepared by an experienced specialist in Endodontics. The specimens were immersed in 2.5% sodium hypochlorite (NaOCl) solution for 30 minutes for surface disinfection and then in saline solution, and finally, placed in Eppendorf tubes (Eppendorfs do Brazil, São Paulo, SP, Brazil). To standardize the teeth to 17 mm, the crowns were worn with a carborundum diamond disk (KG Sorensen, Serra, Brazil). The working length (WL) was measured visually, using a K #08/.02 hand file (Dentsply Maillefer, Ballaigues, Switzerland) under microscope at 20x magnification until the tip of the file was visible through the apical foramen. The WL of the canal was set at 17 mm, i.e. the entire length of the tooth.

The caps of the Eppendorf tubes were removed with scissors. A hole was drilled at high speed into the center of each cap using a #1014 (KG Sorensen, Serra, Brazil) diamond ball drill. The roots were wrapped with Teflon tape and only the apical 1 mm was exposed[19]. The Teflon-wrapped root remnants were inserted into the holes in the caps up to the border of the cementum, leaving 12 mm of the root below the cap and 5 mm of the coronal remnant above. Then, the interface between the cap and the tooth was sealed with cyanoacrylate (Super Bonder, Loctite, Henkel Ltda., São Paulo, Brazil). Fluid resin (FGM, Joinville, Brazil) was applied over the cyanoacrylate to protect it and prevent irrigant solution from entering the tube during root canal preparation. All weights in the following experiment were measured three times on a high-precision electronic balance (RADWAG, Radom, Poland), and the mean values were calculated and recorded.

Each cap/tooth set was weighed. This weight was designated as P1. Then 1.5 mL of 1.5% agar gel (Kasvi, São José dos Pinhais, Brazil) was placed in each Eppendorf tube (uncovered) using a pipette. After the tubes had been filled with the liquid agar, they were covered with the cap/tooth, turned upside down, and placed in a plastic holder so that the roots were completely immersed in the agar solution. The gelled agar was used to simulate periapical tissues.

After the agar had fully gelled at room temperature for 24 hours, each tube/agar/cap/tooth set was weighed. This weight was designated as P2. Thus, P2 (tube/agar/cap/tooth) minus P1 (cap/tooth) provided the weight of the tube with agar, which was designated as P3 (tube/agar).

The Eppendorf tubes were positioned in a holder to prevent the operator from seeing the tooth root during endodontic preparation. The specimens were divided into three groups (n=8): CPH, WGG and RPL, and the glide paths of the mesio-buccal and mesio-lingual root canals prepared using one the following glide path instruments: *Group CPH* - C-Pilot hand file (Dentsply Maillefer, Ballaigues, Switzerland), *Group WGG* - WaveOne Gold Glider

(Dentsply Sirona, Charlotte, USA) or *Group RPL* - R-Pilot (VDW, Munich, Germany). After the glide paths had been prepared, the canals were instrumented with WaveOne Gold Primary files.

During the glide path and mechanical preparation of the canals, a peristaltic pump model EK1960 (Gikfun, China) and a NaviTip 30G 25mm needle (ULTRADENT, Indaiatuba, Brazil) were used to irrigate with double distilled water at a flow rate of 1mL/minute. A total 4 mL of double distilled water was used to prepare each root canal.

Glide path procedure in the tested groups

Group CPH: C-Pilot hand file #15/.02

The first rinse was performed with 1mL of double distilled water in the canal until the needle reached 13 mm (end of the middle third). Patency was achieved with a K#10/.02 (Dentsply Maillefer) file 1 mm beyond the foramen. The glide path was started with a C-Pilot #15/.02 hand file using gentle pushing and pulling movements with amplitudes of no more than 1 mm and a quarter turn to the left and a quarter turn to the right until the WL (17 mm) was reached. Then, the cervical and middle thirds were instrumented with a WaveOne Gold Primary file driven by the IQ motor (Dentsply Sirona) in reciprocating mode, followed by irrigation with 1 mL of double distilled water with a needle inserted into the canal 2 mm short the WL, followed by a K#10/.02 file until patency. Instrumentation was then performed with the WaveOne Gold Primary file until WL. Final irrigation was then performed with 1 mL of double distilled water in the canal, with the needle penetrating up to 15 mm intracanal.

Group RPL: R-pilot 12.5/.04

The roots were prepared in the same way as in group CPH, but with the glide path with the R-Pilot file (VDW) coupled to an X-Smart IQ contra-angle handpiece motor (Dentsply Sirona, Charlotte, USA) in the reciprocating function with smooth in-and-out movements of 3 mm amplitude to a depth of 17 mm.

Group WGG: WaveOne Gold Glider 15/.02

The roots were prepared in the same way as in group RLP, but with the glide path with the WaveOne Gold Glider (Dentsply Sirona).

Extruded material quantification

After completion of mechanical preparation, the Eppendorf tubes were removed from the holder, and the caps/teeth carefully detached from the tubes. The Eppendorf tubes containing the agar, debris and irrigant were weighed and labeled as P4 (tubes/agar/debris/irrigant). The amount of debris and irrigants extruded through the foramen and deposited in the agar gel was calculated as P4 - P3, i.e. tube/agar/debris/irrigants (after instrumentation) (P4) minus tube/agar (before instrumentation) (P3). This weight was designated as P8.

Any remaining debris that might have adhered to the root apex of the cap/tooth removed from the agar was washed with 2 mL of double distilled water, and collected in another Eppendorf tube, which was previously weighed and designated as P5. These tubes were placed in a stove at 37°C for 48 hours to evaporate the liquid, and then weighed again as P6: Weight of the Eppendorf tube with debris collected from the tooth apices of the cover/teeth set. P6 - P5 = P7 was the weight of the debris adhering to the outer surface of the tooth apex (Fig. 2).

Thus, the total amount of debris extruded through the foramen (both deposited on the agar and adhered to the tooth apex) was represented by P7 plus P8 and labeled as P9. Fig. 3 shows the sequence of weighing the extruded dentinal debris.

Statistical analysis

Given the normal distribution, comparison between the glide path files in terms of apical extrusion of debris was performed using one-way analysis of variance. For multiple comparisons, the Games-Howell test was used due to heteroscedasticity. Statistical calculations were performed using the SPSS 23 program (SPSS Inc., Chicago, IL, USA), assuming a significance level of 5%.

RESULTS

One-way analysis of variance showed that apical debris extrusion was significantly influenced by the type of glide path file ($p < 0.001$). Debris extrusion was significantly lower with the WaveOne Gold Glider file than with the R-Pilot file, which in turn produced less extrusion than the C-Pilot file (Table 1).

Discussion

The aim of this study was to investigate the apical extrusion of debris after the use of different glide

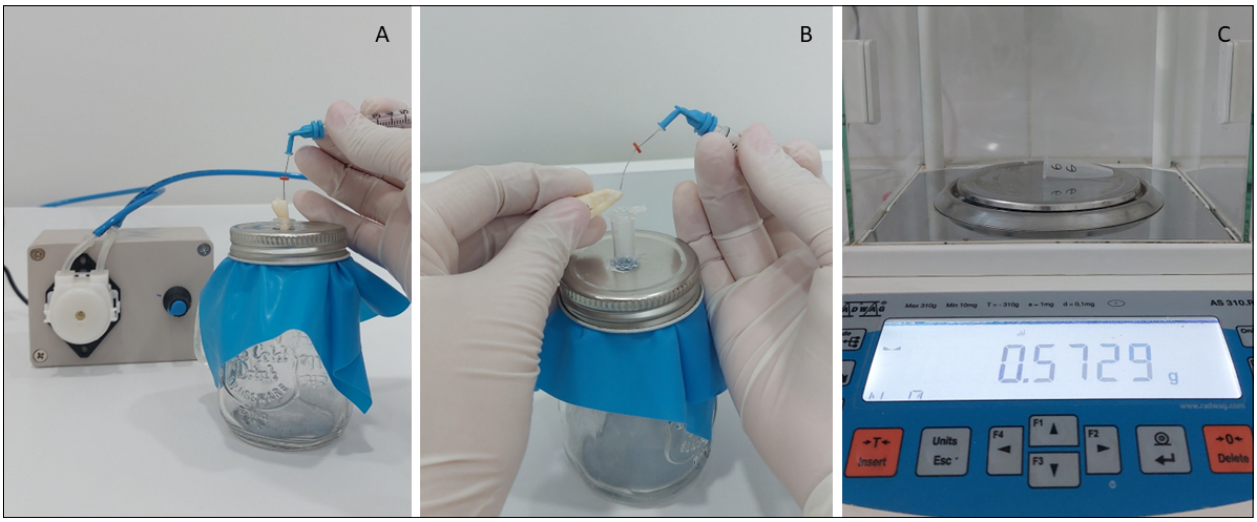

Fig. 2: Representative images of the extruded dentinal debris weighing procedure. A) Shaping and irrigating procedures. B) Washing any remaining debris that might have adhered to the root apex. C) Debris weighing procedure.

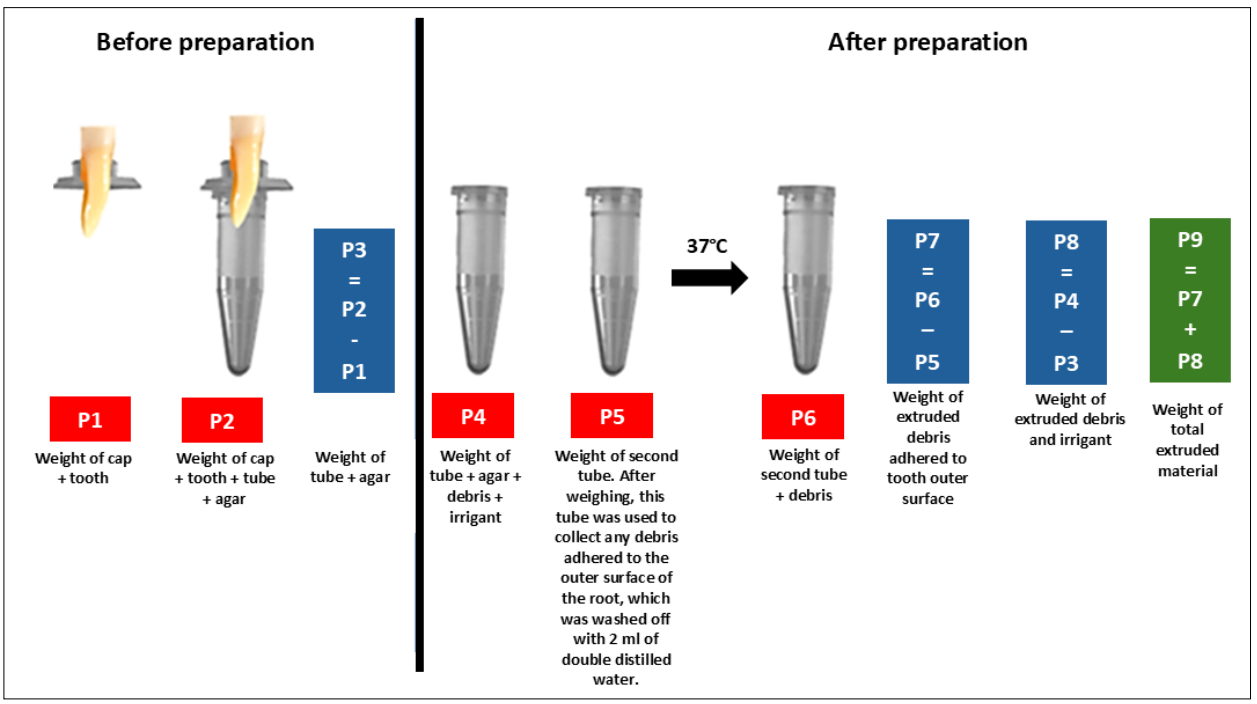

Fig. 3: Schematic figure showing the technical sequence of weighing the extruded dentinal debris.

