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IN MEMORIAM

Fermín Alberto Carranza
1926-2025

With deep sorrow, we announce the passing of Prof. Dr. Fermín A. Carranza on March 9 in Los Angeles, California, at the age of 99.

Dr. Carranza, along with his colleague and friend, Prof. Rómulo Luis Cabrini, was instrumental in the creation of the *Argentine Society for Dental Research* in 1961 and, in 1983, in the founding of the journal *Acta Odontológica Latinoamericana*.

He earned his degree in Dentistry and his Doctorate in dental sciences at the *University of Buenos Aires (UBA)*. He later specialized in periodontology at *Tufts University School of Dental Medicine* in Boston, USA, where he also served as Assistant Professor of Periodontology from 1955 to 1956.

Upon returning to Argentina, he continued his professional and academic career, and became Full Professor of the Periodontology Department at the *Faculty of Dentistry (UBA)* from 1966 to 1974.

In 1974, he was invited to join the *University of California, Los Angeles (UCLA) School of Dentistry* as a Professor and Chair of the Department of Periodontology, a position he held until his retirement in 1994. He was then honored with the title of Professor Emeritus at UCLA. Despite his permanent relocation to the United States with his wife and three children, he remained deeply connected to Argentina, maintaining strong family ties, friendships, and active involvement in scientific collaborations.

His research work resulted in over a hundred scientific publications, and he received numerous honorary appointments and awards from prestigious institutions worldwide.

He authored ten books, including studies on periodontology and historical essays on dentistry and medicine. His most renowned work, *Clinical Periodontology*, has been translated into six languages and is widely used in dental schools across the globe. It is currently in its 14th edition under the title *Newman and Carranza's Clinical Periodontology*.

Beyond his outstanding scientific legacy, his family-now extended with ten grandchildren and two great-grandchildren, along with his many friends, collaborators and disciples, will always remember him as a role model, admired for his warmth and generosity in sharing his knowledge.

María E. Itoiz
Honorary Editor

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Heat generation during osteotomy performed with ultrasonic insert versus rotary instrument in bovine femur: *ex vivo* comparison

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ABSTRACT

Osteotomy procedures in dentistry are usually performed with drills, but piezosurgical instruments have also been used to improve surgical conditions for both the patient and the operator. This ex vivo study uses infrared thermography to analyze heat generation in osteotomies. ***Aim:*** *The aim of this study was to conduct an infrared thermographic comparison of the heat generated by an ultrasonic insert, either with or without an aerosol dispersion control device, in contrast to a conventional bur, during osteotomy procedures performed on bovine femur specimens.* ***Materials and Method:*** *Osteotomies were performed on nine bovine femur blocks, with each osteotomy consisting of a linear cut 12 mm long and 3 mm deep. Each block underwent a single cut from each instrument examined. The osteotomies were divided into three groups according to the instrument used: Group CARB, carbide bur #701; Group INS, #SFR4 ultrasonic insert coated with diamond-like carbon (DLC); and Group INS-S, #SFR4 ultrasonic insert coated with DLC in combination with an aerosol dispersion control device ("spray control"). All incisions were standardized using an automated device. Thermal variations (ΔT) were assessed using an infrared thermographic camera. The maximum (Tm) and minimum (T0) temperatures recorded were utilized to calculate ΔT, following the equation: ΔT = Tm – T0. Statistical analyses were conducted using Kruskal-Wallis test and Dunn's test for multiple comparisons ($p < 0.05$).* ***Results:*** *The T0 and Tm recorded for INS (21.5°C ± 0.7°C and 23.2°C ± 0.7°C) and INS-S (20.8°C ± 0.4°C and 21.8°C ± 0.4°C) were significantly higher ($p < 0.05$) than for CARB (14.9°C ± 0.8°C and 17.6°C ± 1.1°C, respectively). The observed ΔT for INS (1.7°C ± 0.4°C) and INS-S (1.0°C ± 0.4) were significantly lower ($p < 0.05$) than for CARB (2.7°C ± 1.1°C). No significant difference in ΔT was observed for the other comparisons.* ***Conclusion:*** *INS and INS-S produced significantly higher temperatures than CARB. Use of the "spray control" device resulted in a reduction of the temperature variation observed for the piezoelectric insert.*

Keywords: *dental implants - osteotomy - piezosurgery - femur - thermography.*

Geração de calor durante a osteotomia realizada com inserção ultrassônica versus instrumento rotatório em fêmur bovino: comparação *ex vivo*

RESUMO

Procedimentos de osteotomias em odontologia são comumentes realizados com brocas, porém intrumentos piezocirúrgicos também vem sendo utilizados com o intuito de se melhorar as condições cirúrgicas tanto para o paciente quanto para o operador. Este estudo ex vivo analisou a geração de calor em osteotomias usando termografia infravermelha. ***Objetivo:*** *Este estudo conduziu uma comparação termográfica infravermelha do calor gerado por insertos ultrassônicos, com ou sem dispositivo de controle de dispersão de aerossol, em relação à broca convencional em osteotomias em fêmures bovinos.* ***Materiais e Método:*** *As osteotomias foram realizadas em 9 blocos de fêmur bovino com cortes lineares medindo 12 mm de comprimento e 3 mm de profundidade. Os instrumentos foram divididos em grupos: Grupo CARB, broca carbide #701; Grupo INS, inserto ultrassônico #SFR4 revestido com carbono tipo diamante (DLC); e Grupo INS-S, inserto ultrassônico #SFR4 revestido com DLC com dispositivo de controle de dispersão de aerossol ("controle de spray"). Todas as incisões foram padronizadas usando um dispositivo automatizado. As variações térmicas (ΔT) foram avaliadas usando uma câmera termográfica infravermelha. As temperaturas máximas (Tm) e mínima (T0) registradas foram utilizadas para calcular ΔT: ΔT = Tm – T0. As análises estatísticas foram conduzidas usando o teste Kruskal-Wallis e teste de Dunn para comparações múltiplas ($p < 0,05$).* ***Resultados:*** *O T0 e o Tm registrados para INS (21,5 °C ± 0,7 °C e 23,2 °C ± 0,7 °C) e INS-S (20,8 °C ± 0,4 °C e 21,8 °C ± 0,4 °C) foram significativamente maiores ($p < 0,05$) do que para CARB (14,9 °C ± 0,8 °C e 17,6 °C ± 1,1 °C, respectivamente). O ΔT observado para INS (1,7°C ± 0,4°C) e INS-S (1,0°C ± 0,4) foi significativamente menor ($p < 0,05$) do que o de CARB (2,7°C ± 1,1°C). Não foram observadas diferenças significativas no ΔT para as outras comparações.* ***Conclusão:*** *Os grupos INS e INS-S produziram temperaturas significativamente maiores em comparação ao grupo CARB. O uso do dispositivo "spray control" resultou em uma redução da variação de temperatura observada para o inserto piezoelétrico.*

Palavras-chave: *implantes dentários - osteotomia - piezocirurgia – fêmur - termografia.*

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INTRODUCTION

Osteotomy is a surgical procedure often used in dentistry for applications such as tooth extractions, implant placement, bone grafting and orthognathic surgery. It has traditionally been performed with handpieces and rotary surgical burs, but in recent years, ultrasonic instruments have emerged as an alternative. They provide better visualization of the surgical site, selective cutting of hard tissues, and less surgical trauma[1].

When osteotomies are performed with conventional rotary instruments, there are often postoperative complications such as pain, swelling, trismus and paresthesia. Conventional instruments can also reach exceedingly high temperatures during the procedure, thereby predisposing to local osteonecrosis and hindering bone regeneration and repair[2]. Piezoelectric devices are a promising alternative to traditional instruments. Piezoelectricity is a physical phenomenon that generates mechanical vibrations in ceramics or quartz crystals, facilitating the separation of solid interfaces, including bone tissue[3,4].

Owing to its selective cutting capability, piezosurgery has been employed to mitigate trauma to hard tissues and prevent soft tissue injuries. However, the surgical time required is longer than for conventional approaches[5]. In response to this concern, manufacturers have developed coatings such as diamond-like carbon (DLC) to increase the efficiency of piezoelectric instruments. DLC increases bur hardness, heat resistance, and decreases the coefficient of friction between the bur and tissue, thereby reducing heat generation during the procedure and mitigating instrument wear, darkening and corrosion[6].

Another relevant factor is the control of aerosol generation during dental procedures conducted with rotary, ultrasonic or piezoelectric instruments. The importance of aerosol generation has been highlighted, particularly as from the COVID-19 pandemic, when the American Dental Association reported that SARS-CoV-2 particles can be dispersed via the aerosols generated by dental equipment, thereby amplifying the risk of cross-contamination. To satisfy increasingly strict biosafety standards, manufacturers are producing dental equipment designed to mitigate aerosol dissemination[7].

The temperature increase and the heat conveyed to the tissues as a result of an osteotomy depends on various factors, including bone pattern, cutting speed and pressure, bur design and longevity, and irrigant temperature and volume, among other factors. All these variables should be managed to ensure better postoperative outcomes[8,9]. However, there are few studies comparing the heat generated by piezoelectric devices versus conventional rotary instruments, and few devices that control aerosol generation adequately. There is therefore a need for research to determine which instruments are best to ensure that osteotomies are precise and safe, thereby fostering more predictable and uneventful postoperative periods. The aim of this study was to conduct an infrared thermographic evaluation of the heat produced by an ultrasonic insert, with or without an aerosol dispersion control device, in comparison to a conventional bur, throughout the execution of osteotomies in bone blocks sourced from bovine femurs.

MATERIALS AND METHOD

Experimental design

This study was exempt from evaluation by the Institutional Research Ethics Committee (registration no. 2021-1158) because it did not involve human subjects or experimental animals. The study used nine bovine femur bone blocks approximately 6 cm long and 1 cm wide, obtained from a slaughterhouse. Each bone block underwent three osteotomies, resulting in a total 27 osteotomies performed for the study.

The sample size of 9 bovine femur blocks provided test power above 95%, with a significance level of 5%, for the effect sizes found in the trial. These calculations were performed using R[10] and G*Power[11] software.

Osteotomy procedure

Three linear incisions 12 mm long and 3 mm deep were made – one with each instrument – on the cortical surface of each bone block (Fig. 1). The experimental groups were:

- **Group CARB**: incisions made with carbide bur #701 (Komet, Santo André, SP, Brazil),
- **Group INS**: incisions made with #SFR4 ultrasonic insert coated with DLC (CVDentus, São José dos Campos, SP, Brazil), and
- **Group INS-S**: incisions made with #SFR4 ultrasonic insert coated with DLC, in conjunction with an aerosol dispersion control device ("spray control"; CVDentus).

Fig. 1: Bovine femur bone blocks after performing the osteotomies.

Fig. 2: Carbide bur #701 used in the study.

In Group CARB, the #701 carbide bur was driven by a high-speed turbine (Extra Torque 505C; Kavo Kerr, Biberach, Germany) under water cooling (Fig. 2). In Groups INS and INS-S, the incisions were made by a previously trained operator, and temperature was assessed by two independent evaluators. The #SFR4 inserts, either with or without "spray control" (Fig. 3), were driven by a piezoelectric motor (DentSurg PRO; CVDentus), set to operate in cortical bone surgery mode and level 5 irrigation, following the manufacturer's recommendations. Cooling during the procedure was performed using 0.9% saline solution at room temperature (21°C).

In Group CARB, the turbine was attached to a device designed to automate the controlled horizontal motion of the bone block. The incisions were executed over a duration of 30 s, with a constant pressure of 57.85 g applied to the bur tip. The pressure was standardized by placing metal nuts along the stem of the automated device. Two nuts were employed to ensure the stability of the turbine during motion, and the overall weight of the turbine assembly was gauged using a precision scale. The turbine was operated by a foot pedal, the specimens in this group were irrigated with filtered water at room temperature.

In Group INS, the handpiece of the motor was connected to the same cutting automation device. Bone blocks were positioned in the device, and preliminary perforation markings were initiated with the insert, without pre-set pressure, to delineate the

Fig. 3: #SFR4 ultrasonic inserts, with and without "spray control," used in the study.

Fig. 4: Position of the insert with "spray control" in the automated cutting device.

intended incision paths on the bone. The incisions were executed over a period of 30 s, with a constant pressure of 57.85 g. Pressure was standardized as in Group CARB.

In Group INS-S, the same protocol was implemented (Fig. 4). A new #SFR4 ultrasonic insert was allocated to each of the INS and INS-S groups. In all experimental groups, Evaluator #1 numbered the bone blocks and placed them on the device in a randomized sequence generated through www.random.org, while Evaluator #2 took videos and photographs.

Laboratory ambient temperature was maintained constantly at 21°C using an air conditioning system.

Thermographic analysis

Thermal variations (ΔT) were assessed with an infrared thermographic camera (FLIR C5; Teledyne Flir, Wilsonville, OR, USA), and computed by determining the difference between the maximum (Tm) and minimum (T0) temperatures recorded (ΔT = Tm – T0).

The camera was fixed to a tripod, perpendicular to the bone block, at a distance of 30 cm (Fig. 5). Throughout the osteotomy procedure, the thermographic camera gathered ΔT data in °C, while a conventional video camera recorded the readings displayed on the thermographic camera. This procedure was adopted because the rapid fluctuation of values on the camera display made it difficult to record them manually.

The camera also acquired thermal images of the procedure at 10 and 30 s, with temperature recordings taken at both timepoints. The infrared thermographic measurements were conducted with

Fig. 5: Position of the infrared thermographic camera during the experiment.

specified parameters, including emissivity (ε) 0.90, reflected temperature 20°C, relative humidity 50%, and ambient temperature 21°C. The crosshairs of the camera were precisely aligned over the point of intersection between the images of the bone block and either the bur or insert (Figs. 6 and 7).

Statistical analysis

Data distribution was assessed with the Shapiro-Wilk test, indicating a non-normal distribution. Consequently, the Kruskal-Wallis non-parametric test was used to compare data across the experimental groups. Multiple comparisons were conducted utilizing Dunn's test. Statistical analyses were performed using SPSS v. 23 (SPSS, Chicago, IL, USA) and BioEstat v. 5.0 software (Mamirauá Foundation, Belém, PA, Brazil). The significance level was set at 5%.

RESULTS

The T0 and Tm values recorded for INS (21.5°C ± 0.7ºC and 23.2ºC ± 0.7ºC, respectively) and for INS-S(20.8ºC ± 0.4ºC and 21.8ºC ± 0.4ºC, respectively) were significantly higher than for CARB (14.9ºC ± 0.8ºC and 17.6ºC ± 1.1ºC, respectively). ΔT for INS-S was significantly lower than that for CARB ($p < 0.05$). ΔT was 1.7ºC ± 0.4ºC for INS, 1.0ºC ±

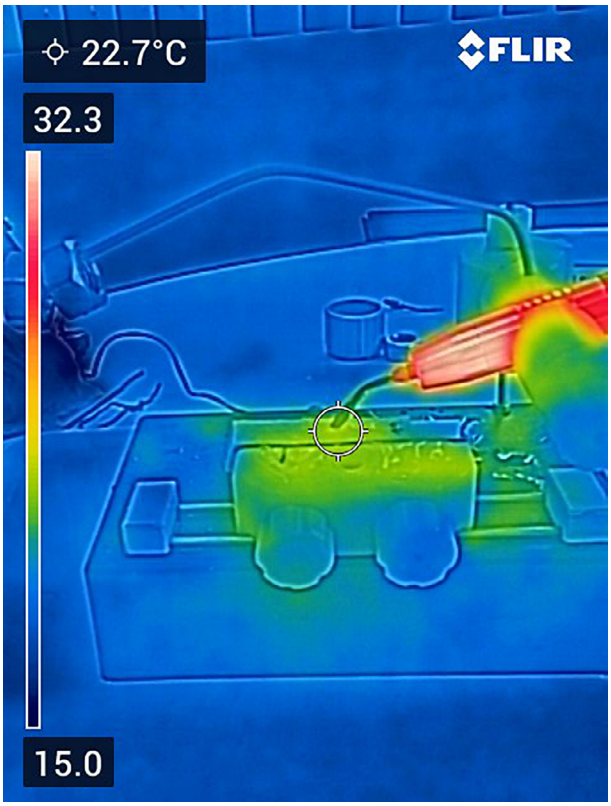

Fig. 6: Thermal image obtained during the osteotomy performed with the ultrasonic insert. Piezoelectric insert positioned over the bone block, which was attached to the automated cutting device. The photo was taken by the thermographic camera at the time the osteotomy was performed and shows the temperature of the delimited target.

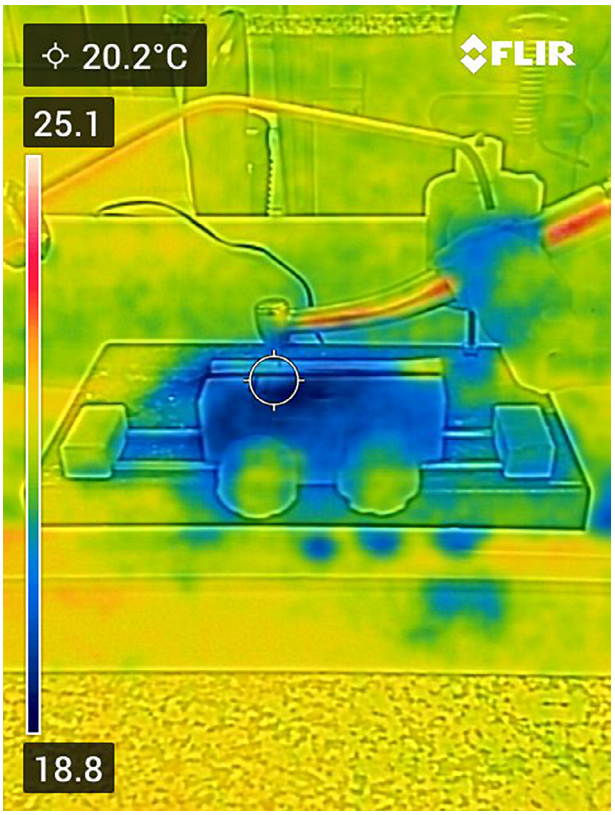

Fig. 7: Thermal image obtained during the osteotomy performed with the carbide bur. Carbide bur positioned on the bone block, which was attached to the automated cutting device. The photo was taken by the thermographic camera at the time the osteotomy was performed and shows the temperature of the delimited target.

0.4 for INS-S and 2.7ºC ± 1.1ºC for CARB. INS attained an intermediate ΔT level, and did not differ significantly from the ΔT values for the other groups ($p > 0.05$); (Table 1).

DISCUSSION

Twenty-seven osteotomies were conducted on bovine femurs, and the heat generated by the instruments was quantified through infrared thermographic analysis. The aim of this study was to find data that would help improve understanding of the range of available osteotomy instruments and the intraoperative temperature elevation they induce.

Bovine femur was used because of its structural resemblance to human bone, and its high density and uniformity in the cortical region, which provide a high level of friction between the instrument and the bone surface[1,9,12-14]. Bone density is a significant factor influencing the thermographic variations observed during osteotomy procedures[15].

In the literature, there is a lack of consensus regarding the optimal *in vitro* model for investigating heat generation in osteotomies. Various authors[4,16-18]

Table 1. Mean, standard deviation and median values of minimum temperature (T0), maximum temperature (Tm) and temperature variation (ΔT; in °C) observed for the experimental groups.

Experimental groups		T0	TM	ΔT
CARB	Mean (SD)	14.9^{A} (0.8)	17.6^{A} (1.1)	2.7^{B} (1.1)
	Median	14.7	18.1	2.9
INS	Mean (SD)	21.5^{B} (0.7)	23.2^{B} (0.7)	1.7^{AB} (0.4)
	Median	21.8	23.3	1.6
INS-S	Mean (SD)	20.8^{B} (0.4)	21.8^{B} (0.4)	1.0^{A} (0.4)
	Median	20.9	21.9	0.9
p-value		$p < 0.001$	$p < 0.001$	$p = 0.001$

CARB: carbide bur #701; INS: #SFR4 ultrasonic insert coated with diamond-like carbon (DLC); INS-S: #SFR4 ultrasonic insert coated with DLC and utilized in conjunction with a "spray control" device. Means followed by different capital letters within the column denote a statistically significant difference between groups (Kruskal-Wallis and Dunn tests; $p < 0.05$).

have used different experimental models, including rabbit tibia, porcine rib, bovine rib, porcine mandible, and synthetic bone blocks with varying densities. In most of these studies, the osteotomies were perforations made with implant burs, rather than linear cuts, which justifies a greater concern of these authors in simulating in several models of different bone densities found, such as bone types I, II, III, and IV. Considering the clinical applications of osteotomies with linear incisions in more cortical areas, further studies are needed to standardize the ideal model.

Infrared thermography can be used for non-invasive monitoring of temperature alterations on the outer surface of the bone and the visible portion of the bur. It is often used in research to evaluate thermographic variations in animal tissues and organs[6,19,20]. Other authors have used digital thermometers and thermocouples for temperature-related research[18,21,22].

Thermocouples can measure temperatures at a single point, but require attachment perforations in the bone, rendering them relatively more invasive. Conversely, infrared thermography provides a thermal profile of the material, including leakage heat, but can only detect surface temperature[23]. Accordingly, Harder et al.[24] compared infrared thermography to thermocouples for measuring heat generation and concluded that thermography is more accurate for measuring changes in intraosseous temperature.

In the current study, osteotomy cut standardization was based on Delgado-Ruiz et al.[25], and thermographic analysis methodology was based on Gabrić et al.[26] and Scarano et al.[27]. In Group CARB, the cutting process was characterized by greater instrument vibration, particularly at the beginning, when the cutting path was being delineated, whereas the cuts executed with the ultrasonic inserts were more accurate and linear.

The mean T0 and Tm values recorded for INS (21.5°C ± 0.7°C and 23.2°C ± 0.7°C, respectively) and INS-S (20.8°C ± 0.4°C and 21.8°C ± 0.4°C, respectively) were significantly higher than those observed for CARB (14.9°C ± 0.8°C and 17.6°C ± 1.1°C, respectively). However, they remained considerably lower than the temperature threshold associated with osteonecrosis, which manifests when the bone is subjected to a temperature of 47°C for one minute[28]. Sagheb et al.[29] report temperatures of 37°C ± 1°C for conventional burs and 36°C ± 1°C for piezosurgical inserts in cortical bone, based on photos taken by a thermographic camera. Although these values are higher than those recorded in the present study, heat generation did not differ significantly among the experimental groups.

Aquilanti et al.[16] used a thermographic camera to assess heat generation during initial osteotomies for implants in synthetic bone blocks, reporting averages of 19.58°C ± 1.11°C for rotary burs and 84.10°C ± 40.98°C for piezosurgical inserts. These values are substantially higher than those observed in the present study, and surpass the clinically safe threshold. The authors concluded that piezosurgical inserts induced a significantly greater temperature increase compared to burs, which is consistent with the results of the present study. Conversely, Rashad et al.[30] used thermocouples to compare sonic and ultrasonic inserts to conventional burs in bovine ribs, finding that both ultrasonic and sonic osteotomies generated significantly lower heat than conventional osteotomy did. None of the instruments tested in their study exceeded the critical heat threshold.

Gabric et al.[26] compared Er:YAG laser, piezosurgical inserts, and conventional burs, reporting average Tm values of 79.1°C ± 4.6°C for laser, 29.1°C ± 0.2°C for inserts, and 27.3°C ± 0.4°C for burs. While the average Tm values for inserts and burs were higher than those observed in the present study, the ΔT observed for the inserts was consistent.

One limitation of the current study was that although bovine femur was used due to its high density, the study did not quantify specimen bone quality, which may influence the temperatures generated. In this regard, Sagheb et al.[29] used ultrasound transmission velocity, which is considered reliable and safe for bone quality testing, especially in *ex vivo* samples.

Another limitation of the current study is that the osteotomies were performed on bone blocks at room temperature, after storage under refrigeration and removal one day before the procedure. In other studies[31], the bone blocks were stored in a water bath prior to the procedure, and heated to 36°C to simulate body temperature.

In the present study, the temperatures recorded in all three groups remained below the limit considered safe for performing osteotomies. Although variations in heat generation were observed between the instruments tested, it can be argued that the clinical significance of this finding is limited, since this is

an *ex vivo* study, with limitations inherent to this type of model, which does not include the variables present in an *in vivo* study.

In Group INS-S, ΔT was significantly lower than in Group CARB, while in Group INS, ΔT remained at an intermediate level, statistically similar to that observed in the other groups. This suggests that the device developed with the primary aim of safeguarding the operative field against generated aerosols not only increased intraoperative biosafety, but also guided the irrigation drip of the inserts, thereby effectively managing temperature levels. Consequently, the aerosol protection device utilized in INS-S can be deemed effective in contributing to temperature regulation in clinical scenarios in which piezoelectric ultrasound is used. This device has a silicone cover attached to the rod of the ultrasonic insert, extending to its working tip[7], and can be autoclaved.

While the conventional bur employed in CARB yielded the lowest temperatures, its ΔT was significantly higher. This can be attributed to three factors: (1) the substantially greater irrigation it provides compared to piezoelectric inserts; (2) occasional deviations in the direction of the water jet emitted from the handpiece due to inadequate calibration; and (3) the dispersion of the aerosol during use.

Bernabeu-Mira et al.[6] posit that instruments without DLC coating may undergo considerable wear over time, thereby inducing greater friction and heat generation. In the present study, although the inserts utilized in Groups INS and INS-S had DLC coating, their temperatures were higher. Nevertheless, these temperatures remained constant, and the corresponding ΔT levels were lower than those observed in Group CARB, which lacked a DLC coating.

The initial contact between the bur and the bone tissue typically occurs in the cortical region, which generates more heat due to the pressure required to produce the initial rupture of the bone plate[4]. This trend was observed during the current study, as evidenced by the highest temperatures being recorded at the beginning of the procedures.

There is still a need to develop a device integrating all the properties required to ensure a swift, safe surgical procedure. Although piezosurgery ensures safety, it requires longer surgical intervention time. Hence, the system should be selected based on the operator's expertise. Combining both systems may prove advantageous, depending on the nature of the procedure to be conducted[2,5].

The DLC-coated piezoelectric inserts, with or without "spray control," employed in the present study produced significantly higher temperatures compared to the carbide bur. Nevertheless, incorporation of the aerosol dispersion control device in conjunction with the insert yielded a significantly lower ΔT compared to the conventional bur. Further research may provide a more comprehensive evaluation of this device and its functionalities.

CONCLUSION

The DLC-coated piezoelectric inserts generated significantly higher temperatures compared to the high-speed carbide bur. However, use of the aerosol control device mitigated the temperature variation associated with the inserts.

CONFLICT INTERESTS

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

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Empathy: A challenge for the new generations of dentistry students at the University of Buenos Aires

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ABSTRACT

Empathy is the ability to be aware of and understand the emotions, feelings and ideas of others. Assessing empathy levels among dental students is essential for improving educational strategies and patient outcomes. ***Aim:*** *The aim of this study was to assess the levels of empathy among dental students at the University of Buenos Aires (UBA) and analyze differences based on academic year and gender.* ***Materials and Method:*** *The study involved third- to sixth-year dental students at UBA. Participants provided informed consent and completed a sociodemographic questionnaire: the Jefferson Scale of Empathy – Health Care Provider Student version (JSE-HPS). Data analysis included descriptive statistics, t-tests for gender and academic year differences, and assessment of empathy dimensions.* ***Results:*** *Among 424 participants, the average JSE-HPS score was 108.2 (SD = 15.0), ranging from 36 to 134. The dimension of emotional and compassionate care had a mean of 35.7 (SD = 5.5), perspective taking had a mean of 59.0 (SD = 9.7) and standing in the patient's shoes had a mean of 13.4 (SD = 3.6). Empathy scores increased from the third year (100.9, SD = 22.4) to the fifth year (111.5, SD = 10.0), with a slight drop in the sixth year (110.6, SD = 12). Females (mean 109.0, SD = 15.5) displayed higher empathy than males (mean 104.4, SD = 12.2).* ***Conclusion:*** *The study revealed high levels of empathy among dental students at UBA, with variations by academic year and gender. These findings underscore the importance of incorporating empathy into dental education and suggest the need for curricular adjustments to further enhance empathy-related skills. Future research should explore interventions to sustain and improve empathy levels among dental students and faculty, ultimately benefiting both patients and healthcare providers.*

Keywords: *empathy - dental student - dentist patient relations - Argentina – education - dental*

Empatía: Un desafío para las nuevas generaciones de estudiantes de Odontología en la Universidad de Buenos Aires

RESUMEN

La empatía es un atributo fundamental en las profesiones de la salud, especialmente en odontología, donde la comunicación efectiva y la comprensión de las emociones del paciente inciden directamente en la calidad de la atención. Evaluar los niveles de empatía en estudiantes de odontología es clave para mejorar las estrategias educativas y los resultados clínicos. ***Objetivo:*** *Este estudio tuvo como objetivo evaluar los niveles de empatía entre los estudiantes de odontología de la Universidad de Buenos Aires (UBA) y analizar las diferencias en función del año académico y el género.* ***Materiales y Método:*** *El estudio incluyó a estudiantes de odontología de tercer a sexto año en la UBA. Los participantes completaron la versión para estudiantes de la Escala de Empatía de Jefferson para Proveedores de Salud (JSE-HPS), un cuestionario sociodemográfico y dieron su consentimiento informado. El análisis de datos incluyó estadísticas descriptivas, pruebas t para establecer diferencias según género y año académico, y la evaluación de las dimensiones de la empatía.* ***Resultados:*** *En un total de 424 participantes, el puntaje promedio en la JSE-HPS fue de 108,2 (DE = 15,0), con un rango entre 36 y 134. La dimensión de atención emocional y compasiva presentó un promedio de 35,7 (DE = 5,5), la toma de perspectiva de 59,0 (DE = 9,7) y la capacidad de ponerse en el lugar del paciente de 13,4 (DE = 3,6). Los puntajes de empatía aumentaron del tercer (100,9, DE = 22,4) al quinto año (111,5, DE = 10,0), con una ligera disminución en el sexto año (110,6, DE = 12). Las mujeres (promedio 109,0, DE = 15,5) presentaron mayores niveles de empatía que los hombres (promedio 104,4, DE = 12,2).* ***Conclusión:*** *Los resultados evidenciaron altos niveles de empatía entre los estudiantes de odontología de la UBA, con diferencias según el año académico y el género. Estos hallazgos resaltan la importancia de integrar el desarrollo de la empatía en la formación odontológica y sugieren la necesidad de realizar ajustes curriculares para potenciar estas habilidades. Futuros estudios deberían enfocarse en intervenciones que mantengan y promuevan los niveles de empatía entre estudiantes y docentes, con el objetivo de mejorar la atención tanto a pacientes como a profesionales de la salud.*

Palabras clave: *empatía - estudiantes de odontología - relaciones dentista paciente - educación en odontología*

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INTRODUCTION

All dimensions of healthcare focus on satisfying patient needs , which can be influenced by patient feelings, emotions and perceptions at the time. Identifying these emotional and psychosocial factors is one of the challenges faced by students and professionals in Health Sciences, and can be achieved by developing empathy skills and cognitive attributes that will have positive impact on the professional-patient relationship[1]. Empathy as a structure of moral behavior is crucial in dental students. Well-educated, competent dentists sometimes only fulfill their role as such if they have sufficient empathy with their patients[2]. The American Dental Education Association (ADEA) endorses the inclusion of empathy in dental curricula[3].

This paper will adhere to the integrative theory of Davis, who defined empathy as "the ability to be aware of and understand the emotions, feelings and ideas of others"[4]. It is a set of multidimensional, active, strongly adaptive constructions by one person in response to the experiences of another. It involves adopting roles and individual decentralization (suppressing the usual egocentric posture), which implies that the person needs to make a cognitive effort and simultaneously be emotionally aware of it. The author then proposes the "Organizational Model of Empathy", in which the antecedents, processes and consequences of the construct are explained. Some authors cite empathy as an important attribute for dentists, applicants or students at various Universities, where a reliable, valid test is required to assess empathy in students taking courses related to health sciences[5], and the results are taken into account upon admission to these Universities[6]. Evaluating empathy levels in students is relevant because empathy strengthens the professional-patient relationship, generating greater satisfaction in the patient regarding the service received[7], and a state of mental well-being[8] with less stress and burnout for the professional[9].

Empathy involves cognitive, affective and emotional development. The cognitive domain implies the ability to understand the experience of other people's inner world; the affective domain refers to celebrating or participating in the experience of the other people's feelings; and the emotional domain refers to the subjective responses obtained through affinity with other people[10]. Empathy may be affected by the teaching models in some universities that focus on human biology, long working hours, or the lack of resources in the health system.

Considering the topic of empathy provides knowledge about the current state of students' acquisition of non-technical skills, and enables us to propose and develop new strategies to reinforce their training.

The goals of this study were to learn about the state of empathy of dentistry students at the University of Buenos Aires (UBA), evaluate their empathy levels, and analyze differences in empathy levels according to academic year and gender. We hypothesized that there are higher levels of empathy in students as they succeed in their clinical subjects, and different levels of empathy and prosocial behavior according to gender, as in the study by Díaz-Narváez et al.[11], who concluded that women were not necessarily more empathic than men across the populations studied.

