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Immediate single implant placement and loading in the aesthetic zone with a novel dental implant: a 1-year prospective single cohort study

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OBJECTIVE: This prospective single-cohort study aimed to evaluate the clinical and radiological performance of a novel dental implant positioned in post-extraction sockets in the anterior zone of both jaws and immediately loaded after 1 year in function.

MATERIALS AND METHODS: Patients requiring single implant in the anterior zone were treated between January and March 2019 and followed for at least 1 year of function. Minimum values of 35 Ncm or 64 ISQ were required to perform immediate loading. Screw-retained zirconia-based single prostheses were delivered after the healing period. Patients were followed up for 1 year after definitive prosthesis placement to determine implant and prosthetic survival rate, marginal bone loss (MBL), insertion torque, and ISQ values.

RESULTS: 40 implants (BoneLevel X, Straumann AG) were positioned in 60 patients. None of the positioned implants failed accounting for an implant survival rate of 100 %. No definitive prostheses failed to result in a 100% prosthetic success rate. Mean MBL was $0.6 \text{ mm} \pm 0.4 \text{ mm}$. Mean overall insertion torque was 58.9 ± 13.8 while mean overall ISQ at implant placement was 72.3 ± 8.1 .

CONCLUSION: Within the limitations of the present prospective single-cohort study, the investigated clinical and radiological performance of the novel dental implant placed in the anterior zone seems to be a reliable treatment option.

Dental implants were considered a favorable treatment option to rehabilitate partially or complete edentulous jaws (1-6). Different surgical and prosthetic protocols could be applied and were individualized by different implant placement options. Immediate implant placement combined with immediate loading became an attractive solution for patients and for clinicians that may reduce treatment morbidity and time (5). However, immediate implant placement was influenced by several factors that may negatively affect the long-term aesthetic and functional outcomes such as the alveolar anatomy and the buccal bone wall thickness (7-10). Furthermore,

immediate loading strictly depended on insertion torque or implant stability quotient (ISQ) values. Low values of insertion torque (<30) did not affect osseointegration, however high values (>35) could prevent micro-movements that may negatively affect bone healing process(11). It was recommended to achieve primary stability with an insertion torque of at least 30 Ncm or an ISQ value ≥ 60 and a minimal implant length $\geq 10\text{mm}$ to perform immediate loading and to not exceed $150 \mu\text{m}$ of micromotions during the early healing/loading period to prevent soft-tissue encapsulation (12,16). High insertion torque values could provide more confidence to the clinician when

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performing immediate loading; however, it was mandatory to remind that several studies reported bone micro-fracture and higher stress in the cortical zone related to high insertion torque delaying the healing process. The ongoing development of three-dimensional (3D) planning software and the possibility of 3D visualization of anatomical structures near to implant recipient site merged with prosthetic wax-up led to more comprehensive and predictable implant placement in both healed and post-extraction sites. A digital approach may overcome some difficulties related to prosthetically-driven implant placement, especially in the anterior zone of both jaws where the patients' expectations and requirements were significant (17-24). This prospective single-cohort study aimed to evaluate the clinical and radiological performance of a novel dental implant positioned in post-extraction sockets of the anterior zone of both jaws and immediately loaded after 1 year in function.

MATERIALS AND METHODS

Study design

Patients requiring a single implant FDP rehabilitation in post-extraction sites of the anterior zone of both jaws after the signature of the informed consent were enrolled in the study between September and December 2018. Patients were informed of the nature of the study, benefits, risks, and possible alternative treatments and provided consent before inclusion in the study, as well as any follow-up evaluations required for the clinical study. Patients were consecutively treated in one rehabilitation center between January and March 2019 and followed for at least 1 year of function. The study was conducted in compliance with the Declaration of Helsinki for biomedical research involving human subjects as amended in 2008 and according to the industry regulations (the International Conference for Harmonization Guideline for Good Clinical Practice and ISO14155). This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for improving the quality of observational studies (25-30)

Fully tapered implant with self-cutting and self-tapping design, and a condensing core and thread design to optimize primary stability with a core made of titanium-zirconium alloy (Roxolid®) and a chemically modified sandblasted,

large-grit and acid-etched surface (SLActive®) (BoneLevel X, Straumann AG, Basel, Switzerland) were placed in the anterior zone and loaded immediately with a screw-retained FDP. One expert clinician performed all surgical and prosthetic procedures.

Inclusion criteria for participating in the trial were: at least 18 years old, healthy patients, full mouth bleeding and full mouth plaque index lower than or equal to 25%, at least one permanent missing tooth in either the maxilla or the mandible in the anterior zone, the need for FDP, fresh extraction sockets with an intact buccal wall and adequate bone quantity to place an implant with a diameter of at least 3.5 mm and a length of at least 10 mm, minimal insertion torque of 35 Ncm, minimal ISQ mean value of 64.

Exclusion criteria were general medical (American Society of Anesthesiologists, ASA, class III or IV) and/or psychiatric contraindications; pregnancy or nursing; any interfering medication such as steroid therapy or bisphosphonate therapy; alcohol or drug abuse; heavy smoking (>10 cigarettes/day), radiation therapy to head or neck region within 5 years, untreated periodontitis; acute and chronic infections of the adjacent tissues or natural dentition; severe maxillomandibular skeletal discrepancy; high and moderate parafunctional activity, absence of opposite teeth; unavailability to attend regular follow-up visits (31-37).

On the day of the surgery, all patients received antibiotic prophylactic therapy: 2 g of amoxicillin (or clindamycin 600 mg if allergic to penicillin) 1 hour before the surgery and rinsed with chlorhexidine mouthwash 0.2% for 1 minute before the intervention. Local anesthesia was performed using articaine with adrenaline 1:100000. Teeth extractions were performed as gently as possible to avoid trauma on the buccal walls (Fig. 1-3). Surgical templates were realized to guide implant drills' pathways (Implants sites were prepared using a dedicated drill set (Straumann, Straumann Holding AG, Basel, Switzerland) following the manufacturer's guidelines to achieve the highest primary stability. Bone density was assessed during the implant recipient site preparation by clinician experience and tactile perception. The drills were used in their normal sequence to prepare the implant site. Low-speed drilling [40 Rotations Per Minute (RPM)] was performed from the second to the last used drill without irrigation to avoid overheating the bone recipient site and to recover autologous bone as previously described by the authors(38-45) (Fig. 4).

The implant platform was positioned between 1 and 2 mm below the bone crest level according to the prosthetic final design. Mucoperiosteal flaps were raised if any implant site needed guided bone regeneration (Fig. 5). The implant insertion torque was assessed after the removal of the surgical template to avoid any interferences using a surgical unit (iChiroPro Drill Motor Set, BlenAir) and ISQ was recorded utilizing a dedicated device (Osstell, W&H, Göteborg, Sweden). Screw retained temporary



Fig. 1. Tooth 2.4 with old metal-ceramic fixed restoration.



Fig. 2. Periapical X-Ray of hopeless 2.4.



Fig. 3. Tooth extraction

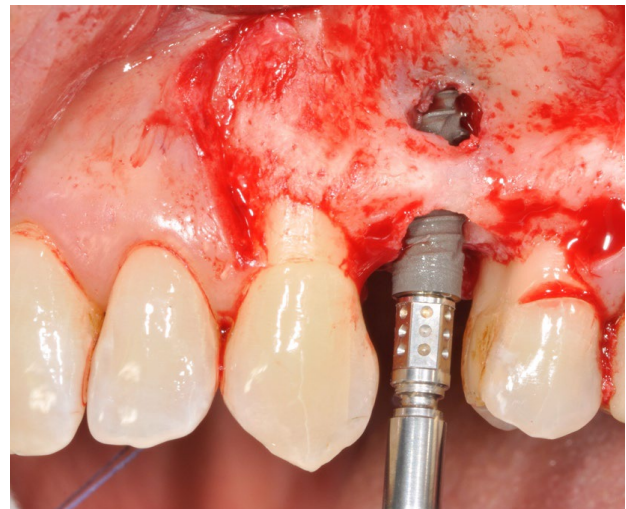


Fig. 4. Implant placement (Straumann BLX 3.75x14 mm, Straumann AG).

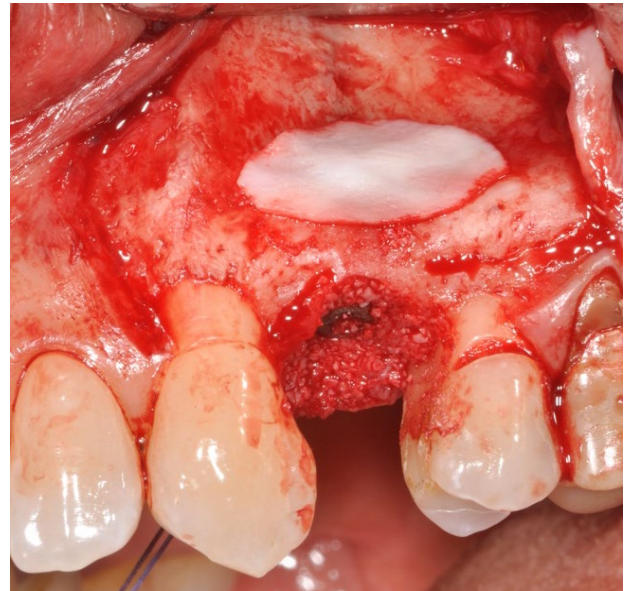


Fig. 5. Guided-Bone-Regeneration after implant placement.

abutment (VarioBase, Straumann) was secured up to 15 Ncm onto the implant. Flap closure was performed using Polyglycolic Acid (PGA) sutures (Vicryl, Ethicon, Somerville, NJ, USA).

Prosthetic protocol

The temporary crown was checked to connect it to the temporary abutment. The abutment and the screw holes access were blocked out with rubber dam and Teflon. The abutment was merged to the temporary crown intraorally with a self-curing resin (Enamel Plus Temp, Micerium, Avegno, Italy) and then screwed off and transferred to the

lab to finish and polish. The provisional crown was screwed to 15 Ncm of torque and the screw channel was sealed using Teflon and temporary filling material (Fig. 6, 7). Occlusion was checked by means of a 40 µm articulating paper to obtain light occlusal contacts uniformly distributed and to avoid lateral contact. Patients were educated to rinse with 0.2% chlorhexidine mouthwash for 1 minute twice a day for 15 days, to have a soft diet for one month, and to avoid any trauma on the surgical sites. Patients were recalled after 7 days to check the occlusion if required suture removal and to deliver oral hygiene instructions. The prostheses were not removed before six weeks from the surgery (46-52). Depending on the surgical site, the healing period ranged from 4 to 6 weeks in the mandible and 6 to 8 weeks in the



Fig. 6. Immediate restoration in-situ.

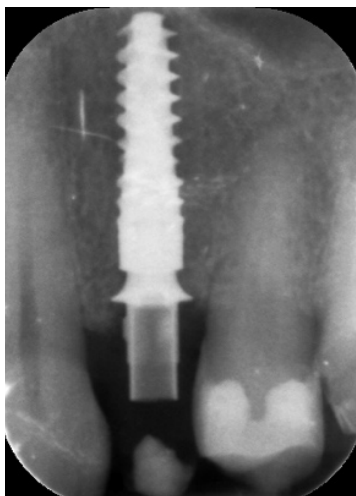


Fig. 7. Periapical x-ray at immediate loading.



Fig. 8. Definitive restoration at 1-year follow-up.



Fig. 9. Periapical X-Ray at 1 year of follow-up.

maxilla. Thereafter, the temporary crown was removed to check implant stability by screwing the temporary abutment to 35 Ncm torque. If the implant had mobility, pain, or soft tissue irritation or suppuration, it had to be removed and classified as “failed”. If not, a digital impression was taken by means of an IOS (Trios3, 3Shape) and dedicated scan-bodies (Mono ScanBody CARES, Straumann AG) to record soft tissues sculpturing and implant coordinates. The definitive restorations were screw-retained lithium disilicate fused on zirconia custom abutments digitally designed (Straumann CARES Abutments, Straumann AG). The definitive crown was screwed up to 35 Ncm as recommended by the manufacturer and the screw channel was filled with composite resin (53-58) (Fig. 8, 9).

Follow-up protocol

Patients were recalled every 4 months for professional

oral hygiene and every 12 months for the annual radiographic and clinical examination. Standardized periapical radiographs were performed by an independent operator in order to assess radiographic peri-implant bone health. Bleeding on probing (BoP) and plaque score (PS) were recorded at each planned visit.

Implant survival rate

An implant was considered stable if there were an absence of subjective complaints such as pain, no mobility, no suppuration when peri-implant mucosa was pressed, a probing Depth $\leq$ of 5 mm. Each implant was examined and classified as a “successfully integrated implant” if it had none of these criteria at the clinical and radiographic examination. In addition, if a biological complication occurred it was reported.

The definitive prostheses in function without complications were considered as a “successful prosthesis”.

Marginal bone loss

Marginal bone levels were assessed using standardized intraoral digital periapical radiographs with the parallel technique by means of customized periapical radiograph holder (Rinn, DentsplySirona, York, PA, USA) at implant placement (baseline) and after 1-year from the definitive prosthesis delivery. The x-ray images were analyzed by an independent operator not informed on the aims of the study. The periapical X-rays were loaded onto Osirix MD 7.5 image diagnosis and analysis software package (Pixmeo SARL, Geneva, CH) on a Mac Pro Workstation (iOS 10.15.3) adjusting the density and contrast for optimal visibility of the crestal bone. MBL was subsequently calculated for paired radiographs from baseline (the day of definitive prosthesis delivery) to last follow up.

Insertion torque

The implant insertion torque was measured after the removal of surgical template to avoid any interferences using a surgical unit (iChiroPro Drill Motor Set, BlenAir) at the moment of the implant insertion. Implant stability quotient (ISQ) was recorded using the RFA (ISQ values, range 1 to 100) at implant placement.

Soft tissue parameters

Soft tissue parameters concerning the implant/abutment interfaces were assessed by means of a periodontal probe

(Hu-Friedy PGF-GFS, Hu-Friedy, Chicago, IL., USA). The BoP was recorded at four sites around each implant (mesial, distal, buccal and lingual) according to the Mombelli Index (15). The probe was carefully inserted 1 mm into the sulcus and the provoked bleeding within 20 s was assessed (0 = no bleeding; 1 = spot bleeding, 2 = linear bleeding, and 3 = spontaneous bleeding). The PS was assessed by passing the probe parallel to the abutment/restoration interface and scored at one site for implants as the presence of plaque (yes/no). A blinded independent dental hygienist who was not involved in the study recorded all the periodontal measurements.

Statistics

Continuous variables were summarized by mean and standard deviation (SD). The implant was used as unit in the statistical analysis.

RESULTS

Clinical data from 40 patients of both sexes (17 men and 23 women), mean age, 55.3 years ranged from 47 to 78 years, rehabilitated with a single-fixed screw-retained prosthesis in the aesthetic zone immediately loaded and followed-up with a minimum period of 1 year were examined and classified. No patient drop-out occurred, and all patients were treated according to the original protocol. A total of 40 dental implants (BLX Regular Base, Straumann AG) were placed and all of them were loaded immediately with a fixed screw-retained interim prosthesis. Twenty-four implants (60%) were placed in the maxilla and 16 (40%) in the mandible. Three different implant diameters were used: 4.5 mm 17 times (42.5%), 4.0 mm 8 times (20%) and 3.75 mm 15 times (37.5%). The most used implant length was 14 mm 19 times (47.5%), followed by 12 mm 15 times (37.5%) and 16 mm 6 times (15%). The mean overall insertion torque was 58.9 ± 13.8 . Mean overall ISQ at implant placement was 72.3 ± 8.1 . None of the 40 failed, accounting for an ISR of 100%. No biological or mechanical complications occurred during the entire follow-up. A total of 40 definitive single screw retained FDPs were delivered and none of them failed during the entire follow-up, resulting in a prosthetic success rate of 100%. At the last follow-up, BoP was

detected around 2 implants (5%). 3 patients showed a superficial amount of plaque around 3 implants resulting a PS of 7.5%. At 1-year of follow-up, mean overall MBL was $0.6 \text{ mm} \pm 0.4 \text{ mm}$ (Table I).

DISCUSSION

The aim of this prospective single-cohort study was to evaluate clinical and radiological performance of a novel dental implant positioned in post-extraction sockets of the anterior zone of both jaws and immediately loaded after 1 year in function. Because it was designed as a single-cohort prospective study, the main limitation of the current investigation was the lack of control group (59-64). Nevertheless, 40 fully tapered implant with self-cutting and self-tapping design, condensing core and double-cutting-thread design to optimize primary stability with core made of titanium-zirconium alloy (Roxolid®) and a chemically modified sandblasted, large-grit and acid-etched surface (SLActive®) (BoneLevel X, Straumann AG) were placed in the aesthetic zone, loaded immediately with a screw-retained FDP and followed up for at least 1 year in function. To the best of Authors' knowledge, this represents the first prospective study investigating clinical and radiological outcomes of a novel dental implant immediately loaded in post-extraction sockets of the anterior zone of both jaws. None of the positioned implants failed, accounting for an ISR of 98.3%. No definitive prosthesis failed, accounting for a PSR of 100%. The results of the present study are in accordance with a recent systematic review, reporting an overall ISR of single implant loaded immediately in the anterior zone at 1 year of follow-up of 97.9% (16). Another systematic review by Pigozzo et al (17) reported an ISR of immediately loaded single implants of 97.3%. Even if high ISR value was reported, it is mandatory to point out that immediate loading of single implants in the anterior zone requires highly sensitive and technical surgical and prosthetic skills in order to avoid implant bad positioning and outcomes (65-68). The dental implants used in this study may have influenced the ISR value recorded. All the implants positioned had core made of titanium-zirconium alloy (Roxolid®) and a chemically modified sandblasted, large-grit and acid-etched surface (SLActive®) surface.

The described characteristics of the type of dental implants used may be crucial when immediate loading is performed. In addition, the variable thread design with self-cutting and self-tapping design enhanced primary stability values even in post-extraction sites resulting in high recorded values of insertion torque and ISQ. High implant insertion torque values may be connected with higher stress on the cortical bone that may cause augmented marginal bone remodeling. In the present study at 1-year of follow-up, mean MBL was $0.6 \text{ mm} \pm 0.4 \text{ mm}$. Similar results were reported in several studies investigating single implant loaded immediately in the anterior zone at 1 year of follow-up (18–20). Even if high insertion torque values were recorded, no augmented MBL values were reported in the present study. The Authors assumed that this may be explained by the reduced neck diameter (3.5 mm, for all the implant diameters involved in the study) with micro-threads that not over stress the cortical bone during implant placement in combination with an in-built platform switching (69-70). In addition, the low-speed drilling sequences and performed in all the surgeries and the flapless or mini-flap approach may have reduced stress and over-heating of the implant recipient sites.

CONCLUSION

Within the limitations of the present prospective single-cohort study, the investigated clinical and radiological performance of the novel dental implant placed in post-extraction sites of the anterior zone seems to be a reliable treatment option. However, several randomized controlled prospective trials with longer follow-up periods are required to assess the effectiveness of this novel dental implant.

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Caries risk, saliva and their relationship

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In the fight against caries, the goal of clinicians must not be focused only on performing conservative or prosthetic restorations but must include the execution of specific protocols for controlling the causal factors of the disease. Caries is a pathology with a multifactorial etiology, in which bacteria, diet, quality of saliva, oral pH, and the patient's lifestyle, hygiene and health play a fundamental role. The CAMBRA method allows us to customize caries care, based on individual risk analysis, and to develop a suitable prevention plan for each patient.

The term “caries” should be used to indicate the disease that attacks the surface of the teeth and that manifests with different signs, including cavitated lesions and carious lesions, and not only for the demineralizing action of bacteria and acids (1-5). The therapy of any pathology must aim to address the causes, not the consequences, through the inclusion of protective factors. Yet, most clinicians often limit treatment to eliminating cavities and take no other action to address the underlying causes of the disease. Certainly, the elimination of carious lesions is fundamental to halt the progression of the lesions but performing a restoration does not address the disease.

Caries is a pathology with a multifactorial etiology, in which bacteria, diet, quality of saliva, oral pH, and the patient's lifestyle, hygiene and health play a fundamental role. The introduction of fluoride, calcium and phosphate compounds is also an important factor to consider. The delicate balance between the presence or absence of the disease is determined by the less or more effective control of pathological factors and protective factors (6-11). The risk level of caries development, defined as caries risk assessment, and the interventions to be implemented to reduce it were the subject of two

distinct consensus conferences organized in California by Featherstone in 2003 and 2007 (5). The author, one of the creators of the Caries Management by Risk Assessment (CAMBRA) method, proposes a strategy for assessing the individual risk of caries of the patient and treatment indications to reduce this risk, if present and high. In this method, the main factors to analyze are saliva, bacteria, diet, fluoride and lifestyle.

In this article, we will examine the risk analysis of caries, using the DMF index, and the role of saliva as a protective factor against carious pathology, as well as the danger of infiltration of conservative and prosthetic restorations.

MATERIALS AND METHODS

The DMF index

The DMF index represents an easy indicator for detecting caries and is useful to assess the patient's caries risk. It entails allocating a point to every tooth that has a cavity (D), is missing due to decay (M) or is filled (F). A value is given to each tooth, excluding the third molars. The minimum value is 0 and the maximum value is 28.

According to the most recent revisions of this index, it is

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possible, in relation to age, to assign a patient a level of risk based on his or her previous history of carious disease, given by the sum of the carious teeth lost due to caries or restored (7-13). Fig. 1. shows two examples of different risk levels.

It must be said, however, that this is an indicator of the patient's past caries risk. In fact, we cannot know whether in the meantime there have been moderate changes to the other factors that may affect the current level of risk. The DMF index must be used as a quick tool for an initial screening, which must however be followed by the analysis of all the other factors, pathological and protective, of the patient: quality and quantity of saliva, oral pH, evaluation of diet, and habitual use of fluorinated compounds or remineralizing products (casein phospho-peptide-amorphous calcium phosphate) (14-20) (Table I).

A patient with a low risk level will have to have a dental check every six to 12 months and will have to use a fluoride toothpaste twice daily. A patient with an extreme risk level will have to be followed more often, will certainly have to undergo salivary tests (for control of the pH and the salivary buffer capacity), will have to use fluoride toothpaste and mouthwash daily, as well as impede excessive development of bacterial biofilm with chlorhexidine. The control visits will be performed every three months, introducing the professional application of fluoride varnishes and, if deemed appropriate, of remineralizing products based on calcium

phosphate (casein phosphopeptide-amorphous calcium phosphate). In these patients, six-monthly radiographic screening via bitewing radiographs is also recommended (21-26). Periodically, a new evaluation will have to be carried out with the aim of verifying whether, after the introduction of the protective factors, the risk class of the patient has changed.