Table 1. Mean and standard deviation of apical debris extrusion, according to the glide path instrument used.

File	Mean	Standard deviation
C-Pilot	0.137 [C]	0.037
R-Pilot	0.089 [B]	0.030
WaveOne Gold Glider	0.042 [A]	0.012

Different letters indicate significant difference between instruments.

path files – C-Pilot #15/.02, WGG and R-Pilot – were used prior to instrumentation with the WaveOne Gold primary file. Double distilled water was used to irrigate the canals, as reported in other studies[20,21], because using sodium hypochlorite may cause salt deposition or crystal formation after the evaporation process, which would lead to overestimation of the mass of extruded material[22]. A peristaltic pump was used in the present study to standardize the total volume and flow rate of the double distilled water to 1 mL/min to ensure controlled conditions, as the

manually controlled flow rates of the rinsing agent may not be sufficiently standardized[23].

This study used agar gel to simulate periapical tissues because its density is similar to that of periapical tissues for apically extruded materials[19,24]. The glide path technique has been associated with a lower incidence and intensity of postoperative pain and less apical extrusion[25]. However, several factors may influence this outcome, including the taper, tip size, preparation technique and kinematics[26]. Tooth-related factors, including the curvature and size of the apical foramen, have been reported to contribute to debris extrusion[6,27,28]. The cross-section of the instrument and the volume of irrigation fluid used during preparation can also influence the extrusion of debris[29].

In this study, the amount of debris extruded using the three different glide path files differed significantly, therefore the null hypothesis was rejected. The hand C-Pilot file caused higher debris extrusion than the R-Pilot and WGG files ($p<0.05$). This may be related to the stiffness of stainless-steel C-Pilot hand files, which can push more debris apically than NiTi alloy files with M-wire[30]. Studies have shown that hand stainless-steel files cause higher extrusion of debris than rotary and reciprocating systems[17,31]. In this study, the C-Pilot hand file also caused higher extrusion of debris, while the WGG file caused lower extrusion than the R-Pilot file. The WGG file is a NiTi file with gold heat treatment, which is more flexible than the R-Pilot file. Flexible instruments may cause lower apical transportation and consequently lower extrusion of debris in curved canals[32]. This could explain the lower extrusion in the WGG group compared to the R-Pilot and C-Pilot groups. In the study by Kirici et al[33], less debris was extruded when WGG was applied before WOG compared to ProGlider, in agreement with the results of the current study.

The results of the present study differ from those of Keskin[1], who investigated the apical extrusion of debris and found that the use of the R-Pilot, WGG and ProGlider followed by the use of the Reciproc Blue R25 file caused a similar amount debris extrusion from the mesial roots of mandibular molars. The fact that debris extrusion with the R-Pilot, WGG and ProGlider files was evaluated after using the Reciproc Blue R25 file may have masked the difference in debris extrusion with the glide path files, as the Reciproc Blue file caused significantly more debris extrusion than the Wave One Gold file when shaping root canals[34,35].

CONCLUSION

Glide path preparation resulted in apical extrusion, regardless of the instrument used. However, since the best results were achieved with the WaveOne Gold Glider (lowest debris extrusion), it could be recommended for use before instrumentation with the WaveOne Gold primary file. It is important to note that, due to the ex vivo nature of the study, the results should be extrapolated with care to clinical situations.

DECLARATION OF CONFLICTING INTERESTS

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

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Effect of resin cements on the bond strength of three types of glass fiber post systems to intraradicular dentin

Sue A Lavareda Correa Uchôa, Flávia L Botelho Amaral

Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Odontologia Restauradora, Campinas, São Paulo, Brazil

ABSTRACT

Rehabilitating teeth after root canal treatment often requires the use of glass fiber posts (GFPs) to retain the final restorations, so the choice of resin cement is critical for bond strength (BS) and treatment success. ***Aim:*** *The aim of this study was to evaluate the effect of different GFP systems on BS to intraradicular dentin using two dual-curing resin cement types.* ***Materials and Method:*** *Thirty bovine incisors with wide canals were filled endodontically with gutta-percha and epoxy resin sealer. Initially, the canal filling was removed, and 4 mm of the apical seal was left intact. The teeth were divided into three groups according to the GFPs used: AP (anatomical posts – prefabricated GFP (Reforpost #2, Angelus®) relined with composite resin (Filtek Z350, 3M ESPE); SPLENDOR (Splendor SAP, Angelus®), and milled CAD/CAM (FiberCAD, Angelus®). Posts were fixed with conventional (RelyX Ultimate, 3M ESPE) or self-adhesive resin cement (RC) (RelyX U200, 3M ESPE), following the manufacturer's instructions. After 48 h, the roots were sectioned into thirds and subjected to push-out BS testing using a universal testing machine. BS data were analyzed using Wilcoxon and Mann-Whitney U tests. Failure modes were assessed with Fisher's Exact test (α=0.05).* ***Results:*** *In the apical and middle root sections, BS was similar in the AP and Splendor groups, both of which performed better than the milled CAD/CAM group (p≤0.05). In the cervical section, BS was significantly higher for the anatomical posts than for Splendor and milled CAD/CAM posts. Self-adhesive RC promoted statistically lower BS compared to conventional RC for the milled CAD/CAM post in the cervical and middle thirds (p≤0.05). Self-adhesive RC provided statistically higher bond strength than conventional RC for the anatomical post in the apical third (p≤0.05). No significant difference in failure modes was observed between resin cements and different root sections (p>0.05).* ***Conclusion:*** *The BS of the GFP system was affected by resin cement type and root section, with composite resin-relined anatomically shaped posts generally performing better.*

Keywords: *dental cements - dental posts - shear strength - intraradicular retainer*

Efeito de cimentos resinosos na resistencia de união de três tipos de pinos de fibra de vidro à dentina intrarradicular

RESUMO

A reabilitação de dentes tratados endodônticamente geralmente requer o uso de pinos de fibra de vidro (PFV) para retenção da restauração final, e, portanto, a escolha do cimento resinoso é crítica para adequada resistencia de união (RU) e sucesso do tratamento. ***Objetivo:*** *O objetivo deste estudo foi avaliar o efeito de diferentes sistemas de PFV na RU a dentina intrarradicular utilizando dois tipos de cimentos resinosos duais.* ***Materiais e Método:*** *Trinta incisivos bovinos com condutos amplos foram tratados endodônticamente com guta-percha e cimento a base de resina epóxica. A obturação do conduto foi removida, e 4 mm de material na porção apical foi mantido intacto. Os dentes foram divididos em três grupos de acorco com o pFV utilizado: PA (pino anatômico – PFV pre-fabricado (Reforpost #2, Angelus®) reembasado com resina composta (Filtek Z350, 3M ESPE); SPLENDOR (Splendor SAP, Angelus®), e PFV fresados em CAD/CAM (FiberCAD, Angelus®). Os PFVs foram fixados com cimento resinoso (CR) convencional (RelyX Ultimate, 3M ESPE) ou autoadesivo (RelyX U200, 3M ESPE), seguindo-se as intruções do fabricante. Após 48 horas, as raízes foram seccionadas em terços e submetidas ao teste de RU por push-out em máquina universal de ensaios. A RU foi na analisada pelo teste de Wilcoxon e Mann-Whitney U. O modo de falha foi avaliado pelo teste Exato de Fisher (α=0.05).* ***Resultados:*** *Nos terços radiculares apical e médio, a RU foi semelhante entre os grupos PA e SPLENDOR, e ambos tiveram RU superior do que o grupo de PFV fresado em CAD/CAM (p≤0.05). Na região cervical, a RU foi significantemente superior para PA do que SPLENDOR e PFV fresado. O CR autoadesivo promoveu RU estatisticamente inferior comparado ao CR convencional para o PFV fresado em CAD/CAM nos terços cervical e médio (p≤0.05). O CR autodesivo promoveu RU estatisticamente superior do que o CR convencional para PA, no terço apical (p≤0.05). Não houve diferença significativa no modo de falhas, considereando-se os diferentes CRs e terços radiculares (p>0.05).* ***Conclusão:*** *A RU de sistemas de PFV foi afetada pelo tipo de cimento resinoso e região radicular, sendo que de forma geral os pinos anatômicos, reembasados com resina composta, tiveram desempenho superior.*

Palavras-chaves: *cimentos de resina - pinos dentários - resistência ao cisalhamento - técnica para retentor intrarradicular*

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Corresponding Author:
Flavia Lucisano Botelho do Amaral
flbamaral@gmail.com

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INTRODUCTION

Teeth that have undergone endodontic treatment and lost a significant amount of their structure are more susceptible to biomechanical failures and fractures. Roots and crowns must be effectively and promptly restored to prevent further complications[1-3].

Rehabilitation protocols to restore the function and appearance of these teeth often involve intraradicular retainers[4,5]. These retainers should ideally mimic the physical properties of natural dentin to ensure seamless integration. The materials should ensure a range of properties, such as biocompatibility, ability to preserve the integrity of the root dentin, good adherence to the existing tooth structure, resistance to corrosion, minimization of stress to the remaining tooth, natural appearance, and affordability[3]. For many years, dental professionals have favored cast metal posts for reconstructing teeth after root canal treatment because of their durability and success rate[5]. However, some drawbacks have limited their popularity, including their less appealing look, lengthy time required for placement, higher cost, need to remove more of the tooth root and crown structure, and greater risk of causing tooth fractures due to stiffness[6].

In recent years, glass fiber posts (GFPs) have become a popular alternative. Their physical properties are similar to those of natural tooth dentin, and they help distribute chewing forces more evenly, thus reducing the risk of root fractures. However, they also involve challenges. They can be difficult to bond securely to the inner tooth dentin, and the bonding process is sensitive to the technique and resin cement employed[7]. Moreover, GFPs do not always fit perfectly in certain types of root canals, particularly those that are oval-shaped or unusually wide, in which case a thick layer of resin cement may form, leading to more shrinkage and bond weakening, and possibly causing the post to become loose over time[8].

Flared root canals clearly require better-fitting retainer posts that will stay in place as a result of increased friction, thereby reducing the need for thick layers of resin cement[8]. The anatomical post technique was developed to address these issues. It involves using composite resin to strengthen fiber posts, thereby enhancing their adaptation and bond strength[8-10]. More recently, CAD/CAM technology has been developed to create customized one-piece fiberglass resin retainers which have better fit and only require a thin layer of cement[9,11].

Recognizing the importance of a precise fit for retainers in root canals, the dental industry has introduced new post systems designed to reduce failures in retention and shorten dental procedure time. One such innovation is the Splendor SAP system, which comprises a GFP and universal sleeve for better adaptation to the canal, thus eliminating the steps of relining the GFP with composite resin[12]. Although it has been demonstrated that bond strength to root dentin is not influenced by the GFP system[13], there is still no consensus in literature about which resin cement strategy performs better in terms of bond strength, especially regarding the Splendor SAP system, on which there are few studies. Lopes et al.[12] observed that the use of either self-adhesive or conventional resin cement did not influence the bond strength of the Splendor SAP system to a flared root canal, while systematic reviews suggest that self-adhesive cements are less sensitive and can improve the bond strength of glass fiber-based posts to root canals[14]. Based on this development, the purpose of this study was to examine the bond strength and failure mode of three types of intraradicular retainers (anatomical, milled CAD/CAM and Splendor SAP posts) cemented into wide root canals using either self-adhesive or conventional resin cements, considering different thirds of the root.