MATERIALS AND METHOD

The protocol of this study was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (CETICA-FOUBA 005/2022).

This study has an analytical, observational, cross-sectional approach. The universe of study comprised third- to sixth-year dentistry students at the University of Buenos Aires (UBA) in 2022, enrolled in the following subjects: Comprehensive Clinic II (3rd year), Epidemiology (4th year), Comprehensive Clinic IV (5th year), and Community Outreach Rotation (6th year). All the students were invited to participate in the study (n= 770).

Participants who did not answer over 5% of the questions were excluded from the sample.

We applied the Jefferson Scale of Empathy – Health Care Provider Student version (JSE-HPS)[12], which consists of 20 Likert-type items on a seven-point scale (1=strongly disagree; 7=strongly agree). All respondents also completed a brief sociodemographic questionnaire (age and gender).

The maximum score is obtained by the direct sum of question points (maximum possible 140, minimum possible 20 points). Higher scores are considered to correlate with a higher degree of empathy[13]. Scores of 20-84 show low empathy, while scores of 85-140 are considered high and reflect a more empathetic behavioral orientation[14]. The questions are grouped

into three dimensions: emotional and compassionate care (dimension 1 - eight items), perspective-taking (dimension 2 - ten questions) and standing in the patient's shoes (dimension 3 - two items). The first dimension, an association of feeling and emotion with empathy and understanding, is considered the core ingredient of empathy and is a relevant aspect of the provider-patient relationship. The second dimension describes the provider's understanding of the patient's concerns. The third dimension indicates an ability to reflect on and comprehend patients' concerns[15].

The JSE has proven to be stable in different groups of physicians, and therefore provides support in the construction validity as well as acceptable reliability. Significant correlation coefficients between JSE-HPS scores and conceptual measures of compassion have confirmed convergent validity. Regarding its discriminant validity, it obtained a lack of significant association with irrelevant conceptual measures such as self-protection[16].

The students were invited to participate and received explanatory information about the research while attending their classes. The design of the form had a heading with informed consent requesting the acceptance to participate in the study voluntarily, ensuring anonymity, protection and confidentiality of data protected by the National Statistics Law No. 17622 and by the Data Protection Law No. 25326. (Argentina), followed by the sociodemographic questionnaire and the measurement instruments via Google Forms.

The data obtained by the questionnaire were analyzed using the Statistical Package for the Social Sciences (SPSS) version 28. To calculate the variables and their dimensions, the averages and sums of the total and the dimensions of the medical empathy scale were calculated with the respective standard deviations.

A descriptive analysis of the Empathy variable was performed, which included the calculation of absolute and percentage frequencies, measures of central tendency and dispersion. The statistical significance level for all tests was set at $p<.05$.

Student's t-test was used to analyze statistically significant differences in the variables of interest (gender and year). The three dimensions of the Jefferson Medical Empathy Scale were analyzed separately, as well as their sum and variability concerning the student's gender and academic year.

RESULTS

Four hundred and twenty-four of the 770 students completed the questionnaire (Table 1). The response rate of women students was slightly higher than that of men; 348 (82.1%) were female, and 76 (17.9%) were male (Table 2).

Table 1. Distribution of students by year

Year	Number of respondents	%	Total number of students
3rd year	101	51.8%	195
4th year	142	67.9%	209
5th year	105	50.7%	207
6th year	76	47.8%	159
Total	**424**	**55.1%**	**770**

Table 2. Sex and age distribution

Gender	n (%)	Mean age ± SD (CI95%: LL - UL)	Range	p value
Female	348 (82.1)	25 ± 4.6 (25.3-26.3)	19 - 62	
Male	76 (17.9)	25 ± 4.1 (24.9-26.8)	21 - 40	0.880
Total	**424 (100.0)**	**25 ± 4.6 (25.4-26.2)**	**19 - 62**	

The average total score for the JSE-HPS was 108.2 (SD = 15.0), with a maximum of 134 and a minimum of 36. The dimensions of the scale were analyzed separately, with the following results: Dimension 1, emotional and compassionate care: mean 35.7 (SD= 5.5), maximum 45, minimum 13; Dimension 2, perspective taking: mean 59.0 (Sd= 9.7), maximum 70, minimum 10; and Dimension 3, ability to put oneself in the other person's place: mean 13.4 (SD = 3.6), maximum 21, minimum 3 (Table 3).

When analyzed according to academic year, scores increase from the third to the fifth year, decreasing slightly in the sixth year. Mean values were 100.9 (SD = 22.4) in the third year, 109.5 (SD = 11.0) in the fourth year, 111.5 (SD = 10.0) in the fifth year, and 110.6 (SD = 12) in the sixth year. In the HSD Tukey analysis, the values for the whole scale and each dimension separately are lowest in the third year.

Regarding the analysis by gender, the means were 109.0 (SD= 15.5) for females, and 104.4 (SD= 12.2) for males, with statistically significant difference between genders (p= 0.016) (Table 4).

Table 3. Scores of the Jefferson Scale of Empathy - Health Care Provider Student version

Dimensions	Year	Mean	SD	CI 95.0% LL	CI 95.0% UL	25%	Median	75%	Min	Max	N	p value ANOVA	p value Kruskal Wallis
Perspective-taking	3rd year	54.5	14.6	51.6	57.4	51.0	60.0	64.0	10.0	69.0	101	<0.001	0.032
	4th year	60.5	7.1	59.3	61.6	57.0	61.5	66.0	36.0	70.0	142		
	5th year	60.5	6.8	59.2	61.8	57.0	62.0	66.0	44.0	70.0	105		
	6th year	60.0	7.1	58.4	61.6	56.0	61.5	65.5	42.0	70.0	76		
	Total	**59.0**	**9.7**	**58.0**	**59.9**	**56.0**	**61.0**	**66.0**	**10.0**	**70.0**	**424**		
Emotional and compassionate care	3rd year	33.7	7.7	32.2	35.2	31.0	36.0	39.0	13.0	45.0	101	<0.001	0.008
	4th year	35.7	4.3	35.0	36.5	33.0	36.0	39.0	23.0	43.0	142		
	5th year	37.0	4.3	36.2	37.8	34.0	37.0	41.0	23.0	44.0	105		
	6th year	36.7	4.7	35.7	37.8	33.0	38.0	40.0	25.0	43.0	76		
	Total	**35.7**	**5.5**	**35.2**	**36.3**	**33.0**	**37.0**	**40.0**	**13.0**	**45.0**	**424**		
Standing in the patient's shoes	3rd year	12.8	3.6	12.1	13.5	10.0	14.0	15.0	4.0	20.0	101	0.065	0.079
	4th year	13.3	3.6	12.7	13.9	11.0	14.0	16.0	3.0	21.0	142		
	5th year	14.0	3.3	13.3	14.6	12.0	14.0	16.0	4.0	21.0	105		
	6th year	13.8	3.6	13.0	14.6	11.0	14.5	16.5	5.0	21.0	76		
	Total	**13.4**	**3.6**	**13.1**	**13.8**	**11.0**	**14.0**	**16.0**	**3.0**	**21.0**	**424**		
Total score	3rd year	100.9	22.4	96.5	105.4	95.0	107.0	118.0	36.0	129.0	101	<0.001	0.011
	4th year	109.5	11.0	107.7	111.3	104.0	110.0	117.0	81.0	134.0	142		
	5th year	111.5	10.0	109.6	113.4	105.0	112.0	118.0	85.0	133.0	105		
	6th year	110.6	12.0	107.8	113.3	105.0	112.0	120.5	79.0	134.0	76		
	Total	**108.2**	**15.0**	**106.7**	**109.6**	**102.0**	**111.0**	**118.0**	**36.0**	**134.0**	**424**		

Table 4. Gender differences in total score of the Jefferson Scale of Empathy – Health Care Provider Student version

		Mean	SD	P value ANOVA	P value Kruskal Wallis
Total score	Female	109.0	15.5	0.016	<0.001
	Male	104.4	12.2		
	Total	**108.2**	**15.0**		

DISCUSSION

This study provides insights into the current state of the acquisition of non-technical skills by dental students, finding that the participants' empathic style is satisfactory. Our results suggest that the dental training curriculum should enable students to acquire bonding skills that help them understand patient emotional condition, in order to ensure that patient experience is as satisfactory as possible. This will enable us to propose new strategies to strengthen professional training. These skills are essential to sustaining an empathic bond to consolidate the professional-patient relationship and, as Howick et al. noted, to reduce medical legal risks[17].

The data collected showed that students in the final year of their studies had lower levels of empathy, which may have been due to the stress of final exams, theses, and the submission of complex papers for the accreditation of subjects, as reported by Hojat et al., who observed a decrease or erosion when the curriculum shifted towards the provision of care after the second or third year of the program[15]. Students' empathic levels may have decreased as a result of time constraints during clinical training or the requirement to fulfil clinical requirements. Even when empathy levels are expected to increase, it is necessary to consider factors related to the educational platform that could generate stress, such as requirements for the accreditation of subjects, the availability of supplies and patients to achieve educational objectives, as shown by other studies that will also be part of this line of research[18]. Although our results showed higher levels of empathy in female students, this finding should be interpreted with caution, since the percentage of male participants was relatively low. This introduces a sampling bias that may limit

the validity of gender comparisons and should be acknowledged as a limitation.

Studying and developing academic interventions to increase and maintain empathy poses a challenge. Other studies, such as those by Rosenzweig et al.[19], have reported a reduction in erosion during clinical practice when teaching activities included more communication with patients and shared decision-making. They used several strategies to improve the empathic levels in dental students, including the use of person-centered educational modules as part of the curriculum. In 2023, the UBA School of Dentistry began implementing relational learning in empathy and prosocial behavior for students who were beginning their clinical practices. The line of research aims in future studies to conduct a longitudinal analysis that will measure whether these new tools have helped improve the levels of these constructs in students as they progress in their studies, and to measure stress levels in participants. In measuring levels of empathy in teachers, Carvajal et al.[20] found that the levels of empathy were higher in teachers than in students, which is relevant to developing strategies for dealing with patients. The academic curriculum should include this kind of knowledge for students and teachers, promoting activities to increase the components of empathy[21].

CONCLUSION

The levels of empathy of our dental students are high, and increase as they advance in their studies. Although higher empathy levels were observed in female students, this result should be interpreted cautiously due to the low proportion of male respondents, which may affect the representativeness of gender comparisons. Based on the findings, it would be relevant to review and rethink the curricular contents in terms of these constructs, considering the extensive development of social practices at the School of Dentistry of the University of Buenos Aires.

CONFLICT OF INTERESTS

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

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Variation in mandibular canal position in different sagittal skeletal patterns: a CBCT study

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ABSTRACT

The mandible presents morphological variations, even in individuals without syndromes. This variability will determine different skeletal sagittal patterns, generally classified as Class I, II or III. The anatomical position of the mandibular canal has been investigated in different skeletal patterns, often using cone-beam computed tomography (CBCT) images, for diagnostic or surgical planning purposes. ***Aim:*** *The aim of this study is to perform a three-dimensional analysis of the position of the mandibular canal (MC) in adults with Class I, II and III skeletal patterns, by means of segmentation and 3D measurements on CBCT images.* ***Materials and Method:*** *75 CBCT images were obtained from a secondary database, and 3D analysis was performed using ITK-SNAP and 3D Slicer software. The 3D evaluation consisted of determining the orientation of the position of the mandible, segmentation of the mandible and the MC, creating 3D models, and establishing anatomical landmarks. Vertical (supero-inferior, SI), transverse (mediolateral, RL,) and 3D measurements were performed.* ***Results:*** *The position of the MC is modified according to the skeletal pattern and by morphological factors of the mandible such as sex and gonial angle. The proximity of the MC to the oblique line is smaller in the SI direction in Class III, and the position of the MC is associated with variation in the gonial angle. It may be closer to the cortical lingual in the central region.* ***Conclusion:*** *The mandibular canal position should be considered in tomographic evaluation during diagnosis and therapeutic planning of mandible surgeries, especially in cases of sagittal ramus osteotomy.*

Keywords: *mandible - malocclusion - cone beam computed tomography - mandibular nerve.*

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Variação na posição do canal da mandíbula em diferentes padrões esqueléticos sagitais: um estudo de CBCT

RESUMO

A mandíbula apresenta variações morfológicas, mesmo em indivíduos sem síndromes. Essa variabilidade determinará diferentes padrões sagitais esqueléticos, comumente classificados em Classe I, II ou III. A posição anatômica do canal da mandíbula tem sido investigada em diferentes padrões esqueléticos, frequentemente em imagens de tomografia computadorizada de feixe cônico (TCFC), para fins de diagnóstico ou planejamento cirúrgico. ***Objetivo:*** *Este estudo visa analisar tridimensionalmente a posição do canal mandibular (CM) em adultos com padrões esqueléticos de Classe I, II e III por meio de segmentação e medições tridimensionais em TCFC.* ***Material e métodos:*** *75 imagens de TCFC foram obtidas de um banco de dados secundário e a análise 3D foi realizada nos softwares ITK-SNAP e 3D Slicer. As etapas da avaliação 3D consistiram na orientação da posição da mandíbula, segmentação da mandíbula e do MC, criação de modelos 3D e marcação de pontos anatômicos de referência. Foram realizadas medidas verticais (súpero-inferior, SI), transversais (mediolateral, RL) e 3D.* ***Resultados:*** *A posição do MC é modificada de acordo com o padrão esquelético e por fatores morfológicos da mandíbula, como sexo e ângulo goníaco. A proximidade do MC à linha oblíqua é menor na direção SI na Classe III e a posição do MC está associada à variação do ângulo goníaco, podendo estar mais próxima da cortical lingual na região central.* ***Conclusão:*** *A posição do MC deve ser considerada na avaliação tomográfica durante o diagnóstico e planejamento terapêutico em cirurgias de mandíbula, principalmente nos casos de osteotomia do ramo sagital.*

Palavras-chave: *mandíbula - má oclusão - tomografia computadorizada de feixe cônico - nervo mandibular.*

INTRODUCTION

The mandible presents morphological variations, even in individuals without syndromes[1]. This variability will determine different skeletal sagittal patterns, generally classified as Class I, II or III. While skeletal Class I represents proximity to mandibular balance in size, shape and position, Class II and III relationships are disharmonious. The reestablishment of facial harmonic balance requires planning and may include surgical procedures such as bilateral sagittal split osteotomy (BSSO)[2,3].

Because of its high potential for injury to the inferior alveolar nerve (IAN), mandibular osteotomy to correct facial disharmonies, particularly BSSO[4], requires attention to noble anatomical structures such as the mandibular canal (MC)[2,3]. The incidence of postoperative sensory disturbances, including paresthesia, dysesthesia, hyperesthesia and hypoesthesia, ranges from 11.5 to 77%, according to subjective or objective evaluation[5-7] important Such surgical procedures therefore require prior knowledge of the region, individually and within characteristics of subtypes of skeletal disharmonies. Cone-beam computed tomography (CBCT) images have often been used to investigate the anatomical position of the MC in different skeletal patterns[8-10] for diagnostic or surgical planning purposes. Sekerci and Sahman[11] studied the position of the MC for surgical purposes, but the patients were not classified according to skeletal discrepancy. Identifying the different MC positions in patients with different skeletal patterns can help diagnose and plan oral and maxillofacial surgery, with influence on the treatment plan, choice of fixation type, and postoperative prognosis. Three-dimensional (3D) segmentation in CBCT can be used to isolate and delimit a specific region, focusing on the anatomical structure of interest with quantitative and qualitative analyses[12].

The aim of this study is to conduct a three-dimensional analysis of the position of the MC in adults with Class I, II and III skeletal patterns by means of segmentation and 3D measurements on CBCT images.

MATERIALS AND METHOD

This cross-sectional observational study was approved by the Research Ethics Committee (42632921.6.0000.5083), following STROBE guidelines[13]. A sample was randomly obtained from a CBCT database from dental radiology clinics. The sample included orthodontic and surgical patients. Images were acquired by I-CAT scans (Imaging Sciences International, Hatfield, PA) with voxel size 0.4mm^3 and PaX-Zenith3D (Vatech, Yongin, Korea) with voxel size 0.12mm^3.

Inclusion criteria were 1) CBCT of patients with Class I, II or III skeletal malocclusions; 2) age ≥ 18 years for males and ≥ 16 years for females; and 3) field of view of 23cm × 17cm, with patients at maximum intercuspation. Exclusion criteria were 1) images suggestive of facial trauma or bone surgeries involving the oral and maxillofacial region; 2) absence of any permanent lower teeth, except third molars; 3) third molars in contact with IAN; 4) intraosseous lesions; 5) poor quality CBCT due to artifacts; and 6) mandibular asymmetry.

An orthodontist selected the sample, and the ANB angle was confirmed by CBCT for assignment to skeletal Class I, II or III[14]. Demographic characteristics were recorded. Cephalometric data, such as ANB angle, SNB angle, mandible length, mandible ramus height and gonial angle[15] were recorded and analyzed (supplementary material S1). Sample size was calculated in G*Power from a pilot study with ten images, using a power of 80% and alpha of 0.05, with a standard deviation estimate equal to 0.1. A 3D analysis was performed by a single examiner who had been previously trained. Data collection began after achieving excellent intra-examiner agreement (ICC). The analysis was carried out in eight stages, similar to those described by Evangelista et al[16].

1. Conversion of DICOM to GIPL by ITK-SNAP (version 2.4.0).
2. Standardization to 0.5 mm^3 voxel size in the original scan with the 3D Slicer (version 4.10.2) to reduce the computational power and time for image analysis[17].
3. Orientation of the mandible using the 3D Slicer for standardizing the position of the mandible and the MC.
4. Semi-automatic segmentation of the mandible and MC
5. Creating a volumetric color map with ITK-SNAP. The landmarks, all perpendicular to the mandibular plane, showed three regions in the MC, as specified in Fig. 1. Navigation in sagittal, axial and coronal sections and

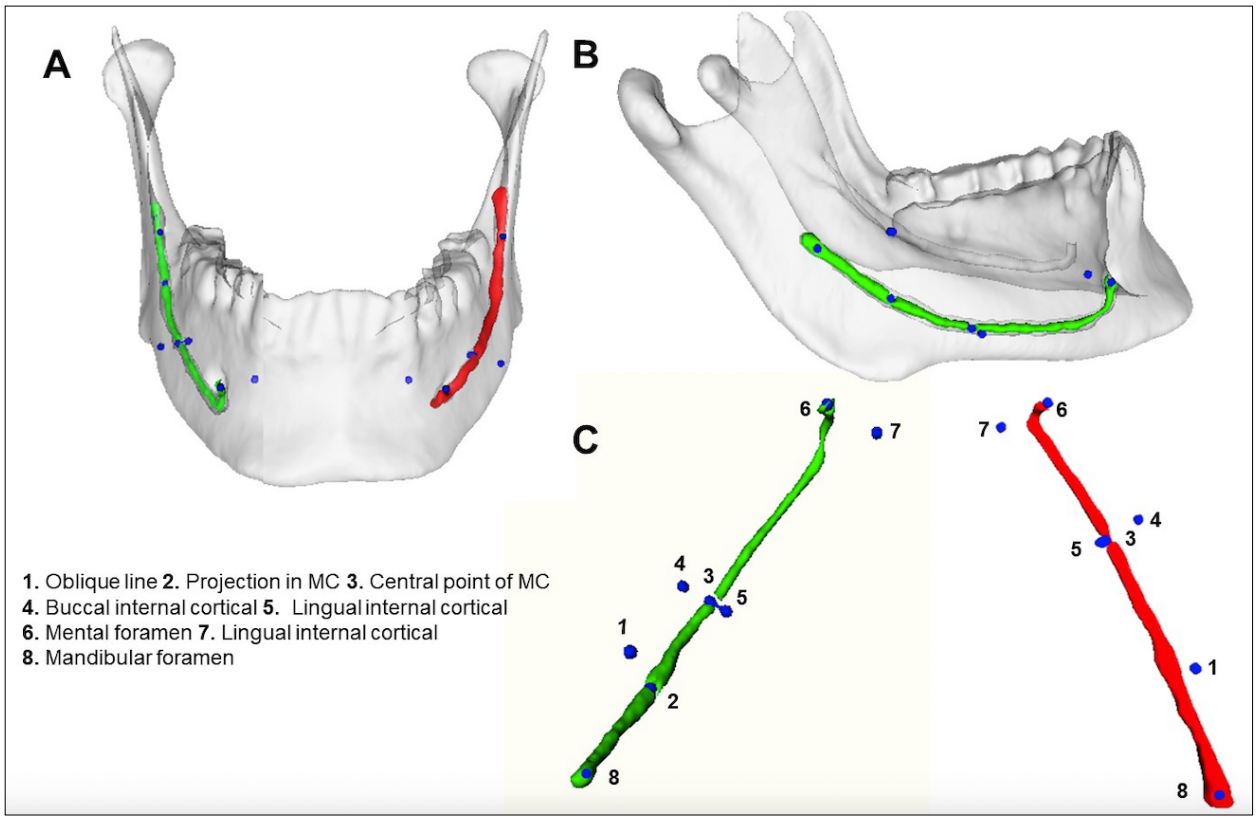

Fig. 1: 3D mandible model, including the right and left MC of a patient with skeletal class III (A) malocclusion. Side view of MC 3D model and landmarks (B). Top view of the MC 3D model and landmarks: oblique line, projection of the line on the MC, the central point of the MC (point projected perpendicularly from the center of the line between the mental foramen and mandible foramen), buccal internal cortical bone of the mandible in the region of the center of the MC, lingual internal cortical of the mandible in the region of the center of the MC, mental foramen, lingual internal cortical of the mandible in the region of the mental foramen and mandible foramen (C).

3D reconstruction were used to position the eight points. The landmarks were identified in ITK-SNAP as follows: mental foramen, mandible foramen, oblique line, projection of the landmark of the oblique line on the MC, landmark in the center of the MC, landmark in the buccal internal cortical of the central region of the MC, landmark on the internal lingual cortical of the central region of the MC, and landmark on the internal lingual cortical of mental foramen region (supplementary material S2).

6. A virtual 3D surface model was generated in 3D Slicer.
7. Landmarks were detected in the original surface models, oriented in a standardized manner, using the Q3DC of the 3D Slicer.
8. Quantitative linear distances and directional changes in the mediolateral (RL), supero-inferior (SI), and 3D axes were generated automatically by the Q3DC. The same points and measurements were performed in the pilot study.

Statistical analysis was performed with SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Variables were tested by the Shapiro-Wilk test, and found to be normal. Differences in the demographic and cephalometric characteristics were calculated using one-way ANOVA (continuous variables) and chi-square (nominal variables). The paired t-test was used to identify the symmetry of measurements between the right and left sides. One-way-ANOVA, together with Levene's test to confirm the homogeneity of variances, was used to compare the mean values obtained by adding the right and left sides of each measurement of the MC between groups, followed by Tukey's post hoc test to indicate the groups that differed from each other. Pearson's correlation was used to verify the association between the MC variables and mandibular position

and morphology. A multivariate regression analysis was performed. ICC detected a systematic error with a 95% confidence level to verify reliability after repeating the placement of all pre-marked landmarks and measurements in one-third of the sample recruited by drawing lots, with a seven-day interval. The level of significance was 0.05.

RESULTS

From a database of 770 CBCT images, 75 were randomly selected and distributed equally according to skeletal Class I, II and III patterns. Table 1 presents the demographic data and morphological and cephalometric characteristics.

All measurements showed good to excellent ICC. No statistically significant difference in linear distances was found between the right and left sides, as shown in Table 2, demonstrating symmetry for all measurements. Standard deviation ranged from 0.7 to 2.7mm. We therefore worked with the means. Table 3 presents the quantitative measurements of the means on the right and left sides for each

Table 1. Demographic data and morphological and cephalometric characteristics

Measurements	Class I (n=25)		Class II (n=25)		Class III (n=25)		*p*
	$\bar{X}$ (SD)	Min/Max	$\bar{X}$ (SD)	Min/Max	$\bar{X}$ (SD)	Min/Max	
Sex (F/M) [b]	13/12		15/10	16/9			0.681
Age (years) [a]	29.6 (7.6) [A]	19.0/45.0	32.2 (9.1) [A]	20.0/54.0	26.6 (4.9) [A]	18.0/38.0	**0.034**
ANB(°) [a]	2.4(1.0) [A]	0.3/3.7	6.2 (2.3) [B]	4.0/12.8	-2.0 (1.7) [C]	-5.5/-0.2	**<0.001**
SNB(°) [a]	80.2 (4.0) [A]	72.6/88.5	77.7 (4.0) [B]	69.2/84.9	85.3 (2.9) [C]	79.3/92.1	**<0.001**
Mandibular length(mm) [a] (Co-Gn)	116.0 (7.2) [A]	100.2/127.8	109.1 (9.3) [B]	81.8/121.7	120.1 (7.2) [A]	104.1/134.9	**<0.001**
Mandibular ramus height (mm) [a] (Co-Go)	60.0 (4.1) [AB]	54.1/66.6	57.7 (5.8) [A]	51.2/70.0	62.3 (5.1) [B]	52.0/72.9	**0.007**
Gonial angle (°) [a] (Co-Go.Go-Gn)	118.4 (6.5) [A]	101.5/130.1	119.0 (9.0) [A]	99.5/134.3	122.6 (8.4) [A]	110.7/140.8	0.141

[a]One-way ANOVA test; [b]Chi-square test; p <0.05; Co: condyle; Gn: gnathion; Go: gonium; Equal letters represent statistically similar results and different letters represent statistically different results; Levene Test p>0.05

Table 2. Linear distance and difference between the right and left sides of the MC in skeletal Class I, II and III malocclusions

Variables	Class I				Class II				Class III			
	Right Side (SD)	Left Side (R-L)	Diff. (R-L)	*p*	Right Side (SD)	Left Side (R-L)	Diff. (R-L)	*p*	Right Side (SD)	Left Side (R-L)	Diff. (R-L)	*p*
Oblique line - MC (mm)												
RL	1.9 (1.4)	2.0 (1.6)	-0.1	0.715	2.6 (1.6)	2.9 (1.8)	-0.28	0.262	1.9 (1.1)	2.0 (1.7)	-0.13	0.666
SI	-14.5 (2.4)	-14.6 (2.6)	-0.14	0.438	-14.5 (2.7)	-14.4 (2.5)	-0.14	0.559	-12.3 (1.4)	-12.1 (1.6)	-0.37	0.371
3D	14.7 (2.5)	14.7 (2.5)	0.08	0.784	14.8 (2.6)	14.8 (2.6)	0.05	0.857	12.5 (1.4)	12.2 (2.5)	0.25	0.514
Central of MC - Buccal Internal Cortical (mm)												
RL	4.1 (1.4)	4.1 (1.4)	-0.04	0.840	4.6 (1.3)	4.5 (1.4)	0.03	0.832	3.6 (1.8)	4.1 (1.7)	-0.49	0.185
3D	4.5 (1.5)	4.5 (1.5)	-0.15	0.180	5.2 (1.5)	5.2 (1.6)	-0.07	0.612	4.2 (1.3)	4.6 (1.8)	-0.36	0.113
Central of MC - Lingual Internal Cortical (mm)												
RL	2.3 (1.0)	2.2 (1.0)	0.05	0.762	2.1 (0.7)	2.2 (1.2)	-0.12	0.560	3.3 (1.3)	2.9 (1.1)	0.45	0.091
3D	2.5 (1.1)	2.4 (1.2)	0.07	0.751	2.3 (0.8)	2.5 (1.2)	-0.21	0.344	3.6 (1.5)	3.2 (1.2)	0.44	0.156
Mental Foramen- Lingual Internal Cortical (mm)												
RL	6.7 (1.6)	6.5 (1.8)	0.23	0.390	6.2 (1.2)	6.0 (1.4)	0.16	0.329	5.5 (1.2)	5.4 (1.4)	0.14	0.487
3D	7.5 (1.7)	7.3 (1.8)	0.18	0.421	6.9 (1.3)	6.9 (1.6)	0.01	0.935	6.1 (1.3)	6.1 (1.5)	0.05	0.792

Paired t-test; p <0.05; : medium; Diff: difference.

Table 3. Linear distance and difference between the right and left sides of the MC in skeletal Class I, II and III malocclusions

	Class I (n=25)			Class II (n=25)			Class III (n=25)			*p*
	$\bar{X}$	SD	CI	$\bar{X}$	SD	CI	$\bar{X}$	SD	CI	
Oblique line- MC (mm)										
RL	2.0 ^{A}	1.4	1.4;2.5	2.8^{A}	1.6	2.1;3.4	2.0^{A}	1.2	1.5;2.4	0.079
SI	-14.4^{A}	1.7	-15.6;-13.7	-14.5 ^{A}	1.9	-15.3;-13.7	-12.1^{B}	1.6	-12.7;-11.4	**<0.001**
3D	14.7 ^{A}	2.5	13.7;15.7	14.8^{A}	2.6	13.7;15.9	12.3^{B}	1.8	11.6;13.1	**<0.001**
Central of MC- Buccal Internal Cortical (mm)										
RL	4.1^{A}	1.3	3.5;4.6	4.6^{A}	1.3	4.0;5.1	3.6^{A}	1.8	2.9;4.4	0.241
3D	4.5^{A}	1.4	3.9;5.1	5.2^{A}	1.5	4.;5.8	4.5^{A}	1.4	3.9;5.1	0.138
Central of MC- Lingual Internal Cortical (mm)										
RL	2.2^{A}	0.9	1.9;2.6	2.2^{A}	0.8	1.8;2.5	3.1^{B}	1.0	2.7;3.5	**0.001**
3D	2.4^{A}	1.0	2.0;2.9	2.4^{A}	0.9	2.0;2.7	3.4^{B}	1.1	2.9;3.9	**0.001**
Mental Foramen- Lingual Internal Cortical (mm)										
RL	6.6^{A}	1.6	6.0;7.3	6.1^{AB}	1.2	5.6;6.6	5.4^{B}	1.2	4.9;6.0	**0.012**
3D	7.4^{A}	1.6	6.7;8.1	6.9^{AB}	1.4	6.3;7.5	6.1^{B}	1.3	5.6;6.7	**0.008**

One-way ANOVA test; $p<0.05$; Equal letters represent statistically similar results, and different letters represent statistically different results. Statistically significant results are highlighted in bold. Levene Test $p>0.05$

skeletal pattern. In Class III, the MC was closer to the oblique line, in the SI direction (12.1 ± 1.6mm) (p<0.001).

In the central region of the MC, the position of the MC in Class III was further from the internal lingual cortical of the mandible and according to the RL (3.1 ± 1.0mm) and 3D (3.4 ± 1.1 mm) distances (p=0.001). In the mental foramen region, the position of the MC in Class III was closer to the lingual cortical of the mandible, expressed in the RL (5.4 ± 1.2mm) (p=0.012) and 3D (6.1 ± 1.3mm) measurements (p=0.008).

Pearson's correlation showed that the greater the ANB angle, the greater the SI and 3D distances from the MC to the oblique line, and the smaller the RL and 3D distances to the lingual internal cortical. The greater the gonial angle, the smaller the SI and 3D distances, with the MC closer to the surface distance in the region of the oblique line, and the smaller the RL and 3D distances from the mental foramen to the lingual internal cortical of the mandible. Same distances from the mental foramen showed a positive correlation with sex, with 0.322 (p=0.005) for the RL dimension and 0.313 (p=0.006) for the 3D dimensions (Table 4). The adjusted R^2 value decreased, demonstrating that the MC distances improved the regression model less than expected by chance (supplementary material S3).

DISCUSSION

It is essential to know the MC position in planning orthognathic surgery because BSSO design begins in the vestibular cortical of the ramus and body of the mandible, passing through the distal region of the molars, close to the oblique line. Postoperative complication of this technique is mainly injury to the IAN at the time of osteotomy[18,19]. Previous studies on the MC position for BSSO purposes used CBCT and evaluated the vestibular and lingual cortices and the MC[11,20]. However, as these studies neither considered the classification of the patients regarding skeletal malocclusion nor assessed the course of the MC, it is difficult for surgeons to apply the results clinically.

The current study used a methodology based on segmentation of 3D models of the mandible and MC, evaluating the course of the MC, with measurements from the mandibular foramen to the mental foramen, adding information about the correspondence of the MC between the right and left sides of each patient, grouped according to skeletal malocclusion.