DISCUSSION

The role of saliva - quantity

In a time-interval of 24 hours, a patient in good general health secretes about 1,500 mL of saliva. During sleep, only 40 mL is produced. For this reason, it is very important to recommend to patients to go to bed with a clean mouth, since the protective power of saliva is lost during the night. (27-30)

The CAMBRA method involves the evaluation of stimulated saliva. The patient is asked to chew a paraffin tablet for 5 minutes and to fill a container with the saliva produced. In normal conditions, the saliva collected with this test should be greater than 5.5 mL. A value lower than this must induce the dentist to intervene regarding this parameter. A value of less than 2.5 mL is related to the condition of xerostomia. (31-33)

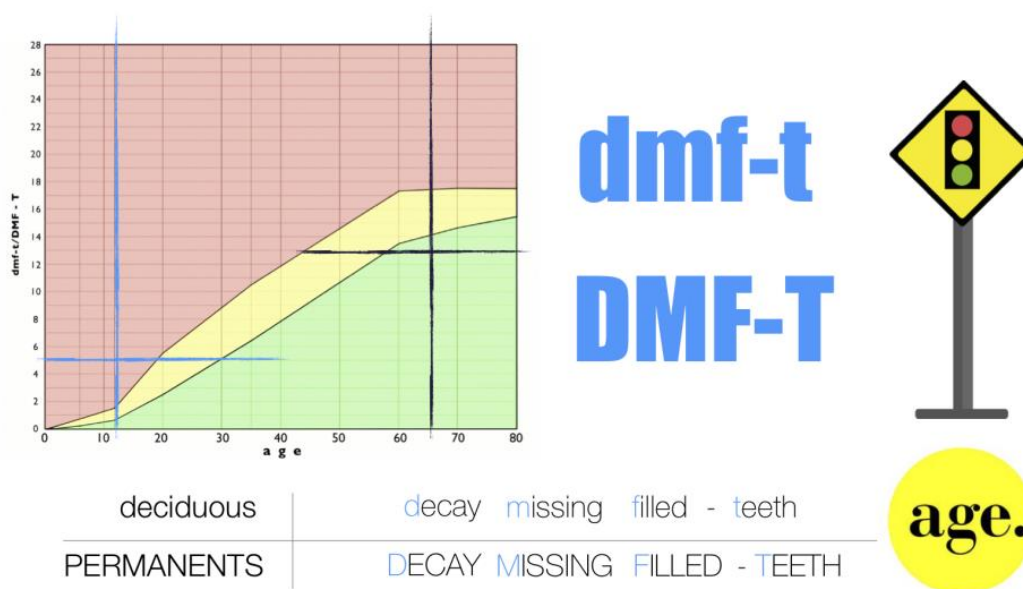


Fig. 1. A 12-year-old child who has a DMF index of 10 is a patient in a high-risk range (red area). The same score is considered normal in a 65-year-old patient (green area).

To encourage an increase in the amount of saliva, it is important to drink plenty of water. Another effective suggestion is the use of xylitol chewing gum (several times per day, for a maximum of 5 to 15 minutes each time) and the controlled eating of fruit, in particular kiwis, oranges and blueberries. These kinds of fruits help increase the quantity of saliva but must be eaten in small quantities only because of their acidity, which can be dangerous for the enamel.

Acidity (pH)

The saliva sample collected with the previous test can be used to measure the pH. It is possible to place a few drops of saliva on a special testing paper and compare the resulting color with a specific reference color scale.

A pH of higher than 6.8 is considered normal and a pH of lower than 5.8 is considered a high-risk factor. (34-38) A pH that is frequently below this level can

promote, in addition to the increased risk of formation of carious lesions, the onset of enamel erosion and consequent exposure of dentin areas. Modern minimally invasive prosthetic restoration techniques allow us to exploit adhesion to the residual enamel to restore patients with erosion problems, avoiding losing further healthy tooth substance (39-41) But it is good to consider this aspect even after restoration, as a constant presence of acidity can result in problems of infiltration of the prosthetic restoration.

Buffer capacity

Buffer capacity is a measure of the ability to counteract a pH change brought about by the addition of a certain amount of a strong acid or a strong base. Owing to the presence of bicarbonates, saliva is a buffer system that corrects the acidity of the oral cavity after food intake. If the buffer capacity of the saliva is good, the resistance of the oral environment

Table I. Behavioral and therapeutic guidelines that can be used according to the patient's risk level, which can be low, moderate, high or extreme.

Risk level	Low 0-5	Moderate 6-10	High 11-15	Extreme 16-27
Salivary Test	Optional	Recommended	Requested	Requested
Antibacterial (CHX/Xylitol)		Xylitol (6-10 g/day) 2 chewing-gums 4 times/day for 5-15'	CHX 0.12% mouthwash for 60 s before bedtime for 1 week/month. Xylitol (6-10 g/day) 2 chewing-gums 4 times / day for 5-15 min maximum 1% CHX gel in tray 5' for 14 days, before phase 2	CHX 0.12% mouthwash for 60 s before bedtime for 1 week/month. Xylitol (6-10 g/day) 2 chewing-gums 4 times / day for 5-15 min maximum 1% CHX gel in tray 5' for 14 days, before phase 2
Fluoride	F toothpaste twice/day F >1000 ppm	F toothpaste twice/day F >1000 ppm F varnish professional initial application, before Phase 2, then after any recall	F toothpaste 2500 ppm, 3 times/day 0,05 % F mouthwash 1-2 times/day. F varnish professional 1-3 applications, before Phase 2, then after any recall. 12500 ppm F before bedtime once a day	F toothpaste 2500 ppm, 3 times/day 0,05 % F mouthwash 1-2 times/day. F varnish professional 1-3 applications, before Phase 2, then after any recall. 12500 ppm F before bedtime once a day
Control Frequency (months)	6-12 months	4-6 months	3-4 months	3 months
RX frequency	BW 24-36 months	BW 18-24 months	BW 6-18 months (until risk changes)	BW 6 months (until risk changes)
Sealant	Recommended	Yes	Yes	Yes
CPP-ACP	Not requested (optional in case of root Hypersensitivity)	Not requested (optional in case of root Hypersensitivity)	CPP/ACP paste 1-2 times/day	CPP/ACP paste 1-2 times/day

to the formation of caries will be high. The analysis of this aspect is another important factor of the CAMBRA method (42-49).

There are tests that, by analyzing saliva samples on specific paper, give us an idea of the buffer capacity in just a few minutes and give us an indication of the specific risk level of the patient so that we can put in place appropriate measures to change this important aspect and lower the risk of developing carious lesions. In the presence of risky pH values or a low buffer capacity, the patient will naturally have a greater risk of developing caries, owing to the prolonged acidity of the oral environment after meals (50-55). When testing detects these problems, one or more of the following modification factors could be recommended to the patient:

1. Use toothpaste containing bicarbonate.
2. Rinse with water, or with water and bicarbonate, at the end of a meal in order to counteract the acidity and improve the buffer capacity.
3. End the meal with a protective food, such as aged cheese.
4. Use buffer substances after meals. There are commercially available bi- carbonate and hyaluronic acid sprays able to improve the buffer capacity.

For reliable results, it is important to perform salivary tests at least 2 hours after the last meal and the last home oral hygiene. It is also important that the patient has not taken antibiotics in the two weeks prior to testing. We also advise patients not to smoke, drink or use mouthwash in the previous 2 hours.

CONCLUSION

In the fight against caries, the goal of clinicians must not be focused only on performing conservative or prosthetic restorations, but must include the execution of specific protocols for controlling the causal factors of the disease, as well as educating the patient that, in order to address caries, it is not enough to eliminate carious lesions; rather, it is fundamental to change his or her lifestyle radically (56-59).

The CAMBRA method allows us to customize caries care, based on individual risk analysis, and to develop a suitable prevention plan for each patient, with the aim of avoiding the onset of new carious

lesions and reducing the risk of marginal infiltration of conservative and prosthetic restorations. Saliva is one of the main controlling factors in patients at high risk of developing caries, and it is the dentist's duty to recommend to the patient the most suitable strategies to improve saliva quality and its protective properties.

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Learning curve of digital intraoral optical scanning for complete arch: an in vitro study

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OBJECTIVE: To evaluate the learning curve by unskilled operator for a complete arch digital impression in terms of scanning time and accuracy.

MATERIALS AND METHODS: Three unskilled operators (OP1, OP2, and OP3) were enrolled to scan for 15 times each one an edentulous maxillary polymethylmethacrylate (PMMA) milled model with six implant analogs in different angulation and depth. The scanning times were recorded for each scan of the randomized sequence. The resulting 45 test files were aligned by the reference one, obtained by a high-precision industrial scanner. Each test file was processed with dedicated software to assess the linear (DX, DY, and DZ axis) deviations, that were summed up to obtain an average value of three-dimensional deviation.

RESULTS: The three operators showed a mean scan time of 169.6 seconds (s), 217,9 s, 193,7 s respectively. Each operators show a statistically significant scan time reduction from the first to last scan: OP1 $p=0,001$; OP2 $p=0,008$; OP3 $p=0,003$. The average three-dimensional (3D) errors reported were OP1 133 microns (mm), OP2 111 mm and OP3 107 mm. Regarding the accuracy, a statistically significant reduction in the average 3D error was reported: OP1 $p = 0.02$; OP2 $p = 0.04$; OP3 $p = 0.04$.

CONCLUSION: Within the limitation of this in vitro study, it seems to be that the repetitive use of IOS significantly affects the investigated variables in an overall scanning time reduction and scan accuracy enhancement.

Nowadays the use of intraoral optical scanning (IOS) increases its interest among dental clinicians (1-3). The success of IOS technologies, which followed the introduction of computer-aided design and computer-aided manufacturing (CAD-CAM) technology in dentistry since 1980, is mainly linked to the optimization of the digital workflow (4-9). Digital impression has benefits over conventional impressions such as improving patient acceptance, linked to reduction gag reflex potential; lower risk of spreading infections; decreased working time; no potential deformation of impression material or expansion of gypsum; real-time visualization; easy repeatability (10-13). Nevertheless, the use of IOSs

still has some limitations: few studies showed that conventional impressions represent a better solution for challenging prosthodontics (e.g., accuracy of full-arch restoration on multiple implants), costs (intraoral scanning systems are still expensive), and last the learning curve of the procedure (14-19). Practitioners are required to invest time in learning to use these devices effectively (20-24). Learning is defined as "an enduring change in behavior or in the capacity to behave in a given fashion resulting from practice or other forms of experience" (25-28). The representation of the map between practice and competence yields the learning curve, that is the rate of learning something over time. Typically, this curve

Key Words: Digital impression; complete arch; learning curve; accuracy

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shows a quick improvement of operators' skills, with subsequent smaller improvements that typify the approach to a plateau.

Different factors affect the learning curves among different practitioners such as innate ability, previous experience, and motivation; the case difficulty and the technology used to also play a role (29-33). Several studies regarding the introduction of new technologies or techniques in medicine, and dental practice too, have determined the learning curve of users. Focused on IOS many studies compared this technology with conventional impression taking, with particular interest regarding the accuracy of the intraoral scanner and clinical acceptance between experienced and inexperienced clinician (34-40). Studies on the learning curve of IOSs are scarce.

One study compared the learning curves of using 2 IOSs among dental hygienists, concluding that scanning time decreased after repeated scanning with both scanners, but the time of scanning, rate of learning and effect of experience differed between the 2 scanners (41-43). In accordance with the previous study Al Hamad KQ⁹ showed in an in vitro trial a decreased scanning time and difficulty level with the repetitive use of IOS, with a learning phase of 5 trials to achieve competence of 80% of their best performance. Róth I. et al in a recent in vivo trial assessed a reduction in scanning time by the practitioners linked to a practice effect of repeated

use, i.e., the students learned to move the scanning tip faster. However, shorter scanning times are associated with poorer coverage quality, with the operator needing to make corrections by adding extra images, so the number of acquired images has not shown a similar decrease too.

The purpose of the present in vitro study was to assess the learning curve of IOS for unskilled operators by scanning time and scanning accuracy. The null hypothesis was that there is no correlation between the sequential use of IOS and the outcomes variables considered in the trial.

MATERIAL AND METHODS

Six implant analogs were placed at the site of lateral incisors (1.2, 2.2), first premolars (1.4, 2.4), and first molars (1.6, 2.6), in an edentulous maxillary polymethylmethacrylate (PMMA) milled model with different angulations and depths (Fig. 1). Six cylindrical-shaped biomedical grade 5 titanium implant scan bodies (ISBs) with an axial incision were produced. The scan region of titanium ISBs was sandblasted with 110 mm powder at 2 bars. Also, a removable soft tissue frame was three-dimensional (3D) printed (Fig. 2). The master model without the removable gingival part was scanned through an industrial structured blue light 3D metrology optical scanner (ATOS Compact Scan 5M, GOM GmbH, Braunschweig, Germany), to achieve a standard tessellation

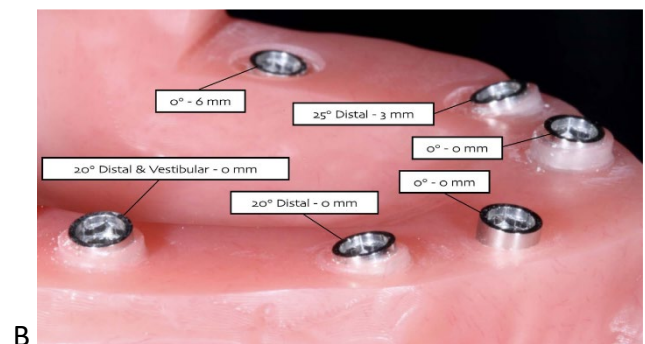
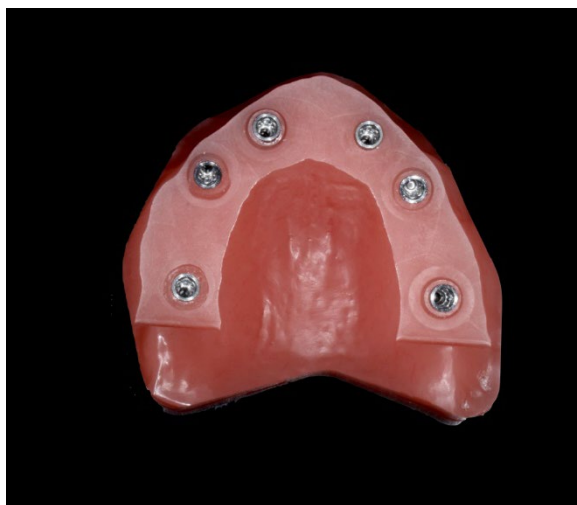


Fig. 1. Master model with six implant analogues (A); and implant angulation and depth (B)

language (STL) file to be used as reference. The emerging geometry of the implant analogs was subsequently coupled by means of a software dedicate (Gom Inspect Professional, GOM GmbH, Braunschweig, Germany) to the native mathematical files of the analogs. Three unskilled operators (OP1, OP2, and OP3) were enrolled for this study, each operator had to make a total of 15 complete-arch scans with, Trios (3Shape A/S) IOS, for a total amount of 45 scans obtained by a randomized sequence.

A fourth operator secured the ISBs into the prosthetic connection by means of torque of 15 Ncm (Fig. 3). The scanning time was assessed immediately after each scansion; to evaluate the accuracy the 45 test files were aligned with the reference one by dedicated software (Geomagic Studio 12, 3DSystems, Rock Hill, SC, USA) and each superimposed file was analyzed by a measurement

dedicated software (Hyper Cad S, Cam HyperMill, Open Mind Technologies, Milano, Italy) in order to assess the linear deviations for each analog. At last absolute values of the linear discrepancies (ΔX , ΔY and ΔZ axis) were summed up to obtain a global measure of absolute error for each analog; the means of the absolute error of the six analogs suggested the absolute mean linear deviation (Δ_{Ass}) for each scansion.

RESULTS

The null hypothesis stated that there was no association between sequential use of IOS and the outcomes variables (total scanning time and scansion accuracy). Based on our results, the null hypothesis was rejected since repeated use of intraoral scanner

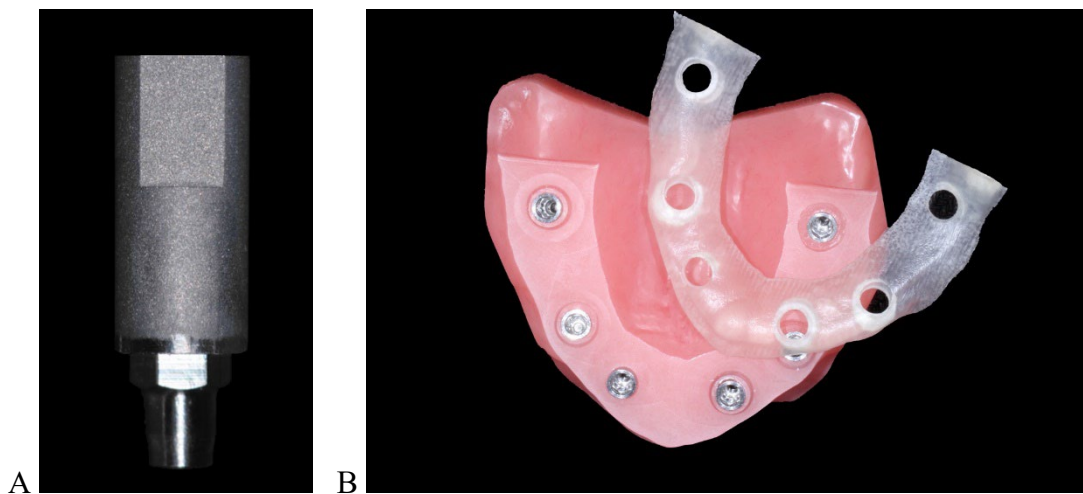


Fig. 2. Titanium ISB (A); and soft tissue 3D printed removable frame (B)



Fig. 2. Titanium ISB (A); and soft tissue 3D printed removable frame (B)

was associated with decreasing scanning times and increasing scanning accuracy, linked to a reduction of average error. The average scan times were: OP1 169.6 seconds (s) (from 230 to 115 s); OP2 217.9 s (from 273 to 160 s); OP3 193.7 s (from 254 to 138 s). Each operator showed a statistically significant reduction in scanning time from the first to last scan (OP1 $p=0.001$; OP2 $p=0.008$; OP3 $p=0.003$). The scan time trend curve is shown in Fig. 4. Concerning the accuracy, the operators showed a statistically significant reduction in the average three-dimensional error, although the significance levels were lower than those relating to the scanning time (OP1 $p=0.02$; OP2 $p=0.04$; OP3 $p=0.04$). OP1 recorded an average 3D error of 133 microns (μm) (from 272 to 40 μm); OP2 111 μm (from 258 to 34 μm); OP3 107 μm (from 237 to 31 μm).

DISCUSSION

This in vitro study investigated the learning curve related to acquisition time and accuracy of the digital impression for complete arch on implants. Three unskilled operators were enrolled in this study, the repeated use of IOS provided a significant reduction in acquisition time and average 3D error, so the null hypothesis was rejected. However, the inherent limitation of this study is intrinsic in its in-vitro design. This does not consider the fact that the performance of an inexperienced operator using IOS in vivo can be adversely affected.

Some patient factors such as presence of saliva, movement of the tongue and cheeks, amount of attached gingiva, shape and length of the edentulous ridge and implant factors such as number, position and inter-implant distance could be reducing its accuracy and increasing scan time (44-50). Few studies have already analyzed the learning curve for the use of IOS, but to the best of our knowledge no one has analyzed this variable in relation to the optical digital impression on implants. Moreover, the clinical situation reproduced in this in vitro study, the complete-arch impression, is still considered one of the most challenging tasks for an intraoral scanner (IOS) device (51-55).

Digital impressions are sufficiently accurate for single tooth gap or partial edentate patients. With edentulous patients, the results are less impressive both in terms of accuracy and scanning time. Stitching, lack of detailed and stable anatomic reference points, the edentulous ridge anatomy (length and shape), quantity of keratinized fixed mucosa and distance between dental implant can influence and lead to a worse performance of the IOS in terms of accuracy, clinical applicability, and scanning time (56-62). Kim J et al in a human subject study focused their attention on the experience curve of two IOS. A total of 29 dental hygienists with more than 3 years of working experience were recruited to learn the iTero and Trios systems. A

Although the learning rate of iTero was rapid compared to Trios, the average scanning time for iTero was longer than Trios, and clinical experience

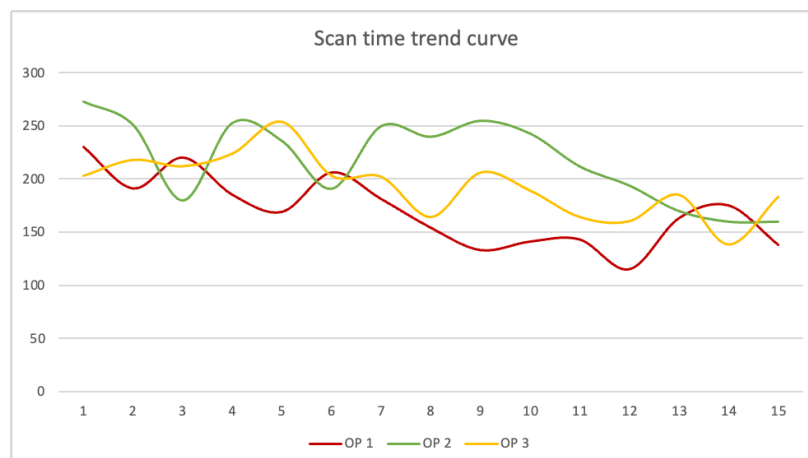


Fig. 4. Scan time trend curve

levels influenced the operator's ability to manipulate the device unlike Trios. In accordance with this study, our in vitro trial using the Trios scanning system showed a low mean scan time but a long learning curve. After the initial oscillations, the curve of the three operators in fact reaches a plateau around the twelfth, thirteenth scan. On the other hand, Al Hamad KQ, in an in vivo study (9) showed how, although inexperienced, operators with high levels of clinical experience show a faster level of learning, and in particular 5 scans were needed to reach 80% of their best performance. At last, Róth I. et al, in their in vivo study described the learning curve of intraoral scanning in terms of scanning time and the image number of digital impressions. Although the variables are different, like what was observed in our in vitro trial, the authors indicate an association between the sequential number of measurements and the outcome variable (63-66). In both studies scanning time decreased along with repeated use of the intraoral scanner. Having also evaluated the average accuracy of the scan as a secondary outcome, we can confirm what the authors of this in vivo trial reported, that is there is no correlation between the number of images acquired and the accuracy of the digital impression. A high image count can lead to longer post-processing times but there is no evidence for decreasing the precision of the virtual cast (67-70). The results of this study therefore confirmed that the learning curve of IOS is influenced by the individual skills of the individual operator, however these user-friendly technologies allow even less skilled operators to achieve a good degree of performance. In relation to the outcomes considered, although operator 1 has an average scan time compared to the other 2, the average level of accuracy is almost unchanged for all operators.