MATERIALS AND METHOD

Ethical Aspects

This in vitro study was approved by the Ethics Committee on Animal Research of the São Leopoldo Mandic School of Dentistry (protocol number 2024/18) because of the use of bovine incisor teeth.

Selection and Preparation of Teeth

Thirty bovine incisors were selected, cleaned of adherent tissue, and sectioned horizontally using a double-sided diamond disc (Microdont, São Paulo, SP, Brazil) under constant cooling with water and air, to achieve a root length of 17 mm from the root apex, as measured by a digital caliper (Mitutoyo Sul Americana, Suzano, SP, Brazil). The crowns were discarded, and the standardized roots were fixed in a 21 x 34 mm acrylic resin matrix filled with condensation silicone (Speedex; Coltene, Altstätten, Switzerland).

A single calibrated operator performed the

endodontic procedures, standardizing the working length at 17 mm. The root canals were prepared using a crown-down technique with a K80 file (Dentsply/Maillefer, Ballaigues, Switzerland) as the primary file. The root canals were irrigated with 1% sodium hypochlorite (Biodinâmica Produtos Químicos, São Paulo, SP, Brazil) at each file change, and 17 % EDTA solution (Maquira, Maringá, PR, Brazil), followed by distilled water.

The root canal was dried with suction tips and absorbent paper, and filled by lateral condensation with gutta-percha cones (Dentsply, Petrópolis, RJ, Brazil) and an epoxy resin-based cement (Sealer 26; Dentsply, Petrópolis, RJ, Brazil). The root canal was sealed temporarily, and the specimens were stored at 37 °C and 100% humidity for 72 hours. After this period, the intraradicular retainer was installed.

The root canals were further prepared under copious irrigation using a Largo #2 drill (Angelus®, Londrina, PR, Brazil), followed by enlargement of the root canal with a conical diamond tip (4137) (Kavo, Joinville, SC, Brazil). A 4 mm thick layer of gutta-percha was used for apical sealing. The canals were irrigated with 16% EDTA (Maquira, Maringá, PR, Brazil), washed abundantly with distilled water, and dried.

Fabrication of the Intraradicular Retainers

The roots were randomly allocated to three different experimental groups: the anatomical post group (AP); the SPLENDOR group, which employed Splendor SAP GFPs; and the CAD/CAM group, which used Fiber CAD System posts.

The anatomical posts for the AP group were fabricated by coating GFPs with composite resin. Initially, the root canals were isolated with a water-soluble gel (KY, Johnson & Johnson, São Paulo, SP, Brazil), using disposable micro-applicators (AllPrime, São José, SC, Brazil). A prefabricated GFP (Reforpost #2, Angelus, Londrina, PR, Brazil) was used. After cleaning and disinfecting the GFP with 70% alcohol, a silane-based bonding agent (Angelus, Londrina, PR, Brazil) was applied using disposable micro-applicators (All Prime, São José, SC, Brazil) for one minute, followed by air drying. Next, the Single Bond Universal adhesive system (3M ESPE, São Paulo, SP, Brazil) was applied, followed by adhesive evaporation with an air jet and light-curing for 10 seconds using a LED light-curing unit (Valo, Ultradent Products, St. Jordan, USA), operating in standard mode with an irradiance of 1000 mW/cm². Subsequently, a small amount of composite resin (Filtek Z350 XT, 3M ESPE, St. Paul, USA) in shade A1 was placed on the apical portion of the post, and the assembly (post and resin) was inserted in the root canal to be molded and made anatomically compatible with the root, followed by initial light-curing for 10 seconds. The post was removed, and light-curing was completed for another 40 seconds (Valo, Ultradent Products, St. Jordan, USA). The water-soluble gel was removed by irrigation with distilled water and suction using a metal cannula, followed by absorbent paper points (Dentsply Maillefer, Catanduva, SP, Brazil).

In the SPLENDOR group, Splendor SAP GFPs (Angelus, Londrina, PR, Brazil) were used, consisting of a post and a sleeve also made of fiberglass, designed to fit into the root canals regardless of their diameter.

In the experimental CAD/CAM group, Fiber CAD system GFPs were fabricated. The root was modeled using Duralay red acrylic resin (Reliance Dental Manufacturing, Worth, USA) and the Pinjet acrylic resin pin for modeling (Angelus, Londrina, PR, Brazil). After lubricating the root with lubricating gel (KY, Johnson & Johnson, São Paulo, SP, Brazil), the acrylic resin (powder and liquid) was manipulated and inserted into the root with a Lentulo drill. Then, the modeling pin was placed, removed, and reinserted several times to eliminate any bubbles and prevent the resin from getting stuck in the root canal until polymerization was complete. After the final polymerization, the Duralay patterns were stored in water and sent to the prosthetic laboratory. In the prosthetic laboratory, the Fiber CAD system fiberglass cores were manufactured using the CAD/CAM system (computer-aided design/computer-aided manufacturing). The process began by scanning the molded pattern in the root canal originally produced with Duralay acrylic resin, using an extraoral scanner (model inEos X, Sirona, Barueri, SP, Brazil). Subsequently, the milling process was conducted with a CEREC MC XL milling unit (Sirona, Barueri, SP, Brazil), using a specific block of the Fiber CAD System – Post and Core (Angelus, Londrina, PR, Brazil).

Cementation of Intraradicular Retainers

The GFPs were fixed using RelyX U200 (3M ESPE, St. Paul, MN, USA) (15 teeth) or RelyX Ultimate

(3M ESPE, St. Paul, MN, USA) resin cements. Initially, the root canals were irrigated with distilled water, and then dried using absorbent paper points (Dentsply Maillefer, Catanduva, SP, Brazil).

In the AP group, the GFPs were cleaned with 70% alcohol. The resin cement was mixed in a mixing block according to the manufacturer's instructions, and inserted into the root canals using the applicator tip of a Centrix syringe (Maquira, Maringá, PR, Brazil), followed by insertion of the GFP. Any excess resin cement was removed with a disposable micro-applicator (All Prime, São José, SC, Brazil). After all the GFPs had been inserted, each was light-cured for 40 seconds, with the tip of the device positioned as closely as possible to the cervical region.

In the CAD/CAM group, adjustments were made to ensure proper fit before cementing the GFPs. This involved liquid carbon Super Film (Kota, São Paulo, SP, Brazil) and a Sof Lex sanding disc (3M ESPE, St. Paul, MN, USA). After the adjustments were made, the GFPs were cleaned with 70% alcohol. A silane-based bonding agent (Angelus, Londrina, PR, Brazil) was then applied with a disposable micro-applicator (All Prime, São José, SC, Brazil), followed by drying with compressed air and waiting one minute. Subsequently, the Single Bond Universal adhesive system (3M ESPE, St. Paul, MN, USA) was applied, followed by adhesive evaporation with compressed air, and light-curing for 10 seconds. The resin cement was then manipulated and inserted into the root canals using a Centrix syringe applicator tip (Maquira, Maringá, PR, Brazil). The GFPs were positioned, and any excess was removed, followed by light-curing for another 40 seconds.

The Splendor SAP GFPs were fixed following a process similar to that of the Fiber CAD GFPs. An additional step involved the surface treatment of the accompanying sleeve. The GFPs were inserted into the canals in two stages: first, the post was positioned, and then the sleeve was carefully accommodated around the post with tweezers. After cementation, the specimens were placed in a humid environment at 37 °C for storage.

Sample Preparation

Forty-eight hours after cementation of the GFPs, the roots were fixed individually in an acrylic resin plate using modeling wax and aligned parallel to one another. They were then sectioned transversely using a double-sided diamond disc (Buehler, IL, USA), attached to a metallographic cutter (Isomet 1000, Buehler, OL, USA), operating at a speed of 300 rpm with constant cooling. This process resulted in a 1 mm thick slice from each root third (apical, middle, and cervical). The slices were mounted on a universal testing machine (EMIC DL2000, São José dos Pinhais, SP, Brazil) to conduct the push-out test at a speed of 0.5 mm/min and a load of 50 Kgf.

After completing the push-out test, the specimens were examined under a stereoscopic microscope to analyze the failure type. Failures were classified as adhesive between the resin cement (RC) and the dentin, adhesive between the resin cement and the GFP, cohesive in GFP, cohesive in RC, cohesive in dentin, or a combination, indicating the presence of two types of failures simultaneously.

Push-out Bond Strength Test

The push-out bond strength test was conducted on the prepared root slices using a universal testing machine (EMIC DL 2000; EMIC, São José dos Pinhais, Paraná, Brazil), using. Each slice was placed in a push-out device consisting of a steel base with an active tip 1 mm in diameter. The test was performed at a speed of 0.5 mm/min and a load cell with a capacity of 500 N. The force data obtained in Kgf were expressed in MPa (MPa = KgF*9.8/area). The area of each section was calculated using the following formula: $\pi * R^2 * h$, where π is the constant 3.1416, R represents the diameter of the pin, and h, the height of the section in mm.

Failure Mode Evaluation

The push-out specimens were observed under a stereomicroscope (Eikonal Equip. Ópticos e Analíticos, model EK3ST, São Paulo, SP, Brazil) at a magnification of 40x. Failures were classified as adhesive failure between RC and fiber post, adhesive failure between RC and dentin; dentin cohesive failure; RC cohesive failure; fiber post cohesive failure, or mixed failure.

Statistical Analysis

Descriptive data analyses were conducted initially. Subsequently, the bond strength variable was examined using a generalized linear model, following a split-plot design. This implies that different thirds were evaluated in the same specimens of the model, while the systems were assessed in different specimens. The analysis of the failure mode was

Table 1. Descriptive analysis and bond strength results for push-out tests (MPa) according to resin cement type, type of retainer and root third

Post	Rely X U 200			Rely X Ultimate		
	Cervical	Middle	Apical	Cervical	Middle	Apical
Milled	2.17 ± 1.09Ba*	0.77 ± 0.30Bb*	0.89 ± 0.92Bb	5.45 ± 2.64Ba#	3.64 ± 2.44Ba#	4.84 ± 5.45ABa
Anatomical	11.08 ± 6.30Aa	14.93 ± 6.47Aa	10.74 ± 5.39Aa*	14.64 ± 3.12Aa	15.66 ± 6.02Aa	1.41 ± 0.46Bb#
Splendor SAP	3.40 ± 4.01Ba	6.38 ± 5.10Aa	10.37 ± 9.60Aa	7.47 ± 8.30AABa	19.98 ± 27.95ABa	9.22 ± 6.65Aa

Means followed by different letters (uppercase in the columns, comparing the GFPs within each third, and lowercase in the rows, comparing the thirds within each type of GFP) indicate significant differences (p≤0.05). Means followed by different symbols indicate a statistical difference between RelyX U200 and Rely X Ultimate resin cements, within the same root third and type of GFP.

conducted using Fisher's Exact Test. All analyses were performed in R software, with a significance level set at 5%.

RESULTS

The results of the bond strength evaluation are presented in Table 1. A comparison of different types of GFPs (milled vs. anatomical post vs. Splendor) showed that the anatomical post and Splendor groups had statistically similar average bond strengths (p>0.05) and were superior to the milled group (p≤0.05) when the RelyX U200 resin cement was used in the apical and middle thirds. In the cervical third, the anatomical post provided statistically superior bond strength compared to the milled and Splendor GFPs (p≤0.05). The latter two were statistically similar to each other (p>0.05).

When RelyX Ultimate cement was used in the cervical and middle thirds, the anatomical post group had a statistically higher average bond strength value than the milled GFP group (p≤0.05). Splendor showed intermediate results that were statistically similar to those of the other groups (p>0.05). In the apical third, average bond strength was statistically higher in the Splendor GFP group than in the AP group, but both were statistically similar to the milled group.