Our results showed differences in MC position in skeletal Classes I, II and III. Nonetheless, in a study by Huang and Liao[9], CBCT evaluation of the position of the MC in skeletal Classes I, II and III with measurements from the buccal and lingual cortical to the root of the first molar found no difference among

Table 4. Correlation between predictor variables and MC distances

Predictor Variables	Posterior Region				Central Region						Anterior Region (Mental Foramen)					
	Oblique line (SI)		Oblique line (3D)		Buccal cortical (RL)		Cortical vestibular (3D)		Lingual cortical (RL)		Lingual cortical (3D)		Lingual cortical (RL)		Lingual cortical (3D)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
ANB	**-0.366**	**<0.001**	**0.304**	**0.008**	**0.253**	**0.028**	**0.253**	**0.028**	**-0.437**	**<0.001**	**-0.431**	**<0.001**	0.125	0.286	0.149	0.201
Mandibular length (Co-Gn)	0.134	0.252	-0.093	0.429	-0.033	0.389	-0.032	0.394	0.181	0.119	0.178	0.127	0.048	0.684	0.143	0.975
Mandibular ramus height (Co-Go)	0.045	0.660	0.143	0.220	0.036	0.380	0.087	0.280	0.162	0.166	0.085	0.471	0.189	0.105	0.155	0.183
Gonial angle (Co-Go. Go-Gn)	**-0.524**	**<0.001**	**-0.536**	**<0.001**	-0.138	0.119	-0.123	0.147	0.077	0.511	0.067	0.565	**-0.285**	**0.013**	**-0.299**	**0.009**
Age	-0.104	0.376	-0.124	0.288	-0.092	0.217	-0.109	0.175	-0.036	0.759	-0,045	0.700	0.063	0.589	0.066	0.574
Sex	0.022	0.851	0.183	0.117	**0.290**	**0.012**	**0.303**	**0.008**	-0.149	0.203	-0.139	0.234	**0.322**	**0.005**	**0.313**	**0.006**

Pearson Correlation test. $p<0.05$. Bold numbers indicate statistically significant values.

groups. A study by Lee and Han[10] reported that upon CBCT evaluation of the anatomical position of the MC concerning the vestibular cortical in skeletal Class III, the participants were divided according to whether or not the MC and cortical made contact. However, due to the divergence of methodologies, it is not possible to make a direct comparison between studies.

Several landmarks have been used in the literature, such as measurements at the mandible foramen, mandible angle, mandible body, and midpoint[8], measurements from the third to first molar, including the distance between the outer surface and the buccal and lingual cortical[11], ramus thickness, MC internal diameter, width from the bone marrow to the buccal cortical and lingual[20], length between the outer margin of the MC and the buccal and inferior cortical, as well as mandible thickness[10]. For our study, we chose to use parameters observed in surgical planning, such as the SI depth of the MC concerning the oblique line. Another important aspect of the BSSO is that it is directed from the lingual region of the ramus to the region of the buccal cortical of the molars. This aspect was evaluated by the distances from the center of the MC to the buccal and lingual internal cortical.

The stability of the anatomical landmarks utilized in Bilateral Sagittal Split Osteotomy (BSSO) is frequently the subject of research. Gaitan-Romero et al.[21] found that both extrinsic and intrinsic factors influence long-term stability, with the amount and orientation of displacement of cephalometric points in the vertical and sagittal planes exhibiting significant angular increases in the ANB angle and backward relapse of SNB, although not exceeding four degrees. Analysis of linear measurements showed that the mean differences in cephalometric landmarks were clinically acceptable, with a value of 2 mm, except for the gonion. Other stable regions have been reported in the literature, such as the posterior region of the ramus of the mandible, located between the gonial angle and the neck of the condyle, and the subcoronoid space, situated inferior to the coronoid process[22].

The stability of landmarks in cases involving different fixation techniques has also been analyzed, yielding satisfactory and comparable results across various fixation groups[23]. Further clinical and prospective studies with medium- and long-term follow-up are needed to assess the stability of the new anatomical landmarks proposed in the current study.

The anatomy of the mandibular canal pathway varies significantly. In the study by Vieira et al.[24], the most common courses of the mandibular canal were identified as straight (74.7%), catenary (19.4%), and progressively descending (6.2%). Nevertheless, the selection and delineation of anatomical landmarks

for orthognathic surgery also vary considerably, and the classification of the mandibular canal reflects this variability[25-27].

Further research incorporating this information and focusing on orthognathic surgery is essential to more accurately evaluate the incidence of neural injuries, particularly in patients undergoing BSSO. The anterior loop of the mandibular canal is frequently observed in the anterior region of the mandible, with prevalence ranging from 0% to 94%. This variation can be attributed to differing definitions, geographic regions, and assessment methodologies[28-30]. Furthermore, understanding the anatomical variations in the mentonian region is crucial for cases involving mentoplasty, as noted by Hui et al[31].

New information from the current study includes the correlation between the distance from the mental foramen to the lingual internal cortical and sex, with smaller size in females, corroborating the findings in the literature, which report dimorphism of the symphysis[32]. It was also observed that Class III has the most superficial MC in the oblique line region, with a correlation to gonial angle. These results reinforce previous findings from a study that demonstrates an association between MC position and gonial angle[33], as well as a method of cephalometric analysis reporting that Class III patients have a greater gonial angle[34].

Another finding was the correlation between ANB angle and RL and 3D distances from the center of the MC to the lingual internal cortical. One study evaluated the same distance in a similar region, reporting greater values in the area distal to the third molar, but did not analyze skeletal classification[11], so data cannot be matched. Our study found a greater RL and 3D distance from the center of the MC to the lingual internal cortical in Class III patients. Thus, MC and neurovascular bundles may be closer to cortical lingual in these patients, indicating an increased risk of injury to the IAN.

Other findings include the correlation of ANB angle and SI and 3D distances from the oblique line to the MC. This finding was analyzed in a recent study using the RL distance, in which the shortest distance from the vestibular bone marrow in the oblique line region was measured. Patients were divided according to ANB angle, with inferences for BSSO. A shorter distance was found in Class III, implying a higher risk of injury to the IAN[35].

The current study is of considerable interest from both surgical and anatomical perspectives. Studying the trajectory of the MC using cone beam computed tomography is regarded as standard for anatomical description of vital structures and their variations, and enables extrapolation to clinical-surgical practices with the aim of preventing irreparable damage[36-37].

It is essential to consider that, although the current trend is to perform orthognathic surgery on increasingly younger patients, observation by CBCT of patients aged 18 in males and 16 in females or younger should not influence the conclusions regarding the anatomical position of the MC in older adult individuals who are candidates for such surgical procedures[38]. Furthermore, from a strictly descriptive anatomical standpoint, the presence of positional or recurring anomalies, which occur in 26% of cases and may be expected in this context, is not emphasized[24].

The clinical applicability of this study is linked to the evaluation and planning of surgical treatment to correct skeletal malocclusions. In our sample, Class III patients had an MC distance closer to the oblique line. This suggests that surgeons should pay careful attention to changes in position and consider the positions of the MC within the different skeletal patterns. A comprehensive evaluation of CBCT images and measurements is essential for preoperative design in orthognathic surgeries using BSSO. Direct injuries to the IAN can be avoided or minimized, thereby reducing discomfort and postoperative complications[39].

The limitations of this study include the use of retrospective data, the absence of complete clinical information, and a sample with skeletal disharmonies of different degrees of severity. Further studies including these parameters, especially the degree of severity, could better clarify the association of MC position in different vertical facial patterns, as well as in patients with facial asymmetry, to enable quantitative and qualitative assessment of intrinsic issues regarding differences in morphological characteristics between mandibular hemiarches.

CONCLUSION

Considering the population studied, the present study suggests that mandibular canal position differs according to the skeletal pattern and

morphological aspects of the mandible. The MC is closer to the oblique line in Class III patients, with a greater gonial angle, and may be positioned closer to the cortical lingual in the central region. Further research including the degree of severity of skeletal disharmonies and anatomical accidents may better elucidate MC position both in vertical facial patterns and in patients with facial asymmetry.

CONFLICT INTERESTS
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Feasibility of using saliva for Cytomegalovirus detection and genotyping in pediatric hematopoietic stem cell transplant recipients

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ABSTRACT

Human Cytomegalovirus (HCMV) is a major viral pathogen that causes severe complications in immunosuppressed individuals, particularly hematopoietic stem cell transplant recipients. In these patients, Cytomegalovirus has been associated with gastroenteritis, pneumonia, hepatitis, and even graft-versus-host disease, and a possible relationship has been identified between Cytomegalovirus genotypes and clinical course, complications and outcome. Early detection of Cytomegalovirus infection or reactivation is important, and previous findings show that it could potentially be evaluated in saliva, where HCMV causes asymptomatic viral shedding. Since saliva can be collected easily and safely, it is important to evaluate its potential for HCMV detection and genotyping, especially in pediatric patients who are receiving hematopoietic stem cell transplantation. ***Aim:*** *The purpose of this study was to evaluate the feasibility of using saliva to detect and genotype HCMV in a cohort of pediatric hematopoietic stem cell transplant recipients (HSCTR).* ***Materials and Method:*** *This study was conducted at Fundación Hospital Pediátrico la Misericordia, in Bogota, Colombia. Stimulated saliva samples were collected once a week and subjected to HCMV detection by qualitative PCR and genotyping by nested PCR followed by sequencing. Finally, a phylogenetic tree was constructed.* ***Results:*** *Twenty patients were enrolled, and 105 saliva samples were collected, of which 29 were positive for HCMV. Twelve patients had at least one positive sample. The gB1 genotype was identified with no coinfection with any other genotype. Phylogenetic analysis showed that some saliva samples were closer to the sequence reported for the Towne laboratory strain, while others were closer to the Merlin strain, with slight differences between them.* ***Conclusions:*** *It was demonstrated that saliva can be used to detect and genotype Cytomegalovirus in pediatric transplant recipients, and that sample collection is easy, with no risk of bleeding or discomfort in the pediatric patients evaluated.*

Keywords: *cytomegalovirus - saliva - genotype - sequencing - stem cell transplant*

Viabilidad de la saliva para la detección y la genotipificación de citomegalovirus en pacientes pediátricos receptores de trasplante de precursores hematopoyéticos

RESUMEN

Citomegalovirus humano (HCMV) es el principal patógeno viral causante de complicaciones graves en individuos inmunodeprimidos, sobre todo en receptores de trasplantes de precursores hematopoyéticos. En estos pacientes, el citomegalovirus se ha asociado con gastroenteritis, neumonía, hepatitis e incluso enfermedad injerto-hospedero, y se ha identificado una posible relación entre los genotipos de citomegalovirus y el curso clínico, las complicaciones y el desenlace en estos pacientes. La detección precoz de la infección o reactivación por citomegalovirus es importante y, según hallazgos previos, la saliva puede ser una herramienta para evaluar esta infección, especialmente en pacientes pediátricos. Dado que el HCMV causa descarga viral asintomática en la saliva y que este fluido se obtiene de forma fácil y segura, es importante evaluar la posibilidad de utilizar la saliva para la detección y la genotipificación de HCMV en pacientes pediátricos que reciben un trasplante de precursores hematopoyéticos. ***Objetivo:*** *El propósito de este estudio fue evaluar la viabilidad de la saliva para detectar y genotipar HCMV en una cohorte de receptores de trasplantes de células madre hematopoyéticas pediátricas (HSCTR).* ***Materiales y Método:*** *Este estudio fue llevado a cabo en la Fundación Hospital Pediátrico la Misericordia, Bogotá-Colombia. Se recolectaron muestras de saliva estimulada una vez por semana, posteriormente se hizo la detección mediante PCR cualitativa y la genotipificación se logró con una PCR anidada seguida de secuenciación y finalmente se construyó un árbol filogenético.* ***Resultados:*** *Se incluyeron 20 pacientes de los que se obtuvieron 105 muestras de saliva; 29 muestras fueron positivas para HCMV y 12 pacientes tuvieron por lo menos una muestra positiva. El genotipo gB1 fue identificado en todos los casos, sin coinfecciones con otro genotipo; el análisis filogenético mostró que algunas muestras de saliva se ubicaron con mayor proximidad a la secuencia reportada para la cepa de laboratorio Towne y otras estuvieron más próximas a la cepa Merlin, con leves diferencias entre ellas.* ***Conclusiones:*** *Se pudo demostrar que la saliva puede emplearse para detectar y genotipificar citomegalovirus en pacientes pediátricos receptores de trasplante, resaltando que la toma de muestras es fácil, sin riesgo de producir sangrado o molestias en los pacientes pediátricos evaluados.*

Palabras clave: *citomegalovirus - saliva, genotipo - secuenciación - trasplante de células madre hematopoyéticas*

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INTRODUCTION

Human Cytomegalovirus (HCMV), a member of the Herpesvirus family, infects 50–100% of the world's population, and is acquired during pregnancy, childbirth or the early years of life[1,2]. The gB envelope glycoprotein encoded in the *UL55* gene is involved in host cell entry, cell-to-cell virus transmission, and fusion between membranes of infected and uninfected cells, leading to the formation of syncytia[3,4]. The variability and polymorphic sequences in this gene have enabled the classification of five main genotypes (gB1 to gB5)[5,6], and they can influence viral tropism due to the interaction with described viral receptors such as EGFR, PDGFR or integrins, to enter different cell types[7–10]. Moreover, gB participates in the activation of Pattern Recognition Receptors such as TLR2, triggering the innate immune response[11].

After primary infection, HCMV establishes lifelong latency[12], and viral reactivation in different tissues or anatomical compartments results in local asymptomatic viral spread, leading to the transient presence of viral particles in bodily fluids such as saliva and urine[13–15].

Viral shedding in saliva is essential because of the ability of the virus to persist and replicate in salivary acinar cells, resulting in salivary aerosols and droplets being effective horizontal transmission mechanisms during speaking, coughing or spitting[13-16]. Thus, saliva is also valuable for monitoring HCMV infection/reactivation in both symptomatic and asymptomatic individuals[17–19].

Genotyping is based on the detection of differences in the sequence of the UL55 gene. Five main genotypes have been recognized: gB1 to gB5. Several genotyping techniques have been proposed based on a nested or semi-nested PCR, followed by restriction fragment length polymorphism. The use of PCR and phylogenetic analysis through a maximum likelihood tree has also been reported[20-23]. Genotyping by sequencing also relies on a variable fragment of the UL55 gene encoding for gB glycoprotein.

It has been suggested that HCMV genetic variability may be associated with the clinical outcome of infection and demonstrated that there is a relationship between genotypes (mainly gB3) and the development of complications such as myelosuppression, gastrointestinal disease, graft-versus-host disease (GVHD) or patient death[20-23].

HCMV genotyping is not a standard diagnostic procedure in transplant recipients. However, it might be advisable to establish the HCMV genotype(s) and whether there is coinfection/mixed infection with more than one Cytomegalovirus genotype in the same patient, given the possible association with complications or higher viral load in such patients[22]. To date, no HCMV genotyping study has been performed in Colombia or in pediatric transplant recipients. Given that collecting saliva samples is safe and easy, the objective of this study was to evaluate the feasibility of using saliva to detect and genotype HCMV in a cohort of pediatric hematopoietic stem cell transplant recipients.

MATERIALS AND METHOD:

Patients

This research was conducted in the Hematopoietic Stem Cell Transplantation Unit of HOMI – Fundación Hospital Pediátrico la Misericordia (Bogota, Colombia), from April to December 2016. The study was approved by the Ethics Committee of the School of Dentistry (certificate No.15 of 2015, National University of Colombia, Bogota) and classified as minimal risk according to Ministry of Health regulations.

The study was explained to the patients and their relatives who were admitted to the transplant unit, and assent and informed consent forms were signed upon their agreement. For each patient, data including diagnosis, transplant type, complications and outcomes were obtained from the medical records. Patients were excluded if their oral or systemic condition prevented more than two samples from being collected.

Saliva samples were collected weekly during hospitalization. For sample collection, each patient was given a block of dental wax to chew for 30 seconds, after which the patient spat out the block of wax and saliva collection began. Patients provided 1 to 1.5 mL of saliva, which was collected in sterile 15 mL tubes containing 450 µL of Dulbecco's Modified Eagle´s Medium (DMEM) supplemented with 100 IU/mL of penicillin and refrigerated until taken to the laboratory. Then, an equal volume of PBS was added, and the tube was centrifuged for 10 minutes at 4 °C (4000 × g), and the supernatant collected and stored at -80 °C until use.

DNA extraction

A 50 µL aliquot of sample was mixed with 150 µL of Chelex 100® (Sigma-Aldrich C7901) resin prepared at 20% in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, and 0.1% sodium azide. The solution was mixed for 10 s and incubated at 56 °C for 20 min, followed by a second incubation at 100 °C for 10 min. The suspension was allowed to cool, and the resin was decanted to obtain the upper aqueous phase, which contained the DNA free of protein contaminants and was used to amplify viral DNA by PCR.

HCMV detection and genotyping

The objective was to detect and genotype Cytomegalovirus in saliva. First, a qualitative PCR was conducted to evaluate the presence of viral DNA, followed by a nested PCR to amplify the gene segment where the UL55 gene is found, and finally, sequencing to determine the genotype.

Viral DNA was detected by PCR amplification using the 5′-GTCAGCGTTCGTTCCCA-3′ and 5′-GGGACACAACCGTAAAGC-3′ primers, amplifying a 283 bp fragment of the *UL83* structural protein gene[24]. The PCR program was as follows: 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 60 °C for 1 min, 72 °C for 30 s, followed by final extension at 72 °C for 5 min[24]. Amplification products were visualized on 2% agarose gels stained with ethidium bromide. The Towne strain of HCMV DNA harvested from the MRC-5 cell line was used as positive control. The multiplex nested PCR protocol previously reported by Tarragó et al.[25] was adapted to start the genotyping of HCMV in saliva samples, based on the amplification of the *UL55* gene. The primers used and the expected products are listed in Table 1. For the first round, 100 µg/µL of DNA was amplified using the following PCR program: 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 60 °C for 1 min, 72 °C for 30 s, and 72 °C for 5 min. For the second round, 2 µL of the product from the first PCR was used, and the first amplification program was used[25]. This protocol was applied to samples that tested positive for HCMV by conventional PCR.

To complete the genotyping, the second-round amplification products were subjected to standard sequencing using an ABI 3730XL sequencer (Macrogen Company, Seoul, Korea). DNA sequence similarity analysis was performed using BLAST in the NCBI database. To determine and verify the genotypes of HCMV in saliva samples, a multiple sequence alignment was performed on MEGA X[26] using Clustal W software and the representative nucleotide sequences for the gB HCMV genotypes. The accession numbers for each genotype were used as follows: Towne (M22343), C327A (M60929), Merlin (NC 006273.2) for gB1; AD169 (X04606), C336A (M60931) for gB2; C076A (M85228), Toledo-p7 (MF783090) for gB3, and C128A (M60924) and C194A (M60926) for gB4[27].

These sequences were compared to those published for each genotype, including those reported for the Merlin and Towne Cytomegalovirus laboratory strains. With these sequences, a Neighbor-Joining tree[28] was constructed using evolutionary distances calculated by the Maximum Composite Likelihood[29]. There were 476 nucleotide positions in the final dataset, after removing all ambiguous positions for each sequence pair (pairwise deletion option).

Table 1. Primers used to obtain the amplification products of HCMV genotypes from the positive saliva samples through nested PCR.

First Round				
PCR	**Polarity**	**Position (nucleotides)**	**Sequence 5' → 3'**	**Product bp**
CMVQ1+	HS5GLYBG+	868-885	TTTGGAGAAAACGCCGAC	
CMVQ1-	HS5GLYBG-	1619-1597	CGCGCGGCAATCGGTTTGTTGTA	751
Second round				
Nested PCR				
CMVGT1+	HS5GLYBG+	1111-1130	ATGACCGCCACTTTCTTATC	420
CMVGT2+	HEHCMVGB+	1074- 1096	TTCCGACTTTGGAAGACCCAACG	613
CMVGT3+	HS5GLYBM+	1341–1359	TAGCTCCGGTGTGAACTCC	190
CMVGT4+	HS5GLYBD+	1057–1082	ACCATTCGTTCCGAAGCCGAGGAGTCA	465
CMVGT5+	AF043721+	307–325	TACCCTATCGCTGGAGAAC	139
CMVQ2-	HS5GLYBG-	1531–1513	GTTGATCCACRCACCAGGC	

Statistical analysis

Data collected from medical records and laboratory results were stored in an Excel 2013 database. Descriptive methods were used to present the general characteristics of all participating subjects, including variables such as HCMV positivity, viral genotype and clinical aspects of the evolution of each patient. STATA 13.0 (College Station, Texas, USA) was used for statistical analysis. Univariate and bivariate analyses were performed on the evaluated clinical features and the relationship between the presence of HCMV in saliva, viral genotype, and the complications that the patient developed during the hospital stay and their outcomes. Fisher's exact test and Spearman's correlation were used to determine the differences between variables, and the p-value was obtained for each analysis.

RESULTS

Patient description: demographics, diagnoses, HCMV serostatus, type of transplant, and complications (Table 2)

Twenty pediatric HSCTR were included. No patient was withdrawn from the study. There were 12 boys and eight girls, mean age 10.3 years (range 4 to 16). The most common pre-transplant diagnoses were acute lymphoblastic leukemia (ALL; 40%) (8 patients), followed by congenital and acquired aplastic anemia (CAA), Hodgkin lymphoma (HL), and Fanconi anemia (FA) (2 patients each). In addition, there was one patient with an initial diagnosis of Fanconi's anemia who developed acute myeloid leukemia (AML), one patient with sickle cell anemia, one patient with a chronic granulomatous disease, and one patient with hemophagocytic lymphohistiocytosis (Table 2).

Regarding transplant type, eight patients (40%) received allogeneic umbilical cord blood transplant (UCB), seven (35%) received matched related donor transplant (MRD), and two (10%) received autologous transplant. Of the remaining three patients, one received a syngeneic transplant, another received an allogeneic haplo-cord transplant, and the third received an initial UCB followed by an MRD transplant (Table 2).

Table 2 shows the complications reported in clinical records. Nineteen out of 20 patients (95%) presented some complication. Four patients had one complication, six patients had two complications, and nine patients had three to six complications. The most common complication was febrile neutropenia (17 patients, 85%). Of the three patients without febrile neutropenia, one had received an autologous transplant, and the other two had received an allogeneic transplant. Skin graft versus host diseases (GVHD) was the second most frequent complication, affecting five patients (25%). Hemorrhagic cystitis occurred in 25% of the patients, and pneumonia was diagnosed in 15% of the patients, two of them reported as multilobar pneumonia. Oral mucositis was noted in two patients, but the duration or severity of the mucositis did not preclude weekly sampling. Three patients died during hospitalization.

HCMV detection by PCR

Twenty patients were enrolled during the study period, and saliva samples from 12 patients were positive for HCMV DNA by PCR in at least one sample (60%). A total 105 saliva samples were collected, of which 29 (27.6%) were positive (median saliva sample 5 IQR 4-6). Nine saliva samples were collected from patient 6, and the virus was detected in all samples. Eight saliva samples were collected from patients 5 and 13, and the first two samples were positive for HCMV. In some cases, such as patient 2, the low number of samples is explained by difficulties in obtaining saliva, usually due to the general condition of the patient; however, this patient was not excluded from the study because only two saliva samples were not taken. Table 2 shows the salivary viral shedding data for the enrolled patients.

HCMV genotypes

Sequencing identified the HCMV genotype in the saliva samples of the 12 positive patients. Sequence analysis revealed infection by the gB1 genotype in all positive patients, and other genotypes were not identified (Table 2). Analysis of the UL55 gene fragment showed that all samples were most closely related to gB1 genotype, but samples from patients 5, 6 and 11 were more closely related to Merlin strain and samples from patients 9, 17, 18 y 20 were more closely related to Towne strain. The sequences were deposited in the GenBank database (accession numbers OP781314 - OP781325) (Fig. 1).

The median age of HCMV-positive patients was 10.9 ± 3.6 years. No association was found between genotype and sex (p=0.16), age (p=0.36), or the number of complications (p=0.87).

Table 2. Patient description: demographics, diagnoses, serology for HCMV, transplant type, complications, HCMV detection and genotyping.

Patient number	Gender	Age/ years	Prior diagnosis	Type of transplant	Complications during hospitalization	Outcome	# saliva samples/ HCMV positive	HCMV Genotype
1	F	7	Fanconi anemia	MRD	Febrile neutropenia	Discharged	5/0	ND
2	M	7	ALL	MRD	Febrile neutropenia, GVHD, Amebic colitis	Discharged	2/0	ND
3	M	13	ALL	UCB	Febrile neutropenia, pulmonary aspergillosis, pulmonary tuberculosis, septic shock	Deceased	4/0	ND
4	F	5	Hemophagocytic lymphohistiocytosis	UCB	Febrile neutropenia, mucositis, GVHD	Discharged	6/0	ND
5	M	16	Sickle cell anemia	allogeneic haplo-cord	Convulsive syndrome, Gastrointestinal GVHD	Discharged	8/1	**gB1**
6	M	9	ALL	UCB	Febrile neutropenia, bacteriemia, hemorrhagic cystitis	Discharged	9/9	**gB1**
7	M	8	ALL	UCB	Febrile neutropenia, GVHD, amebic colitis, sepsis, pneumonia, multiorgan failure	Deceased	6/4	**gB1**
8	F	6	ALL	MRD	Febrile neutropenia, leukemia relapse	Discharged	4/0	ND
9	M	12	ALL	UCB	Febrile neutropenia, hemorrhagic cystitis, skin GVHD	Discharged	5/3	**gB1**
10	F	7	AML	UCB	Febrile neutropenia, hemorrhagic cystitis	Discharged	4/0	ND
11	M	4	Chronic granulomatous disease	MRD	Febrile neutropenia	Discharged	6/3	**gB1**
12	M	16	Hodgkin's lymphoma	Autologous	Febrile neutropenia, gastrointestinal mucositis	Discharged	5/0	ND
13	F	14	Aplastic anemia	MRD	Hypertension, jaundice, hemorrhagic cystitis, sinusitis	Discharged	8/2	**gB1**
14	M	12	ALL	UCB + MRD	Febrile neutropenia, primary graft failure, hemorrhagic cystitis, unspecified fungal infection, sepsis, pneumonia	Deceased	7/1	**gB1**
15	F	14	AML	UCB	Febrile neutropenia, skin GVHD	Discharged	5/0	ND
16	F	10	Hodgkin's lymphoma	Autologous	Febrile neutropenia	Discharged	6/1	**gB1**
17	F	7	Fanconi anemia and AML	UCB	Febrile neutropenia	Discharged	6/2	**gB1**
18	M	16	ALL	MRD	Febrile neutropenia, sinusitis, oral mucositis	Discharged	2/1	**gB1**
19	M	11	Fanconi anemia	MRD	None	Discharged	4/1	**gB1**
20	M	12	Acquired aplastic anemia	Syngeneic	Febrile neutropenia, bacteriemia	Discharged	3/1	**gB1**

F=Female; M=Male; ALL=Acute Lymphoid Leukemia; AML=Acute Myeloid Leukemia; MRD= matched related donor transplant; UCB=Umbilical cord blood transplant; GVHD=Graft Versus Host Diseases; ND=Not Detected; gB1=Cytomegalovirus Genotype B1

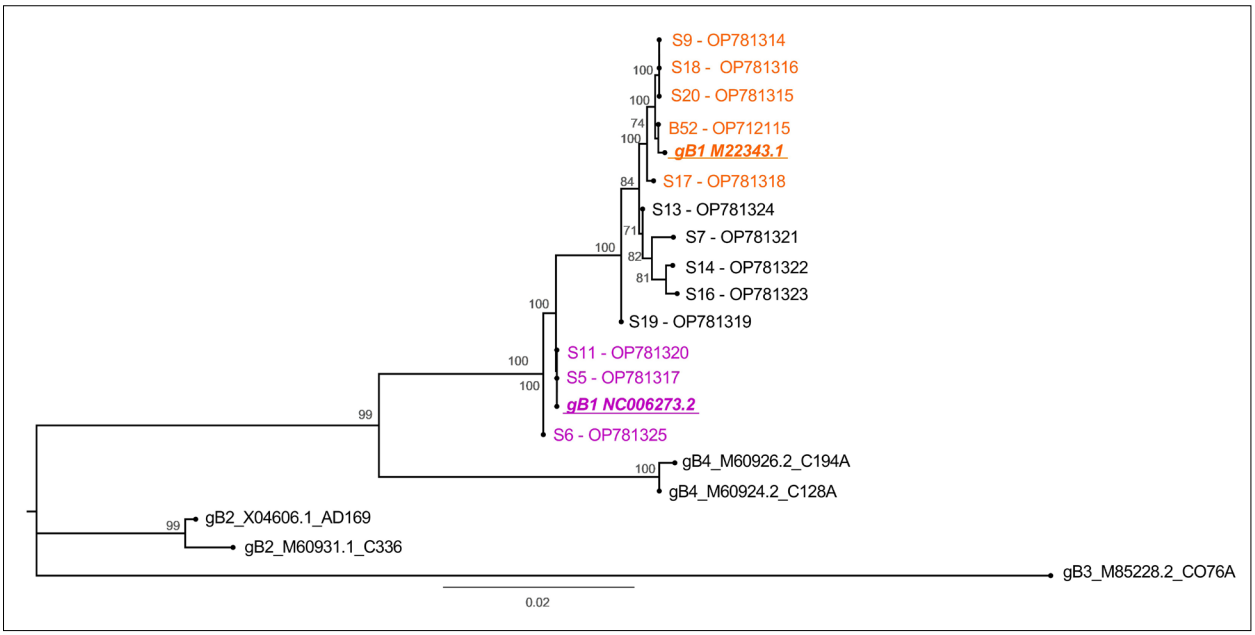

Fig. 1: GenBank database

Among the 12 patients with a positive PCR for HCMV in saliva samples, 50% had three or more complications (7), and no significant difference was observed in the Fisher test (p=0.6). Detailed analysis showed that 75% of HCMV-positive patients presented with febrile neutropenia, with no statistically significant difference (p=0.242).

The patient with primary graft failure and other complications who died was positive for HCMV in saliva. Two other patients died during hospitalization, one of whom was positive for HCMV in saliva.

DISCUSSION

This is the first study of salivary HCMV detection and genotyping in children with HSCT in Colombia. Saliva is an exocrine secretion with essential functions in the oral cavity, and an excellent vehicle for the transmission of HCMV, which can occur by the dispersion of droplets or aerosols in asymptomatic dissemination. Cytomegalovirus infects the acinar cells of the salivary glands, where it persists, so stimulation of salivary secretion may improve viral DNA levels in oral cavity fluid[13,15]. Saliva is currently being used for diagnostic purposes such as tumor marker evaluation, glucose level measurement, virus detection, and many others. The sampling technique depends on the objective or what is to be determined. For virus detection, one of the most important aspects is whether the salivary secretion is stimulated, which may depend on whether the infectious agent is present in the secretion and therefore stimulated saliva increases the possibility of detection, as is the case with Cytomegalovirus. In a previous study of pediatric transplant recipients, we used this technique to collect saliva, and detected Herpes Simplex Virus 2, Epstein Barr Virus and Cytomegalovirus[18].

Saliva is easy to collect with minimal risk to the patient and operator. This is particularly important in pediatric patients, especially as an alternative to blood sampling when the patient does not have venoclysis, or when daily or high frequency sampling is needed. Saliva is easy and safe to transport and does not require highly trained personnel, thus reducing costs. The composition of the medium in which the saliva is transported to the laboratory is intended to maintain the viability of the viral DNA, and the antibiotic inhibits bacterial growth, thus reducing the microbial load in the sample, and facilitating the detection of the viral genetic material. For this reason, it is often used in transplant recipients to diagnose and monitor viruses of the Herpesviridae family, which are one of the main causes of complications[17-19,30].

In this study, 29 of 105 samples collected (27.6%) were positive for HCMV. In some patients, only one sample was positive, while in others, several or all samples were positive. In all cases, the

viral shedding was asymptomatic (no oral lesions of viral etiology were observed) and patients diagnosed with oral mucositis were negative for the virus. Intermittent detection of the virus in saliva may be related to the individual immune status or ganciclovir administration, as suggested by Sarmento et al. upon finding that only four out of 20 kidney recipients had a single positive saliva sample[31]. In addition, because the virus undergoes localized, independent replication in the different anatomical compartments[19], it can sometimes be detected in one type of sample (saliva) but not in the blood, as reported by Correa-Sierra et al. in their evaluation of asymptomatic viral shedding in 27 pediatric solid organ transplant recipients at 32 weeks post-transplant[32]. They also observed that 70.4% of the patients were positive for HCMV in saliva. In another study, the virus was only detected in 10 of the 27 blood samples evaluated[33]. These and other studies have shown that the behavior of salivary virus shedding in HSCTR is variable, dynamic, and may depend on several factors such as immunosuppression status, bone marrow reconstitution and antiviral therapy[18,19]. The above reasons may explain why HCMV was not detected in all the specimens from the patients who were positive for HCMV in the current study.

The gB glycoprotein (encoded by the *UL55* gene) is important for HCMV fusion and entry into cells, and influences viral tropism by interacting with different receptor molecules[7–10]. We genotyped the HCMV using the *UL55* gene from saliva samples and determined the gB1 genotype in the 12 patients. This is the most frequent genotype reported in studies worldwide, although several studies on HSCT recipients have reported coinfections with gB2, gB3 or gB4 genotypes[20-23]. The detection of more than one genotype can be explained by viral infection and/or reinfection[22], but this was not the case in the 12 patients studied here. This is probably due to the size of the sample, which included only 20 patients who were admitted during the proposed period. Other genotypes might have been detected in a larger sample, but in Colombia there is no report suggesting that any HCMV genotypes other than gB1 are in circulation.