Within the limitation of this in vitro study, comprehensive conclusions can be drawn: 1) The repetitive use of IOS affected the variables considered in the study, scanning time and scan accuracy, with a decrease of both parameters in time; and 2) the learning curve, by unskilled operator in a stressed condition such as a complete arch digital impression, request 12 or 13 scans to achieve a stable data of scan time.

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Stomatognathic system rehabilitation: “the gnatological pre-treatment”

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The case report shows the need for proper “gnatological pre-treatment” to be taken in any dental treatment plane, especially where the functional rehabilitation of the stomatognathic joint system is necessary. The re-coordinated movement of TMJ, the resolution of the joint block and of the reciprocal and bilateral click, result in a clear improvement of either mouth opening, both stability in occlusion centric and eccentric movements.

The relationship between occlusion and TMJ has long been the focus of important discussions in the national and international scientific field (1-5). The role of orthodontics is fundamental to contribute to the correct development of the maxillary bone bases during the patient's growth, therefore the TMJ, except for the presence of congenital structural anomalies, will follow this positive evolution in case of harmonious development of the jaws.

In the adult patient, before going through orthodontic treatment, the orthodontist must be fully aware of the joint condition of the patient who is about to treat. Hence the concept: TMJ dysfunction must be considered on the same level as malocclusion. We will not be able to say that we have solved a case of malocclusion in an adult patient if we have not first treated the condition of the TMJ. Therefore, we wanted to describe our approach of “gnathological pre-treatment”, which precedes any subsequent rehabilitation treatment in the adult subject. The concept of dental rehabilitation to a re-evaluation at the end of the growth, or in any case in the adult subject, cannot be separated from a rehabilitation of the joint function (6-10).

During our dental practice it is common to meet patients who demonstrate signs and symptoms of different pathologies of the oral cavity and of the stomatognathic system. At a careful clinical examination with gnathological evaluation, we focus on the function of the temporomandibular joints, on the action of the masticatory muscles, as well as on the periodontal and dental state, also using the latest generation diagnostic imaging and ultrasound for the study of joint movement.

The presence of dental or skeletal malocclusions, dental wear, fractures, migrations, caries and neck injuries, gingivitis, periodontitis etc., and clicks or joint noises and various symptoms such as tinnitus, buzzing, dizziness, bruxism, headache, and muscle pain associated with muscle tension pathology with difficulty in opening and closing and during the movement of the mouth, places our clinical attention towards the search for the triggering causes (11-14). The concept of dysfunctional patient also includes patients in whom the aforementioned alterations suffered over time by the dental elements and the periodontium (dental wear, fractures, migrations, caries of the neck, gingivitis, periodontitis) are the

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expression of an adaptation of the stomatognathic system to perform functional work, even in conditions of pathology of the mandibular movement (15-20).

The ultimate purpose of a careful functional analysis of the occlusal relationships is to obtain dental elements that have a shape and a position such as to allow a physiological movement of the temporomandibular joints, led by the action of the relevant muscle, to obtain shapes capable of supporting the muscular load exerted in the specific moment, that is when the contact between the arches occurs. As Harry Sicher teaches us “when muscle and bone fight, muscle never loses”, and as Peter E. Dawson confirms “when muscle and tooth fight, muscle never loses” (21-23).

The introduction of an alteration in the shape or position of a tooth can appear as an interference to the patient’s usual occlusion up to that moment.

If a new reconstruction or coronal shift of the position of an element is configured, this involves a change in the occlusion and therefore in the movement of the mandible as a whole by adaptation (24-27). The interference during the instinctive movement of swallowing determines a pre-contact capable of changing the occlusal relationship. Thus, the alteration, however due to a malocclusion, can occur in a distributed manner over time and therefore can chronically alter the muscular articulation functions.

In the case of reconstructions or of restorations or of orthodontic movements, it can take place more quickly and thus destabilize the usual occlusion immediately. This also implies a change in the joint, a dislocation of the meniscus, a change in the action of muscle fibers as a contracture, a change in the shape and position of the teeth, resulting in an altered work of the temporomandibular joints (28-32). The temporomandibular joint is a third-degree lever, therefore unfavorable (33-35), so that the three components of strength, fulcrum and resistance must be well positioned to perform its task.

As part of the resistance exerted on the teeth, the lever must be unfavorable otherwise our muscles would be able to fracture the teeth (36-40). Since the most unfavorable point of support of the lever during lateral movements cannot be exerted on the posterior teeth as they are located in a position between

the fulcrum and the power, and since the teeth of the anterior group serve to guide the movement protruding of the mandible, the teeth most suitable for expressing eccentric movements are the canines, which determine canine guidance (41-44). The latter avoids the contact of the posterior teeth in lateral movements by determining the first phase of the chewing movement, guiding the movement of the jaw until the end of the chewing movement, then up to closure, allowing the posterior elements to meet the cusps of the upper teeth with lower teeth (45-48).

In fact, the very shape of the back teeth shows that they are responsible for shredding. So, their contact, following the slipping caused by the canine guide, allows the chewing cycle to be terminated with adequate shredding of the food (49-51).

Therefore, re-establishing the shapes and functions of the dental elements is a pre-eminent condition to obtain the re-coordination of the joint movement, therefore the repeatability of the function expressed by the teeth muscles and the temporomandibular joint, such as to allow the physiological stability of all the districts: muscle, joint, bone, periodontal, and also of the integrity of the teeth themselves and guaranteeing occlusal stability over time without the need for any orthodontic retention aid. In the light of the evident presence of many of the symptoms and effects already mentioned, our clinical case demonstrates the need to carry out what we define as “gnathological pre-treatment”, conceived from our clinical experience, to be adopted in the context of any dental treatment plan.

The “gnathological pre-treatment” is proper especially in cases where it is necessary to rehabilitate the joint function of the stomatognathic system of our patients, ie in dysfunctional patients. We use an acrylic resin plate as a support, for upper or lower arch, appropriately modeled, by an adequate and suited forms of teeth, on which our patient can perform proper movements with the chewing muscles. In this way we can obtain the desired articular position of centric relationship, the only stable and repeatable situation over time, able to make our work appropriate (52-56).

We present the case of a 48-year-old male patient with alterations characterized by severe wear and dental migration (fig. 1). In addition the patient complained of muscle pain and difficulty in chewing,

buzzing, dizziness, headaches with recurrent tingling of the right limbs and tinnitus. At our clinical observation we highlighted the presence of: midline deviation, absence of dental elements, presence of several prosthetic and conservative restorations, gingival recessions, posterior right open bite and increased overjet.

On palpation of the chewing muscles, pain is evident, localized on the: external pterygoid muscle, internal pterygoid muscle and right masseter muscle. This is a clear symptom of a contracture, that also involves the sternocleidomastoid muscle, with recurrent tingling of the right limbs. The auscultation of the joint highlights a mutual click in both joints, greater on the left. During the opening movements, the click first arises to the left and then to the right and is reduced first to the right and then to the left. Analyzing the mandibular movements, a lateral deviation towards the right of the mandible in the opening of the mouth was noted.

Thanks to gnathological therapy, following careful mandibular manipulation, we detected the presence of a left posterior occlusal interference, responsible for the abnormal conduction of mandibular movement, joint block and reciprocal and bilateral clicks. We therefore diagnosed the presence of a temporomandibular joint dysfunction, associated with dental malocclusion.

Therefore, we are faced with a reducible intra-articular pathology, associated with a condyle-disc incoordination and a tension muscle pathology. We can appreciate noises from structural irregularity due to deformation of both articular discs, not associated with an arthrosic process, so state no MRI examination is required. The case is part of the intracapsular disorders that are not reversible, but because of adaptive changes, can function comfortably if occluso-muscle harmony is re-established (57-59).

Alginate impressions of both dental arches were taken and cast with type III gypsum. Moreover starting joint registrations were taken, using a deconditioning

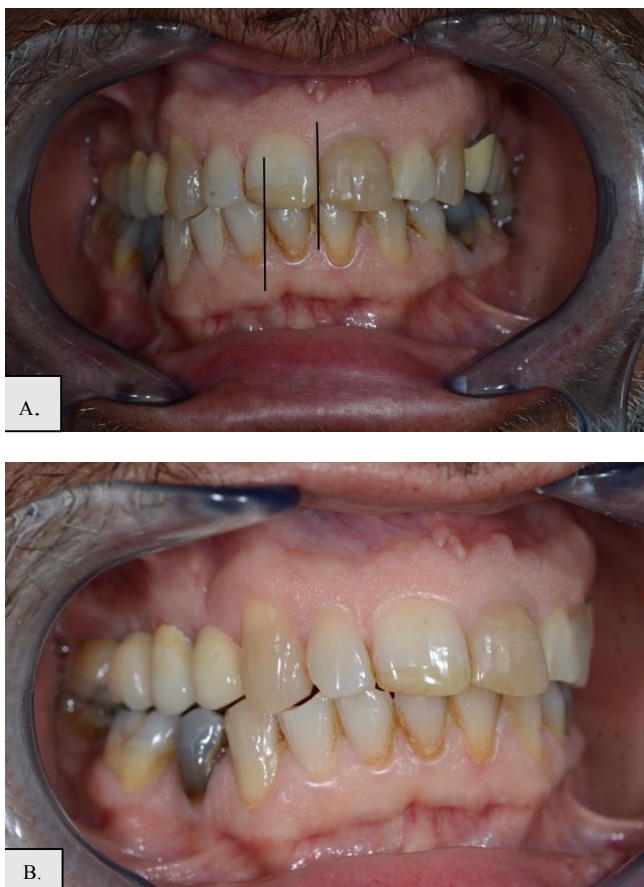


Fig. 1. A, B, C): Pretreatment intraoral images

maneuver so tending to a more stable and repeatable joint position. A face bow recording and an ultrasonic axiography was performed (Fig. 2).

Thanks to the detection of the face bow and the joint recordings, the plaster models were mounted on an articulator at average values (Fig. 3).

Based on the assembly in the articulator, an acrylic resin brace was made with hinted tooth shapes. Subsequent models are carried out in the chair directly on the brace worn by the patient while exercising the functional protrusive and lateral movements. Using our gnathological brace (fig. 4), morphologically customized on the patient in subsequent sessions, we can obtain, through the modeling of stylized tooth shapes but increasingly suitable for the correct conduction of the mandibular movement,

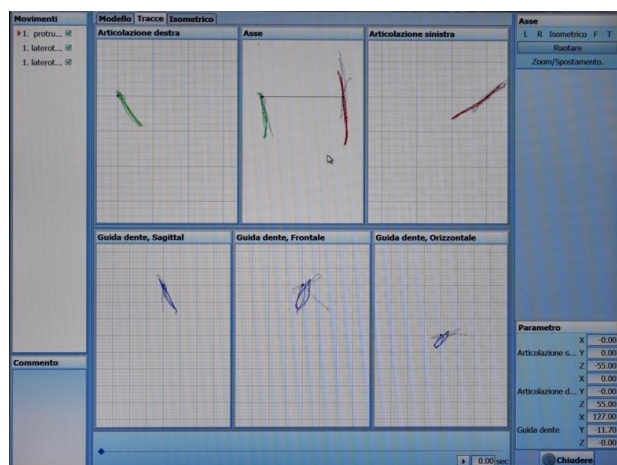


Fig. 2. The axiographic exam shows abnormal conduction of mandibular protrusive movement and joint block and reciprocal and bilateral clicks.



Fig. 3. Tutor made on average values of the articulator.



Fig. 4. Resin adjustments of the tutor during mandibular movements.

a deconditioning of the facial muscles, which had contracted due to the need to adapt to continuous and repeated support on elements that may be badly positioned or no longer intact.

Rearranging the mandibular movement to a physiological movement means obtaining a physiological positioning of the mandible whose condyles will find the right seat in the glenoid cavity and therefore the restoration of the centric relationship, the only stable and repeatable position both physiological and adapted, as in the case of intracapsular damage.

The shapes deriving from the modeling of the brace lead the mandibular movement and determine the area of movement of the mandible, deconditioning the facial muscles to adapt the movement by leaning on poorly positioned or no longer intact elements. By resting the dental elements of the lower arch on the brace positioned on the upper arch, the mandible was thus able to find the right supports on the teeth, to express the physiological movement of the muscles. The first modeling was then improved during the delivery session of the brace, by manipulating the mandible.

The patient wears the brace strictly during the night and progressively more and more hours during the day. The improvements in the joint position and the relaxation of the muscles are consolidated on the brace with subsequent sessions every 15 days (Fig. 5).

At each session a further improvement is induced, which will be perfected in the following session, where the shapes on the brace are changed by adding



Fig. 5. *Final stage: a stable joint position is obtained.*

or removing the resin. The sessions last until a stable joint position is obtained and the patient can and must wear the brace as much as possible within 24 hours (Fig. 6, 7). With the appropriate daily use of our brace for a period of about two months, a new joint position is now more physiological and repeatable.

This new joint position, with its occlusal relationship, is recorded using waxes and a facebow and transferred again to an articulator at average values (Fig. 8) and verified with ultrasonic axiography. The transfer of the new joint situation, free from dysfunctions, pre-contacts and, or dental interference, will be preparatory to the technical realization of a correct dental work program, that will



Fig. 6. *Completed modelling: stable contacts in centric relationship and repeatable movements conducted on the slopes built in resin.*

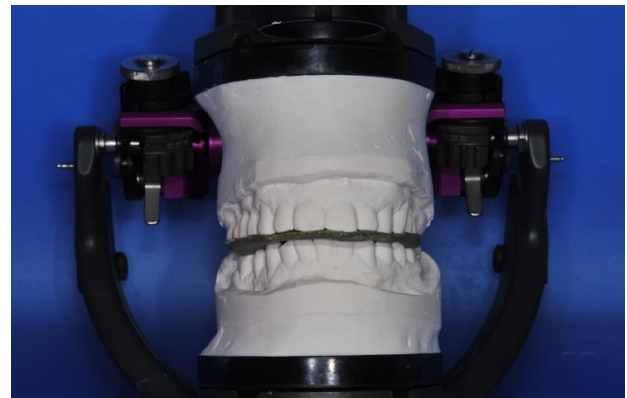


Fig. 7. *Axiographic joint recordings of the new situation, free from dysfunctions, waxes and face bow, that have been transferred to the articulator at average values and verified by ultrasonic axiograph.*



Fig. 8. *A, B): Assembly in the articulator of the new joint position obtained with the pretreatment.*

be stable and lasting. Therefore, this pre-treatment allows an accurate diagnosis, which allows to define the most suitable work program, achievable through a multidisciplinary dental approach.

At the end of the treatment with the brace, the condyles find the right seat in the glenoid cavity and so we obtain the restoration of the centric relationship. This is the only stable and repeatable position both physiological and adapted, as in the case of intracapsular damage. The maximum opening of the mouth is thus obtained (Fig. 8, 9).

With the analysis of this case, we describe the eventuality for which it is necessary to proceed with a rehabilitation through a prosthetic phase, as several elements are involved in wear, fractures or inadequate shapes. The transfer on the articulator will be preparatory to the realization of a diagnostic wax-up and temporary crowns that will be therapeutic. Following a diagnostic wax-up of the dental areas to be modified (Fig. 10),

the conditions are created for the fabrication of the temporary resin crowns (Fig. 11).

Our temporaries suitably modeled on an average value articulator are positioned in a single session on the patient, upper and lower arch together (Fig. 12), with the intention of transferring the same position packaged on the articulator, immediately allowing the patient's stomatognathic system to further improvement of the atm functions, assuming the same function of the brace used at the end of the modeling. Thanks to the placement of the provisionals, the patient will be able to take advantage of that modeling during all 24 hours of the day. The use of the provisionals of both arches, whose occlusal relationships are verified and preformed, allows the stomatognathic system to stabilize the improvement of the TMJ functions. The harmonization between the shapes and functions of the dental elements supports the correct synergistic movement of the muscles and of the atm, suitably

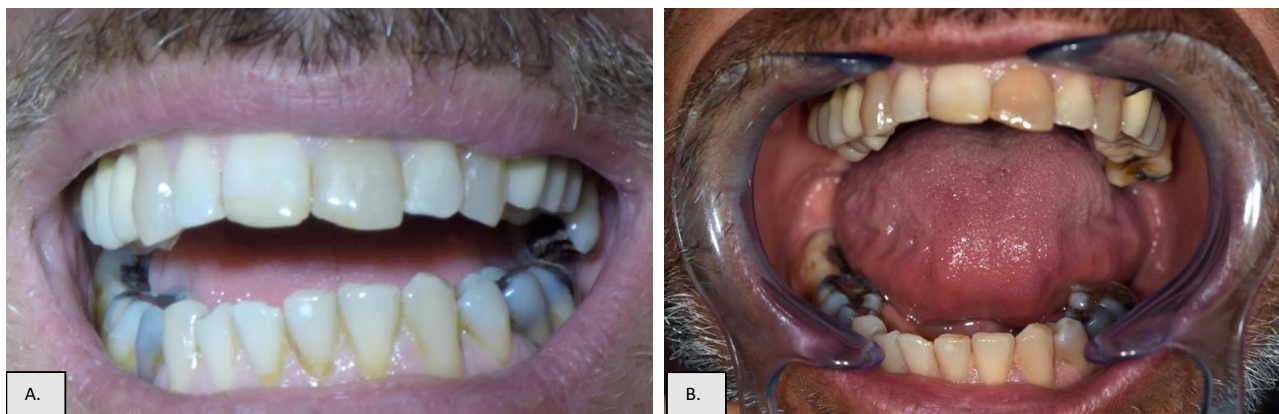


Fig. 9. A, B): Assembly in the articulator of the new joint position obtained with the pretreatment.

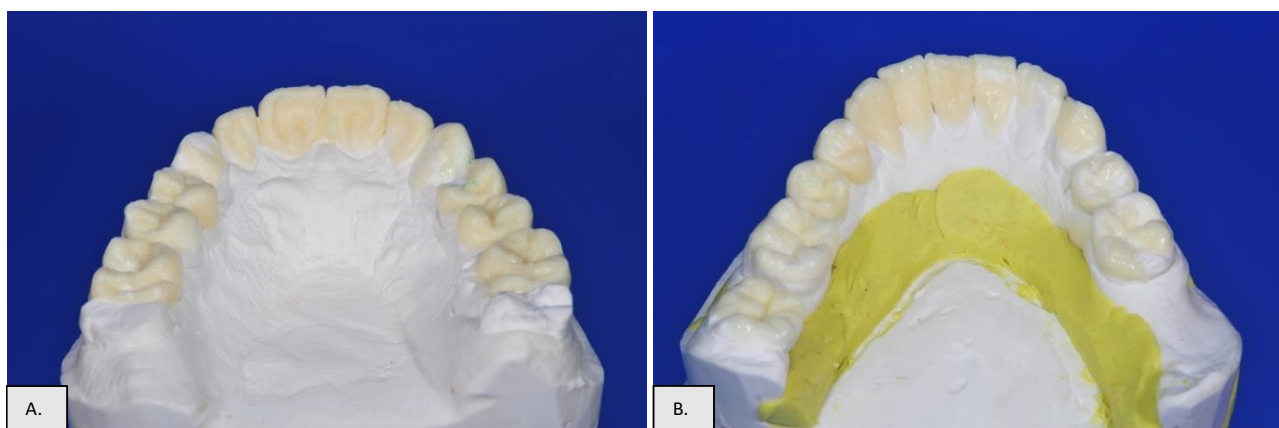


Fig. 10. A, B): Diagnostic gnathological wax up.

supported by these new tooth shapes. It will be precisely the harmonization between the shapes and functions of the dental elements to support the correct synergistic movement of the muscles and of the TMJ, appropriately supported by these new tooth shapes (Fig. 13).

The protrusive and lateral movements are linear and carried out without difficulty in a repeatable way. The routes show the linearity and the repeatability



Fig. 11. *Temporary crowns.*



Fig. 12. *Temporary crowns after 15 days.*



of the movement, conducted by the muscles (Fig. 14). Stability and reproducibility of movements will be guaranteed during the performance of all daily functions, such as phonation, swallowing and masticatory cycle. By means of appropriate sessions of selective grinding and suitable reconstructions in resin, of ridges and slopes with different inclinations, the shapes and positions of the various temporary elements are perfected.

These modeling allow the tripod meeting between the rear antagonist elements and the right disclusion during eccentric movements.

Through these careful interventions on the dental elements, it is possible to follow and to promote the improvement of the movement and the occlusal position. That is to say that in every point of space the patient dislocates his mandible will now find the supports on the dental elements, such as to support the muscular load.

All that in the ambition, as far as clinical and physiological possibilities are concerned, to rehabilitate the system from intra and extra capsular damages that have occurred over time, which are inevitably produced, in the event of incoordination of the muscles-teeth -atm system. Only after having achieved this result, will it be possible to finalize the treatment with the creation of definitive single crowns, using less usable materials, suitable for obtaining stable resolution over time.

RESULTS

There are different types of treatments for diseases



Fig. 13. *A, B): Control and verification of centric occlusion and the regularity and stability of the movements sliding on the teeth.*

related to the temporomandibular joint. There are treatments with aids of release plates, of bite planes, of rigid or soft consistency, mounted on the upper or lower arch, or in any case equipment that modify the occlusal relationships by inducing in a coercive and static way a position of the mandible different from the usual one, but often forced.

There are types of resin equipment built with a randomized shape, which exclude the contact of the arches inducing another occlusal relationship, and therefore a different muscular and articular working relationship. All of these causes an alteration of the state of the stomatognathic system which can give different results but usually does not induce the restoration of the physiological muscular work, and therefore a refocusing action on the position of the meniscus; they are mostly mouthguards, which can induce a momentary relief of the muscle tension pathology or apparently reduce the wear of the teeth only at the time of use (60-62).

Both rigid resin and soft silicone aids of various kinds are used, mostly generated based on the patient's tooth impressions, therefore perfectly fitting, but with absolute unpredictability and unrepeatability of a stable and repeatable occlusion as little is done in terms of a rearrangement of intracapsular physiological conditions.

The first phase of our treatment, manifested using the personalized brace, led to the resolution of joint

and muscular problems thanks to the restoration of the musculoskeletal centric relationship in centric occlusion and the guidance of eccentric movements, when the brace was worn by the patient. In the second phase of the treatment with the positioning of the temporary crowns, the new centric position was confirmed with the stabilization of the posterior contacts and the incisive and canine guidance, definitively ensuring the resolution of the muscular-articular problems.

The treatment performed, therefore, allowed to obtain a coordinated movement of both temporomandibular joints, the resolution of the joint block and of the reciprocal and bilateral click. This led to a marked improvement in mouth opening and occlusal stability in centric and eccentric movements. The improvement of the physiology of the movement of the chewing muscles has caused by adaptation, a clear improvement of all the other muscles that participate in the posture of the whole organism, the remission and disappearance of symptoms such as headaches, muscle pains, joint clicks, dizziness, tinnitus and buzzing present. For a long time and complained by the patient daily.