Regarding resin cements, RelyX U200 had statistically lower bond strength than RelyX Ultimate for the milled post in the cervical and middle thirds (p≤0.05). RelyX U200 resin cement provided statistically higher bond strength than Ultimate (p≤0.05) for the anatomical post in the apical third. In the other comparisons between the two resin cements, considering the same GFP and root third, the means did not differ statistically.

Comparison of root thirds showed that the milled GFP cemented with RelyX U200 self-adhesive resin had statistically lower bond strength in the apical and middle thirds than in the cervical third (p≤0.05), with no statistical difference between apical and middle thirds (p>0.05). However, the averages for the milled pin cemented with RelyX Ultimate conventional resin showed no significant difference among the apical, middle and cervical thirds (p>0.05).

Regarding the anatomical post cemented with RelyX U200 self-adhesive resin, no statistically significant difference was observed between the average bond strengths across the apical, middle and cervical thirds (p>0.05). However, for the same post cemented with RelyX Ultimate conventional resin, the middle and cervical thirds had statistically higher bond strength than the apical third (p≤0.05), with no significant difference between middle and cervical thirds (p>0.05). Finally, there was no statistically significant difference in bond strength for the Splendor post in the apical, middle or cervical thirds, regardless of the resin agent used (p>0.05).

The results of the failure mode analysis for the RelyX U200 and RelyX Ultimate 3M cementation systems, stratified by cervical, middle and apical thirds, are presented in Fig. 1. There was no significant difference between the resin cements or among the thirds in terms of failure modes (p>0.05 in all cases), suggesting a similar distribution of failure modes among the evaluated groups.

For the RelyX U200 resin cement, most specimens exhibited adhesive RC/D failures (3 for AP, 4 for Splendor, and 3 for Fiber CAD) in the cervical third, followed by cohesive RC failures (1 for AP, 2 for Fiber CAD), and one mixed-type failure (1 for AP). In the middle third, most failures were also mixed type (2 for AP, 5 for Splendor, 2 for Fiber CAD),

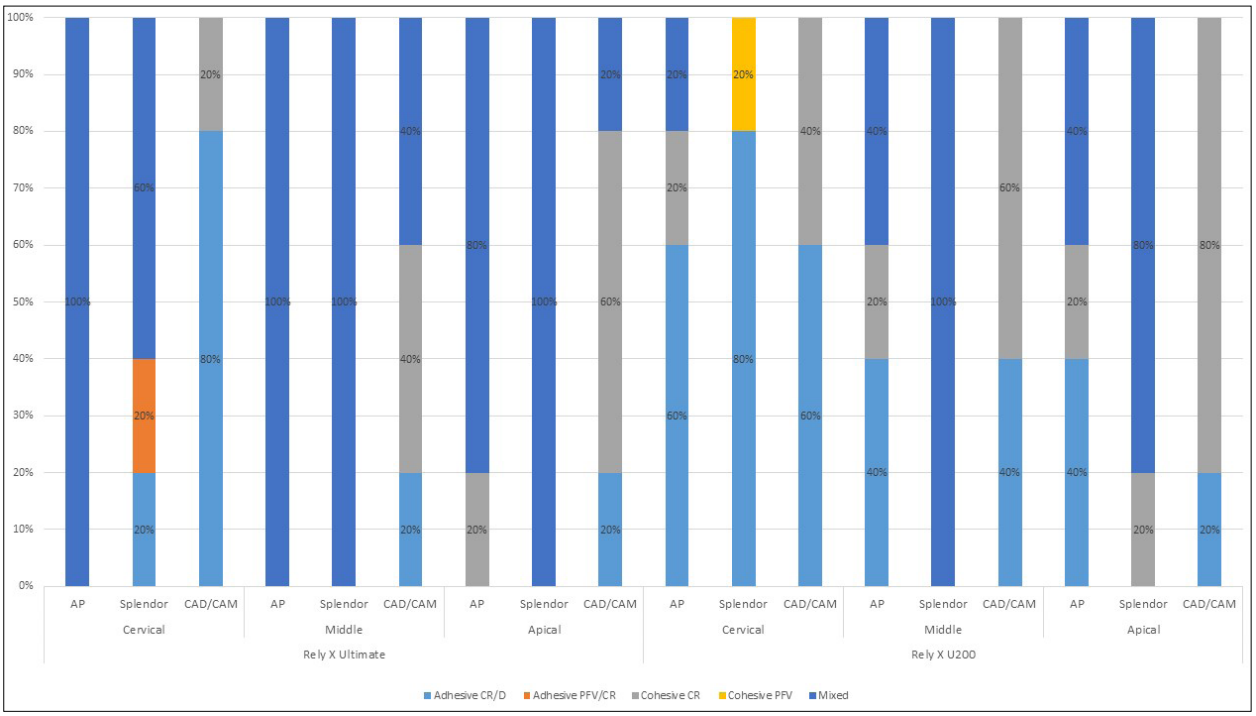

Fig. 1: Distribution of failure modes (%) relative to the experimental groups.

followed by cohesive GFP failures (1 for Splendor), cohesive RC failures (1 for AP, 3 for Fiber CAD), and adhesive RC/D failures (2 for Fiber CAD). In the apical third, mixed-type failures predominated (2 for AP, 4 for Splendor, 2 for Fiber CAD), followed by cohesive RC failures (1 for AP, 1 for Splendor) and adhesive RC/D failures (2 for AP, 1 for Fiber CAD).

Regarding RelyX Ultimate resin cement, a similar distribution of failures was observed in the cervical third, with a predominance of adhesive RC/D failures (4 for Fiber CAD), followed by mixed-type failures (5 for AP) and one cohesive RC failure (1 for Fiber CAD). In the middle third, again, most failures were mixed type (5 for AP and Splendor, 2 for Fiber CAD), followed by cohesive RC failures (2 for Fiber CAD) and one adhesive RC/D failure (1 for Splendor). In the apical third, mixed failures were the most common (4 for AP, 5 for Splendor, 1 for Fiber CAD), followed by adhesive RC/D failures (1 for AP, 1 for Splendor) and cohesive RC failures (3 for Fiber CAD).

DISCUSSION

The results of the present study demonstrated that there were significant differences in the bond strength of the GFP systems depending on the resin cement and the root third, so the null hypothesis was rejected.

Bond strength with RelyX U200 resin cement in the apical and middle root thirds was lower for CAD CAM posts than for anatomical or Splendor SAP posts, possibly due to the fit of CAD CAM posts. The first step in the CAD CAM process is to scan the acrylic resin pattern, which can introduce more variables in the digital process of a single-piece GFP[15]. The difficulty in fitting a post using the acrylic pattern to replicate the post space has been reported in a previous study[16]. Poor fit may have increased the thickness of resin cement in the middle and apical thirds, thereby affecting the degree of resin cement conversion. Indeed, it has been demonstrated that self-adhesive cements are viscous, which may affect the ability of radicals to migrate and complete the setting reaction, thereby decreasing the degree of conversion[17].

Comparisons of root thirds showed that the milled CAD CAM posts promoted lower bond strength in the apical and middle thirds than in the cervical third, when cemented with Rely X U200 resin agent. In this regard, it has been demonstrated that the self-polymerizing reaction of the dual-polymerized self-adhesive cement might not fully compensate for the inadequate light polymerization[18]. As mentioned

before, the method for obtaining the replica of the post space by scanning an acrylic resin pattern may have led to more poorly fitting posts and the need for adjustments. Added to the low light transmission in middle and apical regions, this may have had an undesirable effect on the mechanical properties of the resin cement, especially if the cement was thicker due to poor post fit.

In contrast, bond strength in the CAD CAM posts cemented with Rely x Ultimate resin cement was statistically higher than in the group that received Rely X U200 resin cement, without differences among root thirds. The need for a universal adhesive system during the adhesive procedures of the conventional resin cement, Rely X Ultimate, may have compensated for any lack of fit promoted by the technique used to obtain the post space replica. The universal adhesive contains a functional monomer (10-MDP monomer), which establishes chemical bond to tooth structure[19], and silane, a coupling agent that strengthens the bond between the inorganic fillers in the post and the organic matrix in the resin[20]. This is especially important in the comparisons between resin cements, where it was found that in general, the cementation of milled CAD CAM posts with conventional resin cement promoted higher bond strength values, especially in the cervical and middle root thirds, compared to the self-adhesive resin cement, Rely X U200.

Comparison of the bond strength of different GFPs cemented with Rely X Ultimate cement showed greater strength for the anatomical post than for the CAD CAM milled post in the cervical and middle thirds. The anatomical post is used to enhance frictional retention between the GFP and the dentin walls, produce a more uniform resin cement layer, and reduce resin cement thickness, thereby enhancing the strength of the bond to root dentin[21]. However, specifically in the apical third, the bond strength was lower for the anatomical post than for the Splendor SAP post. This may be because the anatomical post is relined with composite resin, which may have acted as a barrier preventing adequate light transmission, thereby reducing resin cement polymerization[22].

For the self-adhesive cement, bond strength was also lower for all root thirds, though without statistical difference. The Splendor SAP post, which is prefabricated with a glass fiber sleeve, may have allowed more light transmission to the apical region and indeed, for this post system, there was no statistical difference in bond strength among root thirds.

This study presents promising solutions from a scientific perspective for daily clinical practice, highlighting the ease of fabrication of anatomical posts, the practicality of adaptation with Splendor SAP GFPs, and the use of advanced digital techniques such as CAD/CAM manufactured posts. The research aimed to identify a combination of simplicity and effectiveness among the rehabilitation techniques. Further studies are needed to confirm the stability of these findings, and gain further insights.

CONCLUSION

Based on these findings, it was concluded that:

- Bond strength to intraradicular dentin was influenced by the type of glass fiber post, resin cement and root third;
- In general, the anatomical post relined with composite resin performed better than the milled CAD/CAM post;
- The Splendor SAP post and the anatomical and milled CAD-CAM posts showed comparable results;
- The conventional resin cement (Rely X Ultimate, 3M ESPE) had similar or better bond strength than the self-adhesive resin cement (Rely X U200), except for the anatomical post in the apical third.

CONFLICT INTERESTS

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

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Resin composite color change by spatula manipulation, effects on surface and color stability

Samara R dos Santos Franco[1], Jorge E da Silva Junior[2], Waldemir F Vieira Junior[1], Fabiana Mantovani Gomes França[1], Cecilia Pedroso Turssi[1], Roberta Tarkany Basting[1]

1. Faculdade São Leopoldo Mandic, Campinas, São Paulo, Brazil.
2. Innovare Odontologia Especializada, Maceió, Alagoas, Brazil.