Regarding the genotyping technique, DNA sequencing is also a complementary technique for HCMV genotypic variants based on gB cleavage site (CLS) genotypes (gBCLS 1 to 4), since gB exhibits variability at both the N-terminus (gBN 1 to 4) and the C-terminus (gBCLS 1 to 2), which is highly conserved in each gBCLS genotype. Therefore, this approach is based on sequencing a variable fragment of *UL55*, after PCR amplification. In addition, sequencing is very useful for detecting unknown variants. Although the most common genotypic variants are gB1 to 4, there is strong evidence for homologous recombination among gBCLS. In fact, different gBN sequences have been described but not assigned to a genotype numbering system[27]. In these cases, the use of conventional methods, such as specific multiplex PCR, restriction length polymorphism analysis or microarrays are not useful, and sequencing it is recommended.

Genotype confirmation by sequencing provides more robust data, as genotype detection by size can be inaccurate in cases where nested PCR products are similar in size. Currently, the cost of sequencing is low, so the use of both approaches is highly recommended.

HSCT recipient clinical features

More than 100 saliva samples from this cohort of 20 pediatric patients diagnosed with neoplastic and non-neoplastic conditions were analyzed to perform an outcome analysis during hospitalization. ALL was the most frequent pre-transplant diagnosis in the cohort, and the second most common were aplastic anemia (congenital or acquired), HL, and only one case of AML, in contrast to information published by the European Society for Blood and Marrow Transplantation (EBMT), where one third of the cases were AML[34]. The current study also included patients with non-neoplastic diseases such as aplastic anemia, chronic granulomatous disease, hemophagocytic lymphohistiocytosis, and pathologies for which HSCT is recommended by the EBMT, among others[35].

In this cohort, 17 patients (85%) had febrile neutropenia, which is one of the most common complications in patients receiving myeloablative therapy. Empirical antimicrobial therapy is initiated early in patients, with the assumption that the causative agent is bacterial or fungal. However, in some cases, these agents cannot be identified, and viral etiology may be suspected, but HCMV is not frequently reported[35-38]. The second most frequent complication was GVHD (n = 5). The risk of HCMV infection or reactivation after transplantation is

increased by the immunosuppression associated with GVHD prophylaxis and/or treatment. The use of corticosteroids also interferes with immune reconstitution and contributes to increased risk of reactivation. The relationship between HCMV and GVHD is bidirectional, as immunosuppression and the consequent possibility of viral reactivation and dissemination also contribute to the development of GVHD and other serious complications associated with Cytomegalovirus, such as pneumonia, gastroenteritis, and even transplant failure or leukemia relapse[37,38].

HCMV infection or reactivation is frequently associated with morbidity and mortality in HSCT patients, although sensitive methods of detection and early therapy have significantly improved patient prognosis. Viremia detection is followed by ganciclovir or valganciclovir therapy, although these antivirals induce or exacerbate myelosuppression, and contribute to the occurrence of infectious complications[38-40].

Two of the three fatal cases tested positive for HCMV in saliva. Since all the HCMV patients had the gB1 genotype, it was not possible to establish associations with the observed outcomes. Eleven of 12 patients who were HCMV-gB1 positive in saliva had complications that were likely related to HCMV infection/reactivation or other factors such as pre-transplant diagnoses and type of transplant. Although all individuals in this cohort were gB1, genotyping of infectious HCMV to search for coinfections may help to understand the outcome of patients or infection/reactivation episodes[22].

The present study demonstrated the feasibility and usefulness of saliva as a specimen in which to detect HCMV in hematopoietic stem cell pediatric patients. The collection of saliva samples to detect Cytomegalovirus infection or reactivation can be recommended, given the ease of sample collection, patient convenience and reliable results using qualitative PCR. The study also shows that nested PCR followed by sequencing is a suitable approach for determining HCMV genotype. These techniques are accessible and inexpensive at present.

There is no information on the genotypes of Cytomegalovirus in Colombia. It can therefore be assumed that the predominant genotype is gB1, as in other countries. In this cohort, all the patients were gB1 positive. The study demonstrated the feasibility of using saliva to detect HCMV reactivation and genotyping with highly sensitive techniques such as sequencing. In this cohort, patients found it easy to cooperate with the saliva collection, and were not uncomfortable with the procedure.

Several studies have attempted to determine the relationship between HCMV genotypes, complications and outcomes in recipients of hematopoietic precursor and solid organ transplants[20–23], with variable results. Moreover, the correlation between complications and outcomes has not been observed in all groups studied. It is crucial to identify mixed infections with more than one genotype. These coinfections are associated with higher viral loads, which persist longer and are difficult to control[22,23]. It has also been reported that coinfections may be associated with the development of the Cytomegalovirus associated syndrome and the earlier onset of clinical manifestations of reactivation[40].

Although the current study included a small number of patients and did not allow inference of these associations, it reinforces the importance of evaluating this association and determining Cytomegalovirus genotype(s).

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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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Color change and surface degradation of esthetic brackets after exposure to cigarette smoke and two cleaning treatments

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ABSTRACT

Aim: *During orthodontic treatment, the presence of brackets increases the accumulation of biofilm, which can increase the surface degradation of brackets. Thus, cleaning methods must address removal of both biofilm and stains, specially acquired due to cigarette smoke. Therefore, color change and surface texture of esthetic brackets subjected to cigarette smoke were evaluated before and after use of different cleaning treatments.* ***Material and methods:*** *Three types of conventional esthetic brackets (slot size 0.022" x 0.028" and Roth prescription) were evaluated: polycarbonate/P (Composite/Morelli), polycrystalline ceramic/PC (Iceram/Orthometric) and monocrystalline ceramic/MC (Iceram-S/ Orthometric). They were exposed to cigarette smoke (Marlboro Red Box) for 5 days in a machine that simulated the oral conditions of a smoker. Then, they were assigned to one of two different cleaning treatments (n=10): a) bicarbonate jet (sodium bicarbonate particles 4 µm in diameter, at pressure 2.3 bar, distance 5 mm, for 10 seconds), or b) Robinson brush, pumice stone and water. Color analyses (CIEL*a*b*, WI_D, ΔE_{ab}, ΔE_{00} and ΔWI_D) and surface micromorphology (500 x magnification) were performed before and after exposure to smoke, and after the cleaning treatments.* ***Results:*** *Mixed generalized linear models (α=0.05) showed that after exposure to smoke, all brackets showed a significant decrease in L* (p<0.0001) and WID (p<0.0001), and a significant increase in a* (p<0.05) and b* (p<0.0001), with greater staining for the P brackets (p<0.0001).* ***Conclusion:*** *After the cleaning treatments, it was not possible to recover the initial color of the P brackets with the use of a Robinson brush. Although the cleaning treatment partially or completely removed the surface staining, the P brackets showed more extensive surface degradation, especially with use of the bicarbonate jet.*

Keywords: *orthodontic brackets - color, cigarettes - smoke*

Alteração de cor e degradação superficial de bráquetes estéticos após a exposição por fumaça do cigarro e dois tratamentos de limpeza

RESUMO

Objetivo: *Durante o tratamento ortodôntico, a presença de bráquetes aumenta o acúmulo de biofilme, o que pode aumentar a degradação da superfície desses bráquetes. Assim, métodos de limpeza devem ser utilizados para remover tanto o buofilme como as manchas, especialmente aquelas advindas do cigarro. Assim, a alteração de cor e a textura superficial de bráquetes estéticos submetidos à fumaça de cigarro foram avaliadas antes e após o uso de diferentes métodos de profilaxia.* ***Materiais e Método:*** *Foram avaliados bráquetes estéticos de sistema convencional (tamanho de slot 0,022" x 0,028" e prescrição Roth): tipo policarbonato (Composite/Morelli), cerâmica policristalina (Iceram/ Orthometric) e cerâmica monocristalina (Iceram-S/Orthometric). Estes foram expostos à fumaça de cigarro (Marlboro Red Box) durante 5 dias em uma máquina que simulava as condições bucais de um fumante. Cada tipo de bráquete foi subdividido de acordo com os diferentes métodos de profilaxia (n=10): a) jato de bicarbonato; b) profilaxia com escova Robinson, pedra-pomes e água. Análises de cor (CIEL*a*b*, WI_D, ΔE_{ab}, ΔE_{00} e ΔWI_D) e micromorfologia de superfície (ampliação de 500 x) foram realizadas antes, após a exposição à fumaça e após a profilaxia.* ***Resultados:*** *Modelos lineares generalizados mistos (α=0,05) mostraram que, após exposição à fumaça, todos os bráquetes apresentaram diminuição significativa em L* (p<0,0001) e WID (p<0,0001), em a* (p<0,05) e b* (p<0,0001), sendo o manchamento mais exacerbado para os bráquetes de policarbonato (p<0,0001).* ***Conclusão:*** *Após a limpeza, não foi possível obter a mesma cor inicial dos bráquetes de policarbonato com o uso da escova Robinson. Embora a profilaxia tenha minimizado ou removido manchas superficiais, os bráquetes de policarbonato apresentaram degradação superficial mais extensa, principalmente com o uso do jato de bicarbonato.*

Palavras-chave: *braquetes ortodônticos - cor - cigarros - fumaça*

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INTRODUCTION

Adults are increasingly interested in undergoing orthodontic treatment, often for esthetic reasons, and since appearance is a cause for significant concern there is growing demand for minimally visible appliances[1,2].

Esthetic brackets can be made of polycarbonate or ceramic. Ceramic brackets may be polycrystalline or monocrystalline. Polycrystalline ceramics, especially those made by milling or machining procedures, are rougher and have a more porous surface than monocrystalline types[3,4] and therefore enable greater biofilm deposition and potential staining[4-6]. Polycarbonate brackets appear to be less resistant to staining than ceramic brackets due to their high capacity for water absorption, and their more extensive surface irregularities[7-9]. One of the issues in this regard is that bracket color may change over time as a result of staining by extrinsic substances from smoking or food and drinks containing dyes[2,10-12].

Smoking is relatively common among adults. The World Health Organization has estimated that there are approximately 1.3 billion smokers in the world[13]. Tobacco is one of the main agents that are toxic to humans. Tobacco smoke involves a complex matrix composed of a particulate phase and a gaseous phase, introducing several negative influences into the oral cavity[14], many of which have been extensively studied. However, there are few studies on cigarette smoke staining esthetic brackets. Some studies have shown that tobacco smoke causes chemical and mechanical changes in dental materials, especially composite resins[15,16], which can change the color and texture of the surface of composites[17] by deposition of yellow and black pigments[18].

It is worth emphasizing that orthodontic treatment increases the accumulation of biofilm, which can increase the surface degradation of brackets. Thus, cleaning methods must address removal of both biofilm and stains acquired due to eating habits[19]. One widely used cleaning method is a sodium bicarbonate jet, as it requires only a short time, and does not generate heat compared to cleaning with a rubber cup or Robinson brush and prophylactic paste[20]. Cleaning methods should be effective in removing pigmentation and biofilm without altering the surfaces of the tooth enamel or the orthodontic accessories[21,22].

Considering that adult patients undergoing orthodontic treatment with esthetic appliances may smoke, it is important to assess the degree of staining that occurs on different types of esthetic bracket materials, as well as the influence of stain removal methods on degradation of the surface of these materials.

The null hypotheses of this study were: H01) the color of esthetic brackets does not change after exposure to either cigarette smoke or cleaning methods; H02) the surface of esthetic brackets is not altered by cleaning methods.

MATERIALS AND METHOD

Bracket specifications and initial color evaluation

The sample consisted of 60 esthetic brackets of different brands and compositions: 20 each of polycarbonate (Composite/Morelli), polycrystalline ceramic (Iceram/Orthometric) and monocrystalline ceramic (Iceram-S/Orthometric). All brackets used in this study were of the conventional system, slot size 0.022" x 0.028", Roth prescription, and indicated for use on the maxillary right central incisor[2,11,12].

Initially, the brackets were immersed in Eppendorf tubes containing artificial saliva[23] (1.5 ml), and stored at 37 ºC for 24 hours. After this, initial color was determined using a spectrophotometer (VITA Easyshade V, Vita, Baden-Württemberg, Bad Säckingen, Germany). The brackets were positioned in a reading chamber with a white background, with the active tip of the spectrophotometer positioned at an angle of 90 degrees to the buccal surface of the bracket[24]. The L*, a* and b* values according to the CIEL*a*b* system were measured three times in a row, and recorded in a spreadsheet to calculate the average.

Protocol of exposure to cigarette smoke and color assessment after staining

A smoke machine (registered under No. 01810012043 INPI - National Institute of Industrial Property) was used[18] to impregnate the brackets with the pigments and substances contained in cigarettes, with the aim of replicating *in vitro* the conditions in the oral cavity of smokers. The machine aspirated and conducted smoke through compartments to create a flow of smoke from the environment, thereby enabling the deposition of chemicals on the brackets. The cycle was programmed with a 3-second time interval,

simulating normal smoker inhalation. The timer allowed ambient air to be inhaled every 10 seconds, thereby simulating the exhaustion and subsequent elimination of smoke.

Brackets were fixed with wax in a plastic holder with ten niches, and placed in the smoke machine[7]. Each niche corresponded to one of the holes where cigarettes were fastened to the machine. When the cigarettes were lit, these holes "smoked" them. The brackets were subjected to smoke from one packet of cigarettes (Marlboro Red Box, Philip Morris Brasil Ind. and Com. Ltda., Santa Cruz do Sul, RS, Brazil), corresponding to 20 cigarettes per day, for a total of 5 days[24]. Between simulations, the brackets were stored in artificial saliva at 37 °C. Every 24 hours, they were washed with distilled water and re-immersed in a new artificial saliva solution to prevent pigment sedimentation[25].

After staining, bracket color was evaluated again, following the protocol described.

Procedures for removing pigmentation (cleaning procedures) and final color assessment

After exposure to smoke, brackets of the same composition were divided into two subgroups (n=10), each of which was subjected to cleaning by either (a) bicarbonate jet or (b) Robinson brush, pumice stone and water.

Cleaning by bicarbonate jet was performed with a sodium bicarbonate device (Gnatus, Prophy Jet Gold Line, Barretos, SP, Brazil) using sodium bicarbonate particles (Maquira Airon, Maringá, PR, Brazil) 4 µm in diameter. The reservoir was filled to 50% of its total capacity with sodium bicarbonate. Airborne particle abrasion was applied perpendicularly to the brackets in a standardized manner at a pressure of 2.3 bar, from a distance of 5 mm, for 10 seconds.

Cleaning with pumice and water was performed using a Robinson brush (3R Ind. e Com. EIRELI, São Paulo, SP, Brazil) with a micromotor and contra-angle (Intramatic I 181D/ Kavo, Joinville, SC, Brazil), at a constant speed of 5,000 rpm for 5 seconds on each bracket. Pumice powder (Maquira, Maringá, PR, Brazil) with extra-fine particles was placed in a Dappen pot up to half its capacity, approximately 1.5 ml of its volume, together with 5 ml of water, to form a cleaning paste. The paste was distributed evenly over the bracket surfaces with the aid of a tamper-type spatula (Calcador 6337 Nº 02, FAVA, São Paulo, SP, Brazil), and then the brackets were brushed. One Robinson brush was used to brush 5 brackets. According to McCracken et al.[26], two minutes are sufficient for brushing 28 teeth of a patient, resulting in an approximate time of five seconds for each tooth/bracket. After this, the brackets were washed with distilled water and stored in artificial saliva for subsequent evaluation of the final color.

Color Assessment

Color changes were evaluated according to the CIEL*a*b* color space. L* represents the lightness or color value (from black to white) of an object, with pure black having an L* value equal to 0. When the object fully reflects the color, L* is equal to 100, which is pure white. The a* axis measures the values from green to red, with a+ (positive a*) being values that reflect red and a– (negative a*) values that reflect green. The b* axis measures the values from yellow to blue, where b+ (positive b*) is yellow and b- (negative b*) is blue.

After this, ΔL*, Δa* and Δb* were calculated for each group and time, and used to evaluate color change ΔE_{ab}) estimated by the formula[27]: $\Delta E_{ab} = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)}$. The limits of perceptibility and acceptability considered for ΔE_{ab} were 1.2 and 2.7, respectively[27,28]. Color change was also evaluated by CIEDE2000 (ΔE_{00}), which uses h (hue) and C (chroma) values[29]. ΔE_{00} values of 0.8 and 1.8 were adopted as the perceptibility and acceptability limits[28]. Tooth staining was evaluated by the Whiteness Index for Dentistry (WI_D), in which the parameters L*, a* and b* were used in the following equation[30]: $WI_D = 0.511L^* - 2{,}324a^* - 1.100b^*$ Differences in WI_D between the initial and final assessments were calculated (ΔWI_D), using threshold values for ΔWI_D of 0.72 for perceptibility and 2.60 for acceptability[31].

Evaluation of surface micromorphology by scanning electron microscopy (SEM)

The surface micromorphology of three specimens of each type of bracket, chosen at random, was examined at baseline, after staining and after the cleaning treatments. The brackets were sputter coated with gold (gold layer thickness estimated at 200 Aº) using a sputter coater (Sputter Coater, EMITECH, K450, Kent, United Kingdom). Images of the surface micromorphology were acquired at 500x magnification with a high-resolution scanning

electron microscope (Thermo Fisher Scientific, Model Quattro S, Thermo Scientific UltraDry, Brno, Czech Republic) with a voltage of 20 kV and a spot size of 32 pA. Qualitative analyses of the surface were performed to determine whether there was presence of erosion and/or irregularities.

Statistical Analysis

After descriptive and exploratory data analysis, mixed generalized linear models were applied for repeated measures over time for the L*, b* and WI_D values. Generalized linear models were also adjusted to analyze the variables ΔE_{ab} and ΔE_{00}. The variables of a* and ΔWI_D were analyzed by the Mann Whitney test to compare the two cleaning methods. Kruskal Wallis and Dunn tests were used to compare bracket types, and Friedman and Nemenyi tests were used to compare the values recorded at the three measurement times. The analyses were performed in the R Program (2022), with a level of significance of 5%.

RESULTS

Baseline L* was significantly lower for polycarbonate brackets than for the other types of brackets ($p<0.05$) (Table 1). After staining, L* decreased significantly for all brackets ($p<0.05$), and subsequently increased after cleaning ($p<0.05$).

Table 1. Mean (standard deviation) of L*, b* and WI_D values considering bracket composition, cleaning method and time

Parameter	Cleaning method	Composition of brackets	Time		
			Baseline	After staining	After cleaning
			Mean (standard deviation)		
L*	Bicarbonate jet	Polycarbonate	86.73 (5.13) Ab	55.41 (8.65) Bc	92.52 (7.34) Ab
		Polycrystalline ceramic	97.87 (2.54) Aa	75.95 (6.92) Ba	99.41 (0.97) Aa
		Monocrystalline ceramic	98.29 (1.75) Aa	65.90 (5.53) Bb	98.33 (1.92) Aa
	Robinson Brush	Polycarbonate	90.81 (4.25) Ab	* 69.64 (6.21) Cb	* 83.38 (4.26) Bb
		Polycrystalline ceramic	97.46 (2.19) Aa	78.22 (3.48) Ba	* 97.23 (3.14) Aa
		Monocrystalline ceramic	98.86 (1.75) Aa	63.85 (6.54) Cb	* 90.38 (10.41) Ba
b*	Bicarbonate jet	Polycarbonate	6.84 (1.31) Ca	21.65 (2.33) Aa	9.31 (3.86) Ba
		Polycrystalline ceramic	3.04 (0.70) Cc	14.57 (2.58) Ab	4.92 (0.85) Bc
		Monocrystalline ceramic	4.05 (0.66) Cb	16.33 (2.32) Ab	7.23 (1.17) Bb
	Robinson Brush	Polycarbonate	7.58 (0.56) Ca	20.69 (3.71) Aa	* 15.04 (2.46) Ba
		Polycrystalline ceramic	3.06 (1.03) Cc	13.10 (1.67) Ac	5.18 (1.83) Bb
		Monocrystalline ceramic	4.65 (0.51) Cb	17.45 (2.81) Ab	6.75 (2.29) Bb
WI_D	Bicarbonate jet	Polycarbonate	44.43 (2.17) Ab	- 10.73 (8.97) Bc	42.27(10.87) Aab
		Polycrystalline ceramic	52.42 (2.08) Aa	14.71 (8.50) Ca	48.01 (2.34) Ba
		Monocrystalline ceramic	52.67 (1.73) Aa	5.62 (6.20) Cb	44.83 (3.17) Bb
	Robinson Brush	Polycarbonate	45.75 (2.06) Ab	* 3.69 (10.17) Cb	* 24.52 (7.45) Bb
		Polycrystalline ceramic	52.06 (2.93) Aa	18.86 (5.24) Ca	46.40 (5.28) Ba
		Monocrystalline ceramic	51.95 (0.95) Aa	1.28 (6.78) Cb	40.17 (10.41) Ba

*Differs from the bicarbonate jet under the same bracket conditions and time within each variable ($p\leq0.05$). Different letters (capitals in horizontal and lower case in vertical) comparing the brackets within each cleaning method) indicate statistically significant differences ($p\leq0.05$).

For all types of brackets, L* was significantly higher after bicarbonate jet cleaning than after Robison brush cleaning ($p<0.05$) (Table 2). At the final time, once again, L* values were significantly lower for the polycarbonate brackets than for the other types of brackets ($p< 0.05$).

Baseline b* was higher for polycarbonate brackets than for polycrystalline ceramic brackets ($p<0.05$) (Table 1). For all three bracket types, b* increased significantly after staining and subsequently decreased after cleaning ($p<0.05$). Final b* for polycarbonate brackets was lower with bicarbonate jet treatment than with Robinson brush treatment ($p<0.05$). Final b* was higher for polycarbonate brackets treated with bicarbonate jet than for polycrystalline ceramic brackets ($p<0.05$). After cleaning with Robinson brush, b* was higher for polycarbonate than for the two ceramics ($p<0.05$).

Baseline WI_D was significantly lower for polycarbonate than for the two ceramics ($p<0.05$) (Table 1). WI_D was significantly lower after staining for all bracket types, and increased after cleaning ($p<0.05$). Final WI_D for polycarbonate brackets was higher with bicarbonate jet than with Robinson brush treatment. When bicarbonate jet treatment was used, final WI_D was higher for polycrystalline ceramics than for the monocrystalline types ($p<0.05$). When the Robinson brush was used, WI_D was lower for polycarbonate than for the two ceramics ($p< 0.05$).

Baseline a* was more negative for polycarbonate than for polycrystalline ceramic brackets ($p<0.05$) (Table 3). After staining, a* was significantly higher for all bracket types ($p<0.05$). Final a* for polycarbonate brackets was lower when cleaning was performed with bicarbonate jet than with Robinson brush. After bicarbonate jet cleaning, a* was lower for polycarbonate brackets than for monocrystalline ceramics ($p<0.05$). When cleaning was performed with Robinson brush, a* was higher for polycarbonate brackets than for polycrystalline ceramic brackets ($p<0.05$).

After bicarbonate jet cleaning, the change in color measured by ΔE_{ab} was significantly greater in polycarbonate brackets than in polycrystalline ceramic brackets ($p<0.05$) (Table 4). When Robinson brush was used, the variation in color after cleaning was greater in monocrystalline ceramic and smaller in polycarbonate brackets ($p<0.05$). For polycarbonate brackets, the variation in color after cleaning was significantly lower when a Robinson brush was used ($p<0.05$). After bicarbonate jet cleaning, the change in color measured by ΔE_{ab} was significantly greater in polycarbonate brackets than in monocrystalline ceramic brackets ($p<0.05$). When the Robinson brush was used, the variation in color was greater in monocrystalline ceramic than in the other bracket types. For polycarbonate brackets, ΔE_{00} was higher after treatment with bicarbonate jet than with Robinson brush ($p<0.05$).

After bicarbonate jet cleaning, ΔWI_D (57.28) was higher for polycarbonate than for polycrystalline ceramic ($p<0.05$). After Robinson brush cleaning, ΔWI_D was higher for monocrystalline ceramic than for polycarbonate brackets ($p<0.05$). Also for the polycarbonate brackets, ΔWI_D values were significantly higher following treatment with

Table 2. Median (minimum maximum) of ΔWI_D values considering bracket composition, cleaning method and time

Cleaning method	Composition of brackets	Time	
		After staining – Baseline	After cleaning- After staining
		Median (minimum; maximum)	Median (minimum; maximum)
Bicarbonate jet	Polycarbonate	-54.28 (-75.47; -43.76) b	57.28 (28.81; 68.98) a
	Polycrystalline ceramic	-39.82 (-48.19; -19.17) a	34.53 (20.39; 45.32) b
	Monocrystalline ceramic	-47.11 (-57.05; -38.81) ab	38.63 (30.94; 50.61) ab
p-value		0.0018	0.0049
Robinson Brush	Polycarbonate	* -40.90 (-61.85; -23.80) ab	* 20.22 (12.34; 32.86) b
	Polycrystalline ceramic	-32.70 (-45.24; -23.63) a	27.56 (15.93; 37.16) ab
	Monocrystalline ceramic	-48.26 (-61.26; -44.17) b	42.09 (18.61; 59.27) a
p-value		0.0002	0.0101

*Differs from the bicarbonate jet under the same bracket conditions and time ($p\leq0.05$). Different letters in the vertical (comparing the brackets within each cleaning method) indicate statistically significant differences ($p\leq0.05$).

Table 3. Median (minimum; maximum) a* values considering bracket composition, cleaning method and time

Cleaning method	Composition of brackets	Time			p-value
		Baseline	After staining	After cleaning	
		Median (minimum; maximum)			
Bicarbonate-jet	polycarbonate	-3.28 (-3.60; -2.95) Bb	6.58 (4.55; 9.25) Aa	-2.25 (-4.35; 0.50) Bb	0.0004
	Polycrystalline ceramic	-2.60 (-3.00; -1.65) Ba	3.68 (0.90; 5.05) Ab	-1.35(-1.95; -0.05) Bab	0.0001
	Monocrystalline ceramic	-2.90(-3.30; -2.70) Bab	4.33 (2.70; 5.90) Ab	-1.13 (-2.20;-0.45) Aba	<0.0001
p-value		0.0002	0.0002	0.0357	
Robinson Brush	Polycarbonate	-3.33 (-3.60; -2.80) Bb	* 4.03 (0.60; 7.20) Aab	* 1.05 (-1.90;1.95) ABa	<0.0001
	Polycrystalline ceramic	-2.60 (-3.25; -1.10) Ba	2.86 (1.65; 4.45) Ab	-0.85 (-2.25; 0.80) ABb	<0.0001
	Monocrystalline ceramic	-2.85 (-3.05; -2.50) Ba	5.10 (3.80; 7.20) Aa	2.0 (1.70; 1.70) ABab	<0.0001
p-value		0.0002	0.0009	0.0095	

*Differs from the bicarbonate jet under the same bracket conditions and time (p≤0.05). Different letters (capitals in horizontal and lower case in vertical) comparing the brackets within each cleaning method) indicate statistically significant differences (p≤0.05).

Table 4. Mean (standard deviation) of ΔE_{ab} and ΔE_{00} considering composition of the brackets, cleaning method and time

Parameter	Cleaning method	Composition of brackets	Time	
			After staining – Baseline	After cleaning - After staining
			Mean (standard deviation)	
ΔE_{ab}	Bicarbonate-jet	Polycarbonate	36.40 (10.24) a	40.20 (12.14) a
		Polycrystalline ceramic	25.57 (6.13) b	25.86 (6.73) b
		Monocrystalline ceramic	35.50 (6.27) a	34.21 (6.47) ab
	Robinson Brush	Polycarbonate	* 26.26 (6.45) b	* 15.43 (±5.53) c
		Polycrystalline ceramic	22.38 (4.51) b	21.01 (4.26) b
		Monocrystalline ceramic	38.32 (4.74) a	29.42 (12.18) a
	p-value		p(bracket)=0.0001; p(cleaning)= 0.0141; p(interaction)=0.0070	p(bracket)=0.0001; p(cleaning)= 0.0007; p(interaction)=0.0001
ΔE_{00}	Bicarbonate jet	Polycarbonate	28.52 (8.45) a	30.31 (9.55) a
		Polycrystalline ceramic	18.33 (4.48) b	17.59 (4.81) b
		Monocrystalline ceramic	25.46 (4.75) a	23.80 (4.93) ab
	Robinson Brush	Polycarbonate	* 19.10 (4.89) b	* 11.05 (4.45) b
		Polycrystalline ceramic	16.07 (3.12) b	14.37 (3.00) b
		Monocrystalline ceramic	27.51 (4.10) a	21.18 (8.61) a
	p-value		p(bracket)=0.0001; p(cleaning)= 0.0074; p(interaction)=0.0021	p(bracket)=0.0007; p(cleaning)<0,0001; p(interaction)=0.0001

*Differs from the bicarbonate jet under the same bracket conditions and time (p≤0.05). Different letters in the vertical (comparing the brackets within each cleaning method) indicate statistically significant differences (p≤0.05).

bicarbonate jet than with Robinson brush (p<0.05). The microscopy images (Fig. 1) show that the monocrystalline ceramic bracket had the greatest surface smoothness and uniformity, followed by polycrystalline ceramic brackets and polycarbonate brackets. In polycarbonate brackets, glass fibers were observed both before and after cleaning. After staining with cigarette smoke, the monocrystalline ceramic maintained its appearance of surface smoothness, contrarily to the polycarbonate and polycrystalline ceramic brackets, in which the appearance of the surface became more textured.

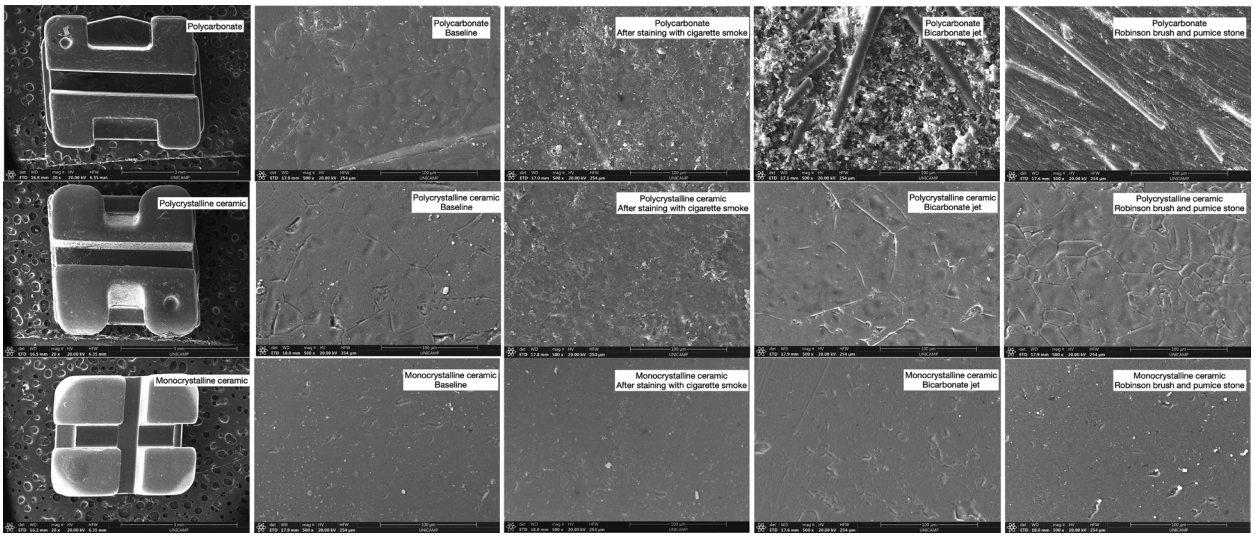

Fig. 1: Images of the surface micromorphology of brackets at different evaluation times.

Irrespective of the type of cleaning used, the smoothness and uniformity of the monocrystalline bracket surfaces did not change. For polycarbonate brackets, however, the bicarbonate jet led to more significant change on the surface, with evidence of glass fibers due to the removal of the most superficial resin matrix and caused porosity in this resin matrix. Cleaning with a Robinson brush and pumice stone not only produced more significant evidence of glass fibers on the surface, but also promoted wear of the resin matrix, including scratches. Although no scratches or irregularities were observed on the polycrystalline ceramic brackets, cracks appeared between the alumina crystals, possibly due to the absorption of water by the material.

DISCUSSION

The different types of esthetic brackets differ in terms of composition, and this is reflected in the differences found in the parameters L*, a*, b* and WI_D. In general, the polycarbonate brackets had the lowest values for L* (less lightness than the other brackets), a* (more "greenish") and WI_D (less bright), and the highest values for b* (more "yellowish" than the other brackets). The monocrystalline and polycrystalline ceramic brackets had similar values for L*, a* and WI_D, but b* was higher for the polycrystalline than for the monocrystalline brackets. These results may be explained by the structural characteristics of the brackets, since the polycarbonate types are composed of a polymeric matrix reinforced with glass fibers that produce less lightness and more yellowing, as well as a more irregular porous surface. Ceramic brackets are smoother because of the aluminum oxide in their composition. Moreover, both ceramic types are lighter than the polycarbonate, with monocrystalline brackets being even lighter and more translucent than polycrystalline brackets because they consist of a larger size of ceramic grains and contain fewer impurities[32].