DISCUSSION

It is evidently noted how chewing, phonation, swallowing has improved, how stable and

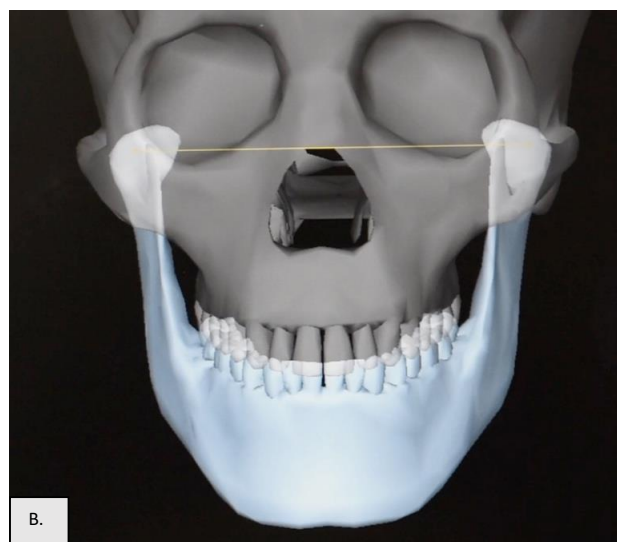
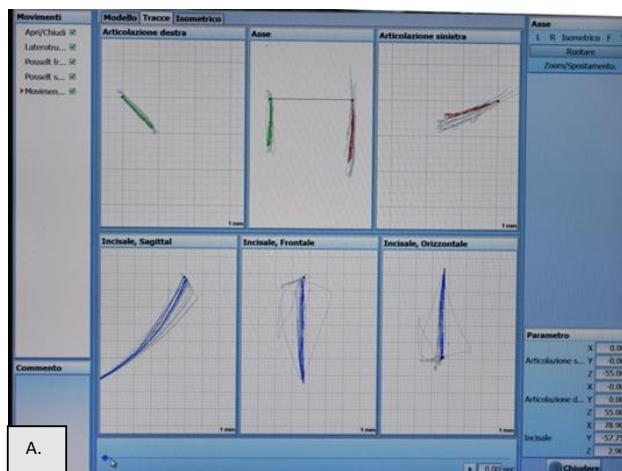


Fig. 14. A, B): Control and verification axiography.

homogeneous the periodontium is with excellent appearance, tone and trophism, already from the temporary state obtained with the temporary crowns. The patient confirmed the sensation of a clear improvement in the chewing capacity and therefore of the subsequent digestion, all obviously guaranteed by the stability and therefore by the renewed muscular capacity and by the repeatability of the movement that guarantees the ideal performance to the stomatognathic system.

CONCLUSIONS

The presentation of this case confirms how the concepts dictated by classical gnathology, implemented by the regularization of the extension of the movements, are still necessary and sufficient for the stable and lasting resolution of dysfunctional pathologies.

The gnathological pre-treatment is a necessary and sufficient action to allow the most accurate and predictable diagnosis, to ensure the stability over time of our dental care. The obvious need to carry out this gnathological pre-treatment highlights, in the light of our clinical experience, a concept that is fundamental for us: the displacement of the TMJ is to be considered a pathology as well as malocclusion.

Gnathology is movement: little can be drawn from static images, even if the same images presented, however static, are already demonstration of a recovery of periodontal health, which together with the restoration of the functional and aesthetic shape of the dental elements, are evidence of a physiologically functioning system.

Conflicts of interest

The authors declare that there are no conflicts of interest

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Antimicrobial effects of Photodynamic and high-power laser endodontic therapy on patients with necrotic pulp and periapical lesion

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The aim of the present study was to test the combination of conventional endodontic therapy followed by antimicrobial Photodynamic Therapy (PDT) or high-power laser in a clinical trial in patients requiring endodontic treatment. 50 patients were recruited for this study. For each patient a single tooth with clinical symptoms of pulp necrosis and periapical periodontitis was selected. The samples were randomly allocated into two experimental groups (n = 25 per each): group 1 (n = 25): high-power diode laser and group 2 (n = 25): photodynamic therapy (PDT).

All samples of the two groups were treated with an intermediate dressing with calcium hydroxide and provisionally filled; after one week the dressing was removed and they underwent further PDT and high frequency laser treatment. After each step, a microbiological sample was taken. Finally, the elements were filled using the MicroHeat root canal filling technique. The culture method was chosen for the microbial count of common aerobes, facultative anaerobes, and microaerophilic bacteria. During the process, specific microbial flora was not identified, but general microbial count of colony forming units (CFU) was performed.

The count of the number of CFU indicates a very wide range for the different samples. The tested photodynamic laser treatment shows positive and clinically relevant results when used as an adjuvant to traditional mechanical and chemical cleansing. There is a further reduction after a second treatment with photodynamic therapy. The decrease in CFU post photodynamic treatment is similar before and after dressing with calcium hydroxide. Photodynamic endodontic treatment as an adjuvant to traditional mechanical and chemical cleansing has shown greater efficacy in controlling the contamination of the treatment with diode laser. The first photodynamic treatment causes a reduction in the microbial count, and the second, after intermediate dressing, is comparable to the first. This is probably due to a lower complexity of the biofilm created after the intermediate dressing with calcium hydroxide compared to the initial intracanal biofilm. This therapeutic adjuvant is, therefore, more suitable in clinical situations where an intermediate dressing is necessary.

The clinical management of microorganisms and their elimination from the endodontic space represent the primary objectives of the endodontic treatments (1-3). Many failures are caused by inaccurate control or elimination of the infection (4-6).

Success rates in endodontics are higher (94%) in bacteria-free canals; instead, in the presence of bacteria this percentage decreases to 68% (7-8).

Mechanical procedures combined with chemical irrigation have been shown to remove most

Key words: endodontics, antimicrobial Photodynamic therapy, high-power laser, microbial count

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microorganisms (4). Due to the high variability of endodontic spaces, some bacterial residues can persist in important regions, such as the lateral and accessories channels. The antimicrobial susceptibility or resistance of the microflora, which includes common anaerobes, facultative anaerobes and aerobes bacteria can lead to endodontic failure (9-11). *Enterococcus faecalis* has an important role in persistent infections and endodontic reinfections because it can resist and remain in the dentinal tubules of the canal walls. In association with mechanical cleansing, the removal of instrumental debris and irrigation allow a good cleaning of the dentinal walls of the canals.

Therefore, the need to obtain ever more efficient disinfection methods has led researchers to experiment and apply new and more efficient technologies in endodontics.

Many irrigants have been proposed in the literature, and currently the most used are NaOCl and EDTA (5). New root canal disinfection technologies have been developed; the antimicrobial effect of the laser has been reported by many authors as well as the use of its technology as a method of root canal disinfection (6-7). There are different types of lasers on the market: solid state lasers, such as Er: YAG lasers (2940 nm) and Nd: YAG lasers (1064 nm), which have high affinity for hard tissues; CO₂ gas lasers; KTP lasers (532 nm), which have a high affinity for hemoglobin; and semiconductor lasers or Diode Lasers (630 nm, 810 nm to 980 nm), which for the different wavelengths and compact dimensions are widely used in the dental field.

Most studies to test high-power laser technology in endodontics have been carried out on planktonic models, polymicrobial or not, and on biofilms in vitro models, but there are still very few clinical studies (12-14).

In fact, the high-power laser, if used at incorrect dosages, can cause various damages such as dentin charring, ankylosis, cementum melting, root resorption, and periradicular necrosis (15-20). The tissue with a high-water component strongly absorbs laser radiation, creating heat. The water evaporates quickly, and the biological material is vaporized.

Photodynamic antimicrobial therapy (aPDT) is a new antimicrobial strategy (21-23) based on

the interaction between a nontoxic photosensitive agent (photosensitizer: methylene blue or toluidine blue) and a low-power laser light. The photosensitizer accumulates between the cell wall of the microorganisms and absorbs photons from the emitted laser light beam, becoming excited; in this way free radicals and reactive oxygen species are produced which, although having a very short life, before being eliminated quickly attack any organic compound, causing irreversible damage to the cellular membrane, proteins, and nucleic acids.

There are now numerous in vitro and ex vivo studies in the literature showing an increase in root canal disinfection after the use of aPDT. Clinical studies evaluating the efficacy of the aPDT used as an adjuvant at the end of conventional endodontic cleansing and shaping treatments are still limited.

The purpose of the following research protocol is to test, through a clinical study, the combination of conventional procedures followed by photodynamic antimicrobial treatment (aPDT) or high-power laser treatment in patients with acute periapical periodontitis and pulp necrosis who need an endodontic treatment (24-27).

MATERIALS AND METHODS

This study protocol was approved by the Ethics Committee of the University of Rome "Tor Vergata" (n. 46/17). For this clinical study 50 patients (26 males and 24 females) in good health and who need endodontic treatments, aged between 20 and 60 years, were selected.

This study was performed at the UOSD-Emergency Dental Department of the Unit of Conservative, Restorative and Endodontic Dentistry of the "Tor Vergata" Polyclinic, Rome, Italy. These patients had symptoms of pulp necrosis and/or acute periapical periodontitis in one or more dental elements with closed apex. Patients with cognitive deficits, treated with psychotropic drugs and/or narcotic substances, pregnant women, and patients with allergy to local anesthetics were excluded from the study.

During the first meeting, an anamnestic questionnaire and informed consent relating to the present trial were delivered to the patient. For each patient, a dental element with clinical symptoms of pulp necrosis and/or periapical periodontitis with single straight canal (maxillary and mandibular anterior

teeth and mandibular premolars), negative for the pulp vitality test, with no endodontic treatment, which requires endodontic treatment, was selected. A periapical traditional x-ray was performed to evaluate the presence of a single channel, closed apex and periapical radiolucency. After local anesthesia, the tooth was isolated with the rubber dam, in order to minimize contamination during treatment. The access to the pulp chamber was performed with a sterile high-speed diamond shaped conical bur number 16 (Komet, Rock Hill, SC, USA) under water coolant. If tooth decay was present, it was removed with a sterile round carbide bur (Komet, Rock Hill, SC, USA).

Once the pulp chamber was opened, the working length was determined using a Kfile 10 (Maillefer Instruments, Dentsply Sirona, Italy) and an electronic apex locator (Root ZX, Morita Corp, Japan). The K-file was removed with small backwards and forwards motion to remove the necrotic tissue, then the canal was irrigated with 1 ml of sterile saline solution and dried with 3 sterile paper points, kept inside the canal for 1 min. The three paper points were placed in a sterile test tube containing the Moller's VMGA III transport medium and used to determine the number of colony-forming units (CFU). This procedure provided the first microbiological sample that reproduces the initial bacterial contamination of the root canal.

Successively, the root canals were cleansing and shaping with Mtwo (Sweden & Martina, Italy) mechanical instruments at a rotational speed of 250rpm and maximum torque, using the basic sequence 10.04, 15.05, 20.06 and 25.06. During the preparation, the canal was generously irrigated with 2 ml of 5.25% sodium hypochlorite and 10 ml of EDTA to remove smear layer during and after instrumentation. The apical gauging was verified using a NiTi T-file 25 (Maillefer Instruments, Dentsply Sirona, Italy); therefore, a further apical finishing step was carried out using the mechanical instrument Mtwo apical n. 2 25/40 (Sweden & Martina, Italy). The root canal was irrigated with 5 ml of sterile saline solution to remove the antimicrobial agents and dried with three other sterile paper points from which the second microbiological sample was obtained. At this point the teeth were divided into two study groups (n = 25) based on the laser treatment received.

Group 1: high-frequency (or high-power) laser endodontic treatment

The root canals of this group (n = 25) were irradiated

with diode lasers (LaserHF, HagerWerken, Duisburg, Germany) for 20s by repeating the procedure three times with 10s pause.

The laser was used with the following parameters: wavelength $\lambda = 975$ nm, power = 2 W, t-on (time on, laser beam operative) = 5 ms, t-off (time off, laser beam inoperative) = 25 ms, with continuous timer.

For the emission of the laser light, an optical fiber of 320 μ m was used inserted at 1 mm from the working length and pulled back with motions in crown-apical direction according to the manufacturer's indications.

At this point the canal was further irrigated with 10 ml of sterile saline and dried with 3 sterile paper points which were used as the third microbiological sample.

Group 2: Photodynamic (aPDT) laser endodontic treatment

The root canals of this group (n = 25) were filled with toluidine blue dye ($155 \mu\text{g ml}^{-1}$) up to the endodontic access cavity. The dye was agitated with a Kfile 15 and left in the root canal for 1 min and then it was irradiated by diode lasers (LaserHF, HagerWerken, Duisburg, Germany) with a wavelength $\lambda = 660$ nm and power = 100 mW. For the emission of the laser light, an optical fiber of 320 μ m was used inserted at the working length and pulled back with motions in crown-apical direction, according to the manufacturer's indications. At this point, the root canal was further irrigated with 10 ml of sterile saline solution to remove the dye and dried with 3 sterile paper points which were used as the third microbiological sample.

All the teeth of the two groups were treated with an intermediate medication with calcium hydroxide and a provisional restoration. After a week, patients were called back to perform new microbiological samples before and after further laser treatment according to the group where they were assigned (forth and microbiological samples). Each tooth was finally obturated/sealed using a warm gutta-percha system (MicroHeat, Sweden & Martina, Italy) and restored with composite resin (Estelite Asteria, Tokuyama Dental, Japan).

Microbiological analysis

The microbial analysis was done for the evaluation of the microbial count of common aerobes, facultative anaerobes, and microaerophilic bacteria such as *Enterococcus* sp, *Candida* sp, *Lactobacillus* sp and *Phorphyromonas* sp. During the process, specific

microbial flora was not identified, but a general microbial count was performed.

The collected samples were removed from the transport medium (VMGA III) and placed inside a 1.5 mL microcentrifuge with BHI (Brain-Heart-Infusion) culture broth and centrifuged for 30 seconds. The samples were placed in 96-well plates in a chamber with an atmosphere of 5% oxygen, 15% of carbon dioxide, 80% of nitrogen and incubated for 72 hours at 37 ° C. The count of the number of colonies forming units (CFU) was performed at each treatment phase (initial, after conventional endodontic treatment, after laser treatment) and reported in Table I and II.

Statistical analysis

Statistical processing was performed on the logarithmic transform of the original variables as they are not normal. The logarithmic transform of these variables, on the other hand, satisfy the condition of normality (Gauss curve) required for the significance tests adopted (Kolmogorov-Smirnov and Shapiro-Wilk). Statistical comparisons between means were performed with a paired t test using

Microsoft Excel (Redmond, WA), The significance level for all analyses was set at $p < 0.05$.

RESULTS

The periapical x-rays confirmed the diagnosis of pulp necrosis and the presence of periapical lesions; the analysis of the first post-scouting samples showed the presence of infection in all the root canal (Table I, II).

Table I presents the distribution of the results (mean, standard deviation) before and after the high-power laser treatment. In this group we can see a statistically significant reduction in CFU after traditional endodontic treatment. Instead, the high-power laser treatment does not show a statistically significant reduction in CFUs ($p = 0.455$). Table II presents the distribution of the results (mean, standard deviation) before and after PDT treatment. The reduction in the number of CFUs after the treatment protocol was highly significant for this group ($p < 0.001$).

Table I. *Count of CFU before and after endodontic treatment with High-power laser (n=25)*

TREATMENT	CFU COUNT	STANDARD DEVIATION
Post scouting	81545,96	87085,56
Post traditional cleaning and shaping procedures	9289,84	12422,28
Post first laser treatment	8402,04	12958,53
After calcium hydroxide removal	7890,36	7339,667
Post second laser treatment	7417,64	8015,75

Table II. *Count of CFU before and after endodontic treatment with Photodynamic (PDT) laser (n=25)*

TREATMENT	CFU COUNT	STANDARD DEVIATION
Post scouting	66133,88	76300,18
Post traditional cleaning and shaping procedures	10322,52	16093,61
Post first laser treatment	7149,4	9658,844
After calcium hydroxide removal	4541,4	5244,662
Post second laser treatment	2350,12	4046,587

Instead, there was a further CFU decrease after the second laser treatment in both groups, but statistically significantly in the PDT group ($p < 0.001$).

DISCUSSION

The intracanal disinfection procedures (chemical and mechanical) have proved to be highly effective in removal endodontic biofilm and endodontic microorganisms (28-31).

A new approach to eliminate endodontic microorganisms is the use of laser in the root canal during endodontic therapy. It has been widely demonstrated in literature that a combination of conventional endodontic therapy followed by antimicrobial PDT was highly effective in reducing bacterial count in an ex vivo model of infected human root canals in extracted teeth (32-38).

The CFU count of the reported a very wide range of different species for the different samples of both study groups, statistically confirmed by high standard deviation values.

This substantial variability for the first post-scouting samples is probably caused by changes in the endodontic anatomy of the different teeth, by the duration of the infection, and by the presence or absence of infiltrated decay at the beginning of therapy.

Previous studies have also suggested the potential of the aPDT as an additional disinfection technique to eliminate root canal bacteria after standard chemomechanical debridement (39-46).

The antimicrobial photodynamic laser treatment tested in this study shows positive and clinically relevant results when used as an adjuvant to traditional chemomechanical procedures. The first photodynamic treatment causes an initial reduction in the microbial count, and the second, after intermediate dressing, gives even better results. This is probably due to a lower complexity of the biofilm created after the intermediate dressing with calcium hydroxide compared to the initial intracanal biofilm. In an alkaline environment the photoreaction is higher than in a neutral or acidic environment, as it increases the presence of microorganisms reactive to oxygen and especially to singlet oxygen. This therapeutic adjuvant is therefore more suitable in

clinical situations where an intermediate dressing is necessary (47-53).

There is a further reduction after a second treatment with photodynamic therapy, indicating this therapeutic adjuvant as more effective in clinical situations where we need to do a multi-step therapy, where, as described in the literature for intermediate medication, there is a phenomenon of contrasting recolonization.

As for the treatment with high-power laser ($\lambda = 975$ nm), the data collected in the present study are in line with what is present in the literature and indicate the ineffectiveness of this work scheme in the reduction of intra-canal bacterial contamination, such as it can be seen from the Tables 1 and 2 (54-58).

Our results shown that PDT laser therapy was effective in reducing microbial CFU in samples from infected root canals. The efficacy of PDT laser decontamination resulted positively affected by number of applications and, in a significant way, using an intermediate dressing with calcium hydroxide between laser application.

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Conflicts of interests

None of the authors have any business or financial interests in the corporations that produce or market any of the tested devices.

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Evaluation of the marginal and internal fit of provisional crown fabricated with two different 3D printing technologies

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CAD/CAM technology represents a well-established and always improving tool for the realization of dental prosthetics, appliances and clinical devices. The aim of this paper is to evaluate the marginal and internal fit of provisional crowns realized with two different technology modern 3D printers. 60 patients needing a single crown prosthetic restoration were enrolled for the study and for each case two resin 3D Printed temporary crowns were produced on the same CAD project file realized on an intraoral digital scan. All digital impressions were taken with Trios 3 Color Pod Scanner (3 Shape) and the two 3D Printers tested were: DLP-SLA (Moonray D75) and MSLA (Structo Velox).

Assessment of marginal and internal fit was carried out by a single blind VAS quality assessment by the operator fitting the temporaries on the patients and by a qualitative analysis of the gap thickness in sections obtained from silicon imprints of the fitted temporary crown on the relative tooth measured in four different points.

Both 3D printing technologies gave promising results with measured gaps always under 130 µm but failed the comparison with traditional techniques in terms of cost-effectiveness and time saving. In every case, adjustments and finishing was always needed for a correct fitting a clinical function of the temporary restoration. Nowadays, other 3D CAM procedures such as precured resin blocks milling, appear to be more indicated for the fabrication of temporary prosthetic crowns.

Since its introduction, CAD/CAM technology in dental field has been rapidly acquired and vastly developed (1-4). In the last few years, the use of intraoral digital 3d scanners, as an alternative to traditional impressions, and the use of 3D printers, introduced new clinical perspectives in all dental specialties, speeding up production processes and reducing costs (5-7). In order to realize correct prosthetic restorations, accuracy must be maintained in the production process in every step: from the impression to the modeling, then to their realization.

Accuracy in digital impression, combined with the correct CAM production process, results in an excellent internal and external adaptation of the prosthetic product (11-13). Not only the quality of the

impression but the precision of the model is essential to create a prosthesis with a correct fit (14-15).

The dental arches can be digitally acquired with two methods: the conventional impression using elastomeric materials, and the digital impression using an intraoral scanner. Although the conventional impression has been used successfully for years in the dental field, it can be perceived as uncomfortable by patients and can suffer distortions and dimensional variations during creation of the plaster model. The digital impression shows to be a valid alternative thanks to some advantages, such as reduced chair time, greater patient acceptance, ease of storage and reproduction of virtual model (16-19).

The direct intraoral impression is increasingly widespread but has not yet replaced the conventional

Key words: CAD/CAM technology, provisional crowns, 3D printers, intraoral digital scan

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impression in clinical practice. Intraoral digitization permits to acquire the necessary information to produce prosthetics without the need of a physical model and with minimal manual laboratory procedures.

The clinical case 3d model and the subsequent CAD modeling produce a digital project of the prosthesis. The project can currently be used as a source to produce in subtractive technique by milling (CAM technology) and in additive technique with 3D printing process. The technology 3D based on stereolithography, identified with the acronym SLA (Stereo Lithography Apparatus), uses the photopolymerization/solidification process of a liquid resin (photosensitive resin composed of polymers) through a laser. DLP (Digital Light Processing) and Mask SLA printers differ from SLA printers in light source. The laser is replaced by an HD projector in DLP and an LCD/LED matrix in the MSLA. SLA, DLP, MSLA printers are commonly referred to "additive fusion", with substantial difference in the time of realization of the object (20-25).

CAM systems are currently the most widely used for zirconia and ceramic materials but can also be used for resin-based materials. These systems do have a large waste of material during the milling process, a high purchase cost and long working times. On the other side, the advantages of 3D printing include the possibility of printing artifacts using a smaller amount of material with obvious economic advantages but currently is limited to fewer materials. 3D printing in dentistry has recently increased its fields of use: after an intraoral digital impression, it is possible to quickly create implant surgical guides, program and produce aligners for orthodontic treatments and create prosthetic temporaries (26-30).

In the rehabilitation of a single tooth with indirect full-coverage restoration, the temporary resin provisional is a fundamental step as it guarantees protection of the prepared tooth and periodontal tissues. Its correct function is directly related to the degree of marginal precision (31-34).

The creation of prosthetic temporary restorations can take place through a totally digital workflow from taking an intraoral digital impression to realization of the final product with 3D printing procedures, reducing time and costs.

The purpose of this project is to evaluate and compare the precision in terms of marginal fit, internal fit, occlusal adaptation and correct adjacent teeth contact areas, in temporary prosthetic restorations made with digital impression with Trios 3 shape intraoral scanner and printed using two different technologies DLP-SLA (Moonray D75) and MSLA (Structo Velox). Through this prospective clinical study, we wanted to verify the possibility of speeding up the work process in the realization of the temporaries while maintaining clinical quality and optimizing costs (35-38).