ABSTRACT

Aim: *To evaluate the influence of spatulas on resin composite color stability, and characterize the surface of both the spatulas and the resin composites before and after manipulation.* ***Materials and Method:*** *Discs (ø6 mm x 2 mm) of suprananofilled resin composite (Palfique LX5/Tokuyama) and nanohybrid resin (Empress Direct/Ivoclar Vivadent) were fabricated. They were divided into groups (n=10), and manipulated with different spatulas: non-manipulated (control), metal spatulas (Almore Millennium/ Golgran, Almore #3/ Quinelato, LM Arte Modella/ Quinelato), or plastic spatulas (Jon). Manipulation involved lightly pressing the spatula 50 times against the resin composite on waterproof paper. Color was analyzed at three time points: immediately, after finishing and polishing, and after 24-hour immersion in distilled water, with CIELab* parameters, ΔE_{ab}, ΔE_{00}, and ΔWI_D. Spatulas and resins were submitted to micromorphological and energy-dispersive X-ray spectroscopic (EDS) analysis. Generalized linear models or Kruskal-Wallis and Dunn tests (α=5%) were applied.* ***Results:*** *Resin color changed after manipulation with a metal spatula, but there was no significant difference in ΔE_{ab} and ΔE_{00} for the resin composites according to the spatula ($p>0.05$). The suprananofilled resin varied more than the nanohybrid resin over time ($p<0.05$). Abrasive wear was observed on the spatulas after manipulating the resin composites, with greater wear for the spatulas used with suprananofilled resin. EDS showed different spatula and resin composite compositions.* ***Conclusions:*** *Metal spatulas influenced resin composite color stability, with greater color change for suprananofilled resin. The spatulas exhibited abrasive wear, attributable to the difference in hardness between the spatulas and the resin composites.*

Keywords: *color - composite resins - scanning electron microscopy*

Alteração de cor de resina composta após manipulação com espátula, efeitos na superfície e estabilidade de cor

RESUMO

Este estudo avaliou a influência das espátulas na estabilidade da cor da resina composta e caracterizou a superfície das espátulas e das resinas compostas antes e após a manipulação. Foram confeccionados discos (ø6mm x 2mm) de resina composta suprananoparticulada (Palfique LX5/Tokuyama) e nanohíbrida (Empress Direct/Ivoclar Vivadent). Cada grupo foi manipulado com diferentes espátulas (n=10): não manipuladas (controle), metálicas (Almore Millennium/ Golgran, Almore #3/ Quinelato, LM Arte Modella/ Quinelato) e espátula plástica (Jon). A manipulação foi realizada pressionando levemente a espátula 50 vezes contra a resina composta sobre papel impermeável. A análise de cor foi avaliada em três momentos: imediato, após acabamento e polimento e após 24 horas de imersão em água destilada, com parâmetros CIELab, ΔE_{ab}, ΔE_{00} e ΔWI_D. Espátulas e resinas foram submetidas a análises micromorfológicas e espectroscópicas por raios X por energia dispersiva (EDS). Foram aplicados modelos lineares generalizados ou testes de Kruskal-Wallis e Dunn (α=5%). Houve alteração de cor após a manipulação das resinas com as espátulas metálicas, mas ΔE_{ab} e ΔE_{00} não apresentaram diferenças significativas para as resinas compostas em relação ao tipo de espátula ($p>0,05$). As variações foram maiores para a resina suprananoparticulada do que para a resina nanohíbrida durante todo o período de tempo ($p<0,05$). Foi observado desgaste abrasivo nas espátulas após manipulação das resinas compostas, notando maior desgaste para a resina suprananoparticulada. A EDS mostrou diferentes composições de espátula e resina composta. As espátulas metálicas influenciaram a estabilidade da cor da resina composta, destacando maior alteração de cor para a resina suprananoparticulada. As espátulas apresentaram desgaste abrasivo atribuído à diferença de dureza entre as espátulas e as resinas compostas.*

Palavras-chave: *cor - resinas compostas - microscopia eletrônica de varredura*

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Corresponding Author:
Roberta Tarkany Basting
Email: rbasting@yahoo.com

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INTRODUCTION

Mimicry and longevity are desirable features in restorative treatments[1]. Resin composites replicate the optical characteristics of dental tissue and have satisfactory physical properties, making them suitable for the purpose[2-6]. Resin composites consist of an organic matrix made primarily of Bis-GMA (bisphenol glycidyl methacrylate) monomers, which are highly viscous. This can be corrected by incorporating other monomers such as TEGDMA (triethylene glycol dimethacrylate) and UDMA (urethane dimethacrylate) as diluents[7,8]. The material is strengthened by adding fillers, particularly in the form of nanoparticles and nanohybrids, with excellent outcomes for resistance, handling, polishing, and long-term gloss retention[3,9-12]. Over time, fillers have undergone modifications to address issues of low mechanical strength, especially when used for posterior teeth[6,13-15]. These alterations have involved changes in the shape, composition, size and concentration of particles[16], particularly in nanofilled and nanohybrid resins[17]. Some manufacturers add particles such as zirconia (also known as zirconium dioxide) and barium glass to the resin composite to enhance its mechanical properties[18].

Resin composites for clinical applications require the use of specific instruments, such as spatulas, which are available in various shapes, thicknesses, lengths and compositions. Spatulas are used to remove the resin composite from its packaging, and place it in the cavity[19]. When the material is used, particularly for aesthetic procedures involving the vestibular surfaces of anterior teeth, it is smoothed with spatulas and brushes to achieve the proper contour and dental anatomy[20,21].

When the resin composite is manipulated with a spatula, the friction between the two surfaces in relative motion creates abrasion. The result of this process is determined by the roughness, geometry, coefficient of friction, velocity, load, distance, and hardness of the surfaces in contact. The difference in hardness between the materials can result in a phenomenon generally referred to as wear, based on the principles of tribology, the science used to describe the phenomena of friction, wear and lubrication[22]. Consequently, the interaction of the tribological pair of "resin composite and spatula" during manipulation could alter the surface of both the resin composite and the spatula, according to their hardness, leading to abrasive wear between them[23]. In resin composites, this wear-related interaction could interfere with color stability, leading to aesthetic problems, especially for treatments with high aesthetic demands in anterior teeth.

The aim of this study was therefore to evaluate the influence of spatula types on the color stability of resin composites, and to characterize the surface of the spatula and resin composite before and after manipulation. The following null hypotheses were tested: H01) The spatulas used do not alter the color of the resin composites; H02) There is no difference between the surfaces of the spatulas before and after manipulation of the resin composites; H03) There is no difference between the surfaces of the resin composites before and after manipulation by the spatulas.

MATERIALS AND METHOD

Preparation of specimens and manipulation of resins with spatulas

The resin composites and spatulas evaluated are presented in Table 1. A total of 100 Teflon molds (2 mm high and 6 mm internal diameter) were used to prepare 50 specimens with suprananofilled resin (LX5 Palfique/Tokuyama/WE enamel color), and 50 specimens with nanohybrid resin (Empress Direct/ Ivoclar/BL-L enamel color), yielding 10 specimens of each resin allocated to each type of spatula. The resin composites were manipulated with the different metal spatulas tested (Almore Millennium/ Golgran, Almore number 3/Quinelato, LM Arte Modella/Quinelato,) or plastic spatula (Jon), and then placed in the Teflon mold. Fifty manipulations were standardized with the respective spatula designated for each specimen, with movements similar to those used to flatten the resin on a cavity when performing a direct veneer. This number was determined after observations in collaboration with clinical professionals. The specimens were manipulated on a disposable impermeable paper block. The same face of a previously unused spatula was always employed for each specimen, thus eliminating the possibility of any potential manipulation-related influence between different types of resin composites. A plastic spatula (plastic spatula/Jon) was used for the specimens in the control group (non-manipulated), just to remove the resin from its packaging, and place it directly into the mold (without any manipulation).

Table 1. Specification of the materials.

Commercial name/ manufacturer	Description	Composition	Manufacturer's recommended use
Palfique LX5/ Tokuyama Shade WE	Suprananofilled resin composite	Organic phase: BisGMA (Bisphenol A-glycidyl methacrylate); TEGDMA (Triethylene glycol dimethacrylate) Inorganic phase: Silica-zirconia Particles: Spherical Average size: 0.2 µm Monodisperse = all particles have uniform dimensions Weight concentration: 82% Volume concentration: 71%	Photocuring for 20 seconds
Empress Direct/ Ivoclar Shade BL/L	Nanohybrid resin composite	Organic phase: Dimethacrylates (20-21% by weight, opalescent 17% by weight); Copolymer (77.5 - 79%; opalescent 83% by weight); Additives, catalysts, stabilizers, and pigments (<1.0% by weight) Inorganic phase: Barium glass; ytterbium trifluoride; mixed oxides; silicon dioxides Particle type: Nanohybrid Average size: 550 nm Weight concentration: 75-79% Volume concentration: 52-59%	Photocuring for 20 seconds
Golgran	Metal spatula/ Almore (ALM) Millennium - code 98-14	Metal	Manipulation and application of the material in the cavity/tooth.
Quinelato	Metal spatula/ #3 Almore - code QD.325.03	Metal	
Quinelato	Metal spatula/ LM-Arte Modella - code 442-443 XSi	Metal	
Jon	Plastic spatula/ Jon - code 7165 - color: green	Thermoplastic polymer	

* Information provided on the company websites

Two strips of polyester were used to eliminate any bubbles and achieve a smooth surface: one strip was placed between the glass plate and the mold, and the other, over the mold after resin insertion. A glass slide was then placed over the resin, and a weight of 500 grams was applied for 30 seconds. Subsequently, the glass slide was removed for resin composite photoactivation using a LED light device (Valo, Ultradent, South Jordan, UT, USA) for 20 seconds with irradiance 1000 mW/cm².

The specimens were identified according to the groups (n=10), and to their upper surface (facing the light from the photoactivator). Their surface was evaluated for color (initial), after applying the finishing/polishing protocol, and after storage in humidity for 24 hours in a bacteriological incubator at 37 °C.

Finishing and polishing procedures and storage

Specimen surfaces were finished and polished with fine and extra-fine polishing disks (Sof-Lex Pop-On/3M). The disks were attached to a dedicated mandrel for use at low speed, and gentle pressure was applied to each disk for 15 seconds. The polishing procedure was carried out by fixing the specimens on an acrylic plate with sticky wax, keeping the surface parallel to the horizontal plane. After applying each type of sanding disk, the surface was rinsed with water and air-sprayed for 15 seconds. The sanding disks were replaced after every three applications. Subsequently, the specimens were submitted to surface color analysis, stored in distilled water for 24 hours, and reevaluated.

Color evaluations

Specimen surfaces were dried briefly with absorbent paper, and color analyzed by placing them a box with a white background to standardize the lighting. The spectrophotometer (VITA Easyshade® Advance, Vita, Germany) was previously calibrated automatically, by using the handpiece of the device positioned on the calibration block holder, with the measuring tip supported and pressing the calibration block at a 90° angle. Color was then evaluated with the measuring tip supported and fully seated, perpendicular to the surface of the specimens, always in the same position, in the central region. This measurement process was performed in duplicate to support the data used in the analysis. Specimen color was evaluated at three different times: immediately after fabrication, after finishing and polishing, and after 24 hours of immersion in distilled water.

The color of the resin composites was evaluated using the CIELab* parameters. The ΔL*, Δa* and Δb* values were measured for each group at each time, and used to assess color change (ΔE_{ab}) with the following formula[24]: $\Delta E_{ab} = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)}$. The perceptibility and acceptability thresholds considered for ΔE_{ab} were 1.2 and 2.7, respectively[24,25]. The color change was also evaluated using CIEDE2000 (ΔE_{00}), which uses h (hue) and C (chroma) values[26]. ΔE_{00} values of 0.8 and 1.8 were adopted as the perceptibility and acceptability limits, respectively[25]. Dental staining was monitored using the Whiteness Index for Dentistry (WI_D), where the L*, a*, and b* parameters were used in the following equation[27]: $WI_D = 0.511L^* - 2.324a^* - 1.100b^*$. Differences in WI_D (ΔWI_D) were also assessed between the evaluations, using threshold values of 0.72 for perceptibility and 2.60 for acceptability[27].

Scanning electron microscopy (SEM) and relative chemical composition analyses of the spatulas and resin composites

The surfaces of the spatulas (n=2) and resin composites (n=2) were evaluated for micromorphology before and after manipulation by SEM (Thermo Fisher Scientific, Model Quattro S, Thermo Scientific UltraDry, Brno, Czech Republic), and for relative chemical composition by energy-dispersive X-ray spectroscopy (EDS) (6070, LEO Electron Microscopy/Oxford, Cambridge, England). All resin surfaces were gold sputtered (gold layer thickness estimated at 200 Å) using a sputter coater (Sputter Coater Emitech K450, Kent, United Kingdom).