After staining, L* and WI_D decreased significantly for all brackets, while b* and a* increased significantly. Thus, the first null hypothesis (H01) was rejected, as all the parameters evaluated underwent statistically significant changes. These results corroborated those found by Borges et al.[33] who reported change in the color of esthetic brackets exposed to cigarette smoke. In smokers, the oral cavity is exposed to cigarette smoke, which consists of toxic substances such as carbon monoxide, ammonia, nickel, arsenic, tar, lead and cadmium[34]. The components present in cigarette smoke impregnate tooth surfaces and materials in the oral cavity, and consequently, yellow, red/brown and black pigments can be incorporated into these materials[18], explaining the decrease in lightness (L*) and "whiteness" WI_D), and the increase in values on the b* axis (greater yellowing of the brackets). Due to their high water absorption capacity (considering that they were immersed in artificial saliva before and during the staining simulation protocols) and greater surface irregularities (Fig. 1), polycarbonate brackets were more susceptible to staining than ceramic brackets[7,8]. Furthermore, changes in the surface of polycarbonate and polycrystalline

ceramic brackets were observed, considering that originally, there were differences between them since the polycarbonate type is made of a polymer, and the polycrystalline type is made of alumina oxide. This may explain the greater deposition of cigarette smoke components on polycarbonate brackets[18]. Polycarbonate brackets change color when immersed in vitro in coloring solutions such as red wine, coffee and tea[10], and staining may increase over time. Among the ceramic brackets, polycrystalline alumina brackets (composed of aluminum oxide crystals fused at high temperatures and produced by means of a less complex industrial process[3,4]) have rougher, more porous surfaces than monocrystalline ceramic brackets[4]. This agrees with the microscopy images of the present study, in which the monocrystalline ceramic bracket did not exhibit perceptible changes in surface texture, even after cleaning (Fig. 1). Nevertheless, the parameters L* and WI_D showed that polycrystalline ceramic brackets had the highest lightness and whiteness after staining, although their surface was rougher than the monocrystalline type. Polycrystalline ceramic brackets yellowed less (lower b* values), even with the deposition of smoke pigments. This could be explained by the initial differences (baseline) between the brackets with regard to these parameters.

Smokers need more reinforcement of the cleaning methods than do non-smokers to reverse the extrinsic staining of brackets and teeth. Nicotine, which is present in high concentration in tobacco leaves, can produce salts with acids that are generally soluble in water and can be absorbed by brackets and adhesive materials[14]. Cleaning with sodium bicarbonate jet is quick and practical, but prolonged use can increase bracket surface roughness[35,36]. After the cleaning procedures, all brackets showed a significant increase in L* and WI_D, and a significant decrease in a* and b*, enabling us to state that as an immediate result, these procedures promoted the removal of pigments, especially those on the surface. For all brackets, bicarbonate jet cleaning led to L* values that were statistically similar to baseline, and significantly higher than those achieved with the Robinson brush. For polycarbonate brackets, the bicarbonate jet also achieved significantly higher a* and WI_D values and significantly lower b* values than did the Robinson brush. The pressure of the jet and the impact of the sodium bicarbonate particles against the structure of the bracket provided more effective dispersion and penetration into inaccessible regions, with greater power of abrasion and stain removal. However, depending on bracket material, degradation of the sandblasted surface was observed in the scanning electron microscopy images, which was more severe on the polycarbonate bracket. Thus, even though bicarbonate jet cleaning minimizes or removes surface staining, it can considerably increase surface degradation of polycarbonate brackets, and even lead to less color stability over time. The second null hypothesis is therefore rejected.

For polycarbonate brackets, after the use of airborne particle abrasion, a considerable loss of part of the polymeric matrix was observed, triggered by the force of the jet when it reached the surface, generating a significant increase in water sorption and, possibly, greater instability of color throughout the course of treatment. For polycrystalline ceramics, there were cracks on the surface between the molten grains of the crystal structure. When airborne abrasion is performed with another type of particle (such as aluminum oxide), ceramics begin to show greater retention of cements and a larger number of surface irregularities[37].

After bicarbonate jet cleaning, the change in color determined by ΔE_{ab} and ΔE_{00} was significantly greater in polycarbonate brackets than in monocrystalline ceramic brackets, followed by the polycrystalline type (Table 3), which were less stained.

Perez et al.[30] proposed a "whiteness" index (WI_D), also based on CIEL*a*b* coordinates, with the aim of avoiding the subjectivity of the visual factor in measuring color. Its advantage is that it provides a very simple analysis: higher values indicate whiter samples and lower values (including negative) indicate darker samples.

In the current study, after bicarbonate jet cleaning, ΔWI_D was higher for the polycarbonate than for the polycrystalline ceramic brackets. With the Robinson brush, ΔWI_D was higher for monocrystalline ceramic than for polycarbonate brackets. For the polycarbonate brackets, ΔWI_D was significantly higher with the bicarbonate jet than with the Robinson brush. The results suggest that polycarbonate brackets are more sensitive to cigarette smoke, since their color changed more by staining in comparison to the ceramics. Among the ceramics, the monocrystalline were the most resistant, in terms both of staining and the deleterious effects on

the surface considering the cleaning methods used to remove stains, since their smoother, more regular surfaces were maintained.

In view of the findings presented, we suggest that ceramic brackets may be a better alternative for smoking patients due to their greater resistance to staining and better tolerance of the effects of both cleaning methods.

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

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Dental biofilm in children with normal weight, overweight and obesity: a pilot study

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ABSTRACT

Microorganisms attached to surfaces form intricate colonies known as biofilms. Dental plaque is the biofilm formed on the tooth surface, including the gingival sulcus. Plaque staining makes it easier to see which areas need more brushing time, and which have higher risk of periodontal disease or caries. Dental plaque is largely influenced by diet, becoming more and more dependent on diet-related carbohydrates as it ages. Inadequate dental care and bad eating habits are frequently associated with the presence of visible bacterial plaque. ***Aim:*** *The aim of this study is to evaluate the percentage of dental plaque according to body mass index (BMI) and to identify whether the plaque is newly deposited, mature or acidified.* ***Materials and Method:*** *Twenty-five patients from the Pediatric Dentistry Clinics at the University of Guadalajara were evaluated. The percentage and type of plaque were identified using a disclosing gel. The percentage of plaque was compared among children with normal weight, overweight and obesity.* ***Results:*** *14 children were normal weight, six were overweight, four were obese, and one was underweight. Average percentage of total plaque was 70.92%. The most predominant plaque was newly deposited (pink staining), followed by mature plaque (purple staining), and a lower percentage of acidified plaque (light blue staining).* ***Conclusion:*** *The high percentage of plaque indicates lack or inefficacy of tooth brushing, highlighting the importance of using plaque disclosure for diagnostic and educational purposes for children and parents.*

Keywords: *dental plaque - normal weight - overweight - obesity*

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Placa bacteriana dental en niños con normopeso, sobrepeso y obesidad: un estudio piloto

RESUMEN

Los microorganismos adheridos a las superficies forman colonias intrincadas conocidas como biopelículas. La placa dentobacteriana es la biopelícula formada sobre la superficie del diente incluso en el surco gingival. La tinción con placa hace que sea más fácil ver qué áreas necesitan más tiempo de cepillado y qué áreas tienen un mayor riesgo de enfermedad periodontal o caries. La placa dental se acumula en los dientes y está influenciada en gran medida por la dieta. La placa se vuelve cada vez más dependiente de los carbohidratos relacionados con la dieta a medida que envejece. Además, un cuidado dental inadecuado y malos hábitos alimentarios se asocian frecuentemente con la presencia de placa bacteriana visible. ***Objetivo:*** *Este estudio tiene como objetivo evaluar el porcentaje de placa dental según el índice de masa corporal (IMC) e identificar el tipo de placa presente, ya sea placa recién depositada, madura o acidificada.* ***Materiales y Métodos:*** *Se evaluaron veinticinco pacientes de las Clínicas de Odontología Pediátrica de la Universidad de Guadalajara. Se identificó el porcentaje y tipo de placa mediante un gel revelador. Se comparó el porcentaje de placa entre niños con peso normal, sobrepeso y obesidad.* ***Resultados:*** *14 niños presentaron peso normal, seis tenían sobrepeso, cuatro eran obesos y sólo un niño tenía bajo peso. Un porcentaje medio de placa total del 70,92%. La placa que más predominó fue la placa de reciente depósito (tinción rosa), seguida de la placa madura (tinción violeta) y en menor porcentaje la placa acidificada (tinción azul claro).* ***Conclusión:*** *El alto porcentaje de placa indica falta de cepillado dental o ineficacia del cepillado si se practica, destacando la importancia de utilizar la divulgación de placa con fines diagnósticos y educativos para niños y padres.*

Palabras clave: *placa bacteriana - normopeso - sobrepeso - obesidad*

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INTRODUCTION

Biofilms are complex communities of microorganisms adhered to surfaces. A community of microbial cells embedded in an extracellular matrix forms dental plaque, a biofilm that grows at the interface between two phases of matter, such as the tooth surface and saliva or gingival crevicular fluid (GCF)[1-3].

Plaque staining helps identify areas with higher caries or periodontal risk and areas requiring more brushing time. It is one of the best strategies for teaching tooth brushing, refining brushing techniques, and establishing this habit[4].

A study that examined plaque staining with gentian purple in children aged 6 to 48 months (4 years) reported findings with practical implications[5]. Dental plaque was highest on the primary molars, followed by the anterior teeth. These results, which are consistent with previous studies using plaque-disclosing tablets, suggest that these methods can effectively identify areas requiring more brushing time[6,7].

Diet and eating habits significantly affect dental plaque and related conditions such as dental caries and periodontal diseases[8]. As plaque matures, it relies on dietary carbohydrates for sustenance[9]. The presence of visible bacterial plaque is often linked to poor nutritional habits and inadequate oral hygiene practices[10].

Children with a high BMI are more likely to consume excess sugars in food such as sweets, grains, and processed/refined cereals[11]. In turn, sugar consumption has generally been associated with reduced diet quality, increased energy consumption and body weight gain[12].

The rate of childhood overweight and obesity in Mexico is currently 17.5% according to the WHO 2022. According to ENSANUT 2020-2022, overweight in schoolchildren was 19.2%, and obesity 18.1% [13].

BMI is calculated based on a person's weight and height, and children are considered normal weight, overweight or obese for their age according to reference tables[14]. Waist circumference is also used as an indicator to assess abdominal fat and the risk associated with metabolic diseases, providing a more complete view of the child's physical condition.

Thus, diet, weight and plaque are related. Plaque is linked to the occurrence of caries and periodontal disease[15], and is thus an indicator of oral health. It is therefore important to evaluate whether there is an association between percentages of dental plaque and BMI.

A disclosing gel that stains dental plaque pink, purple or light blue according to its age and acidogenicity is commercially available. Only one study has employed this gel to identify plaque in patients with or without asthma and their caries risk based on plaque acidogenicity[16].

Dental plaque index and caries progression improve with educational reinforcement[17]. Proper tooth brushing and use of a plaque-disclosing dentifrice can reduce biofilm within a week.

The aims of this study were thus to determine differences in dental plaque percentage according to dentition type, body mass index and diet by evaluating the total dental plaque index and the percentages of pink (recently deposited plaque), purple (plaque with 24 hours' deposition), or light blue (acidic plaque with more than 48 hours' deposition), and to identify areas with higher cariogenic and periodontal pathogenic risk.

MATERIALS AND METHOD

A cross-sectional study was conducted at the Pediatric Dentistry Specialty Clinic of the University of Guadalajara. Pediatric patients were invited to participate, and the objective of the study was explained to them and their parents. Once they agreed to participate, written informed consent according to the Helsinki Treaty of 2013 was obtained from all children and their parents or guardians. This study was reviewed and approved by the Bioethics and Research Committees of the University of Guadalajara under approval number CI-05323.

This study evaluated 25 children aged 4 to 10 years who visited the Pediatric Dentistry Clinic of the University of Guadalajara.

Selection criteria

Inclusion criteria: Patients who visited the Pediatric Dentistry Specialty Clinic at the University Center for Health Sciences, University of Guadalajara. Patients with normal weight, overweight or obesity. Patients aged 4 to 10 years. Exclusion criteria: Patients with any systemic disease or syndrome. Patients with orthopedic or orthodontic appliances. Patients with dental organs affected by any pathology

(caries, trauma, periodontal disease, amelogenesis, dentinogenesis). Patients with allergic reactions to the plaque-disclosing gel during the pre-reactivity test. Withdrawal: Patients who decide to withdraw from the study during the evaluation process.

Identification of dental plaque

A pediatric dentist recorded the clinical history. The following aspects were evaluated: type of dentition (deciduous, mixed, permanent), molar relationship type (Class I, II or III), canine relationship, brushing frequency, dental plaque index, body mass index (BMI), type of diet, weight, height, and waist circumference.

Dental plaque was stained using the Tri Plaque Gel GC Three-Tone Plaque Disclosing Gel [CG, Tokyo Japan]. The Tri Plaque Gel was placed in a dispensing tray and applied to all dental surfaces using a swab. Subsequently, the patient was asked to rinse their mouth gently with water.

The observed results following plaque staining were interpreted as follows: Pink or red plaque on the tooth surface indicates recent plaque accumulation. Purple indicates mature plaque that has been present for at least 48 hours. Light blue indicates mature plaque with more than 48 hours' deposition and strong acid production.

After staining the plaque, all patients, accompanied by their parents or guardians, were shown the staining using a mirror, and each color was explained to them. After recording all plaque-stained areas and their respective colors, both the child and the parent or guardian were instructed on how to perform proper oral hygiene. They were provided with a toothbrush with soft, smooth bristles and toothpaste.

Evaluation of the dental plaque index

To assess the total dental plaque, the number of surfaces per tooth in the mouth and the number of surfaces with stained plaque were recorded to calculate the percentage.

$$\frac{\text{number of stained tooth surfaces} * 100}{\textit{total number of dental surfaces}} = \text{total staining \%}$$

Similarly, the number of surfaces stained with each color (pink, purple or light blue) was counted, and the percentage of each color was calculated relative to the total number of surfaces.

$$\frac{\text{number of stained tooth surfaces with a color} * 100}{\textit{total number of dental surfaces}} = \textit{dental plaque of one color \%}$$

It should be noted that the amount of plaque per surface was not evaluated; only surfaces stained with any of the three colors were counted. Therefore, the sum of the percentages of each color may exceed 100%, as a dental surface may have all three plaque colors distributed on it.

Anthropometric and dietary assessment

A nutritionist assessed BMI, waist circumference, and dietary intake. BMI was based on the child's sex, age, height and weight. The growth charts from the Centers for Disease Control and Prevention (CDC) were used to classify BMI as underweight, normal weight, overweight, or obesity.

Body weight was measured using the OMRON® HBF-514C body composition monitor [OMRON HEALTHCARE, INC., Illinois, U.S.A]. Height was measured with the Seca 213 portable stadiometer [Seca, Hamburg], and waist circumference was assessed using the SECA 201 anthropometric measuring tape for waist circumference [Seca, Hamburg].

Children's diet was evaluated using the validated instrument "Food Estimation and Consumption Scale in Children" [18], which identifies the frequency of foods in children's diets. The frequency of consumption per week of different foods was evaluated, including bread, sweets, sugary drinks such as black or clear sodas, fruits, vegetables, seeds and red meat. The diet was classified as healthy, fairly healthy or unhealthy.

Statistical analysis

Statistical analysis was conducted using SPSS v.25. Normality was assessed using the Shapiro-Wilk test, showing normality in the various variables. Subsequently, the Kruskal-Wallis and Mann-Whitney U test were applied to identify differences in the percentage of dental plaque according to dentition type, body mass index and nutritional status. A X_2 test was performed to analyze the frequencies of food consumption in all study groups and the type of nutrition. A Spearman correlation test was conducted to evaluate the relationship between age, BMI, and the percentage of new (pink), mature (purple), and acidified (light blue) plaque. A p-value

≤ 0.05 was considered significant.

RESULTS

Identification of dental plaque

The study included 25 patients who visited the Pediatric Dentistry specialty clinic for dental care. Most of the participants were male. Average age was 6.68 years. Seven patients had temporary dentition, 16 had mixed dentition, and 2 had permanent dentition. The predominant molar and canine classes were class I. There were 17 patients with molar class I, four with molar class II, and four with molar class III. Canine class I predominated with 17 patients, and eight patients had canine class II. No significant difference was observed among the study groups for these variables (Table 1).

Anthropometric evaluation and frequency of food consumption

Mean weight was 26.42 kg, and significantly higher in the obese group than in the normal weight group. Mean height was 121 cm, with no significant difference among study groups. Mean waist circumference was 60.32 cm, and significantly higher in the obese group compared to the normal weight group. Mean BMI was 17 kg/m². One patient was underweight, 14 patients were evaluated as normal weight, 6 as overweight, and 4 as obese. Significant differences in BMI were observed among all study groups (Table 2).

The frequency of food consumption was evaluated weekly by six questions about food groups. The most frequently consumed foods per week were fruits, bread, cookies, juices, clear soft drinks, and seeds. The most frequently consumed foods per day were vegetables and fruits. In Table 3, the first row of each food group shows the frequency of consumption per week in the underweight, normal weight, and obesity groups. A significant difference is only observed in the frequency of consumption of clear soft drinks between the normal-weight and overweight groups, with normal-weight children

Table 1. General data and dentition type

	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	P
Age (years)	6.68 ± 2.15	11	6.29 ± 2.12	7.17 ± 2.31	6.25 ± 0.95	*P= 0.249*
Gender M/F	13/12	0/1	8 (57)/ 6 (43)	4 (67)/ 2 (33)	1 (25)/ 3 (75)	*P= 0.429*
Dentition type T/M/P n (%)	7 (28)/ 16 (64)/ 2 (8)	Permanent	5 (36)/ 9 (64)/ 0 (0)	1/ (17)/ 4 (66)/ 1 (17)	1 (25)/ 3 (75)/ 0 (0)	*P= 0.061*
Molar Class I/II/ III n (%)	17 (68)/ 4 (16)/ 4 (16)	I	10 (72)/ 3 (21)/ 1 (7)	3 (50)/ 1 (17)/2 (33)	3 (75)/ 1 (25)/ 0 (0)	*P=0.749*
Canine Class I/ II/III n (%)	17 (68)/ 8 (32)/ 0 (0)	I	9 (64)/ (36) 5/ 0 (0)	3 (50)/ 6 (50)	3 (75)/ 1 (25)/ 0 (0)	*P=0.824*

Age data are shown as mean and standard deviation; the other variables are shown with the sample size by subgroup and (percentage). Gender M (male); Gender F (Female); Dentition T (Temporary); Dentition M (mixed); Dentition P (permanent); P (Statistical significance). A chi-square (χ^2) test was performed for qualitative variables, and a Mann–Whitney U test was used to analyze age. A p-value ≤ 0.050 was considered statistically significant.

Table 2. Anthropometric evaluation

	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	*N vs Ov*	*N vs Ob*	*Ov vs Ob*
Weight (Kg)	26.42 ± 10.40	32,8	21.43 ± 5.73	28.83 ± 10.10	38.65 ± 14.57	*P= 0.099*	***P= 0.011***	*P= 0.201*
Height (cm)	121 ± 0.15	151	117 ± 14	123 ± 16	124 ± 11	*P= 0.364*	*P= 0.288*	*P= 1.000*
Waist circumference (cm)	60.32 ± 10.3	59	55.79 ± 5.02	63.17 ± 9.72	72.25 ± 16.74	*P= 0.096*	***P= 0.019***	*P= 0.517*
BMI	17.44 ± 3.99	14,3	15.33 ± 1.18	18.36 ± 2.27	24.2 ± 5.15	***P= 0.006***	***P= 0.003***	***P= 0.019***

Data are shown as means and standard deviation. Kg (Kilograms); cm (Centimeters); BMI (Body Mass Index). N (Normal Weight); Ov (Overweight); Ob (Obesity); P (Statistical significance), and n (sample size). A Mann–Whitney U test was used to analyze age. A p-value ≤ 0.050 was considered statistically significant.

Table 3. Frequency of food consumption per week

		Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	*N vs S*	*N vs OB*	*S vs OB*
Bread, Cookies, and Sweets	x̄	2.72 ± 2.62	-	2.92 ± 2.78	2.66 ± 2.33	2.75 ± 3.090	0.893	0.822	0.823
	Never n (%)	3 (12)	1 (100)	1 (7.1)	0 (0)	1 (25)	χ^2		
	1 per week n (%)	11 (44)	0 (0)	7 (50)	3 (50)	1 (25)			
	3 per week n (%)	5 (20)	0 (0)	2 (14.3)	2 (33.3)	1 (25)	*P*= 0.345		
	Every day n (%)	6 (24)	0 (0)	4 (28.6)	1 (16.7)	1 (25)			
Black Soft Drinks	x̄	2 ± 2.48	-	1.78 ± 2.42	2.83 ± 3.43	1.25 ± 1.25	0.829	0.955	0.824
	Never n (%)	9 (35)	0 (0)	5 (35.7)	3 (50)	1 (25)	χ^2		
	1 per week n (%)	7 (29)	0 (0)	5 (35.7)	0 (0)	2 (50)			
	3 per week n (%)	5 (20)	1 (100)	2 (14.39	1 (16.7)	1 (25)	*P*= 0.463		
	Every day n (%)	4 (16)	0 (0)	2 (14.3)	2 (33.3)	0 (0)			
Juices and clear Soft Drinks	x̄	0.96 ± 1.51	-	1.14 ± 1.74	0.16 ± 0.40	1 ± 1.41	**0.029**	0.766	0.236
	Never n (%)	11 (44)	0 (0)	4 (28.6)	5 (83.3)	2 (50)	χ^2		
	1 per week n (%)	11 (44)	0 (0)	9 (64.3)	1 (16.7)	1 (25)			
	3 per week n (%)	2 (8)	1 (100)	0 (0)	0 (0)	1 (25)	*P*= 0.052		
	Every day n (%)	1 (4)	0 (0)	1 (7.1)	0 (0)	0 (0)			
Vegetables	x̄	4.16 ± 2.62	-	3.71 ± 2.75	5 ± 2.19	4.25 ± 3.40	0.289	0.780	0.724
	Never n (%)	3 (12)	0 (0)	2 (14.3)	0 (0)	1 (25)	χ^2		
	1 per week n (%)	2 (8)	0 (0)	2 (14.3)	0 (0)	0 (0)			
	3 per week n (%)	9 (36)	0 (0)	5 (35.7)	3 (50)	I (25)	*P*= 0.884		
	Every day n (%)	11 (44)	1 (100)	5 (35.7)	3 (50)	2 (50)			
Fruits	x̄	4.64 ± 2.62	-	4.42 ± 2.76	5 ± 2.19	4.25 ± 3.40	0.590	0.863	0.724
	Never n (%)	1 (4)	0 (0)	0 (0)	0 (0)	1 (25)	χ^2		
	1 per week n (%)	4 (16)	0 (0)	4 (28.6)	0 (0)	0 (0)			
	3 per week n (%)	7 (28)	0 (0)	3 (21.4)	3 (50)	1 (25)	*P*= 0.294		
	Every day n (%)	13 (52)	1 (100)	7 (50)	3 (50)	2 (50)			
Seeds	x̄	0.96 ± 1.51	-	0.85 ± 1.02	1.5 ± 2.73	0.75 ± 0.5	0.929	0.814	0.724
	Never n (%)	11 (44)	1 (100)	6 (42.9)	3 (50)	1 (25)	χ^2		
	1 per week n (%)	11 (44)	0 (0)	6 (42.9)	2 (18.2)	3 (75)			
	3 per week n (%)	2 (8)	0 (0)	2 (14.3)	0 (0)	0 (0)	0,566		
	Every day n (%)	1 (4)	0 (0)	0 (0)	1 (24)	0 (0)			

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Table 3. Frequency of food consumption per week

Red meat	x̄	2.32 ± 1.88	-	2.64 ± 2.23	1.83 ± 1.32	1.75 ± 1.5	0.534	0.529	0.908
	Never n (%)	5 (20)	0 (0)	3 (21.4)	1 (16.7)	1 (25)	χ^2		
	1 per week n (%)	5 (20)	0 (0)	2 (14.3)	2 (33.3)	1 (25)			
	3 per week n (%)	13 (52)	1 (100)	7 (50)	3 (50)	12 (50)	*P*= 0.976		
	Every day n (%)	2 (8)	0 (0)	2 (14.3)	0 (0)	0 (0)			

In each food section, the first row shows the mean and standard deviation of the times each food is consumed per week except in the underweight column because there is only one participant in that subgroup. The frequencies and percentages shown are the number of participants who consume each food either always, 3 times a week, once a week, or never. A chi-square (χ^2) test was performed for qualitative variables, and a Mann–Whitney U test was used to analyze age. A p-value ≤ 0.050 was considered statistically significant.

consuming more. Chi squared analysis of the distribution of consumption of all students according to BMI showed no significant difference (Table 3).

According to the results of the dietary evaluation, 11 children consumed healthy foods: 2 obese, 3 overweight, 5 normal weight, and the participant with malnutrition. Thirteen had a fairly healthy diet: 9 normal weight, 2 overweight, and 2 obese. Only one of the participants in the overweight group had an unhealthy diet. No significant difference was observed (Table 4).

Prevalence of dental plaque in children with normal weight, overweight and obesity

More than 50% of the tooth surfaces presented dental plaque, and the percentage of dental plaque was 70.92%. No significant difference was observed among percentages of total plaque according to BMI. However, a trend was observed where the percentage of total plaque was 73.49% in children with normal weight, which was higher than the 70.82% recorded in overweight children.

Regarding type of plaque, the most predominant was stained pink, with 36.29%, followed by purple, with 30.89%, and light blue, with 14.12%. Children with normal weight had significantly more newly-formed plaque (pink, 35.72%) than acidified plaque (light blue, 11.76%) and higher prevalence of mature plaque (purple, 32.95%) than acidified plaque (light blue).

Overweight children had more newly-formed plaque (pink) than mature plaque (purple), but did not show a significant difference. Obese children had considerably more pink plaque (33.16%) than violet (26.15%) or light blue plaque (13.3%) (Table 5).

Correlations of newly formed, mature and acidified dental plaque with body mass index

There was a positive correlation between total plaque and newly formed dental plaque (pink), but a negative correlation between mature plaque (purple) and height. A positive correlation was observed between consumption of vegetables and percentage of pink plaque, and between consumption of seeds and percentage of light blue plaque.

There were positive correlations between age and weight, age and height, age and waist circumference, weight and height, weight and waist circumference, weight and BMI, height and waist circumference, and waist circumference and BMI. Vegetable consumption was related to fruit consumption (Fig. 1).

DISCUSSION

Dental plaque is a biofilm inhabited by various oral microorganisms. Its maturation over time

Table 4. Nutrition

Type of feeding	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	*P*
Healthy n (%)	11 (44)	1 (100)	5 (37.7)	3 (50)	2 (50)	
fairly healthy n (%)	13 (52)	0 (0)	9 (62.3)	2 (33.3)	2 (50)	*0.480*
Unhealthy n (%)	1 (4)	0 (0)	0 (0)	1 (16.7)	0 (0)	

The data are shown using the sample size by subgroup and (percentage). n= sample size according to whether or not the type of diet of children with normal weight, overweight, obese, and the participant with underweight is healthy or not. A chi-square (χ^2) test was performed. A p-value ≤ 0.050 was considered statistically significant.

Table 5. Dental plaque

	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	*N vs S*	*N vs OB*	*S vs OB*
% Total plaque	70.92 ± 15.80	32	73.49 ± 17.04	70.82 ± 10.44	71.80 ± 4.52	*P*= 0.386	*P*= 0.425	*P*= 0.831
% Pink plaque	36.29 ± 16.95	34	35.72 ± 19.86	40.07 ± 13.20	33.16 ± 15.92	*P*= 0.283	*P*= 0.957	*P*= 0.240
% Purple plaque	30.89 ± 23.38	39	32.95 ± 20.88	27.9 ± 36.65	26.15 ± 12.49	*P*= 0.479	*P*= 0.456	*P*= 0.829
% Light blue plaque	14.12 ± 10.54	6	11.76 ± 9.14	21.52 ± 13.92	13.30 ± 6.71	*P*= 0.114	*P*= 0.873	*P*= 0.286
Pink vs Purple	*P= 0.299*	-	*P= 0.818*	*P= 0.259*	***P= 0.248***			
Pink vs Light Blue	***P= 0.001***	-	***P= 0.001***	*P= 0.065*	***P= 0.029***			
Purple vs Light Blue	***P= 0.006***	-	***P= 0.003***	*P= 0.935*	*P= 0.110*			

The data are shown as the average and standard deviation of the percentage of plaque according to its color (state of maturation and acidification) in the groups of children with normal weight, overweight, and obesity. Regarding the participant who is underweight, the exact value is shown. A Mann–Whitney U test was used to analyze age. A p-value ≤ 0.050 was considered statistically significant.

	% Total Plaque	% Pink Plaque	% Purple Plaque	% Light Blue Plaque	Age	Weight	Height	Waist circumference	BMI	Bread, Cookies, and Sweets	Black Soft Drinks	Juices and clear Soft Drinks	Vegetables	Fruits	Seeds	Red meats
% Total Plaque	1	0.446*	0.292	0.066	-0.087	-0.206	-0.159	-0.034	-0.023	0.123	-0.163	-0.15	0.186	0.148	0.047	-0.227
P		0.026	0.156	0.755	0.678	0.323	0.447	0.872	0.912	0.557	0.436	0.474	0.372	0.48	0.823	0.275
% Pink Plaque		1	-0.248	0.134	0.307	0.128	0.247	0.223	0.048	0.12	-0.015	0.132	0.440*	0.016	0.033	0.046
P			0.232	0.524	0.135	0.542	0.234	0.285	0.821	0.569	0.945	0.528	0.028	0.939	0.874	0.825
% Purple Plaque			1	-0.314	-0.383	-0.352	-0.410*	-0.346	-0.026	0.003	0.282	-0.007	0.091	0.029	-0.248	0.171
P				0.127	0.059	0.084	0.042	0.09	0.901	0.99	0.172	0.973	0.664	0.891	0.231	0.413
% Light Blue Plaque				1	-0.171	-0.121	-0.149	-0.061	0.119	-0.087	-0.256	-0.356	0.076	-0.049	0.526**	-0.349
P					0.414	0.566	0.476	0.772	0.572	0.679	0.217	0.081	0.717	0.817	0.007	0.087
Age					1	0.788**	0.930**	0.608**	0.11	-0.158	0.126	0.063	0.211	0.037	-0.072	0.05
P						0.001	0.001	0.001	0.6	0.45	0.547	0.764	0.312	0.859	0.732	0.814
Weight						1	0.887**	0.801**	0.557**	-0.19	0.097	-0.038	0.112	-0.043	-0.082	-0.025
P							0.001	0.001	0.004	0.363	0.644	0.855	0.595	0.839	0.697	0.906
Height							1	0.661**	0.158	-0.107	0.222	0.039	0.128	0.026	-0.104	0.637
P								0	0.451	0.612	0.285	0.852	0.543	0.901	0.62	0.06
Waist circumference								1	0.604**	-0.21	0.04	-0.017	0.052	-0.048	0.046	0.775
P									0.001	0.313	0.849	0.937	0.804	0.818	0.829	-0.199
BMI									1	-0.102	-0.141	-0.225	0.113	-0.136	-0.028	0.34
P										0.628	0.501	0.279	0.59	0.515	0.893	0.272
Bread, Cookies, and Sweets										1	0.334	-0.088	0.164	0.366	-0.152	0.188
P											0.103	0.675	0.434	0.072	0.469	0.45
Black Soft Drinks											1	-0.003	-0.072	-0.013	-0.271	0.024
P												0.988	0.732	0.95	0.189	0.334
Juices and clear Soft Drinks												1	-0.129	-0.323	0.006	0.102
P													0.538	0.115	0.975	0.196
Vegetables													1	0.456*	-0.129	0.347
P														0.022	0.538	0.15
Fruits														1	-0.185	0.475
P															0.376	-0.215
Seeds															1	0.301
Red meats																1

*Fig 1: Spearman correlation. The value of each correlation is shown with its p value. BMI (Body Mass Index); P (statistical significance); * (P<0.05); ** (P<0.001).*

and excessive accumulation can lead to dysbiosis, resulting in dental caries, gingivitis, periodontitis, and other diseases[2,19]. However, plaque can be removed by effective tooth brushing techniques, flossing, and other adjuncts, thereby preventing such diseases.

In the current study, no significant difference in the percentage of total plaque was observed among the three types of dentition. Recently formed plaque (stained pink) predominated in all children, indicating infrequent or absent tooth brushing, since even a less effective brushing technique will remove some recently deposited plaque (less than 24 hours old). Areas of acidified plaque (stained light blue) suggest infrequent and ineffective brushing that allows plaque to mature and become acidified. It has been reported that the most predominant bacterial species in acidified plaque is *S. mutans*, and that children with more plaque of this type have more caries[20].

The fact that in normal-weight children there was a significant predominance of newly-formed plaque compared to mature and acidified plaque indicates that they did not brush before their appointment.

Overweight children had more pink plaque, although the difference was not significant. They also presented the most acidified plaque, indicating low or no brushing efficiency in the last 48 hours. Obese children presented differences between pink and light blue plaque, showing a similar pattern to children with normal weight.