MATERIAL AND METHODS

This study protocol was approved by the Ethics Committee of the University of Rome "Tor Vergata" (n. 94/19). This study was performed at the UOSD-Emergency Dental Department of the Unit of Conservative, Restorative and Endodontic Dentistry of the "Tor Vergata" Polyclinic, Rome, Italy.

Patients with cognitive deficits, in treatment with psychotropic drugs and / or narcotic substances, and pregnant women were excluded.

Inclusion criteria were: 1) Adult patients; 2) Patients not suffering from psychiatric pathologies and not taking psychotropic drugs; 3) Patients with good oral hygiene; and 4) Patients requiring a single full coverage prosthetic restoration.

The parameters necessary for determination of the size of sample are mean, effect size, standard deviation: a number of 60 patients were considered sufficiently valid for the study (test power up to 80% and statistical significance 0.05%). These parameters have been detected from the literature available on the subject. For the digital intraoral scans, it was used the intraoral camera Trios 3 Color Pod Scanner (3 Shape), a multifunctional digital scanner capable to create 3D digital models, detect the color of the teeth and capture intraoral HD photos, to better record cases. The production of the prosthetic restorations was realized with two different 3D printers: a 3D printer with DLP-SLA technology (Moonray D75) and a 3D printer with Mask-SLA technology (Structo-Velox).

During the first appointment with the patient was asked to fill a complete anamnestic questionnaire and sign

the informed consent related to the trial. All the necessary time was dedicated to give the patient full comprehension of the terms of the study and to complete these forms. After a full visit from the clinician the patient's medical history was fully reviewed and checked. Following inclusion and exclusion criteria, the clinician decided whether to exclude the patient from the study. Only patients in need for a single prosthetic restoration were included in the present trial. Every enrolled patient received two alginate impressions of the two arches were taken, an occlusion wax and record of the teeth Vita shade, in order to get with traditional techniques a "premillling" temporary resin crown by a dental technician.

During second appointment, the operator performed the prosthetic preparation and adapted the conventional resin temporary crown, manufactured by dental technician, and sealed it with temporary cement. The third appointment took place no less than three weeks after the second. The dentist removed temporary crown and took the final impression using Trios 3 Color Pod 3 Shape Intraoral Scanner. Then operator recemented the temporary crown on the treated tooth. The digital impression was processed with ExoCad program, allowing the clinician to virtually model a crown and produce an STL file. The file was sent to the two 3D printers tested, that produced two different resin provisional prosthetic crowns from the same project file. During the fourth appointment, the clinician tried the two printed resin crown in "single-blinded-mode", evaluating 4 parameters for each artifact with a VAS of 100 mm: 1) marginal accuracy; 2) contact points; 3) occlusion; and 4) time for polishing.

Furthermore, marginal precision of both temporary crowns was subjected to a quantitative evaluation by the

clinician who proceeded to create two silicone copy to be analyzed under an optical microscope. The crowns, in sequence, were filled with a high-fluidity silicone (Aquasil Ultra XLV, Dentsply Sirona, Italy), inserted on the prepared tooth and compressed applying a force of 20 N which was verified with a dynamometer. The thin layer of silicone indicates the gap between each of the two crowns and the tooth surface. After removing the crowns, the thin layer of silicone was stabilized injecting into the crown a silicone with firmer consistency (Aquasil Ultra Heavy, Dentsply Sirona, Italy). Once silicone hardened, the two replicas were extracted from the crowns. Specifically, the thickness of fluid silicone layer was measured in four points of the prosthetic crown: on cervical margin, on the long axis of tooth, on the transition point between long axis of the tooth and occlusal plane and on the occlusal plane.

RESULTS

All results for operator evaluation of precision of the tested 3D printed temporary restorations are reported in Table I. All results from the quantitative analysis of precision of the tested 3D printed temporary restorations are reported in Table II.

DISCUSSION

The data collected on adaptation and evaluation of the prosthetic products via VAS scale have demonstrated homogeneity of this evaluation by the operator. Regarding marginal accuracy operator evaluation, the Mask-SLA technology printer (Structo-Velox) received a better evaluation ($86 \pm$

Table I. Precision evaluation of the operator VAS (mm)

3D printer	DLP-SLA		Mask-SLA	
	Mean Value	SD	Mean Value	SD
Marginal fit	78	18	86	15
Interproximal fit	95	15	76	18
Occlusion	67	20	56	26
Finishing time	25	12	45	14

15 mm) rather than DLP-SLA technology printer (Moonray D75) (78 ± 18 mm).

Evaluation of contact points described an excellent friction of the prosthetic models made using DLP-SLA technology (95 ± 15 mm) compared with Mask-SLA (76 ± 18 mm), however positively evaluated. Quality of occlusal contact of the prosthetic products was positively evaluated (averages > 50 mm VAS scale). Therefore, a good occlusal contact was found by the operator especially for the products made using DLP-SLA (67 ± 20 mm) compared with Mask-SLA (56 ± 26 mm) (39-42). Time for finishing confirmed previously listed data, as less chairside time was required to finish the prosthetic crowns printed with DLP-SLA printer (25 ± 12 mm). These crowns showed more precise occlusion and contact points than those milled with Mask-SLA printer, which required more time to be finished and adapted to patient's occlusal system (45 ± 14 mm).

Data regarding quantitative evaluation of provisional crowns made with the two different 3D printing technologies reflected the results described in literature, with gaps <130 μm and a better prosthetic precision for a single provisional crown. Thickness of silicone on prosthetic cervical margin was on average higher in provisionals made using DLP-SLA technology (123.3 ± 41.8 μm) compared with Mask-SLA (101.3 ± 36.4 μm). These data confirmed the clinician's qualitative evaluation using VAS scale on the marginal precision, where DLP-SLA technology resulted slightly less precise than Mask-SLA technology (43-47). Interestingly the quantitative measurement of the fit on the long axis of the tooth was better than other points of the

prosthetic crowns examined for the two 3D printing technologies, without significant differences between the two groups (93.4 ± 36.8 μm and 89.3 ± 41.6 μm).

Compared to the cervical margin, the transition point, the occlusal point and the long axis of tooth were prosthetic areas most easily readable by intraoral scanners and consequently more easily achievable by 3D printers. Higher precision of the new intraoral scanners may lead to a decrease in digital measurement errors resulting in greater precision in 3D printing procedures (48-52).

The main limits of 3D printing for dental prosthetics are represented by scarcity of available polymeric materials certified for temporary or prolonged clinical use. Moreover, the need for multiple production steps in the 3D printing procedure, requiring prolonged curing time after printing and the following cleaning and polishing, still resulted more complicated and time consuming if compared with 3D milling of similar polymers such as PMMA blocks (53-54). Along with the continuous advancements in the technology of intraoral scanners and 3D printing devices, more studies will be required to assess precision and cost efficacy ratio of this kind of restorations. Regarding the marginal fit of the experimental prosthetic temporary restorations tested, results seemed encouraging but with low consistency.

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Conflicts of interests

None of the authors have any business or financial

Table II. Precision measurement on four points in sections. Fit gaps (μm)

3D printer	DLP-SLA		Mask-SLA	
	Mean Value	SD	Mean Value	SD
Margin	123,3	41,8	101,3	36,4
Long axis	93,4	36,8	89,3	41,6
Transition point	109,5	32,6	95,3	34,7
Occlusal plane	119,2	48,4	113	38,5

interests in the corporations that produce or market any of the tested devices.

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The use of advanced medical devices as primary screening for diagnosis of caries aimed at improving oral health

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Occlusal dental decay is still the most widespread disease in the world today. The aim of this *in vivo* study was to validate two different electronic laser fluorescence medical devices for caries diagnosis (Vistaproof and DIAGNOdent pen) in comparison with the gold standard traditional visit with digital x-ray Bite-Wings and the ICDAS II scores. Three expert clinicians, more than 10 years of experience, performed all visits using all three diagnostic methods. A total of 180 patients were enrolled for the study and data were collected from 2044 teeth. The statistical analysis found significant differences between the ICDAS values and the DIAGNOdent values, and between the ICDAS values and the Vistaproof, while there were no significant statistical differences between DIAGNOdent and Vistaproof values. Vistaproof values demonstrated a tendency to overestimate the presence and extension of carious lesions while DIAGNOdent pen gave a slight underestimation in caries detection. From a general point of view and relatively to ease of use and patient comfort, DIAGNOdent was judged better than Vistaproof. The DIAGNOdent pen cannot be a diagnostic substitute for an expert clinician diagnostic evaluation but can be considered an additional help and a valid device improve traditional visit consistency and accuracy. While both tested devices gave under or overestimated results, both are unable by themselves to determine whether or not treatment is required.

Dental decay still represents one of the most common infective pathosis with relevant economic and social impact worldwide. The latest available datasheet published by the WHO on caries disease in Italy, shows values near to 50% prevalence at 12 years in permanent dentition (1-5). This high incidence calls for better prevention strategies on a population base, but also requires new strategies to intercept dental hard tissue initial lesions with the aim of less invasive and more comfortable treatments. Dealing with initial carious lesion often requires simple operations, with low impact on the patient, with short operating times and reduced costs for materials and tools.

This simple assumption, combined with the much wider panorama of therapeutic requests of patients of all ages to the Italian National Health System, gains even more importance in a perspective of optimization of procedures and costs that must fall within the Essential Levels of Assistance (6-9).

The goal must be to minimize the need for complex, expensive and time-consuming procedures for patients by successfully intercepting caries pathologies still in their initial phase. Over the years several indexes have been developed for the evaluation and measurement of carious lesions in order to define the most suitable treatment plan.

Key words: dental decay, caries detection, laser fluorescence, ICDAS II, DiagnoDent pen, Vistaproof

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One of the most used indexes in the world is the DMF (Decayed-Missing-Filled) or DMFT (Decayed, Missing, Filled Teeth) published in the WHO Basic Method in 1997 (10-17), which allows the evaluation of the prevalence of caries disease in the population. This index measures the total number of decayed (D), missing (M) or already treated (F) teeth in an individual and the score for each individual tooth equals 1 (18-25). Unfortunately, this system is not capable to quantify the extent and severity of the carious pathology leading to underestimate or overestimate the initial carious lesions. Today, the reference index used by the international literature is the ICDAS (International Caries Detection and Assessment System) which, unlike the simple DMF, offers a standard for the detection of carious lesions and their extension with the possibility of generating personalized treatment plans related to the severity of the disease (26-33).

The ICDAS I, developed in 2002 and subsequently modified into ICDAS II in 2005, provides for a coding of occlusal coronal carious lesions with a score from 0 to 6 based on the depth and severity of the carious lesion.

The encoding of the ICDAS scores is as follows:

Code 0: healthy tooth surface

Code 1: Primary visible changes in dry enamel (small discolorations white / brown in dry glaze)

Code 2: Visible distinct changes in wet enamel (white / brown spots on wet enamel)

Code 3: Visible microcavities in dry enamel without exposure of dentin or shadows underlying

Code 4: Presence of dark underlying dentin with or without microcavity in dry enamel

Code 5: open cavity with dentin exposure without pulp involvement

Code 6: extended free cavity with dentin exposure with possible involvement pulp

These scores can also be used to describe the presence of caries in the interproximal areas. The standard dental examination for screening and evaluation of the presence of carious lesions includes an accurate examination of the teeth with adequate illumination and magnification and the use of an explorer with a thin tip to test the presence of cavities or discontinuities of the dental

enamel. Beside the intraoral examination, targeted radiographic investigations are often required for the diagnosis of carious pathologies in difficult areas such as interproximal areas (34-40). This method of examination and diagnosis, although apparently simple, is operator-related as it relies on the operator's ability and experience in judging the presence and extent of the decay and the need to treat or not. Both underestimation and overestimation of initial carious lesions can cause considerable discomfort for the patient, exposing him to the failure to intercept small, easy to resolve caries or, on the contrary, to an over treatment.

The advancements in technologies produced new medical devices intended for the early diagnosis of carious diseases. These devices are intended as diagnostic aids for the clinician and could improve the comfort and appropriateness of dental care for the patient reducing the risk of overtreatment. The core technology on which many of these devices are based uses fluorescence light sensors. In general, they have a light source that emits wavelengths capable of exciting metabolites of cariogenic bacterial species (porphyrins) which in response emit light on a different wavelength from healthy enamel and dentin (41-43). This research project, lasting 24 months, tested the sensitivity, specificity and comfort for the patient of two electronic medical devices for caries diagnosis in comparison with the traditional visit with digital Bite-Wings (20-22) and recording of ICDAS scores.

MATERIALS AND METHODS

To compare the three diagnostic methods, (E0) conventional visit with ICDAS II recording and bite-wing x-rays taken with VistaScan Mini device (Dürr Dental, Bietigheim-Bissingen, Germany); (E1) intraoral scan with VistaProof fluorescence camera (Dürr Dental, Bietigheim-Bissingen, Germany) and (E2) intraoral scan with DIAGNOdent pen diagnostic fluorescence laser (KaVo, Biberach, Germany). Three dental clinicians with more than 10 years of experience were enrolled and went through an alignment briefing course. Each operator used each examination method on different patients to minimize any bias coming from previously made diagnosis. A

randomization sample chart for the operators and the examination methods was realized and used for the entire period of the study.

Inclusion and exclusion criteria

This study was approved by the Ethics Committee of the Tor Vergata Polyclinic of Rome (n. 6/20). The patients included in this study were selected within the UOSD of Dental Emergency and Treatment of Vulnerable Social Patients of the Tor Vergata Polyclinic, in good health, aged between 18 and 72 years and who needed at least one treatment of restorative dentistry.

Patients with systemic pathologies, cognitive deficits, in treatment with psychotropic drugs and/or narcotic substances, pregnant women, patients with temporomandibular dysfunction, with severe periodontitis and bleeding at the level of the oral mucosa were excluded. All third molar teeth, teeth with prosthetic and amalgam/composite restorations, and patients in fixed orthodontic treatment or bands in the posterior teeth for orthodontic reasons were also excluded from the study.

Sample size calculation

To determine the sample size starting from the mean and the standard deviation of the ICDAS II score ($M=3.5$; $ds=1.8$) and considering a variation of one point on the scale (variation of $100 \times 1/3.5 = 29\%$), having chosen an α error of 5%, it was decided to have a power of 90% given a range of 30 patients per class of age. Three classes of age were taken into consideration: 1° class 18-36 years-old; 2° class 36.1-54 years-old; 3° class 54.1-72 years-old. The total sample was of 180 patients, 30 per each class of age, equally divided into Female and Male patients. A randomized coupling between three operators and three techniques was performed by a computer program to obtain a sampling chart. For each of the 180 patients recruited for the study, 16 teeth per patient were selected to have an overall total of 2.880 teeth. Of these 2.880 teeth, teeth matching exclusion criteria were excluded from the study. This sample size ($n = 180$) was calculated for a confidence level of 95%.

Operative Procedures

After completing the informed consent and anamnestic form, which shows the age, the country of origin (specifying North, Center and South), the educational qualification and the socioeconomic class, the patient can be included in the

study and proceed with the three exams with the three pre-established operators.

Each patient was assigned the first available sequential number of the Sampling Card based on sex and age class. In the same Card, the sequence of Tests (E0, E1, E2) and the respective Operators (O1; O2; O3) have been assigned to each patient. All dental elements with full-coverage composite restorations and prosthetic crowns were excluded from the study. Before proceeding with the examinations, the first operator assigned to perform the first investigation performed an intraoral scan (Trios Color Pod, 3Shape) and intraoral photos of the teeth affected by decay.

Exam (E0): After cleaning the dental surfaces with a rotating toothbrush, the selected Operator performed the intraoral examination with the use of a mirror, a probe and magnifying devices. Two or more digital x-ray Bite-Wings were performed for the diagnosis of interproximal carious lesions and ICDAS scores were recorded for all teeth included in the study.

Exam (E1): The selected Operator visited the same patient using the diagnostic medical device VistaProof. After having thoroughly cleaned and dried all the dental surfaces, the VistaProof device will be placed on the teeth analyzed. All the acquired images will be processed and stored in the DBSWIN program. The reference color scale of the device (green, purple, red, orange, yellow) and the numerical values with the associated decimals (0: healthy enamel, deep lesion $<1\text{mm}$; 1: initial caries of the enamel, depth of the lesion between 1 -1.5mm; 2: tooth decay in the enamel-dentin junction, lesion depth between 1.5-2mm; 3: dentin caries, lesion depth between 2-2.5mm; 4: deep dentin caries, lesion depth $> 2.5\text{mm}$) provide the data to be recorded for the patient's visit.

Exam (E2): The selected operator visited the same patient using the diagnostic medical device DIAGNOdent pen. After having thoroughly cleaned and dried all dental surfaces, the DIAGNOdent pen device must be set on a healthy tooth surface, as indicated by the manufacturer. All the numerical values obtained for the occlusal surfaces was recorded. Values range from 0-12 (Healthy tooth substance), 13-24 (Initial demineralization), ≥ 25 (Strong demineralization).

All the records collected by the two tested devices and by the ICDAS were collected on a digital database and cataloged for subsequent analysis in respect of the patient's privacy.

Statistical Analysis

All data collected were recorded into a digital Excel database (Microsoft, Redmond, Washington - USA) and analyzed for the statistical analysis with the SPSS software (Windows, version 15.0, Chicago, Illinois, USA).

The descriptive statistics consisted in a mean $\pm$ standard deviation following the normal distribution (with Kolgomorov-Smirnov test) and with the mean and minimum-maximum interval for the variables with non-normal distribution. The comparison between the normal

variables was done with multifactorial ANOVA while for the non-normal variables non-parametric test were done. The Limits of Agreements of Bland & Altman was performed to evaluate a correlation between the score results of the exams and operators. A p value <0.05 was considered statistically significant. All graphs were made with Excel (Microsoft, Redmond, Washington - USA).

RESULTS

The mean age of the examined patients was of 48.7 ± 5.6 years of age for male patients and of 52.3 ± 3.7 years of age for female patients. After taking into consideration the exclusion criteria, the collected data was based on a sample of 2.044 teeth.

The comparison of the means of the normalized data obtained by the three different diagnostic methods, showed no correlation between factor "group of age" and factor "diagnostic technique" (Fig. 1). The age resulted independent to the diagnostic technique factor with no clinical implications. There were significative statistical differences between the ICDAS values and the DIAGNOdent values, and between the ICDAS values and the Vistaproof, while there were no significative statistical differences between DIAGNOdent and Vistaproof values.

The correlation analysis underlined the existence of a good relation between the means of all three diagnostic techniques with a distribution of data that demonstrate

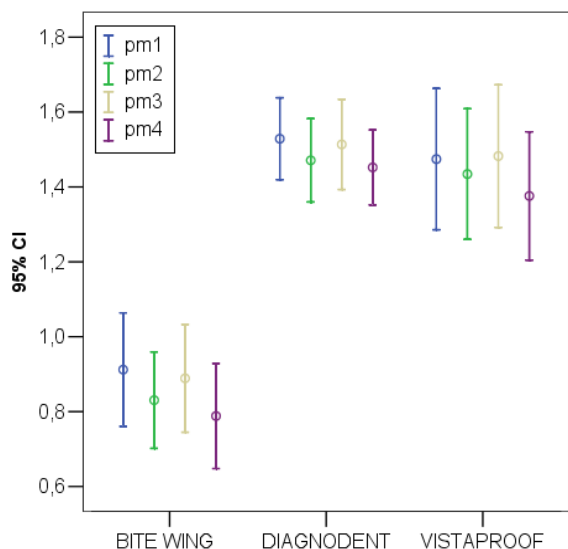


Fig. 1. Normalized means for Diagnostic method of the four evaluated posterior sectors (pm1-pm2-pm3-pm4). There is an evident difference between ICDAS with Bitewings diagnostic method and the two tested devices.

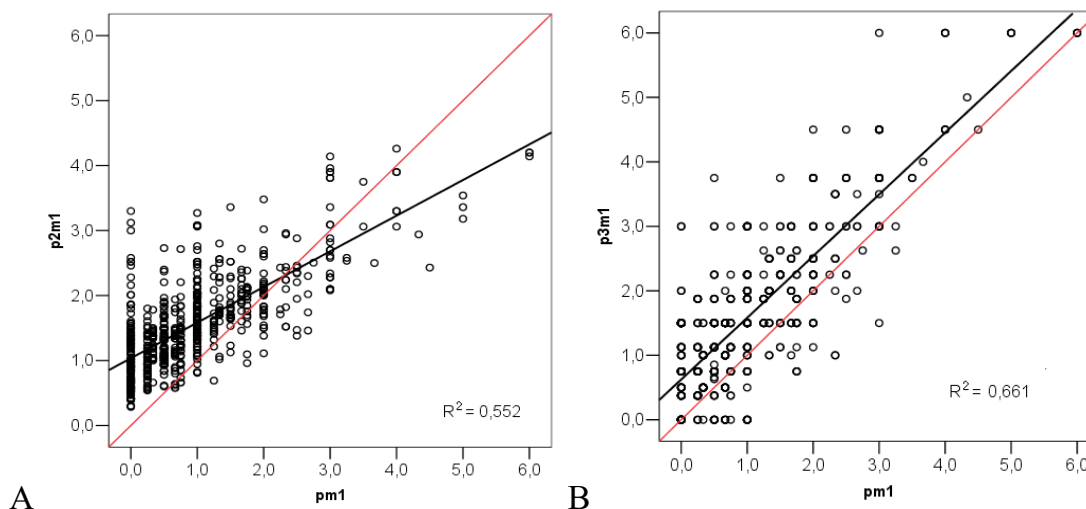


Fig. 2. Identity Diagram: A) DiagnoDent pen and B) Vistaproof.

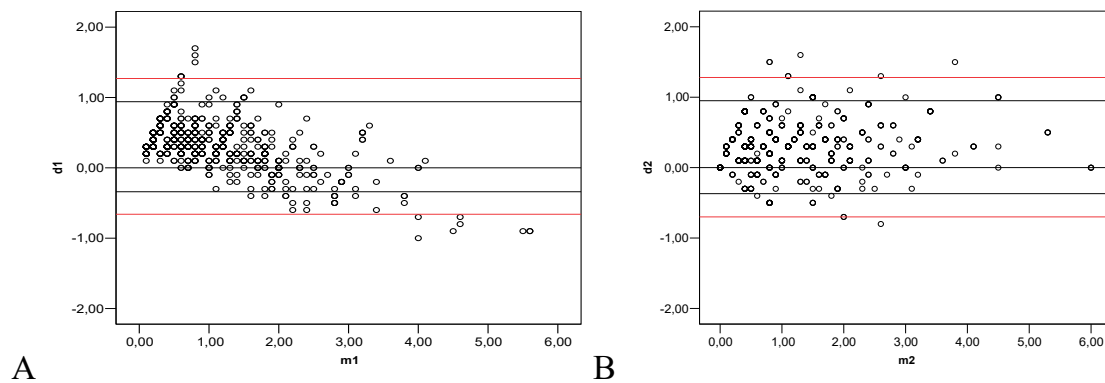


Fig. 3. Limits of agreement of Bland & Altman for **A)** DiagnoDent and **B)** Vistaproof.

a tendency of underestimation for DIAGNOdent, and an overestimation for Vistaproof (Fig. 2).