Images of surface micromorphology were obtained for the spatulas at magnifications of 13 x and 500 x in high resolution, with a voltage of 20.00 kV and a spot size of 100 pA. The resin composite surfaces were assessed using a magnification of 5000 x in high resolution, with a voltage of 20.00 kV and a spot size of 100 pA. Qualitative surface analyses were conducted to assess the presence of scratches and/or irregularities on the surfaces of the spatulas, as well as the presence and morphology of the filler particles in the resin composites. The relative chemical composition was expressed as the percentage of chemical elements present in the central region of the surface of both the spatula and the resin composite specimen.

Statistical analyses

After descriptive and exploratory analysis of all the data, generalized linear mixed-effects models for repeated measures over time were applied to L* and WI_D. Generalized linear models were also fitted to analyze ΔE_{ab} and ΔE_{00}, considering the study factors of resin and spatula, and the interaction between them. Other variables that did not fit a known distribution were analyzed using non-parametric tests, such as Kruskal-Wallis and Dunn for spatula comparisons, Friedman and for time comparisons, and Mann-Whitney for resin comparisons. The analyses were conducted using the R software (R Core Team, 2023) with a significance level of 5%.

RESULTS

For the L* parameter (Table 2), neither resin changed significantly over time ($p>0.05$). The suprananofilled resin composite showed a difference in L* depending on the spatula used ($p<0.05$), with higher luminosity when a plastic spatula was used, and lower luminosity when the #3 Almore or LM-Arte Modella spatulas were used ($p<0.05$). The nanohybrid resin composite showed a significant decrease in WI_D over time ($p<0.05$), with no significant difference between the types of manipulation ($p>0.05$). The suprananofilled resin showed differences in WI_D depending on the spatula used ($p<0.05$), with higher values for the non-manipulated groups and those manipulated with a plastic spatula, at all time points ($p<0.05$),

Table 2. Mean (standard deviation) L* and WI_D according to spatula type, time, and resin

Parameter	Resin composite	Spatula	Time		
			Immediate	After polishing	After 24-hour storage in water
			Mean (standard deviation)	Mean (standard deviation)	Mean (standard deviation)
L*	Suprananofilled	Non-manipulated	91.07 (1.49) Ab	91.18 (1.30) Ab	91.07 (1.99) Ab
		Almore Millennium	90.81 (2.53) Ab	90.89 (2.68) Ab	90.49 (2.86) Ab
		#3 Almore	87.07 (2.30) Ac	87.46 (2.47) Ac	87.95 (2.48) Ac
		LM-Arte Modella	86.30 (1.99) Ac	86.10 (1.95) Ac	86.39 (1.68) Ac
		Plastic	93.60 (2.02) Aa	93.51 (2.03) Aa	93.94 (2.28) Aa
	p-value		p(spatula)<0.0001; p(time)=0.3967; p(interaction)=0.3626		
	Nanohybrid	Non-manipulated	86.00 (0.54) Aa	85.77 (0.68) Aa	85.69 (0.63) Aa
		Almore Millennium	85.47 (2.23) Aa	86.07 (1.82) Aa	86.16 (2.06) Aa
		#3 Almore	84.68 (1.01) Aa	84.77 (1.69) Aa	84.78 (1.10) Aa
		LM-Arte Modella	85.36 (1.11) Aa	85.41 (1.32) Aa	85.46 (1.54) Aa
		Plastic	85.67 (0.47) Aa	85.41 (0.68) Aa	85.46 (0.83) Aa
	p-value		p(spatula)=0.1678; p(time)=0.7942; p(interaction)=0.5271		
WI_D	Suprananofilled	Non-manipulated	44.62 (0.84) Aa	44.02 (1.11) Ba	42.64 (0.86) Ca
		Almore Millennium	39.19 (3.98) Abc	38.37 (3.95) Bbc	36.66 (4.19) Cc
		#3 Almore	40.83 (1.39) Ab	40.59 (1.73) Ab	39.60 (1.31) Bb
		LM-Arte Modella	37.23 (2.13) Ac	37.16 (1.95) Ac	36.35 (1.50) Ac
		Plastic	44.17 (0.65) Aa	43.33 (0.73) Ba	41.97 (0.76) Ca
	p-value		p(spatula)<0.0001; p(time)<0.0001; p(interaction)=0.1830		
	Nanohybrid	Non-manipulated	34.24 (0.45) Aa	32.72 (1.01) Ba	31.36 (0.75) Ca
		Almore Millennium	33.27 (1.05) Aa	32.64 (1.37) Ba	30.83 (1.41) Ca
		#3 Almore	33.55 (0.62) Aa	32.44 (1.09) Ba	30.94 (0.88) Ca
		LM-Arte Modella	34.04 (2.26) Aa	32.52 (1.52) Ba	30.78 (1.44) Ca
		Plastic	33.45 (0.59) Aa	32.60 (0.82) Ba	31.28 (1.20) Ca
	p-value		p(spatula)=0.4937; p(time)<0.0001; p(interaction)=0.6817		

The same letters (uppercase horizontally and lowercase vertically, comparing the spatula types in each resin), indicate that there is no statistically significant difference within each variable ($p > 0.05$).

and lower values for Almore Millennium and LM-Arte Modella ($p<0.05$). There was no significant difference for ΔE_{ab} and ΔE_{00} regarding the spatula used ($p>0.05$) (Table 3). The variations were greater for the suprananofilled resin than for the nanohybrid resin over the entire period ($p<0.05$).

Regarding the suprananofilled resin composite, ΔWI_D differed according to the spatula used ($p<0.05$) (Table 4), with the variation in ΔWI_D being more negative (decrease) with the Almore Millennium than with the #3 Almore spatula ($p<0.05$). ΔWI_D was more negative in the nanohybrid resin than in the suprananofilled resin when non-manipulated or manipulated with #3 Almore and LM-Arte Modella spatulas ($p<0.05$).

The images of the surface micromorphology of the spatulas (Figs. 1 to 4) show that the Almore Millennium, #3 Almore, and plastic spatulas appeared unchanged with non-manipulation, while the LM-Arte Modella spatula already showed some scratches on its surface. After manipulation, all the spatulas showed surface wear, with scratches appearing on the Almore Millennium spatula, and overall surface wear on the other spatulas. The

Table 3. Mean (standard deviation) ΔE_{ab} and ΔE_{00} according to spatula type and resin at each time interval

Parameter	Resin composite	Spatula	Time point	
			After polishing - Immediate	After 24 hours - Immediate
			Mean (standard deviation)	Mean (standard deviation)
ΔE_{ab}	Suprananofilled	Non-manipulated	1.51 (0.58) a	3.02 (0.82) a
		Almore Millennium	0.99 (0.57) a	2.87 (0.74) a
		#3 Almore	1.30 (0.42) a	2.69 (1.00) a
		LM-Arte Modella	1.42 (0.92) a	2.35 (0.93) a
		Plastic	1.11 (0.51) a	2.91 (0.72) a
	Nanohybrid	Non-manipulated	1.32 (0.68) a	*1.90 (0.77) a
		Almore Millennium	1.46 (0.71) a	*2.17 (0.48) a
		#3 Almore	1.43 (0.45) a	*1.67 (0.64) a
		LM-Arte Modella	1.20 (0.75) a	*2.05 (0.83) a
		Plastic	1.13 (0.51)a	*1.58 (0.82) a
	p-value		p(resin)=0.6481; p(spatula)=0.5894; p(interaction)=0.4288	p(resin)<0.0001; p(spatula)=0.4886; p(interaction)=0.2751
ΔE_{00}	Suprananofilled	Non-manipulated	1.03 (0.36) a	2.14 (0.58) a
		Almore Millennium	0.69 (0.35) a	1.89 (0.48) a
		#3 Almore	0.89 (0.26) a	1.89 (0.66) a
		LM-Arte Modella	1.10 (0.97) a	1.72 (0.84) a
		Plastic	0.75 (0.32) a	1.97 (0.47) a
	Nanohybrid	Non-manipulated	0.95 (0.45) a	*1.41 (0.46) a
		Almore Millennium	1.00 (0.47) a	*1.55 (0.32) a
		#3 Almore	0.98 (0.30) a	*1.28 (0.41) a
		LM-Arte Modella	0.96 (0.82) a	*1.61 (0.90) a
		Plastic	0.77 (0.35) a	*1.12 (0.53) a
	p-value		p(resin)=0.5025; p(spatula)=0.3046; p(interaction)=0.5337	p(resin)<0.0001; p(spatula)=0.5047; p(interaction)=0.1821

*Differs from the suprananofilled resin under the same spatula and time point conditions ($p \leq 0.05$). Different letters in the vertical direction, comparing the spatula types with each resin, indicate statistically significant differences ($p \leq 0.05$) in each evaluated parameter.

surfaces of the spatulas used to manipulate the nanohybrid resin showed more scratches, while those used to manipulate the suprananofilled resin showed smoother surfaces.

The evaluations by EDS (Figs. 1 to 4) showed that the composition of the Almore Millennium spatula was predominantly 85% iron (Fe) and 11% chromium (Cr), with 1% carbon, 1% oxygen (O), 1% silicon, and 1% manganese (Mn), while the #3 Almore spatula was composed of approximately 54% nickel (Ni), 44% titanium (Ti), and 2% carbon (C). The LM-Arte Modella spatula was made up of about 77% iron (Fe) and 16% chromium (Cr), with 1% aluminum (Al), 1% carbon (C), 1% nickel (Ni), 1% oxygen (O), 1% manganese (Mn), 1% silicon, and 1% molybdenum (Mo), and the plastic spatula had 67% carbon (C) and 33% oxygen (O) in its composition.

There was no difference between the images of the surfaces of the resin composites (Fig. 5) without manipulation and after manipulation. Regarding the composition of the resins, the EDS analysis showed that the suprananofilled resin was composed of approximately 42% oxygen (O), 29% silicon (Si), 15% carbon (C), 12% zirconium (Zr), 1% sodium (Na), and 1% chlorine (Cl), while the nanohybrid resin consisted of approximately 38% oxygen (O), 25% silicon (Si), 14% carbon (C), 13% barium (Ba),

Table 4. Median (minimum; maximum) ΔWI_D according to spatula type and resin at each time point

Resin composite	Spatula	Time point	
		After polishing - Immediate	After 24 hours - Immediate
		Median (minimum; maximum)	Median (minimum; maximum)
Suprananofilled	Non-manipulated	-0.52 (-1.77; 0.31) a	-1.99 (-2.84; -0.84) ab
	Almore Millennium	-1.03 (-1.97; 0.34) a	-2.85 (-3.15; -1.39) b
	#3 Almore	-0.22 (-2.04; 1.47) a	-1.47 (-2.23; -0.20) a
	LM-Arte Modella	-0.94 (-2.67; 8.27) a	-1.38 (-3.44; 6.05) ab
	Plastic	-1.05 (-1.75; 0.76) a	-2.01 (-4.02; -0.86) ab
p-value		0.7171	0.0172
Nanohybrid	Non-manipulated	*-1.72 (-2.56; 0.62) a	*-3.13 (-4.26; -1.26) a
	Almore Millennium	-0.94 (-2.52; 1.43) a	-2.21 (-4.95; -1.09) a
	#3 Almore	-1.12 (-2.41; 0.24) a	*-2.78 (-3.76; -1.37) a
	LM-Arte Modella	-1.18 (-6.78; 1.03) a	*-2.69 (-9.28; -1.40) a
	Plastic	-0.85 (-2.28; 1.24) a	-2.20 (-4.41; -0.44) a
p-value		0.3997	0.5754

*Differ from the suprananofilled resin under the same spatula and time point conditions ($p \leq 0.05$). Different letters in the columns, comparing the spatula types in each resin, indicate that there is a statistically significant difference ($p \leq 0.05$).