A relationship between the number of cavities and BMI has been reported in adolescents[21] underweight, overweight or obese. Dental caries was diagnosed according to the criteria recommended by the World Health Organization (WHO. Although a meta-analysis published by Angelopoulou et al. found that the results of the studies included were inconsistent, it suggests that overweight or obese children have a higher risk of early childhood cavities[22]. Other studies have also concluded that obese or overweight children may be more likely to have cavities[23,24] if they do not practice good oral hygiene discipline. High BMI and obesity have been found to be associated with the plaque index, which indicates dental plaque and periodontal pocket depth (PD), a metric strongly related to periodontal inflammation and infection[25].

The connection between dental cavities and BMI is biologically plausible because dental plaque accumulation and irregular dietary habits influence the development of both dental decay and obesity[26]. Moreover, caries and obesity share common modifiable factors such diet and lifestyle, highlighting their interconnected nature[27]. Dental caries prevalence has also been related to disadvantaged socioeconomic status[28].

The positive correlation between immature (pink) and total plaque suggests collinearity since there was more pink than purple and light blue plaque. In this sense, it was expected to find a positive correlation with the most prevalent type of plaque, which was pink and the height; however, this correlation was observed with the purple plaque.

The multiple correlations among anthropometric measurements show collinearity; thus, height and age are correlated. The oldest children presented the most significant amount of mature plaque, suggesting that children tend to have less hygiene as their age increases, and highlighting the importance of implementing hygiene education strategies that effectively reduce plaque[29,30]. The highest amount of plaque was observed on upper and lower anterior teeth, in agreement with several other studies[4,5,17]. It has been observed that people who brush their teeth with their right hand have more plaque in the right upper quadrant[31], perhaps because brushing is generally started more energetically in the left quadrant, and becomes less effective when it moves to the right quadrant.

Two participants had permanent dentition. One of them was found to have an early tooth replacement process since he was just ten years old, whereas permanent teeth are usually complete between 11 and 12 years of age[32]. It is worth mentioning that this participant was overweight according to BMI, and it has been reported that permanent dentition is acquired earlier in obese children than in children with normal weight[33]. Similarly, one study reported an association between nutritional status and chronology in the first molar, lateral incisor and first lower premolar in children 8, 9 and 10 years old with obesity[34], and another reported that obesity and overweight were associated to early permanent tooth eruption in males[35].

In contrast, the other child with permanent dentition (age 11 years) had low weight and was referred for nutritional consultation. Because he was underweight, we would have expected that he would not yet have all his permanent teeth; however, this case appears to have been an exception.

Regarding diet, healthy foods such as fruits and vegetables were consumed infrequently by most of the children. Ten of the children were overweight or obese, so although they reported that they did not frequently consume foods high in sugar, possibly when they do consume them, it is in larger quantities. The present study may provide a basis for future studies evaluating the relationship between diet, anthropometric data, percentage of dental plaque, and presence of caries and gingivitis.

It demonstrates the importance of performing dental plaque disclosure for diagnostic and educational purposes for both children and parents, which, if applied consistently, would help reduce the prevalence of caries and periodontal disease.

CONCLUSION

Newly formed plaque (pink) was predominant in all children, regardless of dentition type. A higher

prevalence of newly formed plaque was observed in older children. Based on body mass index (BMI), no significant difference in the amount of plaque was found among children of normal weight, overweight and obese. However, the results indicate that obese children have more acidic plaque accumulation than normal-weight children, suggesting that obesity is a factor related to diet and oral hygiene. Although the results do not indicate that dental plaque accumulation depends on BMI, they demonstrate high levels of plaque in all participants. It is therefore important to teach brushing techniques and use dental plaque staining as a clinical activity, which, if applied consistently, could reduce plaque and caries rates.

CONFLICT OF INTERESTS
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Dental fear and dental anxiety: bibliometric analysis of the 100 most frequently cited papers

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ABSTRACT

Dental fear and anxiety are feelings that are often present at dental appointments and have been studied for a long time. ***Aim:*** *To identify and critically review the 100 most frequently cited papers on dental fear and dental anxiety (DFA).* ***Materials and Method:*** *The 100 most frequently cited papers on DFA were retrieved from Web of Sciences (All databases) using a combined search strategy. Key bibliometric indicators were extracted. The methodological quality of the studies was assessed by the Mixed Methods Appraisal Tool (MMAT) except for reviews and systematic reviews, which were evaluated by the Joanna Briggs Institute (JBI) tool.* ***Results:*** *Citations for the 100 most frequently cited papers ranged from 81 to 882. The largest number of papers was from the United States of America (22 papers; 3850 citations). Community Dentistry and Oral Epidemiology was the journal with highest number of papers (27 papers; 3153 citations). The most frequently cited author was Corah NL (1390 citations). Cross-sectional study design was the most common (67 studies). The topics covered by the studies were diverse, highlighting studies on the development and validation of assessment tools. There were 17 validated assessment tools, of which The Dental Anxiety Scale was the most often used (28 studies). There were five terms used to refer to DFA. Most papers were of intermediate quality.* ***Conclusion:*** *This bibliometric analysis identified the 100 most frequently cited papers on DFA and the topics covered. "Dental anxiety" was the term most often used, although more recent research includes both "fear" and "anxiety". The Dental Anxiety scale was the most frequently used assessment tool. Higher quality papers are encouraged to improve knowledge on DFA.*

Keywords: *bibliometrics - citations - dental anxiety - dental fear - dentistry*

Medo e ansiedade odontológicos: revisão bibliométrica dos 100 artigos mais citados

RESUMO

O medo e a ansiedade odontológicos são sentimentos que estão frequentemente presentes nas consultas odontológicas e são estudados há muito tempo. ***Objetivo:*** *identificar e revisar criticamente os 100 artigos mais citados sobre medo e ansiedade odontológicos (MAO).* ***Materiais e Método:*** *Os 100 artigos mais citados do MAO foram recuperados da Web of Sciences – All databases. Foram extraídos indicadores bibliométricos. A qualidade metodológica dos estudos foi avaliada pelo Mixed Methods Appraisal Tool (MMAT), exceto para revisões e revisões sistemáticas que foram avaliadas pelo instrumento do Joanna Briggs Institute (JBI).* ***Resultados:*** *As citações para os 100 artigos mais citados variaram de 81 a 882. O maior número de artigos foi originado nos Estados Unidos da América (22 artigos; 3850 citações). Community Dentistry and Oral Epidemiology foi o periódico com maior número de artigos (27 artigos; 3153 citações). O autor mais citado foi Corah NL (1390 citações). O desenho de estudo transversal foi o mais comum (67 estudos). Os temas abordados pelos estudos foram diversos, destacando-se estudos voltados para o desenvolvimento e validação de instrumentos de avaliação. Foram 17 instrumentos de avaliação validados, dos quais a Dental Anxiety Scale foi o mais utilizado (28 estudos). Havia cinco termos usados para se referir ao MAO. A maioria dos artigos era de qualidade intermediária.* ***Conclusão:*** *Esta análise bibliométrica elucidou as citações e os temas abordados. Ansiedade odontológica foi o termo mais usado, no entanto, pesquisas mais recentes incluem o termo medo e ansiedade. A Dental Anxiety Scale foi o instrumento de avaliação mais utilizado. Artigos de maior qualidade são incentivados para melhorar o conhecimento sobre medo e ansiedade odontológicos.*

Palavras-chave: *bibliometrica - citação - ansiedade ao tratamento odontológico - medo ao tratamento odontológico - odontologia*

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INTRODUCTION

Dental fear and dental anxiety, which are feelings often induced by dental appointments, have been studied for a long time through research on various aspects such as definition, prevalence, aetiology and management[1-4].

Dental anxiety refers to a state of apprehension that something terrible will happen in relation to dental treatment, with a sense of loss of control[3]. Anxiety is characterised as "the anticipation of future threats and is more associated with muscle tension and vigilance"[5]. Dental fear is a type of anxiety in the face of well-known situations such as injections or dental situations in general[3]. The American Psychological Association (APA) describes fear as "an emotional response to real or perceived threats, usually associated with reactions of excitability preparing for fight or flight"[5]. Dental fear causes a series of effects in the body, generating emotional, physiological and behavioural responses such as defence reactions[3]. A severe, persistent type of dental fear is known as dental phobia[3], since phobia is defined as a "persistent fear of situation, object or activity resulting in huge necessity of avoidance"[5].

Seeking dental care is important in order to support oral health[6]; nevertheless, fear and anxiety often create barriers that prevent people from doing so[7]. Even when patients attend a dental appointment, they may still have feelings and reactions to it[8]. "Dental fear and dental anxiety" (DFA) may be used as a composite term to refer to strong negative feelings related to dental settings[3,9].

DFA can be measured by many different instruments with different names[10-13], which vary regarding the number of questions and the appropriate time at which to assess the feeling[10,11]. Some of them are used for epidemiological studies, and others to measure DFA in the dental office[12-15]. Another difference among assessment tools is what they measure: whether state anxiety, related to how the person feels at the time of the appointment, or trait anxiety, which is assessed outside the dental setting and measures dental anxiety across different procedures or contexts related to dental care[16].

Bibliometric analysis is a scientific method that can be useful to examine academic productivity in different fields such as medicine, dentistry and business[17-19]. It consists of a review of papers published on the topic of interest, and includes all study designs such as descriptive, observational, experimental, qualitative and reviews, to account for all the evidence[20]. Bibliometric reviews differ from systematic reviews. While a systematic review aims to respond to a clear question based on the quality of the evidence[21], a bibliometric review is an enumeration of evidence such as main authors, most impactful papers, collaborations, and countries of the first author in a particular field of existing literature[20,21], enabling the study of publication and collaboration patterns, and exploration of the intellectual structure of a research field or a journal[18]. Moreover, a bibliometric review can build a foundation for advancing research on a certain topic in a new way[18,21]. Recent bibliometric reviews have been conducted on child oral health and outcomes[4,17,22], but there is no equivalent evidence on DFA. While a distinction between fear and anxiety may be made in theory, in epidemiological and clinical settings the definition of these constructs remains subjective.

Citations indicate the level of interest that a scientific study has received from the scientific community, even though they are not a perfect measure of quality. Furthermore, finding a pattern in the use of terminology helps avoid confusion in its use. Thus, the objective of this study is to identify and review critically the 100 most frequently cited papers on the topic of DFA in order to understand and describe aspects related to it.

MATERIALS AND METHOD

This bibliometric study, taking a qualitative approach, retrieved and analysed the papers on DFA. The search was conducted using Web of Science - All Databases (WoS-AD)[17,21]. The search strategy was prepared based on other reviews of DFA with the following terms: ("dental anxiety" OR "dental fear" OR "dental phobia" OR "odontophobia") AND ("pediatric dentistry") AND (child*) OR ("adolescent*") AND ("oral health problem")[9,23,24]. Papers published up to April 2024 were searched with no language restriction. Research papers for which the main outcome was any aspect of DFA were included. Conference papers were excluded.

One researcher (ACP) selected the papers to identify the 100 most frequently cited papers[17,21,25]. The list of the 100 most frequently cited papers on DFA is displayed in descending order, based on the number of citations in WoS-AD. In the event of a draw, the

ranking is based on the number of citations per year (citation density). Citation counts for each paper in Google Scholar and Scopus databases were also collected[17,21,22].

A spreadsheet was created with the following data: title, authors, journal, number of citations, citation density, study design, year of publication, country (first author affiliation), terminology used, assessment tool and sample age. Study designs were classified as follow: clinical trial (randomized or not), cross-sectional, longitudinal, non-systematic review, systematic review, and validation. Papers were grouped into assessment tool development, assessment tool validation, DFA acquisition, DFA management, review of assessment tools, and epidemiological surveys on factors associated to DFA. Spearman's rank correlation coefficient test was performed at the Statistical Package for Social Sciences (SPSS for Windows, version 24.0; IBM Corp) for citations.

The review followed the BIBLIO guideline for bibliometric reviews to enhance transparency[20]. The methodological quality of the validation, cross-sectional, cohort and clinical trials studies was assessed according to the Mixed Methods Appraisal Tool (MMAT)[26]. The MMAT provides a methodological quality criterion for different study designs in a single tool, thereby allowing a direct comparison of quality of evidence in the different types of studies included[26,27,28]. This tool supports critical presentation of quality, rather than an overall score[29]. Systematic and other reviews were evaluated using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Systematic Reviews and Research Synthesis[30].

RESULTS

The search strategy recovered 3,857 references from WoS-AD. Thirty-eight papers were excluded because they did not focus on DFA. The included studies covered all age groups (children, adolescents, adults and elderly people) (Supplementary file 1). Whilst most papers reported local studies (n=64), eleven were national and six were multi-centre.

The 100 most frequently cited papers on DFA were cited 13,957 times altogether (median: 113.5; minimum: 81 citations; maximum: 882 citations) in WoS-AD. Papers were more often cited in Google Scholar than in other databases (median: 280.5; minimum: 117 citations; maximum: 1,990 citations). Positive correlations were found between the number of citations in WoS-AD and Scopus (r = .995) and WoS-AD and Google Scholar (r = .975). The most frequently cited paper on DFA was "Development of a dental anxiety scale" first-authored by Corah NL (1969), which is also the oldest paper published. Corah NL was the most frequently cited author of all publications, with 2 papers and 1,390 citations. The most recent papers were published in 2017 and were authored by Cianetti et al. in Italy, Seligman et al. in the USA and by Lin et al. in Taiwan. The *Community Dentistry and Oral Epidemiology* (27 papers; 3,153 citations) and the *Journal of* the *American Dental Association* (10 papers; 2,056 citations) published the most papers included in the list of the 100 most frequently cited papers on DFA (Supplementary file 1).

Europe was the continent with most papers (54 papers; 7,208 citations) followed by Anglo-Saxon America (30 papers; 4,807 citations). Latin America had only one paper, published in Brazil (84 citations), and the African continent had no paper on the list. At country level, USA had the highest number of both papers and citations (22 papers; 3,850 citations). Papers from the England (11 papers; 1,513 citations), Netherlands (10 papers; 1,265 citations), Sweden (9 papers; 1,586 citations) and Australia (8 papers; 1,046 citations) were cited more than one thousand times. (Fig. 1).

Table 1 presents summary data on the authors with at least two papers included in the list of the 100 most frequently cited papers on DFA. These authors published between 6 and 447 papers and were cited between 425 and 34,899 times in WoS-AD. Armfield JM and Locker D were the authors with the most papers on the list of the 100 most frequently cited papers on DFA.

Most of the papers on the list were epidemiological surveys (67 cross-sectional; 1 cohort, and 3 randomized clinical trials), followed by 18 reviews (4 systematic reviews) and 11 validation studies (4 assessment tool development). Most papers were classified as epidemiological surveys on DFA associated factors. Eight studies were designed to review assessment tools. Different terminologies were found, with "dental anxiety" being the most frequently used term (61 studies), followed by "dental fear" (25 studies), "dental fear and anxiety" (11 studies), "dental phobia" (2 studies) and "dental fear and dental phobia" (1 study) (Table 2). "Dental

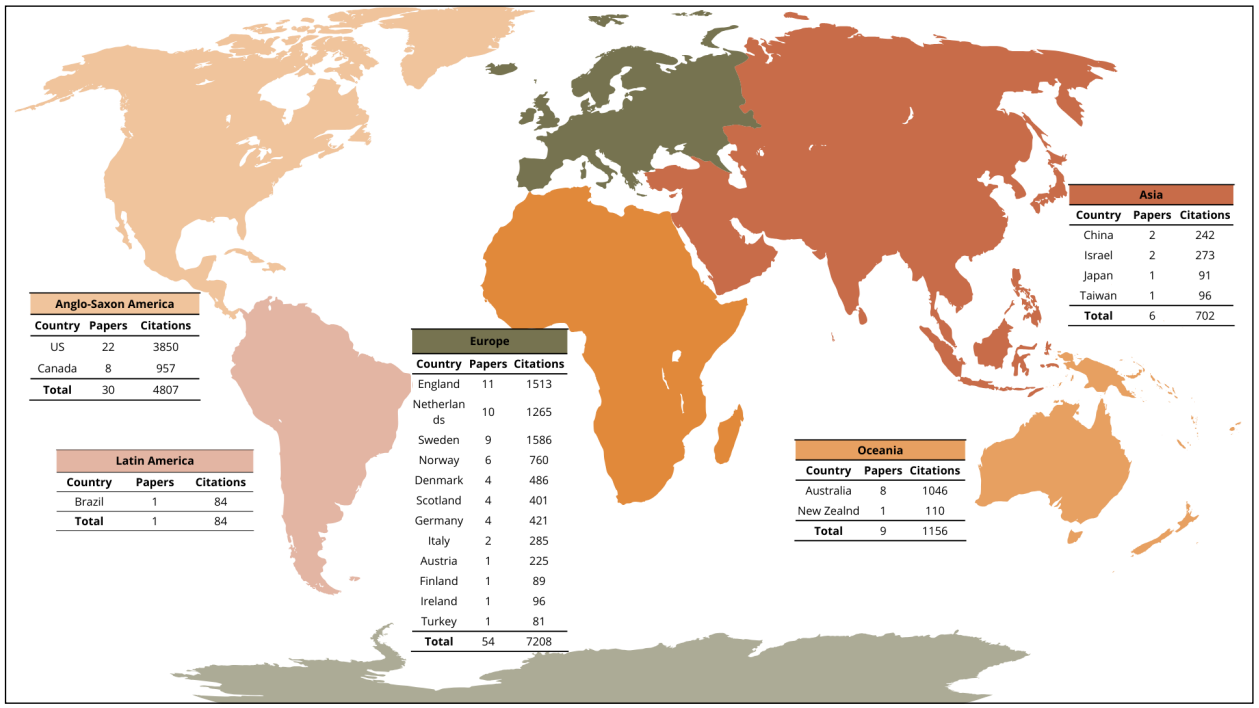

Fig. 1: Global distribution of 100 most frequently cited papers on dental fear and dental anxiety.

Table 1. Bibliometric indicator of authors with at least 2 publications included in the 100 most frequently cited papers on dental fear and dental anxiety.

Author	Number of citations in WoS-AD	Total papers in WoS-AD	h-index WoS	Number of citations in top 100 DFA	Number of papers in top 100 DFA
Milgrom P	34,899	447	63	588	3
Locker D	17,436	415	74	610	5
Humphris G	11,195	360	57	334	3
Berggren U	5,668	195	41	428	2
De Jongh A	5,850	235	42	190	2
Corah NL	2,940	79	26	1390	2
Kleinknecht RA	2,739	54	28	296	2
Armfield JM	2,653	92	26	850	6
Klingberg G	1,930	79	21	599	2
Skaret E	1,382	40	22	182	2
Ten Berge M	1,315	42	20	244	2
McNeil DW	693	65	13	191	2
Moore R	512	14	9	391	3
Oosterink F	425	6	6	312	2

fear and anxiety" as a composite term has been more often accepted in recent papers (Supplementary file 1).

The 100 most frequently cited papers on DFA used 31 different assessment tools, including single instruments or combinations of instruments. The Dental Anxiety Scale was the most often used instrument in the 82 primary research papers (alone in 28 studies; combined with other instruments in 13), followed by the Dental Fear Scale (alone in 5 studies, combined with other instruments in 5) (Table 3). Table 3 also shows the categorization of

Table 2. Characteristics of the100 most frequently cited papers on dental fear and dental anxiety.

	Number of papers	Number of citations	Citation ratio[a]
Study Design			
Cross-sectional	67	8672	129,4
Longitudinal	1	110	110
Clinical trial (randomized)	3	323	107,6
Non-systematic review	14	2406	171,8
Systematic review	4	412	310
Validation	11	2034	184,9
Topic			
Assessment tools validation	7	801	114
Assessment tools development	4	1233	308
DFA acquisition	14	1867	133,3
DFA management	9	1574	174,8
Epidemiological survey	58	7236	124,7
Review of assessment tools.	8	1246	155,7
Terminology			
Dental anxiety	61	8421	138,0
Dental fear	25	3404	136,1
Dental fear and anxiety	11	1664	151,2
Dental fear and dental phobia	1	191	191,0
Dental phobia	2	277	138,5

the studies regarding assessment tool development, assessment tool validation, cross-sectional epidemiological surveys, longitudinal surveys and clinical trials. Seventeen validated instruments were found across the primary research papers, the oldest being from 1952. The Dental Anxiety Scale was the oldest used in the papers on the list. Table 4 presents the evolution of these scales, indicating their year of development and the period of use, according to the studies.

The quality appraisal is presented as a supplementary file (S2 and S3). Most of the studies are classified as having medium quality. The aspect that contributed the most to the lower quality of the papers relates to poor evidence on sample selection. The inclusion and exclusion criteria were unclear in most of the papers. Additionally, incomplete outcome data (<80%), was observed in many studies. Otherwise, almost all studies had appropriate indicators related to the variable measurement. The review papers were classified as medium and low quality. Most of them were not systematic reviews, and there were limitations relating to inclusion criteria, search strategy and analysis.

DISCUSSION

The results of this review show that the 100 most frequently cited papers on DFA were published over a 58-year period (1969 and 2017) and are of intermediate quality. Most of them were conducted in Anglo-Saxon America and Europe, involving primary research exploring factors associated with DFA. “Dental anxiety” was the most common term, followed by “dental fear”, whilst the term “dental phobia” appeared in a few studies. The Dental Anxiety scale was the assessment tool with most citations in the review.

A higher total number of citations was observed in the Google Scholar and Scopus databases than in the WoS-AD. Although the number of citations in the different databases assessed were positively correlated, showing agreement, Google Scholar had a higher number of citations because it retrieves citations from open-access online journal papers, books and non-academic sources.

The 100 most frequently cited papers on DFA were written over a considerable number of years, beginning in 1969. Interestingly, another review showed that the first papers on aspects of DFA date

Table 3. Description of assessment tools used in the studies regarding of number of papers, citation and first authors*

Assessment tool	Number of papers	Citation on WoS-AD	Authors
Dental Anxiety Scale	28	4678	Corah NL (1)[a], Corah NL (2)[b], Berggren U (4)[c], Schuller AA (14)[c], Reisine ST (21)[c], Kent G (24[c], Winocur E (27[c], McGrath C (28)[c], Lidell A (33)[c], Towned E (34)[c], Sohn W (35)[c], Eitner S (38)[c], Locker D (41)[c], Oosterink FMD (43)[c], Maggrias J (47)[c], Moore R (48)[c], Thomson WM (56)[e], Auerbach SM (57)[c], Moore R (58)[c], DeJongh A (61)[c], Doerr PA (64[c], Blomqvist M (65)[c], Sullivan MJL (72)[c], Berggren U (80)[c], Hagglin C (89)[c], Locker D (94[e], Goettems ML (95)[c], Abrahamsson KH (97)[c]
Modified Dental Anxiety Scale	6	777	Cohen SM (19)[c], Humphris GM (25)[b], Hill KB (29)[c], Kritsidima M (37)[d], Buchanan H (71)[c], Humphris G (78)[b]
CFSS-DS	5	690	Klingberg G (11)[c], Migrom P (18)[c], Ten Berge M (41)[c], Ten Berge M (44)[c], Wogelius P (73)[c]
Structured questionnaire	5	777	Milgrom P (6)[c], Lautch H (12)[c], Davey GCL (22)[c], Gatchel RJ (50)[c], Wong M (99)[c]
Dental Fear Scale	5	569	Kleinknecht G (23)[a], Kleinknecht G (30)[b], McNeil DW (63)[c], Mcneil DW (91)[c], Bradley MM (98)[c]
Dental Anxiety Scale, Structured questionnaire	3	538	Hakeberg M (10)[c], Milgrom P (13)[c], Vassend O (17)[c]
Dental Fear Scale, Dental Belief Scale, Geer Fear Scale	2	182	Skaret E (75)[c], Skaret E (86)[c]
Spielberg Trait Anxiety and State anxiety scales	2	331	Lehrner J (7)[c], Stouthard MEA (62)[c]
Structured Clinical Interview for DSM	2	286	Oosterink FMD (9)[c], Ost LG (74)[c]
Single question	2	207	Armfield JM (46)[c]; Pohjola V (83)[c]
Dental Anxiety Scale, GATCHEL, Dental Fear Scale	2	292	Locker D (8)[c], Locker D (93)[c]
Dental Fear Scale, Dental Belief Scale, Dental Anxiety Scale, Geer Fear Scale	1	174	Johnsen BH (15)[c]
Dental Anxiety Question	1	170	Armfield JM (16)[c]
Dental Anxiety Scale, Single question	1	165	Moore R (20)[c]
Visual Analogue Scale	1	149	Facco E (26)[b]
Dental Anxiety Scale, Visual Analogue Scale	1	124	Eli I (39)[c]
Dental Anxiety and Fear IDAC-4C+	1	110	Armfield JM (53)[c]
Visual Analogue Scale, Spielberg Trait Anxiety and State anxiety scale	1	110	Karst M (54)[d]
Dental Anxiety Scale, GATCHEL, Single question	1	110	Locker D (55)[c]
Dental Anxiety Question, Dental Anxiety Scale	1	107	Neverlien PO (60)[a]
Dental Anxiety Scale, Dental Fear Scale	1	101	Mehrstedt M (66) [c]
Dental Anxiety Scale, Modified Dental Anxiety Scale	1	96	Howard KE (70)[b]
The Dental Anxiety Inventory - Short form	1	93	Ng SLW (76)[c]
Modified version of Geer's Fear Survey Schedule	1	93	Bernstein DA (77)[c]
CFSS-DS, Frankl Scale	1	91	Nakai Y (81)[b]
Modified Dental Anxiety Scale, LEO-DEQ	1	89	Humphris G (82)[c]
Venham Anxiety Rating Scale	1	87	Isong IA (87)[d]
Dental Anxiety Scale, Index of Dental Anxiety and Fear	1	87	Armfield JM (88)[a]
Hierarchical Anxiety Scale	1	86	Enkling N (90)[c]
Dental Anxiety Scale, Spielberg Trait Anxiety and State anxiety scales	1	86	Weisenberg M (92)[c]
Modified Dental Anxiety Scale, Dental Fear Scale	1	81	Tunc EP (100)[c]

WoS-AD: Web of Science – All databases; *Review studies were not considered.
[a]Assessment tools development; [b]Assessment tools validation; [c]Cross-sectional epidemiological surveys; [d]Clinical trial surveys; [e]Longitudinal surveys

Table 4. Description of key validated instruments used in the studies regarding development dates, authors and range dates of presentation in the review

Assessment tool	Author of the original scale	Year of publication of the original scale	Range of dates of use in the review
Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)	American Psychiatric Association	1952	1985-2009
Frankl Scale,	Frankl SN et al	1962	2005
Geer Fear Scale/ Modified Geer Fear Scale	Geer JH	1965	1998-2003
Dental Anxiety Scale	Corah NL	1969	1969-2012
Hierarchical Anxiety Scale	Gale EN	1972	2006
Dental Fear Scale	Kleinknecht RA et al	1973	1978-2008
Visual Analogue Scale	Hornblow AR and Kidson MA	1976	2013
Venham Anxiety Rating Scale	Venham LL et al	1980	2014
Children Fear Survey Scale-Dental Subscale	Cuthbert MI and Melamed BG	1982	1995-2005
Spielberg Trait Anxiety and State Anxiety Scales	Spielberg CD et al.	1983	1990-2000
Dental Fear Belief	Smith T	1987	1998-2003
Gatchel Fear Scale	Gatchel R	1989	1999-2003
Dental Anxiety Question	Neverlien O	1990	1990-2006
Modified Dental Anxiety Scale	Humphris G et al	1995	2000-2013
The Dental Anxiety Inventory - Short form	Aartman IH	1998	2008
Level of Exposure-Dental Experiences Questionnaire (LEO-DEQ)	Oosterink FMD	2008	2011
Dental Anxiety and Fear IDAC-4C+	Armfield JM	2010	2010-2013

from the late 1800s and early 1900s[4]. Other research topics, such as oral health-related quality of life and non-pharmacological behaviour management techniques have shorter publication intervals for the most frequently cited papers[17,31], demonstrating that DFA has been important to dental clinical practice for over a century.

The fact that most papers in this study were from Anglo-Saxon America and Europe agrees with other bibliometric reviews in dentistry[31,32]. The papers were published in four of the five continents, with none from Africa and only one from Latin America. Funding for research has a significant impact on publication, with low-income countries participating less in scientific research due to some barriers, including lack of funding[33,34]. This indicates the importance of sharing resources and building collaborations to improve the number of studies published in developing countries.

Most of the papers retrieved in this review were cross-sectional studies (n=67), a similar result to bibliometric reviews[17,32]. Cross-sectional research is important, although some gaps in science will only be filled with different study designs, including randomized controlled trials and cohort studies that follow all the relevant guidelines. Consequently, systematic reviews will help to build up evidence-based science and practice[35].

Studies on DFA use different terms to describe the feeling. The most common is "dental anxiety", followed by "dental fear", and in third place the unique term, "dental fear and anxiety", used in 11 papers, most of which were published after the year 2000, suggesting that the combined term appears to be becoming accepted in recent publications[3,8,9]. "Dental phobia" refers to exacerbated dental fear which is persistent and unreasonable, but was not a common expression[3], having been used in only three studies.

Different assessment tools can be used to measure DFA, which can be self-reported or proxy-reported[16,36]. This review also looked at the development of the assessment tools over the years, and how they are used in the 100 most frequently cited papers on DFA. The most frequently used assessment tool was the Dental Anxiety Scale. Although it is not the first one to have been developed, it was the first one made specifically for dental situations[10]. The Dental Fear Survey was the 23rd most cited paper[11]. The Dental Anxiety Scale

and the Dental Fear Scale are the most frequently used scales in epidemiological surveys nowadays, which accounts for the presence of both on this list[36]. Seven papers on the list referred to validation processes. Validation processes are important to guarantee that an assessment tool has good psychometric properties on the target population, which accounts for the number of citations of the papers on development of assessment tools[21]. The paucity of validation studies compared to tools in this citation list may simply be due to the fact that such studies may not be cited as much as papers reporting the creation and use of an instrument.

A bibliometric review is important to describe how the literature on a specific topic has been covered, by examining publications and the research constituents[21]. Several aspects of DFA were covered by the papers in the 100 most frequently cited list, reflecting the different advances in knowledge that have taken place over the years. Thus, the aim of this review was not to answer a specific clinical question, but to provide an overview of the topic and the gaps in knowledge that need to be covered by future research. This bibliometric review was conducted following the most recent guidelines[18,20,25], and evaluated the quality of the 100 most frequently cited papers on DFA, finding medium to high quality overall. Thus, it may be considered a reliable source of evidence-based information for clinical practice, policy-making or future research. This study provides a guide to help authors develop new research, identify the most significant associations and find opportunities to establish new collaborations, mainly in countries with higher barriers for research. In addition, identifying the authors and research groups that have had the greatest impact may guide clinicians and policymakers to choose the best evidence on which to base their decisions.

The limitations of the study are that (1) a bibliometric review cannot provide conclusive summary of the effect of interventions or robust evidence related to a research question (which is a known limitation of this type of study)[20,21]; (2) the quality of the studies was intermediate overall, suggesting that greater effort is needed to guarantee higher quality research; and (3) important recent papers in the field will take time to achieve the volume of citations that would enable them to be selected, and they thus remain unrepresented.

In conclusion, this bibliometric analysis identified the 100 most frequently cited papers on DFA found in the WoS-AD. The citations of these papers range from 81 to 882 over a 58-year period. They were mainly published by countries in the Global North, and cover different aspects of DFA. Whilst "dental anxiety" is the most common term, there appears to be more emphasis on "dental fear and anxiety" in more recent studies. The Dental Anxiety Scale was the most frequently used assessment tool in this list. The methodological quality assessment suggests the need for higher quality research in this field. Longitudinal and randomized clinical trials should be encouraged in order to produce further evidence.