In respect of the analysis of the limits of Agreement of Bland & Altman for DIAGNOdent vs ICDAS, and Vistaproof vs ICDAS (Fig. 3), a drift of values was seen; in the case of DIAGNOdent averages increased, instead for Vistaproof it was absent. In both cases an error is to be considered very low.

DISCUSSION

The two diagnostic devices tested were seen to be both reliable and required a less clinical experience for the operator. Both devices were seen to have less precision in terms of under or overestimation of the carious lesions. DIAGNOdent resulted the most user friendly and with the least needed technical support in respect to Vistaproof that required longer operating times and a dedicated informatic digital platform. Undoubtedly, less operating time of the DIAGNOdent resulted more comfortable also for the patient (44-48).

Given the good performance of DIAGNOdent, it was considered a more advisable system for the screening of dental decay even though it cannot replace an intraoral examination of a clinical expert together with an intraoral radiographic exam. Complex situations as the presence of prosthetic and amalgam/composite restorations, prevented the efficacy of any other diagnostic systems compared to the radiographic exams (28-33). The best result to detect carious lesions was seen with the use of digital bite-wing radiographs (such as Vistascan) with high resolution images with low exposure to x-rays (49-56).

Further investigations would be useful to evaluate the use of DIAGNOdent or Vistaproof not only as diagnostic devices but also as a mean to plan extension of caries excavation.

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Conflicts of interests

None of the authors have any business or financial interests in the corporations that produce or market any of the tested devices.

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3D printed anatomical models in the preliminary planification and execution of complex implant placement: a case report

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Abstract As times progress and technology advances, new levels of comfort are continually reached in all aspects of life. In medicine, this concept is reached by improving more and more patient's quality of life by reducing treatment times, invasiveness, morbidity and long term stability of medical interventions. Advancing technology helps with new techniques and materials in achieving this goal, applying to all medical fields. In dentistry, combination of more durable materials and finer treatment strategies continues helping patients achieve levels of comfort which were unimaginable just a few decades back. Implantology and the world of implant-supported prosthesis has brought such an advancement that since its advent a stable growth curve is still observed to this day. This has brought patients to request this kind of rehabilitation even in very complex cases which require very skilled surgical techniques and often carry a very high risk of permanent physical damage. It's the case in situations in which very atrophic osseous bases are in correspondence with important anatomical landmarks such as the maxillary sinus or the inferior alveolar nerve. Radiographic advancement has given the opportunity for careful planning with a precise tissue evaluation however, further innovation has brought to the possibility of tridimensional reproduction of these radiographic exams. This provides the professional with the possibility of performing the actual surgical intervention on the stereolithographic model itself and evaluate perfectly the surgical possibility of performing the planned intervention, further aiding professionals in the communication with patients.

Stereolithography is a digital representation of radiographically generated images which are obtained from the tridimensional acquisition of a physical volume. As much as a tridimensional radiographic exam can be viewed, much of the planification performed on such files is purely two dimensional. Radiographic images are imported from a radiographic machine and are saved in a specific format called Digital Imaging and Communication in Medicine(1-3) (.dicom). This format can be then optimized to a more tridimensional format called the Standard

Tessellation Language (4-8) (.stl) which provides a complete representation of the examined volume. This conversion is an essential step to perform in order for the 3D printers to reproduce perfectly in a 1:1 ratio the examined volume into a physical 3D printed anatomical model. Software, hardware and material advancements have brought to a stage in which multiple and different color resins are used to print the model and therefore anatomical landmark are made visible even if their course is completely intraosseous as for the inferior alveolar nerve. The following case report shows physical planification

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of mandibular implant placement performed on the 3D printed model (9-13).

CASE REPORT

Male patient 45 years old presents with a healthy oral condition which, however, does not provide him with the quality of life he would like to achieve. Patient presents with a fixed tooth supported prosthesis in the maxillary arch and a removable metal base prosthesis for the mandible (14-18). Other than the patient's desire to shift to a fixed solution for his mandibular

rehabilitation, the current removable prosthesis had as retention mechanisms a distal attachment on element 4.4 which in time had become mobile and sensible even if it carried an endodontic treatment. Initial radiographic exams (OPG) revealed a very atrophic mandible and, therefore, amount of available bone was evaluated through a CBCT x-ray examination. From this exam, measurements of bone height revealed as little as 6.8mm in 4.7 position and 7mm in 4.6 position. Decision to create a tridimensional model of the patient's hemimandible was taken and the resin model was obtained (Fig. 1) by optimizing the dicom



Fig. 1. 3D printed stereolithographic model of patient's hemimandible

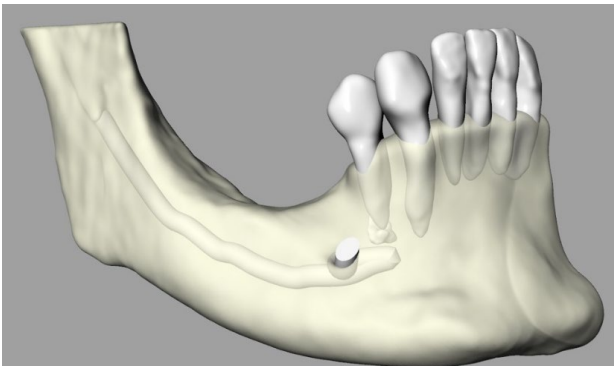


Fig. 2. Virtual hemimandible obtained from DICOM files to STL format



Fig. 3. Surgery planification on the 3D printed model

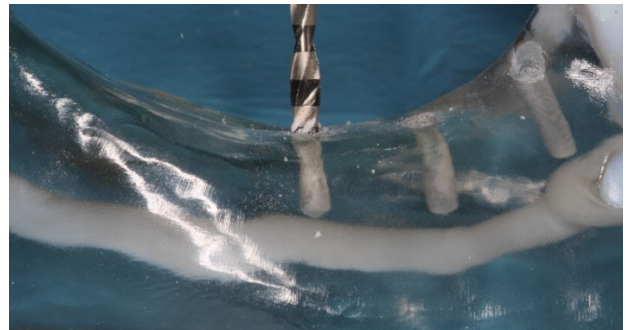


Fig. 4. Surgical bed preparation using surgical burs to visualize implant positioning depth



Fig. 5. Resin guide used to transfer implant position planned on the resin model directly intraorally during the surgery

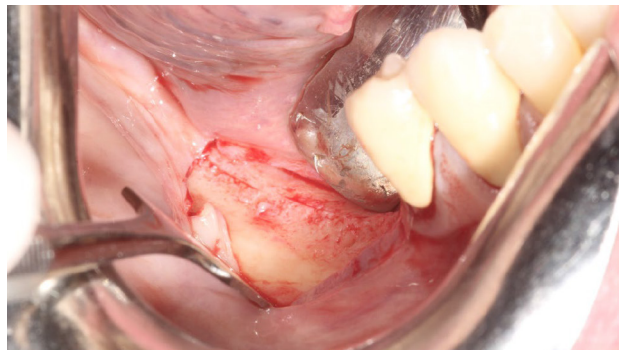


Fig. 6. Full thickness flap to access bone platform

files to stl virtual anatomical model (Fig. 2) (19-25). 3D model was carefully studied and examined, bone height deficiency was evident over the inferior alveolar nerve, also the apical granuloma on element 4.4 was evident and therefore a 3 implant supported prosthesis rehabilitation treatment plan was established. In order to understand surgical complexity, the intervention was performed directly on the resin model (Fig. 3) and implant surgical beds were prepared in the planned positions for the edentulous mandible. 3 implant positions were prepared which helped to exclude the most mesial preparation as it was too close to

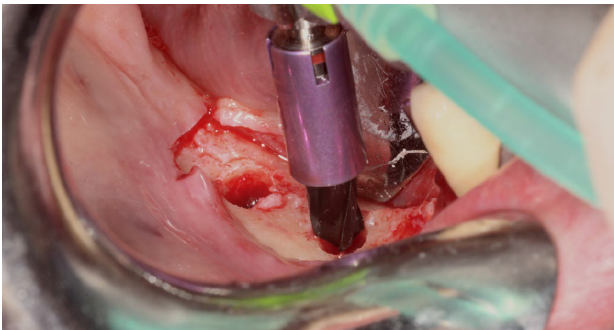


Fig. 7. *Implant bed preparation using the previously planned protocol*

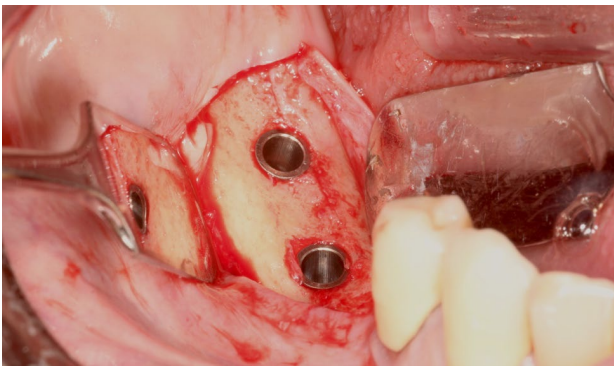


Fig. 8. *Implants*

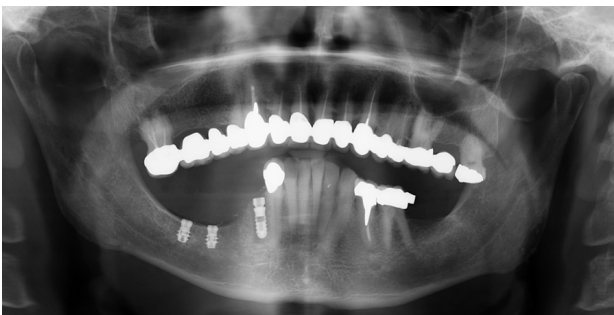


Fig. 9. *Post-Operative X-ray control showing perfect implant placement as planned with absolute Inferior Alveolar Nerve respect*

the mental foramen (Fig. 4). In this way patient was able to view and understand the surgical intervention and patient consent was obtained. Finally, surgery was performed on the patient; a basic resin guide was used to transfer the selected implant positions (Fig. 5). A fully thickness flap was then raised (Fig. 6) and implant beds were prepared using implant bur sequence for the 2 selected 6.5mm short implants (Fig. 7). The implants were then placed (Fig. 8) at bone level implant platform height for the 2 short implants and 2mm below the crest for the post-extractive 4.1mm x 10mm implant in position 4.4. The post-operative x-ray showed perfect surgical execution and planning of the implant placement (Fig. 9) with no neurologic compromise of the inferior alveolar nerve (26-33).

DISCUSSION

The ability to visualize hands-on the patient's anatomy on a physical 3D model gives the operator a completely different feel regarding the intervention that is being planned(34-40). Moreover, the patient being able to see what the intervention really is about can make a more complete informed decision whether he/she wants to undergo such rehabilitation considering the explained risks(41-44). The informed consent will therefore be of greater value since the patient has undergone an actual visual explanation of what the surgery consists of. Such tool therefore has an enormous potential for the professional which is able to physically plan the surgery, for the patient which is able to visualize it and for possible students or young surgeons to acquire initial skills on a resin 3D anatomical model(45-50).

Advancement in medical technology permits for better solutions. In this case a very interesting case of 3D model use was seen, demonstrating this advancement's value in both the surgical field, the patient communication aspect and for the educational potential which it holds.

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An unexpected synovial sarcoma of the parotid gland: a rare localization

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Primary synovial sarcoma of the parotid gland is exceptionally uncommon, with few cases reported in the literature. This is a report of a 46-year-old male with a 7-month history of swelling in the left parotid region occurred after a kidney transplant. Synovial sarcoma clinically and radiologically mimicked a benign lesion and was therefore difficult to diagnose. The patient was treated primarily surgically, followed by radiotherapy. The present case shows that, although uncommon, synovial sarcomas should always be included in the differential diagnosis of a mass in the parotid gland, even if clinical presentation and imaging tend to make a dissimilar diagnosis. It is important to recognize a synovial sarcoma to make a proper management. A multimodality therapeutic approach and a long-term follow-up are necessary because of its late loco-regional and systemic spread.

Synovial sarcoma (SS) is a rare soft-tissue malignant tumour of mesenchymal origin and it represents approximately 5-10% of all soft tissue sarcomas (1-4). Despite its name, tumour cells don't derive from synovial cells but rather from cells of periarticular tissue or from mesenchymal stem cells in every anatomic site. It arises most commonly in the deep soft tissues of lower extremities, it is rare in the head and neck region and exceptionally uncommon in the parotid gland (5-9).

SS has a variable prognosis and favourable and unfavourable histological features have been delineated. It is marked by an expected 5-year survival in adult patients ranging from 50% to 60% (10-14). Current therapeutic management is a multimodal treatment which includes radical surgical resection, radiation and chemotherapy (15-19). Primary SS of the parotid gland is exceptionally uncommon, with few cases reported in the literature.

In the present report, an unusual case of a SS of the parotid gland clinically mimicking a

lymphadenopathy, occurred after a kidney transplant in a young adult, is described.

CASE REPORT

A 46-year-old Caucasian male had a 7-month history of swelling in the left parotid region occurred after a kidney transplant. The patient's medical anamnesis was characterized by a long history of kidney failure and, after years of dialysis, he required a kidney transplant. After surgery, he noticed a painless swelling in the left parotid region. The patient denied any history of trauma or facial surgery.

Physical examination revealed a palpable mass deep in the parotid region, movable to surrounding structures. It had a soft to firm consistency and quite tense regular surface. The mass was well-demarcated and it was no painful nor tenderness. Skin overlying the swelling was normal and neck was clinically negative. Facial nerve function was intact; there weren't others neurological deficits in the facial

Key words: synovial; sarcoma; parotid gland; mesenchymal

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region and opening of the mouth was not restricted.

During preoperative analysis of the patient, a fine-needle aspiration cytology (FNAC) was performed. No cytological criteria of malignancy were found. Results of routine laboratory tests were within normal limits, except for kidney values, as expected.

Magnetic resonance imaging (MRI) confirmed a left parotid mass with a maximum diameter of 1.6 cm and a normal gland profile (Fig.1). MRI revealed an approximately rounded mass, mostly restricted to the deep lobe of the parotid gland. The T1-sequence showed a low intensity mass and irregular borders but well differentiated from the surrounding gland tissue. The mass had high signal intensity in the T2-sequence. After contrast medium administration, the mass showed a marked and inhomogeneous enhancement with an hypointense centre. There were no signs of an involvement of adjacent vascular structures, bones or adipose tissue. Overall, the findings were consistent with a pathological lymph node, although a malignant tumour could not be ruled out.

After completing the diagnostics, because radiological aspects were not diriment in the diagnosis, we performed a total parotidectomy removing the mass completely. The tumour was resected with wide margins (R0) and the facial nerve was preserved. (Fig. 2) The surgical procedure was

followed by a regular post-operative course.

Macroscopically the tumour appeared well circumscribed and firm; the cut surface appeared tan to grey in colour and measured 160 mm in diameter.

Histopathologic examination identified a monophasic mesenchymal proliferation made up by spindle cells (Fig. 3). These spindle elements were relatively small, monomorphic, with scant cytoplasm and ovoid hyperchromatic nuclei. The spindle cells were arranged in fascicles with numerous small branched vessels and variable amount of collagen interposed. Necrosis was absent. The mitotic index was 2 x 10 high power fields (HPF).

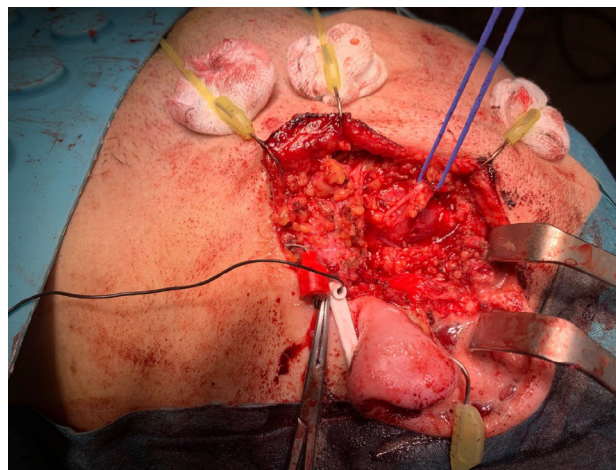


Fig.2 Surgical excision of the tumour with facial nerve preservation.

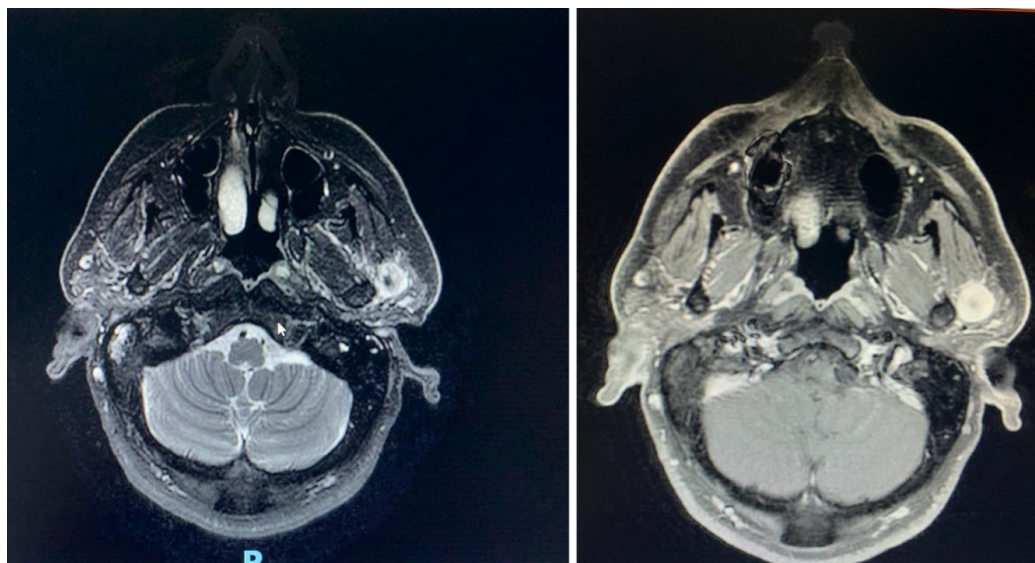


Fig.1 MRI of synovial sarcoma

The immunohistochemical examination showed strong expression of Vimentin, CD99, Bcl2 and CD56; EMA and Cytokeratins AE1/AE3 showed focal expression while immunostainings for CD34,

CD31, Smooth Muscle Actin, Desmin, S100, GFAP, HMB45, CD45, p63 were negative. The proliferation index Ki67 was around 10% (Fig. 4).

Based on the above findings, an unexpected

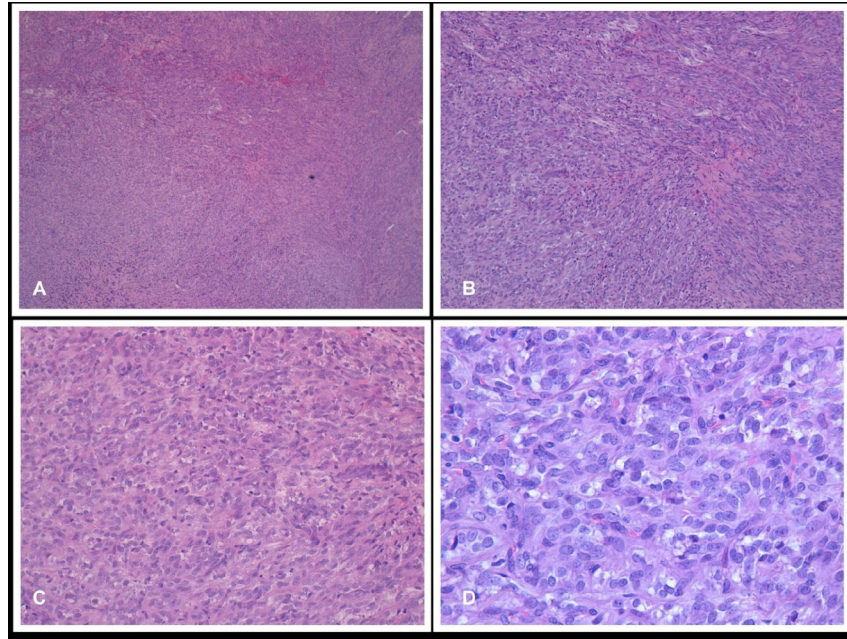


Fig.3 Hematoxylin-eosin staining of specimens. A: Synovial sarcoma: spindle cell background (H&E 4x). B: Spindle cells with variable amount of collagen interposed (H&E 10x). C: Spindle cells arranged in fascicles with numerous small branched vessels (H&E 20x). D: Spindle elements relatively monomorphic, with scant cytoplasm and ovoid hyperchromatic nuclei (H&E 40x).

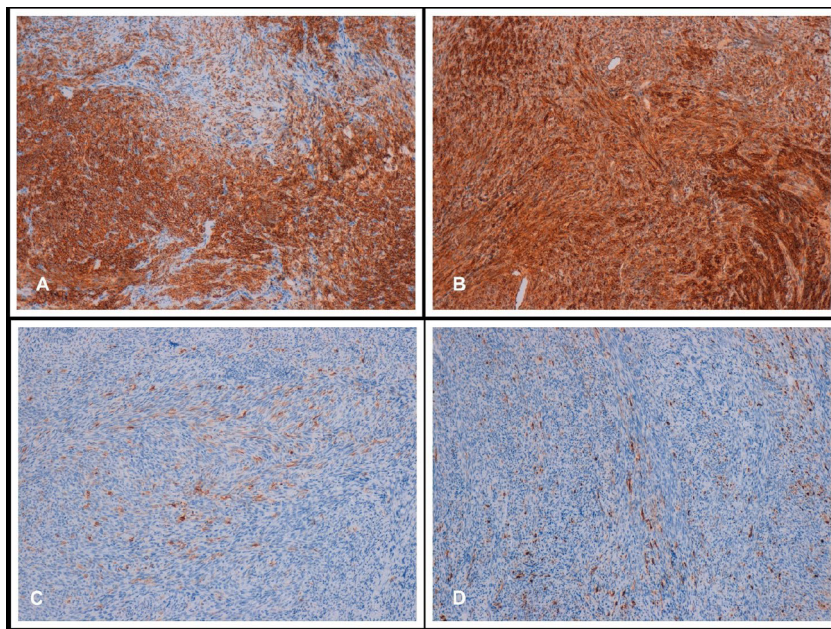


Fig.4 Immunohistochemistry staining of tumour biomarkers. A: CD56. B: CD99. C: EMA. D: CK AE1/AE3.

diagnosis of monophasic synovial sarcoma of the parotid gland, FNCLCC grade 2, was provided.