5% zirconium (Zr), 3% aluminum (Al), 1% sodium (Na) and 1% calcium (Ca).

In addition to determining the composition of each spatula and resin composite, the EDS analysis enabled the Mohs hardness of each component (Table 5) to be related to the Mohs hardness of the spatula constituents and the filler particles of the suprananofilled and nanohybrid resin composites.

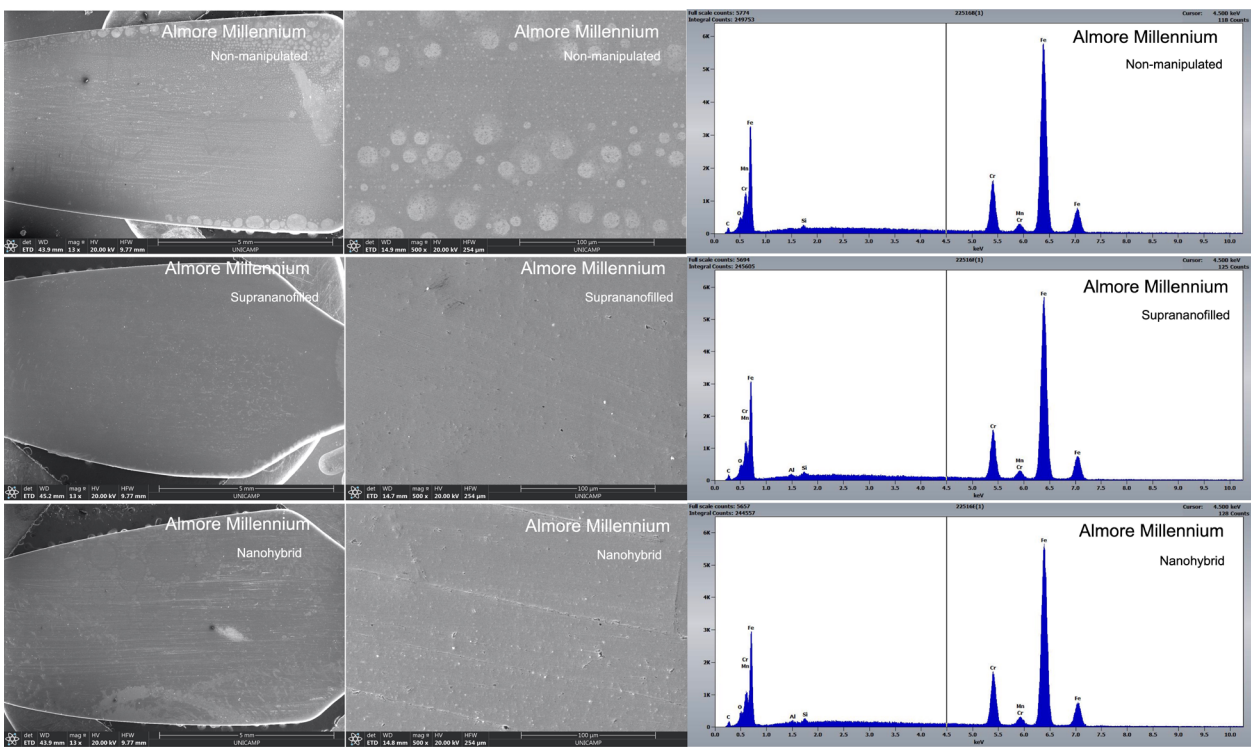

Fig. 1. Images of the surface micromorphology of Almore Millennium spatulas (13 and 500 x) according to different manipulation conditions and EDS analysis

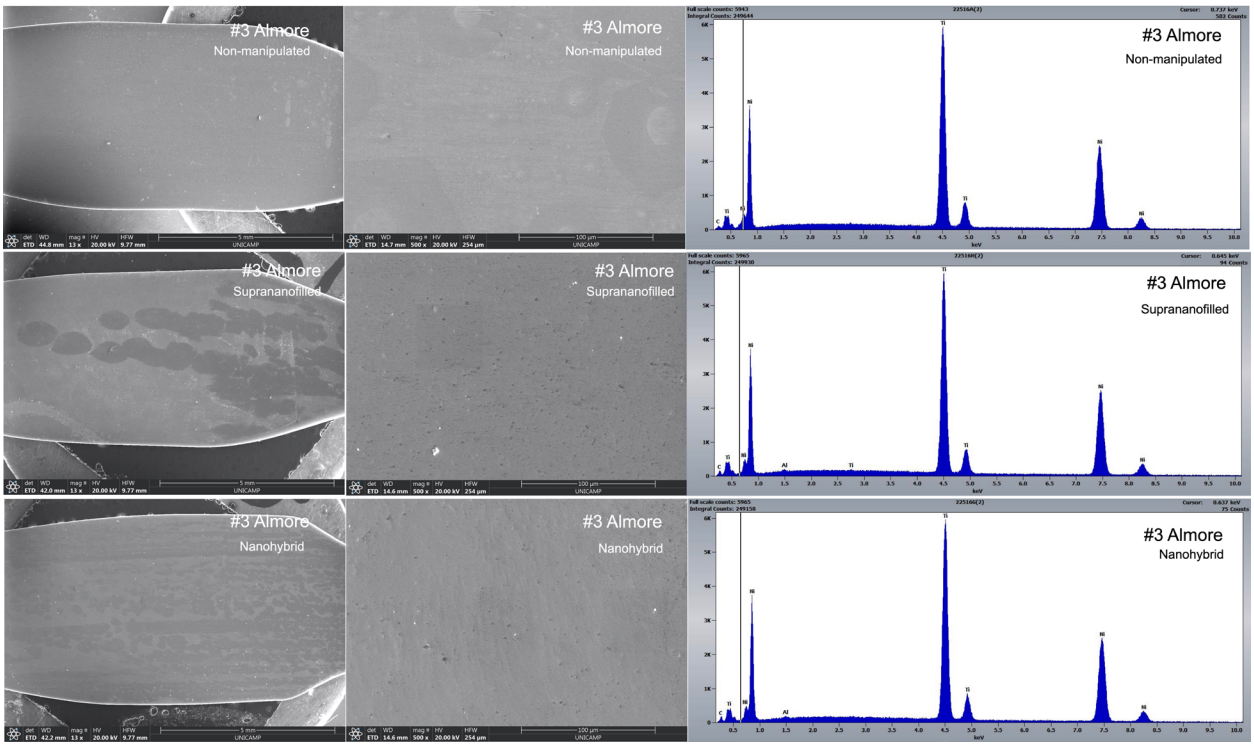

Fig. 2. Images of the surface micromorphology of #3 Almore spatulas (13 and 500 x) according to different manipulation conditions and EDS analysis

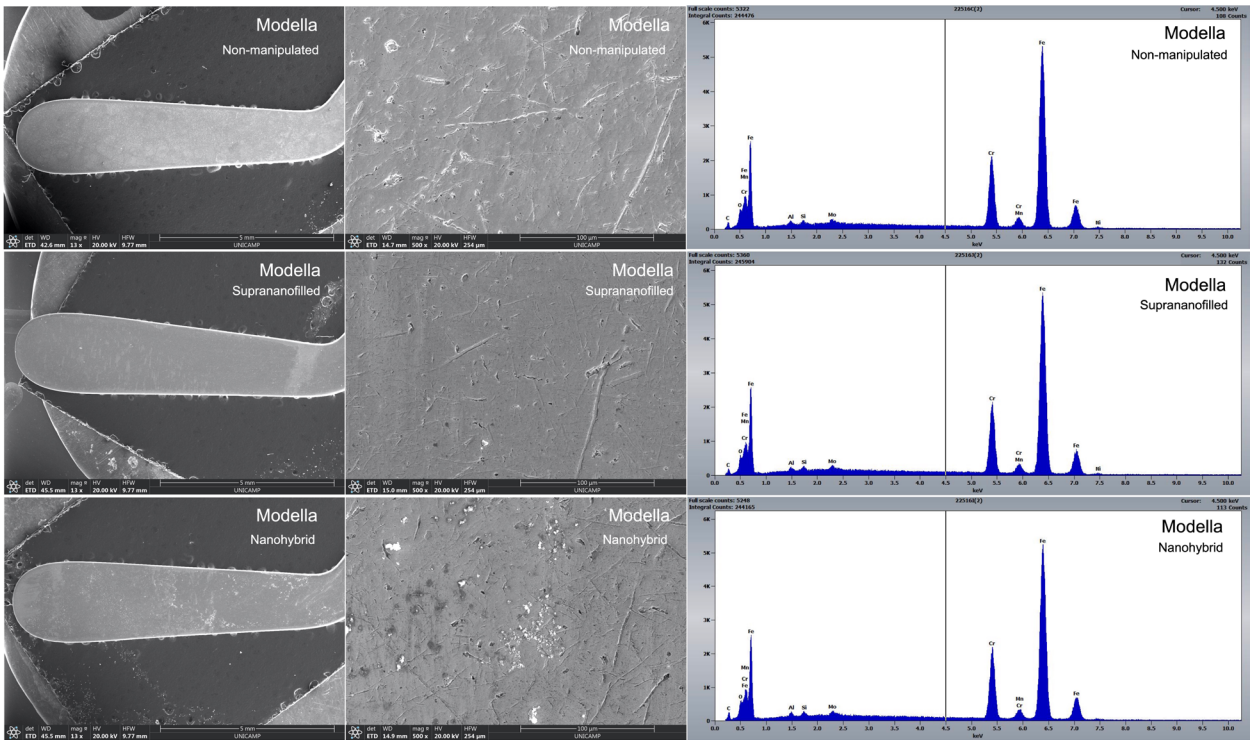

Fig. 3. Images of the surface micromorphology of LM-Arte Modella spatulas (13 and 500 x) according to different manipulation conditions and EDS analysis