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CONFLICT OF INTERESTS

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Effect of thermocycling and surface treatments on the bond strength of a hybrid PICN ceramic

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ABSTRACT

This study evaluated the bond strength of a hybrid PICN ceramic before and after being subjected to 5000 cycles of thermocycling. PICNs are a promising alternative in the field of CAD/CAM dental restorations; however, their adhesive behavior with chemical surface treatments such as silane and 10-MDP, and micromechanical treatments such as hydrofluoric acid and sandblasting is not yet fully understood. ***Aim:*** *To compare the bond strength of a hybrid PICN ceramic treated with different surface protocols before and after a thermocycling process.* ***Materials and methods:*** *An in-vitro experimental study was conducted. Forty PICN ceramic slices were prepared and divided into four groups. Each group received a specific surface treatment (sandblasting or acid etching) and a different adhesive technique (silane + adhesive or adhesive only). Composite cylinders of 3 mm diameter mm were bonded to each slice, and shear strength was measured on a universal testing machine immediately, and after a period of thermocycling. Statistics: Data were analyzed by ANOVA followed by Tukey's post hoc test. All statistics were analyzed with a 95% confidence interval.* ***Results:*** *The group subjected to sandblasting followed by silane and universal adhesive achieved the highest adhesion values, both immediately and after thermocycling (16.3 MPa and 11.2 MPa, respectively), and the group subjected to hydrofluoric acid etching and adhesive had the lowest values, both immediately and after thermocycling (8.6 MPa and 5.4MPa).* ***Conclusions:*** *Cementation of a hybrid ceramic treated with sandblasting, silane and a 10-MDP-based adhesive ensures high bond values, even when aged under hot, humid conditions. Thermocycling significantly reduced the adhesive strength in all groups, the decrease being more noticeable in those that did not include silane.*

Keywords: *tooth adhesion - surface - treatments - ceramics - hybrid*

Efecto del termociclado y tratamientos superficiales en la resistencia de adhesión de una cerámica híbrida PICN

RESUMEN

En este estudio se evaluó la fuerza de adhesión de una cerámica PICN híbrida, sometida a 5000 ciclos de termociclado. Las PICN representan una alternativa prometedora en el campo de las restauraciones dentales CAD/CAM; sin embargo, su comportamiento adhesivo con tratamientos de superficie químicos como el silano y la molécula de 10MDP y tratamientos micromecánicas como el uso de ácido fluorhídrico y arenado aún no se conoce por completo. ***Objetivo:*** *Comparar la fuerza de adhesión de una cerámica PICN híbrida tratada con diferentes protocolos de superficie antes y después de un proceso de termociclado.* ***Materiales y métodos:*** *Se realizó un estudio experimental in vitro en el que se prepararon 40 láminas de cerámica PICN y se dividieron en cuatro grupos. Cada grupo recibió un tratamiento superficial específico (chorro de arena o grabado ácido) y una técnica adhesiva distinta (silano + adhesivo o sólo adhesivo). Se adhirieron cilindros de composite de 3 mm de diámetro a cada lámina y se midió la resistencia al cizallamiento inmediatamente y posterior a un periodo de termociclado en una máquina de ensayos universales. Estadísticas: Los datos se analizaron mediante ANOVA seguido de la prueba post hoc de Tukey. Todas las estadísticas se analizaron con un intervalo de confianza del 95%.* ***Resultados:*** *El grupo que se sometió a arenado seguido de silano y adhesivo universal alcanzó los valores de adhesión más altos, tanto inmediatamente como después del termociclado (16,3 MPa y 11,2 MPa, respectivamente) y el grupo sometido al ataque de ácido fluorhídrico y adhesivo mostro los valores más bajos tanto inmediatamente como después del termociclado (8,6 MPa y 5,4MPa).* ***Conclusiones:*** *La cementación de una cerámica híbrida tratada con chorro de arena, silano y un adhesivo a base de 10-MDP garantiza altos valores de adhesión, incluso cuando envejece en condiciones de humedad y calor. El termociclado redujo significativamente la resistencia adhesiva en todos los grupos, siendo más notoria la disminución en los que no incluyeron silano.*

Palabras clave: *adhesión dental - superficie - tratamientos - cerámicas - híbrida*

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INTRODUCTION

Advances in CAD/CAM have led to the development of new dental ceramics such as VITA ENAMIC, also known as PICN (Polymer Infiltrated Ceramic Network). This is a hybrid ceramic composed of a porous ceramic matrix and a polymeric matrix, which combines the esthetics of ceramic with the strength of resin. Thanks to this unique combination, it offers superior optical and mechanical properties, providing a very natural esthetic appearance and withstanding masticatory forces[1-3]. According to the manufacturer[4], the versatility of VITA ENAMIC makes it suitable for a wide range of esthetic restorations in the anterior sector.

However, adhesion to resin cements is a critical factor in ensuring the durability of these restorations. May et al.[5] state that the long-term success of restorations depends on establishing a reliable bond between the restorative material and the luting agent. A proper luting technique can significantly reduce the risk of fractures and microleakage, and adequate adhesion of dental materials to the tooth structure and the interface ensures the long-term success of dental restorations.

Numerous studies have evaluated the efficacy of different surface treatments, such as sandblasting with aluminum oxide, etching with hydrofluoric acid, and applying silane agents and universal adhesives, especially those containing 10-MDP, known for its chemical affinity with ceramic phases[2,6-9]. However, discrepancies remain as to which protocol is best for hybrid ceramics such as VITA ENAMIC[5].

The aim of this study was to determine the bond strength of a hybrid ceramic to a resinous cement, comparing different surface treatments before and after thermocycling. Few studies include the factor of aging by thermocycling, a standardized technique that simulates oral thermal stress and evaluates bond durability over time.

The working hypothesis was that surface treatment with sandblasting, followed by silane and a 10-MDP-based universal adhesive would provide the highest bond strength to PICN hybrid ceramic, even after aging.

MATERIALS AND METHODS

The materials used in this study are described in Table 1.

Procedure

Sample size: A sample size of 40 was selected to guarantee adequate distribution of the data and minimize random error, considering a standard deviation of up to ± 3 (Table 1).

PICN specimens: Four 12x14x18 mm VITA Enamic blocks (VITA Zahnfabrik, Bad Säckingen, Germany) intended for CAD/CAM use were cut into slices 12 mm long, 14 mm wide and 1 mm thick using a cutting machine (Mini CNC XYZ26187, GreatSolutions) and a diamond cutting disc 0.5 mm thick with a 22 mm radius, to obtain 40 slices.

Surface standardization: To standardize the ceramic surfaces, they were sanded with 600, 1000 and 1200 grit silicon carbide sandpaper sheets for one minute each, following a uniform direction.

Composite resin cylinders: 80 cylinders were made of composite resin (Llis Composite, FGM, Joinville, Santa Catarina, Brazil) (Table 1) using an aluminum matrix with a perforation of 1.5 mm radius and 2 mm long, to ensure that they would all be the same size.

The 40 VITA Enamic slices were distributed randomly into the following 4 experimental groups (n=10):

- **AAC group:** Sandblasting + adhesive + cement
- **ASAC group:** Sandblasting + silane + adhesive + cement
- **AFAC group:** Hydrofluoric acid + adhesive + cement
- **AFSAC group:** hydrofluoric acid + silane + adhesive + cement

The specimens in groups AAC and ASAC were sandblasted with 50 μm Al_2O_3 particles (Bioart, Sao Paulo, Brazil) from a distance of 10 mm from the sandblaster nozzle (STD, Bio-Art, Sao Paulo, Brazil) for 15 s, applied perpendicularly over the entire surface at a pressure of 2 bars.

The specimens in groups AFAC and AFSAC were treated with 10% hydrofluoric acid for one minute, then washed with running water for 60 s, and dried with a jet of oil-free cold air (hair dryer).

Two resin cylinders were cemented to each surface-treated PICN slice. To do so, first, silane was applied in a thin layer with a microbrush for 20 s on half of the sandblasted slices (n=10) and half of the hydrofluoric acid-treated slices (n=10), and left to act for 60 s for its complete evaporation. Then, all 40 specimens were coated with an adhesive

Table 1. Materials used in the study

MATERIAL	MANUFACTURER	BATCH	COMPOSITION
VITA ENAMIC[4]	Vita Zahnfabrik (Alemania)	73340	Ceramic component (86% by weight and 75% by volume): Silicon dioxide (SiO_2), aluminum oxide (Al_2O_3), sodium oxide (Na_2O), potassium oxide (K_2O), boron trioxide (B_2O_3), zirconium dioxide (ZrO_2), calcium oxide (CaO). Polymeric component (14% by weight and 25% by volume): UDMA (urethane dimethacrylate), TEGMA (triethylene glycol dimethacrylate). [4]
SINGLE BOND UNIVERSAL[10]	3M ESPE (USA)	10604[a]	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, water, initiators, silane. [10]
Silane MONOBOND-N (Universal primer)	Ivoclar Vivadent AG Liechtenstein	Z0226T	Alcoholic solution of silane methacrylate, phosphoric acid methacrylate and methacrylate sulfide.[27]
ALL CEM DUAL CEMENT[11]	FGM (Brazil)	190421	Base paste: Bis-GMA, Bis-EMA and TEGDMA, camphorquinone, co-initiators, Barium glass microparticles-aluminosilicates, silicon dioxide nanoparticles, inorganic pigments and preservatives. Catalyst paste: methacrylic monomers and dibenzoyl peroxide and stabilizers, Barium-aluminum glass microparticles. [11]
LIS[28] COMPOSITE	FGM (Brazil)	011021	Bis-GMA, Bis EMA, TEGDMA camphorquinone, and silane, micronized barium-aluminum silicate glass, pigments and nano silica.[28]
ALUMINUM OXIDE SAND	Bio Art	76584	50 µm aluminum oxide particles
CONDAC PORCELANA 10%	FGM	230622	10% Hydrofluoric Acid

containing 10-MDP, rubbed for 20 seconds on the surface and polymerized for 20 seconds with an LED light (Woodpecker). Subsequently, resin cement was applied to one end of each cylinder with a 0.5 mm diameter periodontal probe, and two cylinders attached to each PICN specimen. Excess cement was carefully removed so as not to move the cylinders, and then light-curing was performed using the Woodpecker LED B lamp (Woodpecker, Guilin, Guangxi, China).

Each group of test specimens (consisting of two cylinders) was divided into two subgroups. The first subgroup was subjected to shearing immediately, while the second subgroup was aged with 5000 cycles of thermocycling before shearing. The tests were performed on a universal testing machine (Muver/5053, Muver Cx Server Lite Software) at a speed of 1.0 mm/min (Fig. 1).

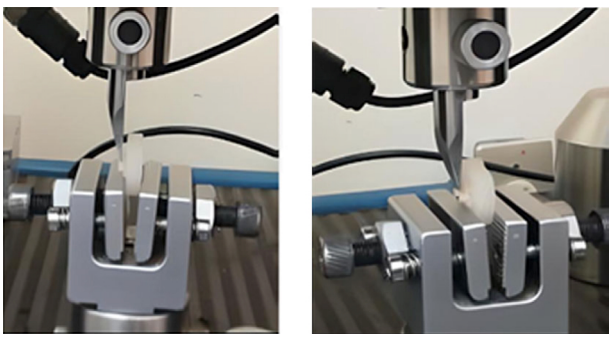

Fig. 1: Universal testing machine used to perform the shear tests.

The shear strength data obtained in newtons were converted to MPa using the following formula:

$$RU = \frac{F}{a}$$

where:

- RU = Bond strength.
- F = Maximum force at which the resin cylinder was peeled off, expressed in newtons
- a = Area of the base of the resin cylinder, expressed in mm^2.

The area of the cylinder was calculated using the formula:

$$A = \pi * r^2 \; [a=\pi r\text{^}2]$$

where:

- $\pi = 3.1416$
- $r2 = 2.25$

Statistics:
Statistics were performed using Minitab 20. software (Minitab Statistical Software. State College. Pennsylvania. USA). Data were subjected to two-way ANOVA (surface treatment and aging) followed by Tukey's post hoc test with a value of $p \leq 0.05$, which was taken as a reference to confirm or rule out statistical differences between groups.

RESULTS

Four groups of n=10 were analyzed; each test specimen with 2 cylinders cemented on its surface, one tested immediately, and the other after simulated aging.

A total 80 ceramic-resin cement interfaces were evaluated. Table 2 shows the average results in MPa and the standard deviations of bond strength for each group.

Adhesion was highest in the immediate ASAC group, at 16.3 MPa. In this group, abrasion with aluminum particles was used as surface treatment, and silane was applied. The lowest value in the immediate group was in AAC, at 9.98 MPa.

Bond strength decreased in all groups after thermocycling (5000 cycles), which is equivalent to 6 months of clinical use in the mouth, according to Alnafaiy S et al[12].

After thermocycling, average bond strength was lowest in AAC, at 6.6 MPa, and highest in ASAC, at 11.2 MPa.

A two-way analysis of variance (ANOVA) was performed to evaluate the effect of surface treatment and aging on bond strength. Tukey's post hoc test was used to determine significant differences between groups (Table 3).

Tukey's analysis with a 95% confidence interval confirmed that after aging, there was no significant difference between groups, regardless of the surface treatment applied (Table 4).

Table 2. Mean and standard deviation of shear bond strength of the AAC, ASAC, AFAC, AFSAC groups tested immediately and after aging

GROUP	Immediate Bond strength (MPa)		Aged bond strength (MPa)	
	Mean	Std. Dev.	Mean	Std. Dev.
AAC	9.98	1.68	6.60	1.14
ASAC	16.30	3.36	11.20	2.19
AFAC	10.58	2.04	6.77	0.98
AFSAC	12.94	3.10	9.73	2.91

Table 3. Two-way ANOVA: showed that both the aging factor (p = 0.000) and the surface treatment factor (p < 0.001) had a significant effect on the differentiation of the groups. However, the interaction between surface treatment and aging was not significant (p = 0.570).

Factor	df	Sum of squares	Mean square.	F	P
Surface Treatment	3	389.70	129.899	23.93	< 0.001
Aging	1	300.73	300.735	55.40	<0.001
Surface Treatment * Aging	3	10.99	3.664	0.67	0.570
Error	72	390.88	5.429		
Total	79	1092.30			

Table 4. Tukey's Analysis

Surface Treatment *Aging	N	Mean	Group *			
ASAC immediate	10	16.30	A			
AFSAC immediate	10	12.94		B		
ASAC aged	10	11.20		B		
AFAC immediate	10	10.58		B		
AAC immediate	10	9.98		B	C	
AFSAC aged	10	9.73		B	C	D
AFAC aged	10	6.77			C	D
AAC aged	10	6.60				D

*Values that do not share a letter differ significantly.

DISCUSSION

Dental material bonding protocols are fundamental to the success of restorative treatment. Many protocols have been developed and are claimed to be the best; however, their efficacy has not yet been fully proven.

This study analyzed the micro-shear bond strength between a hybrid ceramic (VITA ENAMIC) and a resinous cement (AllCem) when different surface treatments were applied.

Our results show that using silane in the bonding protocol is essential to achieve an optimal bond between the hybrid ceramic and resin cement. The group treated with sandblasting, silane and 10-MDP-based universal adhesive exhibited the best bond strength values, while the groups without silane showed significantly lower bonding. These findings support the hypothesis that silane significantly improves bonding in this type of material.

Silane works well for cementing this type of ceramic because it improves the chemical bonds between

silica and methacrylate. The results of the present study agree with those reported by several other authors[7, 13-15].

Silane contains a methacrylate terminal –a small bifunctional molecule, technically called 3-methacryloyloxypropyltrimethoxysilane– which can bind to any ceramic containing silica, as does Vita Enamic (Table 1). It forms covalent bonds at one end (silicon dioxide, Si-O-Si), while the other end binds to the resinous matrix of the cement through the methacrylate group[13].

To achieve this bonding, first, the silane is activated by acetic acid, which converts it to silanol (SiOH). This silanol will bind to an inorganic surface. After the etching reaction, a hydroxyl group (-OH) is produced. The hydroxyl group can lose a proton (H+), facilitating the formation of siloxane bonds (–Si–O–Si–) through condensation.. These covalent bonds are strong when reacted with glass ceramics, as demonstrated in our study. Meanwhile, the organic group (CH3-CH2-) binds to methacrylate-based resins[14, 16].

In the current study, the groups treated with silane only did not have the highest bond strength. The best performing groups were those in which a 10-MDP-based universal adhesive (Single Bond Universal 3M St. Louis, Missouri, USA) was applied after silane. These results confirm the report by the International Academy of Adhesive Dentistry, which suggests that the application of adhesive after silanization could improve resin infiltration into the etched surface[17].

A universal adhesive containing copolymers of methacrylate-modified polyacrylic acid, 10-methacryloyloxyalkyl dihydrogen phosphate (MDP) and silane could bind to both phases (ceramic and resin) of the polymer-infiltrated ceramic network[18]. This occurs because the methacrylate end of the adhesive will bind to the same end which is present in resins and resin cements. The phosphate end of the adhesive will bind to the oxygen at the siloxane end of the previously silanized ceramic. All this determines a strong bond between both materials, which was observed in our study. Even after stress by heat and cold, the strength remained high[19, 20].

One of the most widely used techniques for surface treatment of ceramic materials is sandblasting. According to Fouquet V et al.[17], sandblasting significantly increases the adhesion values for PICN. Abrasion increases the surface roughness of the material, creating irregularities that increase surface energy and improve wettability, thereby improving micromechanical retention[21].

Care is needed for sandblasting. Praisuwanna N [8,14] notes that when a sandblaster is used, pressure, time, distance, and aluminum oxide particle size must be carefully controlled in order to avoid producing microcracks in the ceramic. This was demonstrated by Cevallos[3] and Cascante, et al.[22], who reported that all their in vitro ceramic samples presented fractures when subjected to 50 µm sand. Moreover, abrasion with aluminum oxide particles applied directly on the patient involves the risk of the patient and the practitioner inhaling the dust, which could lead to silicosis, a slowly progressive fibrotic lung disease[19].

Hydrofluoric acid (HF) treatment of a surface selectively dissolves the glassy phase of the ceramic by reacting with the silica, and eliminates the organic part (polymers), producing pores up to 10µ deep, and creating a microstructure that favors bonding strength[20]. The use of low-concentration hydrofluoric acid ensures a gentler surface treatment than sandblasting, and does not produce microcracks, thereby minimizing the risk of subsequent fractures in the ceramic. The results of the groups treated with this technique were similar to those that were sandblasted. Indeed, no significant difference in bond strength was observed between the groups after aging, in agreement with Kömürcüoğlu several other authors[7, 23, 24].

For all these reasons, our findings confirm the Vita Enamic manufacturer's recommendation to treat this ceramic exclusively with hydrofluoric acid. According to Niizuma et al.[25], 4% is an adequate concentration of hydrofluoric acid to avoid damaging the surface of the material and to improve bond strength.

The cementation protocol applied a dual resinous cement whose resinous matrix contains methacrylate monomers such as Bis-GMA, TEGDMA and UDMA, which are also present in the polymeric network of the hybrid ceramic Vita Enamic. The monomers of both materials would therefore be joined by covalent bonds, thereby improving bond strength[24].

Dental restorations are continuously exposed to adverse conditions in the oral cavity, including temperature changes, constant humidity and mechanical forces, which can compromise the durability of the adhesive interface. It is therefore

essential to simulate these conditions during in vitro studies by using standardized aging protocols such as thermocycling, which aims to replicate the thermal stress experienced by restorative materials in the mouth during the ingestion of food and beverages at different temperatures[12].

There is a consensus in scientific literature that thermal cycling between 5 °C and 55 °C adequately simulates intraoral thermal fluctuations. The International Organization for Standardization (ISO TR 11405) recommends a protocol of 5000 cycles to represent approximately six months of clinical aging[26]. During this process, water can penetrate the polymer network due to its small molecular size and high molar concentration, which can lead to phenomena such as plasticization, hydrolysis and degradation of the resin cement. These effects directly impact adhesive strength and compromise the long-term stability of the adhesive system[5,22].

In this context, the present study included thermocycling as a critical variable to evaluate the adhesive behavior of PICN hybrid ceramics. The results show a significant decrease in bond strength after thermal aging, highlighting the importance of selecting surface treatment protocols that provide adhesive stability even under adverse conditions. The group treated with sandblasting, silane and universal adhesive with 10-MDP was shown to maintain superior adhesion after thermal cycling, making it the most effective protocol among those evaluated.

This study has some limitations. Being an in vitro study, the results may not fully reflect clinical conditions. In addition, the number of thermocycling cycles used may not be fully representative of clinical aging.

CONCLUSIONS

Within the limitations of an in vitro study, the following conclusions can be drawn:

- Surface treatment combining sandblasting, silane application and use of a 10-MDP-based universal adhesive provides the highest adhesive strength values in PICN hybrid ceramics, both before and after simulated aging by thermocycling.
- After 5000 cycles of thermocycling, adhesion values decreased in all groups, with no statistically significant difference between them. This could be attributed to hydrolytic degradation of the resinous cement induced by water penetration at the adhesive interface.
- These findings highlight the importance of employing surface conditioning protocols that enhance adhesive stability against thermal challenges in the oral environment. However, long-term clinical investigations are required to validate the efficacy of these treatments under real-world conditions.

CONFLICT OF INTERESTS

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

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Complications in implant-supported full-arch immediate prostheses: a Brazilian retrospective, observational, longitudinal study

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ABSTRACT

Edentulism causes aesthetic, functional, nutritional, phonetic and psychological damage. One of the best treatments for it is implant-supported full-arch prostheses. However, like all techniques, it involves challenges. ***Aim:*** *To evaluate the main complications in implant-supported complete dentures.* ***Materials and Method:*** *This study analyzed the medical records of 140 patients rehabilitated with implant-supported full-arch prostheses with immediate loading using the passive fit technique. The analysis considered the antagonist, and complication location (upper and/or lower jaw). All cases had 1 to 8 years under load.* ***Results:*** *No complication was reported in 115 (82.1%) patients, while 25 presented complications: 14 (56%) prosthetic tooth fractures, 3 (12%) prosthesis retention screw fractures, 3 (12%) loss of cementation of the cylinder, and 5 (20%) implant losses. There were more complications in implant-supported complete dentures in the upper arch or cases of both jaws ($p<0.05$). The success rate (patients without complications during follow-up) was 82.1%.* ***Conclusion:*** *Implant-supported complete dentures made by the passive fit technique were predictable in the long term for rehabilitation of completely edentulous patients.*

Keywords: *implant supported prostheses - passive fit technique - implant loss*

Complicações em próteses totais imediatas implanto-suportadas: um estudo brasileiro retrospectivo, observacional e longitudinal

RESUMO

O edentulismo causa danos estéticos, funcionais, nutricionais, fonéticos e psicológicos. As próteses totais implanto-suportadas são consideradas um dos tratamentos mais adequados para essa situação clínica. No entanto, como todas as técnicas, esta tem seus desafios. ***Objetivo:*** *Avaliar as principais complicações em próteses totais implanto-suportadas.* ***Materiais e Método:*** *Foram analisados os prontuários de 140 pacientes reabilitados com próteses totais implanto-suportadas com carga imediata, utilizando a técnica de assentamento passivo, levando em consideração a característica do antagonista e a localização (maxila e/ou mandíbula). Os casos tinham pelo menos um ano e até 8 anos em função.* ***Resultados:*** *Nenhuma complicação foi relatada em 115 (82,1%) indivíduos. Do total de pacientes, foram observadas 25 ocorrências, nas quais 14 (56%) apresentaram fratura dentária da peça protética, 3 (12%) apresentaram fratura do parafuso de retenção da prótese, 3 (12%) apresentaram perda da cimentação do cilindro e 5 pacientes (20%) tiveram perda do implante. Próteses totais implanto-suportadas na maxila ou em ambas as arcadas apresentaram mais complicações ($p<0,05$). A taxa de sucesso correspondeu a 82,1% dos pacientes, que não apresentaram nenhum infortúnio durante os períodos de retorno.* ***Conclusão:*** *Próteses totais implanto-suportadas confeccionadas pela técnica de assentamento passivo mostraram-se previsíveis em longo prazo para reabilitação de pacientes totalmente desdentados.*

Palavras-chave: *próteses totais implanto suportadas - técnica de assentamento passivo - perda do implante*

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INTRODUCTION

The prosthetic options for completely edentulous patients are conventional dentures, implant overdentures and implant-supported fixed dental prostheses. The advantage of the latter is that it is fully supported by implants, and does not transfer load to adjacent tissues. The masticatory forces are thus transferred to the implants, thereby preventing any further bone resorption of the residual alveolar ridge, as occurs with conventional complete dentures supported by gingival and bone tissue[1,2].

Fabrication and retention methods influence the fit of the prosthetic superstructure. Initially proposed by Sellers in 1989, the passive fit technique consists of three cylinders (castable, brass and titanium). The brass cylinder is larger than the titanium one, but the bases are equal. The calcinable cylinder was designed to adapt to the base of these two cylinders. So, the space between the castable and the brass cylinders is smaller than the space between the castable and the titanium cylinders. The brass cylinder is used as a base for waxing the casting pattern, and after the cast bar, it is cemented on the titanium cylinders. Because there is a size difference between the cylinders, the internal space for cementation will be preserved, and the inherent distortions to the casting procedure will be eliminated[3]. Full-arch implant-supported rehabilitations performed with this passive fitting technique precisely adapt the metallic framework on the abutments, and the immobilization of multiple implants can limit micromotion at the bone-implant interface[4]. The stabilization of implants at initial placement and the limitation of micromotion to 100 µm contribute to successful osseointegration[5,6].

Dental implant loading may be early (1 week to 2 months after implant placement), conventional (more than two months after implant placement), or immediate (less than one week after implant placement)[7]. Several advantages have been related to immediate loading, including primary function and aesthetics, avoidance of a conventional denture during the healing phase, avoidance of second surgeries, and preservation of hard and soft tissue anatomy[8]. According to recent studies, implants loaded immediately with full-arch fixed prostheses achieve high success rates after several years of follow-up in post-extraction bone and healed bone in the maxilla and the mandible[9]. The aim of this research was to evaluate the clinical information reported in the medical records of 140 patients rehabilitated with implant-supported full-arch prostheses with immediate loading, made by the passive fit technique, with 1 to 8 years under load. The study analyzed the main complications, identified their possible association with the antagonist, and compared upper and lower arches.

MATERIALS AND METHOD

This retrospective, observational, longitudinal study was based on a survey of data filed at the Institute of Graduate Studies and Research in Dentistry in Balneario Camboriu, Brazil. Informed consent was obtained from the people involved. The research was approved by the Research Ethics Committee of the São Leopoldo Mandic Institute and Research Center under number 5,501,597.

The sample consisted of 140 patients selected according to the following inclusion criteria; treatments of implant-supported fixed dental prostheses performed between 2013 and 2021, surgeries with implants that received fixed prostheses with immediate loading in at least one arch, produced by the same laboratory (Buche, Curitiba, Brazil), same laboratory technique of passive fitting, and implants and abutments of the same brand (Neodent, Curitiba, Brazil). Exclusion criteria were the following: smokers, prostheses with less than one year under load, ceramic protocols, prostheses without prosthetic components, patients whose surgery and prosthesis were not performed at the same venue (Institute of Graduate Studies and Research in Dentistry in Balneario Camboriu, Brazil), deceased patients, medical records without information or with incomplete data, patients with removable partial denture as antagonist type, and patients who did not sign the free and informed consent form. All complications were recorded and analyzed for correlation with antagonist type and location (upper and/or lower jaw).

Descriptive analysis of variables and hypothesis tests were performed using Fisher's Exact Test and Pearson's test. All tests were performed with a 95% confidence level. Statistical analyses were performed with the software r: Ris, a language and environment for statistical computing and graphics developed at Bell Laboratories (Lucent Technologies).

RESULTS

A total 140 patients were included in the study, 49

male and 91 female, mean age 65. One hundred and forty implant-supported full arch prostheses were analyzed: 100 lower prostheses (71.4%) and 40 upper prostheses (28.6%), with average loading time 60.2 months. This distribution occurred because of the 170 prostheses; 30 patients had bimaxillary prostheses. There were no complications in 20 of them, so they were randomly distributed among ten uppers and ten lowers to achieve one of our objectives: to compare upper to lower.

Over the time analyzed, no complication was reported in 115 (82.1%) individuals, while 25 (17.9%) patients had problems: 14 (56%) with veneer fracture, 5 (20%) with implant loss, 3 (12%) with cylinder cementation loss, and 3 (12%) with fracture of the prosthesis fixation screw. Relative to the total number of patients, the distribution was 10% veneer fracture, 3.57% implant loss, 2.14% cementation loosening and 2.14% screw fracture.

Considering the 40 (28.6%) upper prostheses and 100 (71.4%) lower prostheses, there were complications in 30% (12) of the upper prostheses (10 veneer fractures and 2 implant losses), and in only 13% (13) of the lower prostheses (4 veneer fractures, 3 implant losses, 3 fractures of the prosthesis fixation screw and 3 cementation losses).

Regarding antagonists, 75 (53.6%) patients had conventional full arch dentures, with no complications in 66 (88%) and complications in 9 (12%); 30 (21.4%) had implant-supported full arch prostheses, with no complications in 20 (66.7%) and complications in 10 (33.3%); 18 (12.9%) had natural teeth and implants, with no complications in 16 (88.9%) and complications in 2 (11.1%); and 17 (12.1%) had natural teeth, with no complications in 13 (76.5%) and complications in 4 (23.5%).

Table 1 shows the distribution of complication type in implant-supported full-arch immediate prostheses. Tables 2 and 3 show the results of comparative statistics and the association test. Full arch implant-supported prostheses antagonist resulted in higher complications.

DISCUSSION

This study found an 81.2% success rate (no complication reported in patients' records) for implant-supported full-arch immediate prostheses made by the passive fit technique. Notwithstanding the high survival rate of implants and prostheses, dentists should be aware of the biological and mechanical complications that may occur in implant-supported prosthesis rehabilitation.

Biological complications after installation of the definitive prosthesis include soft tissue inflammation and hyperplasia, peri-implant soft tissue recession, mucositis, peri-implantitis, and implant failure.

Table 1. Complication type in implant-supported full-arch immediate prostheses

Complication	Number of cases	% of complications (n=25)	% of total patients (n=140)
Veneer fracture	14	56%	10%
Implant loss	5	20%	3.57%
Cylinder cementation loss	3	12%	2.14%
Prosthesis fixation screw fracture	3	12%	2.14%
Total with complications	25	100%	17.9%
Without complications	115	—	82.1%

Table 2. Ratio test between antagonists

Comparison	Test	Test P-Value	Conclusion
Natural teeth vs. Natural teeth + Implants	0.2763	0.5992	H 0 not rejected [p-value>α]
Natural teeth vs. Implant-supported full arch prosthese	0.1401	0.7082	H0 not rejected [p-value>α]
Natural teeth vs. Conventional denture	0.7167	0.3972	H0 not rejected [p-value>α]
Natural teeth + Implants vs. conventional denture	0.0000	1.0000	H0 not rejected [p-value>α]
Implant-supported full arch prosthesis vs. Conventional denture	5.2195	0.0223	H0 rejected [p-value≤α]

H_0: There is no difference between the antagonists.

Table 3. Hypothesis testing between all antagonists and the full arch implant-supported prostheses antagonist

	P-Value	Conclusion	Statistic Test
Antagonist	0.0598	H0 not rejected [p-value>α]	Fisher's Exact Test

H_0: There is no association between "Antagonist" and "Complication/No Complication."

	P-Value	Conclusion	Statistic Test
Implant-supported full arch prostheses	0.0267	H0 rejected [p-value≤α]	Pearson's Test

H_0: There is no association between "full arch implant-supported prostheses" and "Complication/No Complication."

Technical/mechanical complications involve wear of the prosthetic material (localized or generalized), fractures of the prosthetic material, loss of material covering the access to the prosthetic screw, loss of cementation, loosening or fracture of the abutment screw, fracture of suprastructure, fracture of abutment and implant fracture[10].

Clinical and scientific evidence supports implant-supported fixed full-arch dentures as a reliable treatment option for rehabilitating edentulous patients. The main focus of previous longitudinal studies, especially in the 1990s, was the success of osseointegration and implant survival[11]. A review by Papaspyridakos et al. claims that studies mainly used implant and peri-implant soft-tissue parameters for measuring success[12]. In contrast, the present study focused mainly on prosthodontic parameters.

The current study analyzed the medical records of 140 patients rehabilitated with implant-supported full-arch prostheses with immediate loading, made by the passive fit technique, with 1 to 8 years under load. It found that 25 patients (17.9%) had problems: 14 (56%) veneer fractures, 5 (20%) implant losses, 3 (12%) cylinder cementation losses, and 3 (12%) fractures of the prosthesis fixation screw. There were more complications in the upper arch than in the lower. Other previous studies corroborate that prosthetic tooth fractures are the main issue after different follow-up periods.

A meta-analysis conducted by Bozini et al. included 19 studies of prosthodontic complication rate for implant-supported fixed prosthesis in edentulous patients after observation periods of 5 to 23 years. The statistical analysis revealed estimated cumulative rates of veneer fractures over an observation period of 5, 10, and 15 years of 30.6%, 51.9%, and 66.6%, respectively. The estimated rates of abutment and prosthetic screw loosening after 15 years were 13.4% and 15%, respectively. The estimated rates of abutment and prosthetic screw fracture after 15 years were 6.3% and 11.7%, respectively. Complications may be influenced by various factors such as parafunctional habits, number of implants supporting the prosthesis, opposing arch condition, and type of suprastructure retention (screw versus cement)[13]. In the referred study, the analysis of various factors potentially influencing complications did not produce any results, and parafunctional habits were not considered.

The retrospective study by Able et al. at the Latin American Institute of Dental Education and Research from 2004 to 2013, on 290 patients rehabilitated with fixed full-arch prostheses on immediate-load dental implants, manufactured according to the passive adjustment technique, with mean follow-up 4.4 years, observed a 98.6% survival rate for prosthetic rehabilitations. Regarding complications, five implants failed and were removed during this period, and the implant survival rate was 99.6%. Prosthetic complications were found in 67 participants (23.1%), with fracture of the prosthetic tooth in 41 (61%), loosening of the prosthetic screw in 15 (22.3%), and cylinder cementation loss in 7 (2.45%)[4]. In the present study, 25 patients (17.9%) had complications, with prosthetic tooth fracture in 14 (56%) and loss of cylinder cementation in 3 (12%).