To complete the staging a total body Computed tomography (CT) scan was performed after surgery. Except for a diffuse enhancement in the left parotid region of probable reactive meaning, there were no signs of local recurrence or metastasis.

After receiving the final pathological results and the exact staging, the necessity of adjuvant radiation therapy was discussed by a tumour board. Confirmed the absence of standardized treatment protocols, a postoperative radiation therapy was performed with the aim of reduce the risk of a local recurrence.

One year after the radiation therapy, the condition of the patient is generally good, the surgical area has healed without complications, the patient had not recurrence of loco-regional recurrence and distant metastasis.

DISCUSSION

Synovial sarcoma (SS) is a rare soft-tissue malignant tumour of mesenchymal origin and it represents only 1% of all malignant tumours and 1% of all head and neck malignancies (20-26). Diagnostic methods are clinical examination, CT or MR imaging and histological examination which is generally a late post-operative finding. Because of the rarity of the primary SS of the parotid gland, it may commonly cause differential diagnostic difficulties, in fact approximately one-third of the patients initially received incorrect pathologic diagnosis. In the parotid gland, it is most likely to be misdiagnosed as a myoepithelial, primary mesenchymal or metastatic neoplasm (27-30).

The pathologic diagnosis of synovial sarcoma remains a challenge because there is histological overlap with other tumour types. Ideally, recognition of the disease should be based upon a combination of findings, including traditional morphology, identification of the chromosomal t(X;18) translocation and a panel of immunohistochemical markers (31-35). SS is a mesenchymal spindle cell tumour which may display variable epithelial differentiation. Three distinct subtypes are described in literature. The monophasic type is characterized

by the predominantly presence of spindle cells. The biphasic type contains spindle and epithelial-like cells, in varying proportions. Finally, there is a poorly differentiated form which seems to occur rarely in a pure form but may be seen in some areas of synovial cell carcinomas; it can be characterized by necrosis, bizarre mitoses, high cellularity and nuclear atypia, but perhaps more often is seen as a proliferation of small, round cells (36-40).

SS has a variable prognosis and favourable and unfavourable histological features have been delineated. It is marked by an expected 5-year survival in adult patients ranging from 50% to 60% and a 5-year metastatic disease-free survival ranging from 40% to 60% (41-44). Distant metastases are not frequent at diagnosis, but they remain the predominant cause of death. Most distant recurrences develop within a few years after initial diagnosis, but late recurrences do occur, even after 10 years [speth] of which 6 (67%.

Many clinical factors have been studied to determine their potential prognostic value. Major prognostic determinants are tumour stage at presentation, tumour size and FNCLCC tumour grade(45-48).

Therapeutic management is based on similar principles to those applied to soft-tissue sarcomas in general. Current multimodal treatment includes radical surgical resection, radiation and chemotherapy. The standard of care for primary SS is wide surgical resection combined with radiation, as appropriate. The role of neoadjuvant chemotherapy is still under refinement and can be considered in patients at high risk of systemic relapse. Patients with nonmetastatic, T1 (<5 cm), superficial tumours in favourable extremity sites may be treated with wide surgical excision alone. Re-excision of unplanned resections is strongly recommended because the high risk (43%) of microscopic disease in T1 tumours beds. In patients with high-risk disease (i.e., high grade, deeply located, >5 cm), the standard treatment of primary, localized SS is represented by the wide surgical resection of the tumour combined with radiation therapy (49-52). For more advanced disease, multimodal treatment that entails surgery, radiotherapy and systemic

chemotherapy may be indicated, even if the efficacy of current chemotherapy is less than optimal. Cytotoxic chemotherapy is often considered in both the neoadjuvant and adjuvant settings for patients with advanced SS(53-56).

In the present case, the age and the sex of the patient were usual, in fact parotid gland SS occurs with a high-prevalence rate in young male adults (> 90% of cases presenting before 50 years of age). SS may be asymptomatic in most cases, or it can present with pain, tenderness, and functional limitation. In the presented case a painless neck mass was the only presenting symptom. Tumour size has been reported in literature to be highly variable (from 0.6 mm to 7 cm): in this case it was within the range (160 mm in diameter). Radiographic findings (in our case MR imaging) are helpful in defining the location and, particularly in deep lobe parotid lesions, they are essential to delineate deep extension, exclude perineural disease and intracranial extension. Imaging may even suggest the correct diagnosis but in the present case it misled us at first to the diagnosis of a lymphadenopathy. MRI findings in SS, in fact, varies and other nonmucosal head and neck masses may appear similar, so no specific imaging characteristics define the entity.

This case fulfils the basic criteria for diagnosis of primary sarcoma of the salivary gland because (1) the patient had not a sarcoma in another site, (2) metastasis was excluded by total body CT, (3) the gross and microscopic appearances were consistent with a primary origin, rather than invasion of the gland from adjacent soft tissue and (4) carcinoma was excluded by means of microscopic study of multiple sections. Criteria above are important because the clinical differential diagnosis for a deep lobe parotid mass is a primary parotid neoplasm, followed by a metastasis or extension of a parapharyngeal tumour.

The tumour was monophasic although usually the biphasic type is more common. Moreover, monophasic subtype is sometimes difficult to distinguish from another spindle cell tumour, such as hemangiopericytoma, neurogenic sarcoma, fibrosarcoma and leiomyosarcoma. In these cases, it is very important to confirm the histological diagnosis of SS by finding the translocation t(X;18) because

it has been identified in SS only (57-58). Because a tumour can be difficult to diagnose based on the histological findings alone, immunohistochemistry can be diriment for the right diagnosis. In our case immunohistochemistry revealed tumour cell positivity for TLE1, EMA and cytokeratin's AE1/AE3. TLE1 is a sensitive marker for the presence of synovial sarcoma. TLE1 expression in context with other immunohistochemical markers (cytokeratin's and epithelia membrane antigen) is not completely specific but can lead to the diagnosis of a synovial sarcoma.

About therapy, information regarding appropriate management for this neoplasm is limited because of its rarity. We performed a complete surgical resection of the mass and postoperative radiotherapy. A prophylactic neck dissection was considered unnecessary because the rare occurrence of lymphatic spread of SS, so it has not benefit for the survival rate. In our case, the monophasic SS was not particularly aggressive, in fact it was Stage I (G2 according to FNCLCC, T1, N0, M0).

Some authors stressed a more aggressive behaviour of the synovial sarcoma of the head and neck than of the same neoplasm in the extremities. However, on the other side, prognosis for patients with synovial sarcoma in the head and neck is considered better than for patients with synovial sarcoma in the trunk and extremities because this tumour in the head and neck is present in younger patients and is treated earlier after the onset of symptoms. Regardless of that disagreement, a long-term (probably a lifelong) follow-up is required for synovial sarcoma because of its tendency for late recurrences and metastasis.

Synovial sarcoma of the parotid gland is a rare malignant tumour of the head and neck region, so there is a global lack of experience in dealing with it and, consequently, diagnosis and clinical management can be a challenge. The present case shows that, although uncommon, synovial sarcomas should always be included in the differential diagnosis of a mass in the parotid gland, even if clinical presentation and imaging tend to make a dissimilar diagnosis. It is important to recognize a SS to make a proper management. A multimodality therapeutic approach and a long-term follow-up

are necessary because of its late loco-regional and systemic spread.

Conflict of interest:

Authors declare that they have no conflicts of interest.

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Early diagnosis of Sjögren's syndrome: the role of biopsy of the minor salivary glands.

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Sjögren's Syndrome is a systemic autoimmune disease that affects the exocrine glands. This pathology causes glandular secretory disorders affecting the patient's quality of life. 61 patients were clinically examined in a 2-year time period (2018-2019), 53 females and 8 males. All patients underwent a biopsy of the minor salivary glands at the San Salvatore Hospital (L'Aquila). The study shows that Sjögren's Syndrome is a disease that mainly involves women, the age group 50-80 years with the major part of the patients graded in G1. Here is a demonstration of the fact that Sjögren's Syndrome is a highly disabling disease that creates a deterioration in the quality of life. It is essential to be able to recognize signs and symptoms for an earlier diagnosis.

Primary Sjögren's syndrome was described in 1926 by Gougerot. It is the second most common multisystem autoimmune disease after rheumatoid arthritis and is characterized by dry eyes and mouth and lymphocytic infiltration of the salivary glands, which are easily accessible and therefore facilitate diagnosis (1-4). Like other autoimmune diseases, it is associated with a specific genetic background, impaired immune response, and environmental factors (5-8). These factors are believed to trigger an innate immune response that leads to the activation of glandular cells, followed by the activation of B and T lymphocytes within the glands.

Sjögren's syndrome is clinically classified into a primary and a secondary form. In the primary form, the exocrine glands are involved with or without systemic involvement; in the secondary form there is an association with other autoimmune diseases (rheumatoid arthritis, SLE). The clinical manifestations at the onset of the disease may be non-specific and appear many years before the definitive diagnosis and are the following: dry eyes, subjective dry mouth, arthralgia, parotid tumor,

Raynaud's phenomenon, fever, dyspareunia, renal involvement (9-13).

MATERIALS AND METHODS

Sample

This study was conducted in the Otolaryngology department of the San Salvatore Hospital (L'Aquila, Italy) from January 2018 to December 2019. The sample includes 61 patients (53 women and 8 men) aged between 45 and 75 who demonstrated symptoms related to Sjögren's Syndrome and who underwent a follow-up visit at the San Salvatore Hospital (L'Aquila, Italy). The patients were identified according to sex, age group and histopathological degree. Biopsy of the minor salivary glands of the lower lip was used as a diagnostic tool (14-20).

Minor salivary gland biopsy

The surgery is performed internally on the lower lip on the normal-looking mucosa (21-23) A 1.5–2 cm incision is made between the midline and the commissure through the mucosa that has just penetrated the epithelium; five or more minor salivary glands are removed and fixed to be sent to the

Key words: Sjögren's syndrome; histopathological diagnosis; minor salivary glands

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pathology, and finally the mucosa is sutured (Fig. 1) (24-27).

In the histopathological examination of the salivary gland, the mononuclear infiltrate shows a periductal distribution; this is common in patients with primary SS, while the perivascular cellular distribution that exists also is found in patients with secondary SS (29-33)

Based on the pathogenesis, the disease can be assessed in 4 grades:

G1: Epithelitis and interstitial T lymphocytosis

G2: Foci of T lymphocytes and appearance of B lymphocytes

G3: Proliferation of B lymphocytes with production of IgG plasma cells

G4: Sclerotic evolution

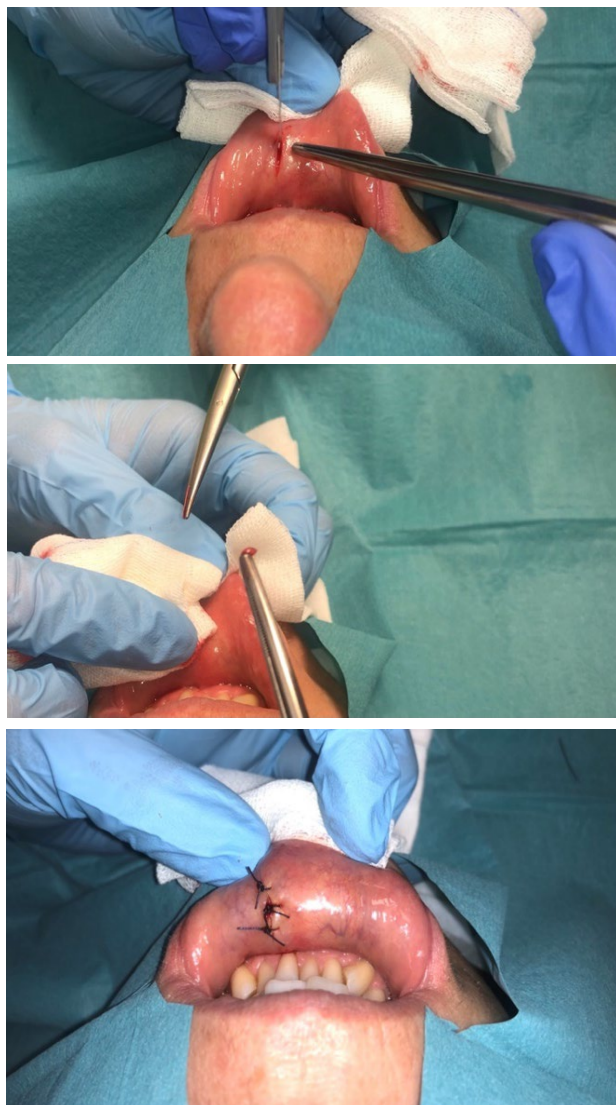


Fig. 1. Procedure for the biopsy of the minor salivary glands

RESULTS

Analyzing the patients of our sample through salivary gland biopsy we can demonstrate that in the years 2018 (Fig. 2) and 2019 (Fig. 3) there was a greater statistical incidence of Sjögren's Syndrome in the female sex and aged between 50 and 80 years. Moreover, a major part of the patients (50% in 2018 and 41,2 in 2019) were graded in G1.

DISCUSSION

The aim of our study was to evaluate the prevalence and clinical relevance of patients with Sjögren's Syndrome in the years 2018 and 2019 (34-40). For many years it has been considered an orphan disease since no specific therapy has been recognized to counter its development.

The treatment of oral and ocular characteristics, as well as the systemic involvement of the organs is entrusted to several medical branches such as ophthalmologists, otolaryngologists, dentists and rheumatologists.

The lack of saliva production (hyposcialia) or the decrease of it leads to an increased susceptibility to the onset of caries, gingivitis and oral candidiasis, traumatic lesions on the mucous membranes and halitosis. All this is accompanied by an impairment of the taste and digestive functions, the ability to chew, swallow, speak correctly and wear removable prostheses (41-45).

The treatment of the disease is based on the use of salivary and tear substitutes (artificial tears or saliva). If tolerated, treatment with drugs that increase saliva and tear secretion such as pilocarpine is indicated (46-50). To control manifestations such as arthralgia and asthenia, anti-malarials such as hydroxychloroquine can be used. For other organ manifestations such as parotidomegaly and neuropathies, steroids and azathioprine are indicated (51-55).

In conclusion, we showed how Sjögren's Syndrome is a highly disabling disease that creates a decline in the quality of life. It often remains unrecognized or diagnosed belatedly. Our experience suggests that we recognize signs and symptoms for an earlier diagnosis.

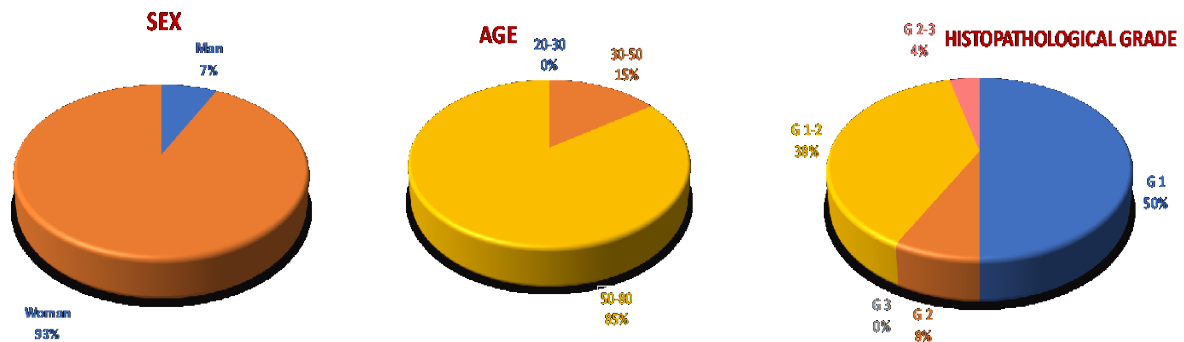


Fig. 2. Statistical evaluation of “Sex”, “age” and “histopathological grade” parameters for patients assessed in 2018.

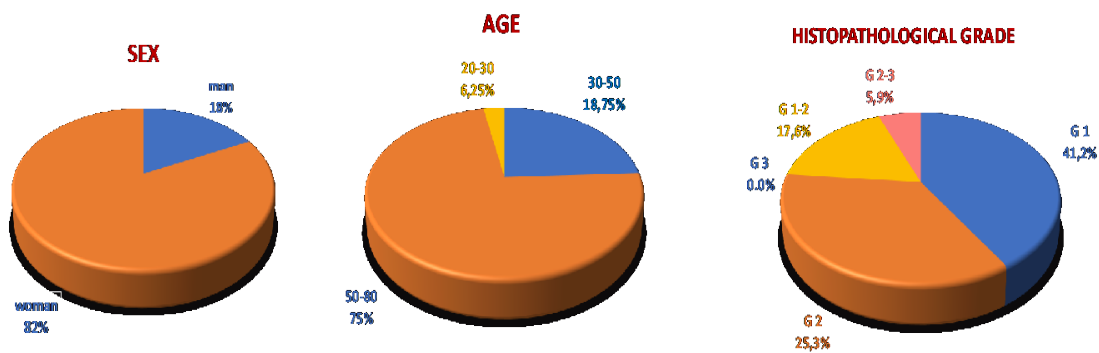


Fig. 3. Statistical evaluation of “Sex”, “age” and “histopathological grade” parameters for patients assessed in 2019.

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Can growth hormone influence dental pulp stem cells differentiation? A puzzle that is going to complete

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Growth hormone (GH) is a powerful and important hormone secreted by the cells of the anterior pituitary gland, influenced by many physiological and pathological conditions. Dental pulp stem cells (DPSCs) may be a source of differentiated cells inducing bone formation and controlled hydroxyapatite crystal growth. These DPSCs have the ability of self-expanding and differentiating in pre-osteoblast producing in vitro autologous bone tissue. The aim of our study is to investigate if GH can influence differentiation of DPSCs in osteoblasts and bone tissue. Gene related to ossification (BMP1, BMP2 BMP3, BMP4, BMP5 BMP6), osteoblast differentiation (BMPR1A, BMPR1B, BMPR2), bone mineralization (TGFB1, TGFB2, TGFB3) and skeletal development (TGFB1 AND TGFB2) were investigated to study the potential effect of GH in osteoblast differentiation and proliferation. After 24 h of treatment all gene investigated were up-regulated in treated DPSCs vs. untreated DPSCs. Significant up-regulated genes (Fold change > 2) were the Bone Morphogenetic Protein BMP2, BMP4, BMP6 and BMP7. GH induce the over-expression of bone related genes after 48 h of treatment too. In this case the significant up-regulated genes were the Bone Morphogenetic Protein BMP3, BMP4, BMP5 and their receptor BMPR1B and BMPR2. Transforming Growth Factor Genes family (TGFB1, TGFB2, TGFB3) and their receptor (TGFB1 and TGFB2) are also significantly up-regulated after GH treatment. GH has demonstrated to influence this process of DPSCs differentiation and expansion in osteoblasts. Further studies are needed to explore this new way of creating bone tissue.

Growth hormone (GH) is a powerful and important hormone secreted by the cells of the anterior pituitary gland, influenced by many physiological and pathological conditions (1-5) GH has a plasmatic half-life between 15 and 20 min after secretion or intravenous injection, and blood concentration of GH reaches its peak 1-3 after subcutaneous or intramuscular administration and is eliminated from the bloodstream after 24 hours (6-10). GH is one of the most important molecules involved in the growth and bone remodeling. Children with various forms

of GH deficiency or other defects in the GH signal transduction pathway do not reach normal skeletal growth. GH supplies were until now rarely used for the treatment of these deficiency diseases, but the advent of commercial applications of recombinant DNA technology has increased the indications for treatment with GH supplies. Nowadays patients with endocrinopathies, diseases associated with AIDS, Turner syndrome, chronic kidney failure, chronic obstructive lung disease and sepsis are treated with GH supplies. This hormone is also

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used illegally to enhance sporting performances of professional athletes, but also amateur. The 5% of the male students in American high school use or used GH as an anabolic agent (11-15). In addition, GH can reverse some of the aging processes, associated with the reduction in the levels of this hormone in the bloodstream, and his administration can lead to increased muscle and bone mass. GH is involved on the proliferation and differentiation of osteoblasts causing bone remodelling, increasing protein synthesis and mineralization and bone matrix proteins. Bone turnover needs differentiated and active osteoblasts to produce the extracellular matrix that will syntetize the mineralizing process. GH has wide applications and well documented anabolic and lipolytic effects on bone metabolism and teeth growth.

It's well known that dental pulp is one of the source of stem cells. In fact in the adult stem cella are present in dental pulp and they are able to produce new dentin after a mechanical or inflammatory stimuli (16-20). The new dentin, called tertiary dentine, presents the same components of primary and secondary dentine, but a different cytoarchitettura. In fact problems in mineralization are common in oral pathologies and the role of stem cells for hard tissue formation has increased interest among reserchers and dentist. Dental pulp stem cells (DPSCs) may be a source

of differentiated cells inducing bone formation and controlled hydrossyapatite crystal growth. DPSCs has been isolated from teeth of subjects up to 30 years and form sporadic dense nodules in vitro, but undergo mineralization and bone or dentin-like formation only when grafted in vivo. These DPSCs have the ability of self-expandig and differentiating in pre-osteoblast producing in vitro autologous bone tissue (21-28).

The aim of our study is to investigate if GH can influence differentiation of DPSCs in osteoblats and bone tissue.

Materials and Methods

The materials and methods have been described in our previous publication (29-31).

RESULTS

Cell cultures were phenotypically characterized by flow cytometric analyses. Cell preparations derived from dental pulp were homogenously CD105⁺, CD90⁺, CD34⁺, CD45⁺, CD14⁺, which is a typical mesenchymal stem cells surface antigen profile.

DPSCs were also characterized by immunofluorescence. The cell surfaces were positive for mesenchymal stem cell marker, CD105, CD90

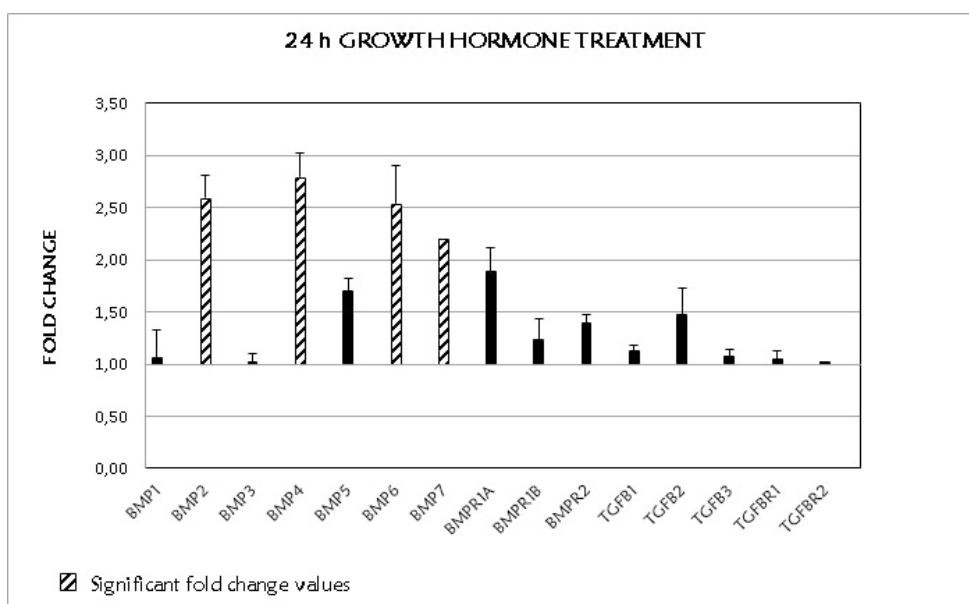


Fig. 1. Bone Morphogenetic Protein BMP2, BMP4, BMP6 and BMP7 were significantly up-regulated

and CD73 and negative for markers of hematopoietic origin, CD34.