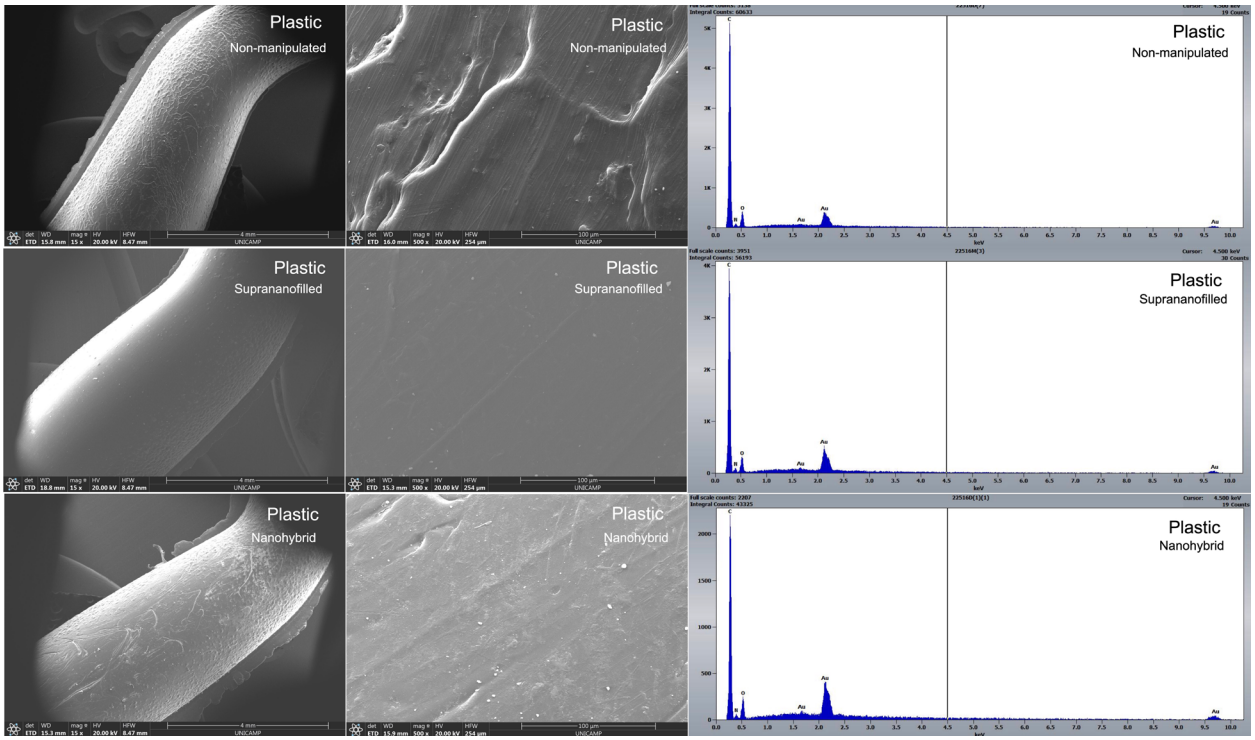

Fig. 4. Images of the surface micromorphology of plastic spatulas (13 and 500 x) according to different manipulation conditions and EDS analysis

Table 5: Percentage of constituents found in spatulas, and Mohs hardness value relationship

Chemical element	Mohs hardness	Constituents of the spatulas (%)			
		Almore Millennium	#3 Almore	LM-Arte Modella	Plastic
Iron	4[28]	85	-	77	-
Chromium	8.5[28]	11	-	16	-
Carbon	-	1	2	1	67
Oxygen	-	1	-	1	33
Silicon	6[28]	1	-	1	-
Manganese	2[28]	1	-	1	-
Molybdenum	5.5[28]	-	-	1	-
Aluminum	2.75[28]	-	-	1	-
Nickel	4[28]	-	54	1	-
Titanium	6 [29]	-	44	-	-

DISCUSSION

When resin composite is manipulated with spatulas, it is important that it should preserve its physical characteristics, especially those related to color stability. The null hypotheses tested in the present study were rejected because they stated that the spatulas used do not influence the color change of the resin composites (H01), and that there is no difference between the surfaces of the spatulas before and after resin manipulation (H02).

This study was able to correlate the color changes found in the resin composites (CIELab*) with the spatula surface alterations (SEM), and to explain these changes according to the constituents of the spatulas and the resin composites (EDS). The friction generated between the tribological "spatula vs. resin" pair resulted in the abrasion of both, due to the difference in hardness between the filler particles of the resin composites and the components

of the spatulas. It could explain the color changes, especially of the suprananofilled resin, in which case the components of the spatula alloy had lower Mohs hardness than the filler particles. When two surfaces have significantly different hardness levels, the micro-coarseness of the harder surface grates against the softer surface like a micro-plowing mechanism. Microscopically, a burr is formed in front of the abrasive particle, and the material is continuously displaced laterally, forming ridges adjacent to the grooves produced. The cyclic movement causes many grooves to be formed parallel to the direction of movement of the abrasive coarse grain, and the proximity of these grooves can weaken the more ductile material, which becomes deformed and is removed by a microfracture mechanism[22,28,29].

Evaluation of the L* and WI_D parameters found no difference between the nanohybrid resin composite manipulated with different types of spatulas and the non-manipulated resin. Nanohybrid resins contain barium, an element with low hardness (Mohs hardness 1.25)[28-30], which is responsible for color stability. The spatulas used to manipulate the nanohybrid resin showed microgrooves on their surface (Figs. 1 to 4), but they were not sufficient to promote significant color alteration in this resin composite.

In contrast, the suprananofilled resin composite contains harder filler particles[31], which promoted greater wear on the surface of the spatulas (Figs. 1 to 4). The suprananofilled resin filler particles contain more zirconium (Fig. 5), which has high hardness (Mohs hardness of 8)[31]. This difference in hardness between the filler particles of the suprananofilled resin and the components of the alloy of the spatulas studied (Table 5) explains the significant color alterations in this resin.

There were significant differences between the L* and WI_D parameters of the suprananofilled resin when it was manipulated with different spatulas, compared to the unmanipulated resin. The L* value is related to the brightness of the resin composite. Only the Almore Millennium spatula did not cause alteration with manipulation. The #3 Almore and LM-Arte Modella spatulas led to lower brightness, while the plastic spatula increased brightness. This suggests that the alterations L* result from the different levels of hardness between the filler particles in the suprananofilled resin and the components of the spatulas (Table 5).

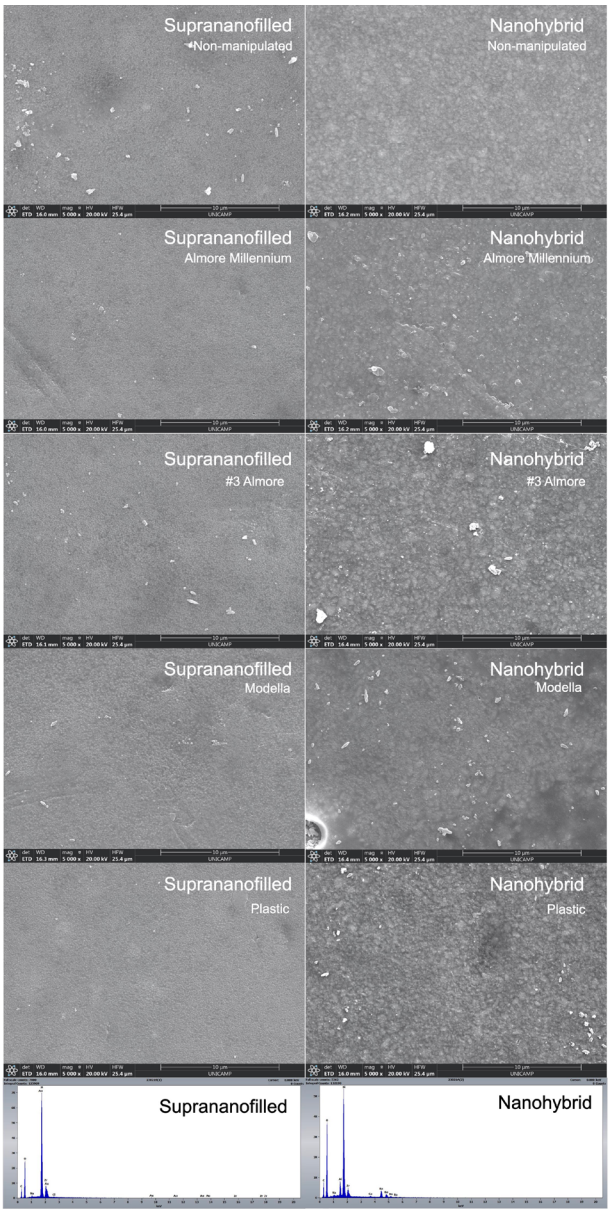

Fig. 5: Scanning electron microscopy images (5000 x magnification) of the surface of the composite resins manipulated by different spatulas according to EDS surface analysis

The WI_D parameter correlates better to visual perception than whitening evaluation indexes do. In this study, although the nanohybrid resin remained stable after manipulation using different spatulas, its color did change over time. Some studies show that resin composites may undergo color changes for up to 14 days after polymerization, after which they attain color stability. However, in this study, the suprananofilled resins underwent not only color change over time, but also significant alterations with manipulation using different spatulas.

Considering ΔE_{ab} and ΔE_{00}, there was no significant

color difference for either resin composite, a result that cannot be attributed either to the manipulation by different spatulas or to the time point. However, even though the lower ΔE_{ab} and ΔE_{00} values for the nanohybrid resin were not significant at the last time point, they indicated better color stability after the time points evaluated. Staining was more pronounced at the immediate time point than at the final time (after 24 hours immersed in distilled water). Although color changes are expected over time for all resin composites, in this study, the color changes were greater in the nanohybrid than in the suprananofilled resin at the evaluated time points. It is important to highlight that the staining values of the suprananofilled composite were significant for the manipulation of different spatulas, but not for the time points.

Regarding the difference in staining values over time (ΔWI_D), the non-manipulated group showed significant staining differences for the suprananofilled resin. This was also the case in the groups manipulated with the different spatulas, except for the #3 Almore spatula. In contrast, the staining differences for the nanohybrid resin were not significant across the evaluated time points.

Concerning the limits of perceptibility and acceptability for ΔE_{ab} and ΔE_{00}, clinically perceptible color changes were found for the suprananofilled resin composite in the non-manipulated, #3 Almore, and LM-Arte Modella groups (at the immediate and after polishing time points), and clinically unacceptable changes were detected in the non-manipulated, Almore Millennium, and plastic groups (at the immediate and 24-hour immersion time points). In contrast, perceptible changes were found for the nanohybrid resin, but they were all clinically acceptable. Further research should be conducted on the color stability of suprananofilled resin, considering that even without manipulation, its color changed significantly between the immediate and the 24-hour immersion time points.

Regarding the analyzed color parameters, the most relevant parameters for color alteration were the L* and WI_D values. These showed a significant color change (lower values) for the suprananofilled resin, noting that the LM-Arte Modella spatula influenced the results more negatively. One of the metals contained in this spatula is aluminum, a ductile lightweight metal with a gray appearance[32-35]. These characteristics may explain the color changes in luminosity and staining. Furthermore, this spatula has the highest number of components, and the nature of these components may also have contributed to the color change in the L* and WI_D values.

The third null hypothesis tested (H03), stating that there is no difference between the resin surfaces before and after manipulation by the spatulas, was not rejected. Visual analysis of the results from SEM images, and EDS composition analysis of the resin composites showed no considerable alteration before or after manipulation. It was expected that the constituent metals of the spatulas would be incorporated into the restorative material; however, the EDS analysis of the resin composites showed no difference in composition before and after manipulation. It is worth noting that the nature of the organic matrix in resin composites may have influenced their color stability[12,16,36-39]. However, based on the results found in this study, the findings of the cited authors could not be corroborated.

This study has shown that the relationship between the composition of the inorganic particles of resin composites and the composition of the spatulas was relevant for the color stability of the restorative material, especially considering the different manipulations. Concerning clinical application, these findings can help understand the color changes caused by manipulation that may affect resin composite, which is particularly important on the vestibular surfaces of anterior teeth. In clinical practice, the authors suggest avoiding excessive manipulation with metal spatulas, especially of suprananofilled resin. Further research in this area would be welcome to enable more precise decision-making. Based on the findings of this research, it is also suggested that certain materials could reduce the friction between the spatulas and resin composite, such as the modeling liquids used to improve the sliding action at the interface between materials. Manufacturers could reconsider spatula composition, including the use of specific coatings for spatulas, such as Teflon films and cold-worked alloys, among other technologies.

In conclusion, the metal spatulas influenced the color stability of the resin composites, considering that the suprananofilled resin underwent greater color change as a result of the different manipulations. The surfaces of the spatulas showed that there was abrasive wear of two bodies, attributed to the difference in the hardness values of the spatulas

and resin composites, considering that abrasion was greater in the spatulas used to manipulate the suprananofilled resin. The surfaces of the resin composites underwent no significant morphological change from the different manipulations.

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

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