Ventura et al. reviewed the literature to identify factors responsible for increasing the incidence of fractures of acrylic teeth in implant-supported rehabilitations. Statistically significant differences were found among the variables; men suffered more fractures than women, maxillary prostheses fractured more than mandibular ones, prostheses that did not have mechanical retention for teeth and acrylic suffered more fractures, prostheses with cantilevers 10 mm or longer fractured less than those with cantilevers shorter than 10 mm, natural dentition caused a greater number of fractures than the full mucosa-supported dentures, and prostheses supported by four implants fractured more than the others (five, six or eight implants). Regarding the arch, maxillary prostheses fractured more than the mandibular ones[14], in agreement with the findings of

the current study and Eliasson et al[15].

In the current study, the following 25 patients presented complications:

- 10 (33.3%) of the 30 patients with implant-supported complete dental prostheses as antagonist, of whom 7 had complications in the upper arch and 3 in the lower arch;
- 9 (12%) of the 75 patients with conventional complete denture as antagonist, all 9 complications being in the lower arch;
- 4 (23.5%) of the 17 patients with natural teeth as antagonist, of whom 3 had complications in the upper arch and 1 in the lower arch; and
- 2 (11.1%) of the 18 patients with natural teeth plus implants as antagonist, both having complications in the upper arch.

The results showed more statistically significant complications when the antagonist was also an implant-supported full-arch prosthesis ($p \leq 0.05$), corroborating Davis et al[16]. In this clinical situation, the maintenance requirements seem to be much greater than with natural teeth or complete dentures as antagonists.

This study was based on data available in clinical records and presented some limitations. The presence of bruxism, alveolar bone loss and wear or aesthetics of the prosthetic material were not evaluated.

CONCLUSIONS

The rehabilitation of completely edentulous patients with implant-supported full arch dentures made using the passive fit technique proved to be predictable in the long term. A greater number of complications were observed in the upper arch, and when the antagonist was also an implant-supported complete denture. The most frequent prosthetic complication was veneer fracture.

CONFLICT OF INTEREST

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

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Randomized controlled trial comparing antibiotics to placebo for single simple dental extractions in diabetic patients

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ABSTRACT

Type 2 diabetes mellitus is a prevalent chronic disease in the adult population, and its complications include delayed wound healing. Dentists often have to decide whether to prescribe antibiotics for tooth extractions in these patients. ***Aim:*** *To compare post-surgical variables for single simple dental extractions from controlled type 2 diabetic patients, administering either antibiotic or placebo.* ***Materials and Method:*** *The study included controlled type 2 diabetic patients requiring a single dental extraction (tooth non-impacted and without acute infection) from April 2021 to May 2023. They were randomized to amoxicillin or placebo prior to surgery. Extractions were performed without raising flaps or bone removal and took no longer than 45 minutes. Before surgery, blood glucose was measured. Age, gender, tooth to be extracted, surgery time, pain, bleeding, trismus, alveolar osteitis, infection, healing, gastric alterations, and number of analgesics taken were evaluated. Patients were checked by telephone call 2 and 14 days after the procedure, and in person after 7 days during the suture removal visit. The data were analyzed using Chi-square, Fisher's exact or Mann-Whitney U tests, as appropriate ($p<0.05$, significant).* ***Results:*** *The analysis included 56 extractions in 56 patients, aged 41 to 81 years (mean SD = 59 +/- 9). During the telephone call at 2 days, no significant difference was found between groups for pain, trismus, edema, hemorrhage, gastric alterations, or analgesics taken. At the clinical checkup at 7 days, no significant difference was found between groups for pain, edema, trismus, alveolar osteitis, hemorrhage, delayed healing, or gastric alteration; but there was a significant difference in the number of analgesics taken ($p < 0.05$), which was higher in the amoxicillin group. During the second telephone call at 14 days, no significant difference was found between groups for pain, edema, trismus, hemorrhage, or gastric alterations; but there was a difference in the number of analgesics taken ($p < 0.05$). Patients in the amoxicillin group took more pain relievers. No case of alveolar osteitis or local or systemic infection was recorded in either group. No patient required additional treatment or hospitalization.* ***Conclusion:*** *No significant difference was found for alveolar osteitis, infection, or healing delay after single simple dental extractions in controlled type 2 diabetics, whether they took amoxicillin or placebo. The data from the present study suggest that antibiotic medication in these cases would not be necessary.*

Keywords: *diabetes mellitus type 2 - antibiotics - placebo - tooth extraction - bacterial drug resistance.*

Estudio clínico aleatorizado de administración de antibióticos para exodoncias simples en pacientes diabéticos

RESUMEN

La diabetes mellitus tipo 2 es una enfermedad crónica de elevada prevalencia que presenta complicaciones en los procesos cicatrizales. Frecuentemente los odontólogos deben decidir la necesidad de prescripción de antibióticos para exodoncias en estos pacientes. ***Objetivo:*** *Comparar variables post quirúrgicas en extracciones simples unitarias de pacientes diabéticos tipo 2 controlados, administrando antibiótico o placebo.* ***Materiales y Método:*** *Se realizaron las exodoncias en pacientes diabéticos tipo 2 controlados que concurrieron al servicio desde abril de 2021 a marzo de 2023, requiriendo la extracción de una pieza dental unitaria, sin retención y sin infección aguda. Se realizó una aleatorización previa de la administración de amoxicilina o placebo. Las exodoncias se realizaron sin la implementación de colgajos ni ostectomías, en tiempos no mayores de 45 minutos de duración. Se evaluaron los siguientes parámetros: edad, género, pieza a extraer, glucemia, tiempo de cirugía, dolor, sangrado, trismus, alveolitis, infección, cicatrización, alteraciones gástricas y cantidad de analgésicos ingeridos. Se realizaron dos controles telefónicos a los 2 y 14 días post-exodoncia y un control presencial a los 7 días post-exodoncia. Los datos obtenidos fueron analizados mediante las pruebas Chi-cuadrado, exacta de Fisher o U de Mann-Whitney, según lo que correspondía ($p<0,05$, significativo).* ***Resultados:*** *la muestra incluyó 56 exodoncias correspondiente en 56 pacientes, edad de entre 41 y 81 años (media y DE = 59 +/- 9). No se encontraron diferencias significativas entre ambos grupos, en el control a los 2 días post-exodoncia, en cuanto a trismus, edema, hemorragia, alteraciones gástricas, analgésicos consumidos, ni dolor. Al control clínico presencial a los 7 días post-exodoncia, no se encontraron diferencias significativas entre ambos grupos, en cuanto a dolor, edema, trismus, alveolitis, hemorragia, retardo cicatrizal, ni alteraciones gástricas; encontrándose diferencia significativa para la cantidad de analgésicos consumidos ($p < 0,05$), siendo mayor el consumo en el grupo amoxicilina. Cuando se realizó el segundo llamado telefónico a los 14 días, no se encontraron diferencias significativas entre ambos grupos, en cuanto a trismus, edema, hemorragia, ni alteraciones gástricas; pero si hubo diferencias en cuanto a analgésicos consumidos ($p < 0,05$). Los pacientes del grupo amoxicilina consumieron más analgésicos. No se registraron en ambos grupos casos de alveolitis, ni de infecciones locales o sistémicas. No se requirieron terapias alternativas, ni hospitalización en ningún paciente.* ***Conclusión:*** *Los datos del presente estudio sugieren que la medicación antibiótica, en pacientes diabéticos tipo 2 controlados, no sería necesaria, ya que no se encontraron diferencias significativas para la alveolitis, infección, retardo cicatrizal al realizar exodoncias unitarias, administrando amoxicilina o placebo.*

Palabras Clave: *diabetes mellitus tipo 2 - amoxicilina - placebo - exodoncia - farmacorresistencia bacteriana*

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INTRODUCTION

Diabetes is a chronic hyperglycemic disease estimated to occur in 8.8% of the adult population[1]. It is usually classified into two main groups: type 1, also known as childhood-onset diabetes, defined by deficiency in insulin production; and type 2, also called the adult-onset diabetes, characterized by insulin resistance. Type 2 is associated with dietary habits and sedentary lifestyle. It develops slowly, and early diagnosis and management are crucial[2].

Diabetes has a range of oral manifestations, including xerostomia, increased predisposition to dental caries, periodontal disease, increased tendency for infections, burning mouth syndrome, taste disturbances, and healing disturbances[3-5]. After tooth extraction in poorly controlled diabetic patients, especially in the early phases, the overall assessment of alveolar healing is slower than in non-diabetics[6]. Uncontrolled patients with poor dental hygiene have been associated with spreading thoracic infections[7,8]. Post-extraction dental infections have been related to tooth, age, gender, degree of impaction, and operator experience[9]. When dental infection spreads, poorer outcomes can be predicted based on trismus, dysphagia, dyspnea, trismus, the severity of the infection, the number of spaces involved, and increased white blood cell count[10].

Diabetes was therefore assumed to be associated to the spread of postoperative infections, and antibiotics were sometimes prescribed preventively. In the USA, 1 in 10 antibiotics are estimated to be prescribed by dentists. However, prescribing antibiotics for invasive dental procedures in diabetic patients has recently been called to question[11].

The massive administration of antibiotics is one of the current and future concerns of the health system, due to the development of bacterial resistance[12, 13]. Although it has been established that it is not necessary to provide antibiotic regimens for simple extractions in healthy patients, evidence is lacking in diabetic patients[13-16].

The aim of this study was to compare post-surgical variables in single simple dental extractions in controlled type 2 diabetic patients, administering antibiotics or placebo.

MATERIALS AND METHOD

This was a prospective, randomized, double-blind (patient and operator), parallel group comparison clinical trial. It was conducted at the Department of Oral and Maxillofacial Surgery I of the School of Dentistry of the University of Buenos Aires (Argentina), from April 2021 to March 2023, on controlled diabetic type 2 patients who required a single simple dental extraction.

Inclusion criteria

The study included type 2 diabetic patients (diagnosed and under medical treatment for at least 6 months), male or female, over 18 years of age, who visited the service for single simple dental extraction of a permanent tooth. A hematological analysis of glycosylated hemoglobin (HbA1c) was requested to evaluate blood glucose levels, and the lower limit was established as 6.5% [17]. Participants signed the medical history and the informed consent designed for the present study (CETICA-FOUBA NUM. 008/19).

Exclusion criteria

Patients with any of the following were excluded:

1. Inherent systemic or local contraindications for performing dental surgery: pathologies that affect blood coagulation, chemotherapy treatments, diseases of connective tissue or bone metabolism, heart conditions or vascular diseases, uncontrolled diabetes, chronic kidney disorders, systemic infections, mental disorders, tumors.
2. Blood glucose levels greater than 180 mg/dl before receiving care[18].
3. Multiple extractions (more than one tooth).
4. Teeth with acute infection, or impacted teeth.
5. Smokers.
6. Patients who took antibiotics in the 7 days prior to the intervention.
7. Type I diabetic patients
8. Inability to receive any standardized study medication, e.g., amoxicillin.
9. Pregnant or lactating patients.
10. Patients who refused to participate.

Exclusion from analysis

The following cases were excluded from analysis:

1. Surgeries that lasted more than 45 minutes.
2. Surgeries that as a complementary resource involved lifting a flap, or bone tissue removal.

Subjects were randomized through the generation of random numbers between zero and one in the Microsoft® Excel computer program. Numbers between 0 and 0.5 were assigned to the placebo group, and numbers between 0.5 and 1 were assigned to the amoxicillin group. In the randomization, the number 0.500000000 did not come up. The results were kept in sealed envelopes in ascending order according to the subject's order of entry into the study, and opened prior to surgery.
Study groups:
Group 1: Dental extraction with administration of antibiotics (Amoxicillin 500 mg, Amixen®, Laboratorios Bernabo, Argentina).
Group 2: Dental extraction with administration of placebo.

Surgical protocol
Pertinent clinical, laboratory and imaging studies were performed. Prior to surgery, the patient's blood glucose was measured (FreeStyle Optium Neo®), and was required to be equal to or below 180 mg/dl with at least two hours of fasting (exclusion criteria). Prior to all surgeries, patients were instructed to rinse their mouth with Chlorhexidine 0.12% solution for 1 minute.
The surgeries were performed according to the following clinical steps:

1. Antisepsis with povidone-iodine.
2. Local anesthesia of the area to be operated on (4% articaine hydrochloride with L-Adrenaline 1:100,000, Totalcaina Forte®, Laboratorios Bernabo, Argentina).
3. Intracrevicular incision with scalpel blade number 15.
4. Slight curettage of the periodontal soft tissue.
5. Dislocation with straight Clev-Dent elevator.
6. Extraction with elevator and/or corresponding forceps.
7. Wound toilette.
8. Simple suture.
9. Post-surgical indications.

Group with antibiotic administration
One 500 mg amoxicillin tablet (Amixen®, Laboratorios Bernabo, Argentina) was administered 1 hour before surgery, and continued every 8 hours for 7 days (21 pills altogether)[19].

Group with placebo administration
One placebo 1 tablet (donated by Laboratorios Bernabo, Argentina) was administered 1 hour before surgery, and continued every 8 hours for 7 days (21 pills altogether).
All tablets were packed in identical bottles, each containing 21 pills. Placebo appearance, size, and odor was indistinguishable from amoxicillin tablets.

In the postoperative period, all patients were prescribed Ibuprofen 600 mg according to pain. Special emphasis was placed on asking patients to maintain blood glucose levels below 180 mg/dl during the postoperative period by regulating the intake of hyperglycemic foods, and continuing with the hypoglycemia medication. Researchers' telephone numbers were provided in the informed consent document so that patients could contact them for any questions or problems they might have.

Parameters evaluated:
Age/Gender; tooth to be removed; blood glucose level.
Patients were evaluated or consulted 2, 7 and 14 days after the intervention. Data were recorded in an ad hoc Excel spreadsheet.

Intra-surgical parameters recorded:
Surgery time (min); intra-surgical complications.

Parameters recorded during phone call after 2 days:
Pain: scale with visual analogue scale (VAS); amount of pain relievers taken; gastric alterations: present or absent; hemorrhage: present or absent; trismus: present or absent; edema: present or absent.

Parameters recorded during clinical checkup and suture removal after 7 days:
Pain: VAS scale; amount of pain relievers taken; gastric alterations: present or absent; hemorrhage: present or absent; trismus: present or absent; edema: present or absent; alveolar osteitis: present or absent; infection: present or absent; delayed healing: present or absent.

Parameters recorded during phone call after 14 days:
Amount of pain relievers taken; gastric alterations: present or absent; hemorrhage: present or absent; trismus: present or absent; edema: present or absent.

Statistical analysis

Categorical variables were described by absolute frequencies and percentages with 95% confidence intervals (CI95), estimated using the Wilson method. The description of numerical variables included minimum (Min), maximum (Max), median, first quartile (Q1), third quartile (Q3), mean and standard deviation (SD). To compare frequencies, Pearson's Chi-square or Fisher's exact tests were used, as appropriate. When all expected frequencies were greater than or equal to 5, Chi-square was used; otherwise, Fisher's exact test. To compare two independent sets of numerical observations, the nonparametric Mann-Whitney U test was used. The Student t-test for independent samples was not used because the conditions of normality and homoscedasticity were not met, analyzed using the Shapiro-Wilk tests with the Royston method and F methods, respectively. Any p values lower than 0.05 were considered statistically significant. The analysis was performed using MedCalc v. 22.021 and R v. 4.3.1 with *DescTools* package. The statistical graphs were created with Calc from LibreOffice.

RESULTS

Patient demographics and clinical characteristics

Patients and interventions

Sixty-six controlled type 2 diabetic patients met the inclusion criteria. Four patients refused to be enrolled in the study, for whom extractions were performed but no data were recorded, and administration of antibiotics was at the discretion of the professional involved in each surgery. Another 6 patients had blood glucose levels higher than 180 mg/dl prior to surgery, so they were excluded from the data analysis. These patients were treated and prescribed antibiotic therapy before and after surgery, and advised to consult the physicians treating their underlying pathology for close follow-up.

The study sample included 56 subjects equally distributed by gender (n=28), age 41 to 81 years (median = 59, Q1-Q3 = 54-65, mean +/- SD = 59 +/- 9. Blood glucose level was between 75 mg/dl and 179 mg/dl (median = 141, Q1-Q3 = 117-168, mean +/- SD = 139 +/- 32). Blood glucose (mg/dl) did not differ significantly (Mann-Whitney U test: U = 377; p = 0.80) between patients on amoxicillin (median = 153, Q1-Q3 = 117-168, mean +/- SD = 141 +/- 31, Min-Max = 75-177) and patients on placebo (median = 135, Q1-Q3 = 115-168, mean +/- SD = 136 +/- 33, Min-Max = 75-179) .

There were 28 patients medicated with amoxicillin and another 28 with placebo. Extractions were performed on all types of teeth except lower first premolars (Fig. 1).

Intra-surgical instance

Fifty-six surgeries were performed, which lasted

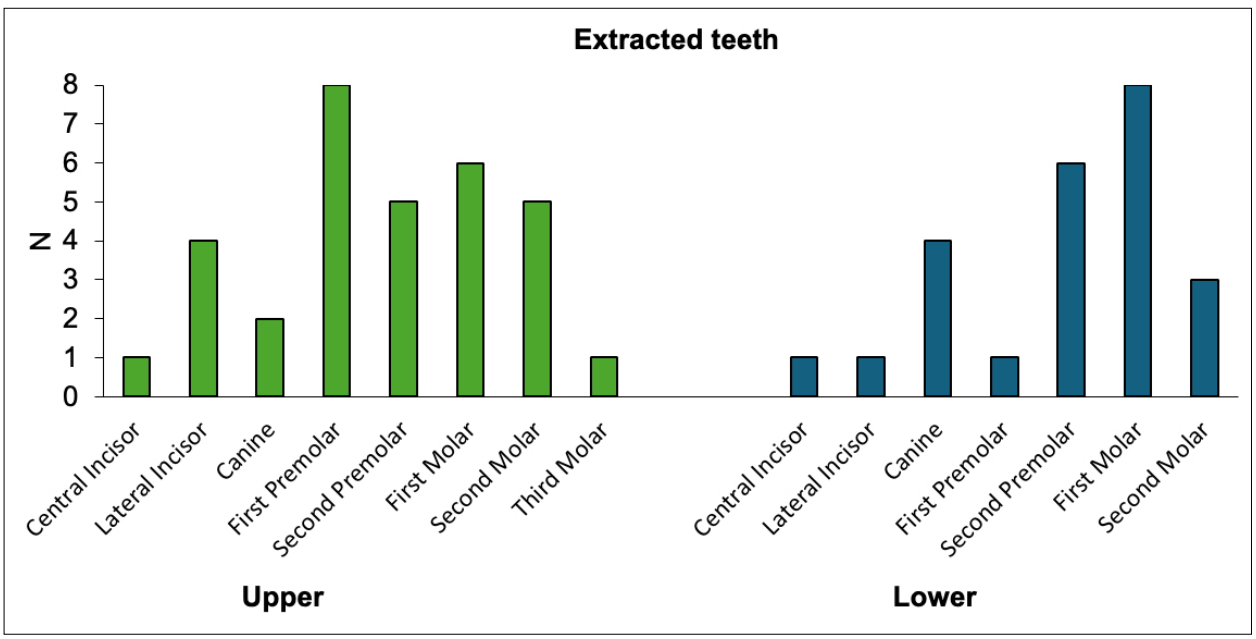

Fig. 1: Number of extractions according to tooth type.

between 5 and 45 minutes (median = 20, Q1-Q3 = 15-25; mean +/- SD = 21 +/- 9). The duration of surgery did not differ significantly (Mann-Whitney U test: U = 356; p = 0.55) between treatments (amoxicillin, median = 20, Q1-Q3 = 14-23, mean +/- SD = 20 +/- 9, Min-Max = 7-40; placebo, median = 19, Q1-Q3 = 15-27, mean +/- SD = 22 +/- 10, Min-Max = 5-45). No surgery took longer than 45 minutes (exclusion criteria for analysis).

Intraoperative complications

All surgeries were completed without the need to apply osteotomy or raise a mucoperiosteal flap. No relevant intra-surgical complication was observed.

Phone interview 2 days after the intervention

Table 1 summarizes the results obtained by telephone interview two days after the intervention. None of the 56 patients presented trismus (100%; CI95: 94% to 100%). Six patients had edema (11%; CI95: 5% to 21%). Hemorrhage was present in 2 patients (4%; CI95: 1% to 12%) and gastric alterations in 6 (11%; CI95: 5% to 21%). Patients took between 0 and 8 analgesic tablets (median = 2, Q_1-Q_3 = 1-4, mean +/- SD = 3 +/- 2). The perception of pain assessed on a visual analogue scale (VAS) was between 0 and 6 (median = 3, Q_1-Q_3 = 1-4, mean +/- SD = 3 +/- 2). There was no significant difference between groups for any of these 6 variables (Table 1).

Table 1. Comparison between patients who received amoxicillin or placebo, according to information collected by telephone interview 2 days after the intervention. Categorical variables, N (%, CI95); numerical variables, median (minimum-maximum).

PHONE INTERVIEW 2 DAYS AFTER THE INTERVENTION			
Variable	Medication		
	Amoxicillin	Placebo	p
Trismus			
No	28 (100%, 88 to 100)	28 (100%, 88 to 100)	1*
Yes	0 (0%, 0 to 12)	0 (0%, 0 to 12)	
Edema			
No	26 (93%; 77 to 98)	24 (86%; 69 to 94)	0.67*
Yes	2 (7%; 2 to 23)	4 (14%; 6 to 31)	
Hemorrhage			
No	27 (96%, 82 to 99)	27 (96%, 82 to 99)	1*
Yes	1 (4%, 1 to 18)	1 (4%, 1 to 18)	
Gastric alterations			
No	24 (86%, 69 to 94)	26 (93%, 77 to 98)	0.67*
Yes	4 (14%, 6 to 31)	2 (7%, 2 to 23)	
Analgesic pills	3 (0-7)	2 (0-8)	0.08#
VAS	3 (0-5)	2 (0-6)	0.79#
N total	28	28	

**Fisher's exact test*
#Mann-Whitney U test

Clinical checkup 7 days after the intervention

All patients were monitored after a week at the visit at which the suture was removed (Table 2). No patient developed alveolar osteitis (dry socket) or infectious processes associated with tooth extraction. It was not necessary in either group to perform any additional clinical maneuvers at the clinical checkup at 7 days. In 18 surgeries there was delayed healing (32%; CI95: 21% to 45%). Gastric alterations were present in 7 patients (13%; CI95: 6% to 14%). The number of analgesic tablets the patients took was between 0 and 5 (median = 0, Q_1-Q_3 = 0-2, mean +/- SD = 1 +/- 2). Pain perception on VAS was between 0 and 5 (median = 0, Q_1-Q_3 = 0-2, mean +/- SD = 1 +/- 1). The number of analgesic tablets taken was the only variable where a significant difference was observed between groups (Mann-Whitney U test: U = 266; $p < 0.05$; Fig. 2): the values were higher with amoxicillin (median = 1, Q_1-Q_3 = 0-3, mean +/- SD = 2 +/- 2) than with placebo (median = 0, Q_1-Q_3 = 0-2, mean +/ - SD = 1 +/- 1).

Phone interview 14 days after the intervention

There was no trismus in any surgery. Two patients suffered edema (4%; CI95: 1% to 12%) and three manifested gastric alterations (5%; CI95: 2% to 15%). The number of analgesic tablets taken was between 0 and 3 (median = 0, Q_1-Q_3 = 0-0, mean +/- SD = 0.2 +/- 0.7).

As occurred 7 days after the intervention, at 14 days, the use of analgesic tablets also varied significantly between groups (Mann-Whitney U test: U = 323; $p < 0.05$; Fig. 3). with higher values for amoxicillin (median = 0, Q_1-Q_3 = 0-0, mean +/- SD = 0.4 +/- 0.9) than for placebo (median = 0, Q_1-Q_3 = 0-0,

Table 2. Comparison between patients who received amoxicillin or placebo, according to the checkup 7 days after the intervention. Categorical variables, N (%, CI95); numerical variables, median (minimum-maximum).

CLINICAL CHECKUP 7 DAYS AFTER THE INTERVENTION			
Variable	Medication		
	Amoxicillin	Placebo	p
Delayed healing			
No	20 (71%, 53 to 85)	18 (64%, 46 to 79)	0.57§
Yes	8 (29%, 15 to 47)	10 (36%, 21 to 54)	
Gastric alteration			
No	24 (86%, 69 to 94)	25 (89%, 73 to 96)	1*
Yes	4 (14%, 6 to 31)	3 (11%, 4 to 27)	
Analgesic pills	1 (0-5)	0 (0-4)	**<0.05**#
VAS	0 (0-5)	0 (0-5)	0.41#
N total	28	28	

§*Pearson's Chi-square test*
**Fisher's exact test*
#*Mann-Whitney U test*

mean +/- SD = 0.1 +/- 0.4). For the other 4 variables compared, no significant difference was found between treatments (Table 3).

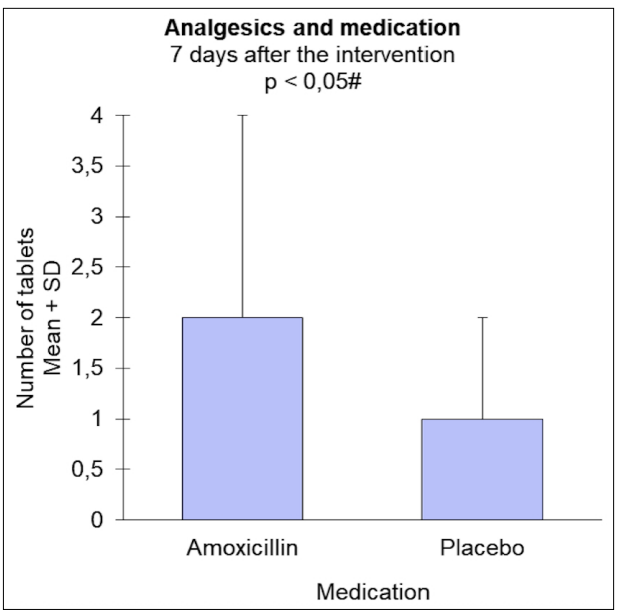

Fig. 2: Analgesics taken (in number of tablets) according to medication, as recorded in the clinical checkup 7 days after the intervention. #Mann-Whitney U test.

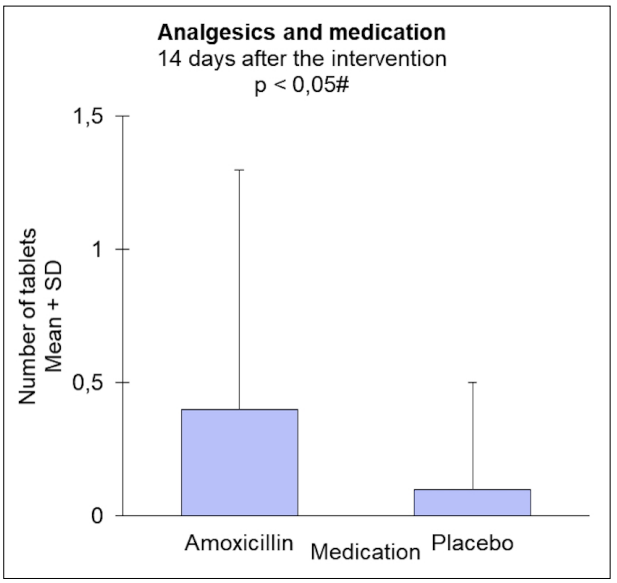

Fig. 3: Analgesics taken (in number of tablets) according to medication, as recorded in a telephone interview 14 days after the intervention. #Mann-Whitney U test.

Table 3. Comparison between patients who received amoxicillin or placebo, according to information collected by telephone interview 14 days after the intervention. Categorical variables, N (%, CI95); numerical variables, median (minimum-maximum).

PHONE INTERVIEW 14 DAYS AFTER THE INTERVENTION			
Variable	Medication		
	Amoxicillin	Placebo	p
Trismus			
No	28 (100%, 88 to 100)	28 (100%, 88 to 100)	1*
Yes	0 (0%, 0 to 12)	0 (0%, 0 to 12)	
Edema			
No	26 (93%; 77 to 98)	28 (86%; 69 to 94)	0.49*
Yes	2 (7%; 2 to 23)	0 (14%; 6 to 31)	
Hemorrhage			
No	28 (96%, 82 to 99)	28 (96%, 82 to 99)	1*
Yes	0 (4%, 1 to 18)	0 (4%, 1 to 18)	
Gastric alterations			
No	26 (86%, 69 to 94)	27 (93%, 77 to 98)	1*
Yes	2 (14%, 6 to 31)	1 (7%, 2 to 23)	
Analgesic pills	0 (0-3)	0 (0-2)	**<0.05**#
N total	28	28	

**Fisher's exact test*
#*Mann-Whitney U test*

None of the patients included in the study (even those medicated with placebo) presented alveolar osteitis or local or systemic infection in the postoperative period. No patient required any additional therapeutic measure, hospitalization, or any other emergency therapeutic measure.

DISCUSSION

Although a 2006 consensus on the use of antibiotics recommended administration for dental extractions in diabetic patients (considered a high-risk group for local and systemic infections)[11], more recent studies cast doubt on this recommendation. Reviews argue that there is no evidence for prescribing antibiotics for diabetic patients if their blood glucose is under control[20,21]. Some studies state that well-controlled diabetic patients do not need antibiotic administration and can be treated as healthy patients[22, 23]. However, diabetic patients with inadequate control should be prescribed antibiotics if surgery is urgent and their blood glucose cannot be properly leveled. It is also recommended to medicate diabetic patients when there is an active infection, or when surgery is complex or prolonged. This is similar to the treatment of non-diabetic patients[11, 20, 23].

Some researchers have compared post-extraction healing in healthy and type 2 diabetic patients. One study that evaluated hematological samples for post-extraction healing in healthy and type 2 diabetic patients found no significant healing difference, and found complications in 10.5% of diabetic patients, and 6.8% of healthy patients. The complications were resolved without need for hospitalization. The authors concluded that it is unnecessary to perform antibiotic prophylaxis for dental extraction in type 2 diabetic patients[24]. Another study in which extractions were performed in type 2 diabetic and non-diabetic patients found that 5% of type 2 diabetic patients and 7% of healthy patients presented a delay in healing beyond one week. No correlation was found between glycemia and healing in diabetic patients. All patients healed completely within four weeks. The authors concluded that there is no difference in the healing process between groups, and preventive medication is not recommended in these cases[25].

The present study provides evidence supporting the premise that antibiotic medication is unnecessary in controlled type 2 diabetic patients. Socket healing after single extractions in diabetic type 2 patients was satisfactory with or without antibiotics. In this study of simple single extractions, there was no relevant post-surgical complication such as alveolar osteitis, or local or systemic infection. Pain, edema, trismus, alveolar osteitis, infection, hemorrhages, delayed healing, and gastric alterations did not differ statistically between groups. To our knowledge, this is the first trial comparing antibiotics and placebo for dental extractions in diabetic type 2 patients. This study design enabled exploration of post-surgical variables of dental extractions, specifically comparing patients with or without antibiotic administration, which is currently a controversial clinical decision. As the study only included single simple dental extractions performed within a maximum of 45 minutes without raising flaps or bone removal, the sample was reasonably uniform.

This trial unexpectedly found significant differences in the intake of analgesic pills (which was higher in the amoxicillin group) at 7 and 14 days after surgery, a characteristic with a non-significant trend at 2 days. This might be because the use of antibiotics such as amoxicillin may be associated with side effects or adverse reactions such as headache, rash, upset stomach, or diarrhea.

Antibiotic prophylaxis in diabetic patients was also observed to be unnecessary in a recent retrospective analysis of diabetic patients. No significant effect was observed on antibiotic prophylaxis with increased odds of post-extraction complication or medical care. The analysis also suggested that it may be necessary to re-evaluate the use of antibiotic prophylaxis in order to minimize unnecessary antibiotic use[26].

In another study, cases of serious infections following dental extractions were reported as clinical cases. Generally, the glycemic data reported were high (218 mg, 305 mg, 428 mg, 378 mg), and in some cases, there was no report of glycemia[11]. These infections spreading to deep anatomical spaces developed in patients with poor glycemic control and poor dental hygiene, and were sometimes associated with other systemic diseases[27-29].

It is important to highlight the disadvantages of unnecessary administration of antibiotics such as side effects and adverse effects (e.g., diarrhea, vaginitis, anaphylaxis), higher costs, and the silent yet important increase in bacterial resistance to antibiotics[9]. Antibiotic resistance is increasing gradually, with new resistance mechanisms

emerging and spreading worldwide, threatening our ability to treat common infectious diseases. A growing number of infections, such as pneumonia, tuberculosis, septicemia, gonorrhea or foodborne diseases, are becoming increasingly difficult to treat as antibiotics lose effectiveness[30, 31].

This trial provides information supporting the premise that antibiotic administration is unnecessary for single simple dental extractions in controlled type 2 diabetic patients. Further studies on larger samples and different clinical situations (e.g., multiple extractions, dental implants[11], endodontic treatment[32]) are needed to clarify the advantages and disadvantages of peri-operative administration of antibiotics in these patients.

In conclusion, the analysis of post-surgical variables suggests that single dental extractions can be performed on controlled type 2 diabetic patients, considering them as healthy patients, without the need to administer antibiotics. Patients receiving placebo did not present greater alveolar osteitis, local or systemic infection, delayed healing, trismus, edema, or pain.

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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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