DPSCs were treated with GH at the concentration of 100 ng/ml for 24 and 48 h. Gene expression in treated DPSCs was compared with untreated cells (control) in order to check the effect of GH on stem cells differentiation.

Gene related to ossification (BMP1, BMP2 BMP3,

BMP4, BMP5 BMP6), osteoblast differentiation (BMPR1A, BMPR1B, BMPR2), bone mineralization (TGFB1, TGFB2, TGFB3) and skeletal development (TGFB1 AND TGFB2) were investigated to study the potential effect of GH in osteoblast differentiation and proliferation (Table I). Table II reported the investigated genes and the correspondent fold change at 24 and 48 h of treatment.

Table I. Primers sequences for SYBR® Green assay

Gene symbol	Gene name	Gene function
BMP1	Bone morphogenetic protein 1	Ossification, Cartilage Condensation, Cell-Cell Adhesion
BMP2	Bone morphogenetic protein 2	Ossification, Osteoblast Differentiation, Bone Mineralization
BMP3	Bone morphogenetic protein 3	Ossification
BMP4	Bone morphogenetic protein 4	Ossification, Osteoblast Differentiation, Bone Mineralization
BMP5	Bone morphogenetic protein 5	Ossification
BMP6	Bone morphogenetic protein 6	Ossification, Osteoblast Differentiation, Bone Mineralization
BMP7	Bone morphogenetic protein 7	Ossification, Osteoblast Differentiation, Bone Mineralization
BMPR1A	Bone morphogenetic protein receptor, type IA	Osteoblast Differentiation, Ossification, Bone Mineralization
BMPR1B	Bone morphogenetic protein receptor, type IB	Osteoblast Differentiation, Ossification, Cartilage Condensation
BMPR2	Bone morphogenetic protein receptor, type II	Osteoblast Differentiation, Ossification, Bone Mineralization
TGFB1	Transforming growth factor, beta 1	Bone Mineralization, Ossification, Calcium Ion Binding and Homeostasis
TGFB2	Transforming growth factor, beta 2	Ossification
TGFB3	Transforming growth factor, beta 3	Bone Mineralization, Ossification
TGFB1	Transforming growth factor, beta receptor 1	Skeletal Development
TGFB2	Transforming growth factor, beta receptor 2	Skeletal Development
GUSB	Glucuronidase, beta	Housekeeping gene

Table II. *The investigated genes and the correspondent change at 24 and 48 h of treatment.*

Gene symbol	24 h	48 h
BMP1	1,06	1,82
BMP2	2,59	1,89
BMP3	1,02	2,42
BMP4	2,79	2,15
BMP5	1,70	2,83
BMP6	2,54	1,39
BMP7	2,20	2,86
BMPR1A	1,89	1,59
BMPR1B	1,23	2,85
BMPR2	1,39	2,49
TGFB1	1,13	2,75
TGFB2	1,48	2,07
TGFB3	1,07	2,18
TGFBR1	1,04	2,97
TGFBR2	1,02	4,54

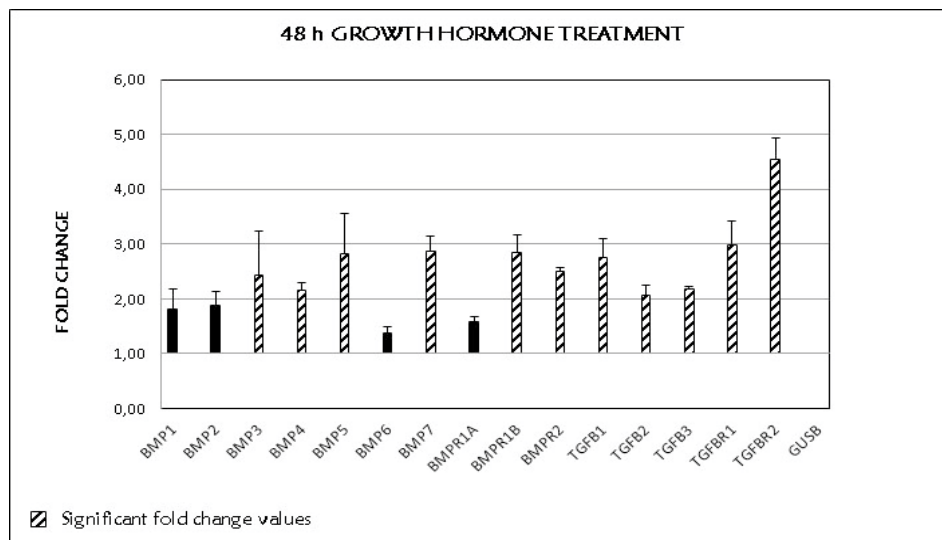
After 24 h of treatment all gene investigated were up-regulated in treated DPSCs vs. untreated DPSCs. Significant up-regulated genes (Fold change > 2) were the Bone Morphogenetic Protein BMP2, BMP4, BMP6 and BMP7 (Fig. 1).

GH induce the over-expression of bone related genes after 48 h of treatment too. In this case the significant up-regulated genes were the Bone Morphogenetic Protein BMP3, BMP4, BMP5 and their receptor BMPR1B and BMPR2.

Transforming Growth Factor Genes family (TGFB1, TGFB2, TGFB3) and their receptor (TGFBR1 and TGFBR2) are also significantly up-regulated after GH treatment (Fig. 2).

DISCUSSION

This study focus action of GH inducing markers of bone formation, provides support of the view that GH influences differentiation of DPSCs in osteoblastic lineage. Bone morphogenetic proteins (BMPs) have been shown to induce DPSCs to proliferate and then differentiate into osteoblastic cells. GH can modulate the expression of various osteogenic markers. Thus, the effects of GH on DPSCs must be examined in light of their apparent maturity in terms of osteogenic potential. Previous studies showing that GH promotes bone growth

**Fig. 2.** *Transforming Growth Factor Genes family (TGFB1, TGFB2, TGFB3) and their receptor (TGFBR1 and TGFBR2) are also significantly up-regulated after GH treatment*

suggest that GH facilitates differentiation as the cell population reaches a more mature, post proliferative stage(31-37).

DPSCs showed differentiation profiles similar to those of bone differentiation and this event make them very interesting as a model to study the osteogenesis. Dental pulp is a good source of stem cells (38-44) that differentiate into osteoblasts, as well as other phenotypes, for hard tissue. During life, bone tissue needs renewal, and dental pulp represents an additional source of mesenchymal cells to bone marrow. Selection and expansion of stem cells are complex procedures commonly used to obtain cells for potential therapy; thus, being able to obtain sorted cells quickly after collection then being able to transplant them directly is an advantage that can lead to easier application of bone-engineering protocols (45-50).

There is a considerable body of work showing osteogenic actions of growth hormone (GH) in cortical bone in several species. In our study we have demonstrated that GH can increase DPSCs and influences differentiation and proliferation of DPSCs in osteoblasts (51-53).

The extreme feasibility to manage the dental pulp stem cells make them ready to use in clinical trial on human patients: autologous stem cells collected from dental pulp of extracted wisdom teeth or from pulpectomy of teeth left in situ are a chance that should be improved in clinical trial. Although in the future dental pulp could play a fundamental role in all human tissue regeneration, the main commitment is bone and bone regeneration is the goal where these cells can find an immediate applications(54-56).

In conclusion, our study has provided evidence that stem cells selected from human permanent dental pulp represent a population of postnatal stem cells able to extensively proliferate and differentiate into mesenchymal cells and mainly osteoblasts. Therefore, these cells constitute a large, ideal source of cells for tissue regeneration, tissue-based clinical therapies, and transplantation.

GH has demonstrated to influence this process in production of DPSCs and differentiation and expansion in osteoblasts.

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Circulating tumor cells of oral cancer: a narrative review

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Early during the formation and growth of a primary tumour (e.g., breast, colon, or prostate cancer), cells are shed from the primary tumour and then circulate through the bloodstream. Many of the major recent advances in targeted therapies have relied on the acquisition of tumour tissue via biopsy before initiation of therapy or after the onset of resistance. The advantage of physical properties is that they allow circulating tumour cell (CTC) separation without labelling. Methods based on physical properties include density gradient centrifugation, filtration through special filters. In addition to using somatic point mutations as markers for the detection of tumor DNA, strategies to detect tumor-derived rearrangements and chromosomal copy number changes in the plasma of patients with cancer have been developed. Several studies have shown that metastatic cells might have unique characteristics that can differ from the bulk of cancer cells in the primary tumor currently used for stratification of patients to systemic therapy. In conclusion, the molecular and functional analysis of CTCs and circulating nucleic acids can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.

Early during the formation and growth of a primary tumor (e.g., breast, colon, or prostate cancer), cells are shed from the primary tumor and then circulate through the bloodstream. These circulating tumor cells (CTCs)(1-6) can be enriched and detected via different technologies that take advantage of their physical and biological properties. CTC analyses are considered a real-time “liquid biopsy” for patients with cancer.

The presence of nucleic acids (DNA and RNA) circulating in the plasma of patients with cancer is a phenomenon of growing interest in the past two decades, especially from two perspectives: its clinical utility for diagnosing and following cancer patients and its possible biological value.

Currently, the diagnosis of most of solid tumors relies on the study of the tumor specimen and the use of tumor markers specifically, in the detection of tumor antigens in blood, although these markers have an important limitation in specificity and sensitivity. Plasma detection of gene sequences of the tumor itself, by means of high-sensitivity molecular techniques, is proposed as a promising alternative, due to sensitivity and specificity and the low invasiveness of the technique. In fact, in recent years, encouraging results have been obtained that led to propose a new term, “liquid biopsy,” a non-invasive form of diagnosis through the study of circulating nucleic acids in plasma(7-11).

The investigation of circulating RNA in plasma

Key words: tumour; liquid biopsy, DNA, genetic study

of started later, although today there is already a number of previous studies. In general, the results were surprising, especially since it has been possible to amplify and quantify it, despite the high concentration of RNAses that are in the serum of cancer patients.

Initially reported by Mandel and Metais(12-16) in 1948, the clinical utility of circulating cell-freeDNA (cfDNA) in the serum and plasma has been an area of active research in many disciplines of medicine. Evaluation of fetal DNA in the circulation of expecting mothers has seen the most success. Investigation of fetal DNA can now uncover germline fetal changes weeks after conception, including point mutation and aneuploidy, and is likely to become part of the standard of care in prenatal assessment in highrisk patients (17-21).

Tissue versus liquid biopsy

Many of the major recent advances in targeted therapies have relied on the acquisition of tumor tissue via biopsy before initiation of therapy or after the onset of resistance. The availability of tissue for molecular analysis has been instrumental in understanding the primary mechanism of action of agents such as trastuzumab, imatinib, cetuximab, and vemurafenib (22-26). Likewise, access to tumor tissue after clinical resistance has helped to define mechanisms of secondary resistance to these targeted agents, often in the very pathway or gene involved in their responsiveness. Although tumor tissue is the gold standard for clinical and investigational sequencing, major barriers exist in terms of acquisition and utility. Circulating tumor DNA can in principle provide the same genetic information as a tissue biopsy necessary to interrogate key companion diagnostics. Accessing the bloodstream has clear advantages. For one, it is a source of fresh DNA, unhampered by preservatives. Sampling the blood from a needle stick is minimally invasive and avoids the dangers of biopsies (27-30).

Physical properties

The advantage of physical properties is that they allow CTC separation without labeling. Methods based on physical properties include: density gradient

centrifugation and filtration through special filters.

A new versatile label-free biochip that exploits the unique differences in size and deformability of cancer cells, (larger and stiffer than blood cells); a photoacoustic flow cytometer; a microfluidics device that combines multi orifice flow fractionation and the di-electrophoresis (DEP) cell separation technique. A DEP field-flow fractionation device allows isolation of viable CTCs by their different response to DEP owing to their differences in size and membrane properties.(31-36)

Pare and digital karyotyping

In addition to using somatic point mutations as markers for the detection of tumor DNA, strategies to detect tumor-derived rearrangements and chromosomal copy number changes (ie, amplifications) in the plasma of patients with cancer have been developed,(37-42) Two novel methods to quantitate levels of tumor DNA in circulation include personalized analysis of rearranged ends (PARE) and digital karyotyping. PARE is an approach to identifying DNA rearrangements in human tumors and using these alterations for development of tumor biomarkers. Digital karyotyping is a genome-wide method for detection of copy number alterations associated with such chromosomal changes. Initial analyses have demonstrated that the sensitivity of this approach (ie, ability to detect tumor DNA in a mixture of tumor and normal DNA) is lower than 0.001%. Furthermore, this approach can be modified to detect amplifications across the genome based on the fact that amplified segments of genomic DNA result in chromosomal rearrangements. Therefore, when whole-genome sequencing of plasma DNA is performed, paired-end sequence data may reveal aberrantly mapped paired-end reads. The breakpoints of these regions are mapped to genes of interest. Tag counts in the aberrantly mapped regions and pooled normal plasma from healthy individuals are then compared to determine copy number variations in amplified regions (43-46).

DISCUSSION

In general, patients with cancer have much higher

levels of normal circulating cell free DNA than healthy individuals(47-51)As the tumor increases in volume, so too does the cellular turnover and hence the number of apoptotic and necrotic cells. Under normal physiologic circumstances, apoptotic and necrotic remains are cleared by infiltrating phagocytes. This does not happen efficiently within the tumoral mass, leading to the accumulation of cellular debris and its inevitable release into the circulation (52-55).

Several studies have shown that metastatic cells might have unique characteristics that can differ from the bulk of cancer cells in the primary tumor currently used for stratification of patients to systemic therapy. These characteristics can be explained by the facts that (a) the metastatic sub-clone within the primary tumor might be small and easily missed and (b) metastatic cells may gain additional genomic characteristics over time and develop independently from the primary tumor. Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, and antibody therapy) might provide unique information for the future clinical management of the individual cancer patient and might serve as surrogate marker for response to therapy (56-60). Elevated concentrations of cell-free circulating tumor DNA (ctDNA) fragments have been found in blood plasma and serum of cancer patients with various tumor types and associated with unfavourable outcome(61-64) ctDNA fragments mainly originate from apoptotic or necrotic tumor cells which discharge their DNA into the blood circulation.

With the development of next generation sequencing technologies, the field of ctDNA analysis and which originally started more than 20 years ago has revived and focused on genomic aberrations relevant to therapy resistance in patients with metastatic cancer (e.g., k-ras mutations for EGFR inhibition in colorectal cancer)(65-67).

More research is needed to establish if these applications will be feasible in tumor types beyond those already studied. In scenarios where only low levels of ctDNA are present (eg, early-stage disease and minimal residual disease), novel genomic methodologies, based on digital PCR, have made

such applications possible. A number of promising CTC-detection techniques have been developed in recent years, and they continue to be improved. These new approaches must be validated in multicenter clinical studies with defined end-points, such as disease-free or overall survival. In addition, we must identify the most aggressive subset of CTCs that are the metastasis-initiating cells.

In conclusion, the molecular and functional analysis of CTCs and circulating nucleic acids can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.

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Gabapentin and healthy gingival mucosa

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Gabapentin is a drug used to treat postoperative pain with antihyperalgesic properties and a unique mechanism of action, which differentiates it from other commonly used drugs. In our study fragments of gingival tissue of healthy volunteers were collected during operation. Gene expression of 29 genes was investigated in gingival fibroblasts cell culture treated with gabapentin, compared with untreated cells. Of the different chemokines and interleukins studied only 10 were statistically significant (CCL1, CCR1, CCR4, CCR5, CCR6, IL1A, IL1B, IL5, IL6R, TNFSF10). Gabapentin causes gingival hyperplasia in a limited number of patients by increasing expression of cytokines. This group of patients have probably a genetic background. Since or sample derived from healthy patients, we do not see an increase of cytokine expression. Additional studies are needed to better understand the interaction between gabapentin and gingival hyperplasia.

Gabapentin is a drug used to treat postoperative pain with antihyperalgesic properties and a unique mechanism of action, which differentiates it from other commonly used drugs. Various studies have shown that the perioperative use of gabapentin reduces postoperative pain.

Gabapentin acts by reducing the hyperexcitability induced by the lesions of the posterior horn neurons responsible for central sensitization (1-4). The mechanism of the antihyperalgesic action may be the result of the postsynaptic binding of gabapentin to the alpha 2-delta subunit of the voltage-dependent calcium channels of the dorsal horn neurons, causing a decrease in calcium entry into the nerve endings and therefore decrease of neurotransmitter release. Other possible cellular mechanisms include the effects of gabapentin on NMDA receptors, sodium

channels, monoaminergic pathways and the opioid system (5-7).

Gabapentin was initially introduced in 1994 as antiepileptic drug (AED), mainly due to partial seizures. It is an anticonvulsant whose side effects are well tolerated and well absorbed after oral administration with the maximum plasma concentration observed after 2-3 h (8-10). Some of the most commonly reported side effects of gabapentin include dizziness, drowsiness, fatigue, ataxia and peripheral edema (11-14). Gabapentin was also beneficial treatment of neuropathic pain related to post-herpetic neuralgia (PHN), postpoliomyelitis neuropathy and reflex sympathetic dystrophy. Furthermore, it has been shown to play a role in the treatment of diabetes-related pain neuropathy in placebo-controlled clinical trials. The mechanism of

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action of gabapentin for treating neuropathic pain depends on its binding to the α -2/delta subunit of neuronal voltage-gated calcium channels and possibly on its interference with neuronal Ca influx (15-18).

In this article we study in vitro the effects of the gabapentin molecule on “Inflammatory Cytokines and Receptors” pathway by using human fibroblasts of healthy volunteers to better understand the possible effect of the drug on inflammation and gingival hyperplasia (19-26).

MATERIALS AND METHODS

Primary human fibroblast cells culture

Fragments of gingival tissue of healthy volunteers (11-year-old and a 20-year-old male and a 68-year-old female) were collected during operation. The pieces were transferred in 75 cm² culture flasks containing DMEM medium (Sigma Aldrich, Inc., St Louis, Mo, USA) supplemented with 20% fetal calf serum, antibiotics (Penicillin 100U/ml and Streptomycin 100 micrograms/ml-Sigma Aldrich, Inc., St Louis, Mo, USA).

Cells were incubated in a humidified atmosphere of 5% CO₂ at 37°C. The medium was changed the next day and twice a week. After 15 days the pieces of gingival tissue were removed from the culture flask. Cells were harvested after additional 24h of incubation.

Cell viability test

A stock solution of gabapentin 1mg/mL was prepared. Further dilutions were made with the culture medium to the desired concentrations just before use. Cell lines were seeded into 96-well plates at a density of 104 cells per well containing 100µl of cell culture medium and incubated for 24h to allow cell adherence. Serial dilution of gabapentin (5000ng/mL, 2000ng/mL, 1000ng/mL, 500ng/mL, 100ng/mL) were added (three wells for each concentration). The cell culture medium alone was used negative control (27-33).

After 24h of incubation, cell viability was measured using PrestoBlue™ Reagent Protocol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Briefly, the PrestoBlue™ solution (10µl) was added into each well containing 90µl of treatment solution. Plates were then placed back into the incubator for 1h, after which absorbance was measured at wavelengths of 570nm

excitation and 620nm emission by an automated microplate reader (Sunrise™, Tecan Trading AG, Switzerland). The percentage of viable cells was determined by comparing the average absorbance in drug treated wells with average absorbance in control wells exposed to vehicle alone. The results were presented as the mean $\pm$ standard deviation of three measures.

Cell treatment

Cell lines were seeded at a density of 1.0×10^5 cells/ml into 9cm² (3ml) wells and subjected to serum starvation for 16 hours at 37°C. Cells were treated with 1000ng/mL gabapentin solution for 24h. This solution was obtained in DMEM supplemented with 2% FBS, antibiotics and aminoacids. Cell medium alone was used as control negative. The cells were maintained in a humidified atmosphere of 5% CO₂ at 37 °C. After the end of the exposure time cells were trypsinized and processed for RNA extraction.

RNA isolation, reverse transcription and quantitative real-time RT-PCR

Total RNA was isolated from cell lines using GenElute mammalian total RNA purification miniprep kit (Sigma-Aldrich) according to manufacturer's instructions. Pure RNA was quantified at NanoDrop 2000 spectrophotometer (Thermo Scientific). cDNA synthesis was performed starting from 500 ng of total RNA, using PrimeScript RT Master Mix (Takara Bio Inc.). The reaction was incubated at 37 °C for 15min and inactivated by heating at 70°C for 10sec. cDNA was amplified by Real Time Quantitative PCR using the ViiA™ 7 System (Applied Biosystems).

All PCR reactions were performed in a 20µl volume. Each reaction contained 10µL of 2x qPCRBIO SYGreen Mix Lo-ROX (PCRBIO SYSTEMS), 400nM concentration of each primer, and cDNA. Custom primers belonging to the “Inflammatory Cytokines and Receptors” pathway were purchased from Sigma Aldrich. All experiments were performed including non-template controls to exclude reagents contamination. PCR was performed including two analytical replicates.

The amplification profile was initiated by 10min incubation at 95°C, followed by two-step amplification of 15sec at 95°C and 60sec at 60°C for 40 cycles. As final step, a melt curve dissociation analysis was performed.

Statistical analysis

The gene expression levels were normalized to the expression of the reference gene (RPL13) and were expressed as fold changes relative to the expression of the untreated cells. Quantification was done with the delta/delta Ct calculation method (34-37).

Table I. Selected genes used in Real Time PCR belonging to “Inflammatory Cytokines and Receptors” pathway. In bold the Fold change of significant gene expression level.

Gene	Fold change	Gene function
CCL1	2.20	Chemokine
CCL2	1.02	Chemokine
CCL2D	0.74	Chemokine
CCL5	1.63	Chemokine
CCL8	1.09	Chemokine
CXCL5	0.69	Chemokine
CXCL10	1.41	Chemokine
CCR1	0.29	Chemokine receptor
CCR4	0.22	Chemokine receptor
CCR5	0.47	Chemokine receptor
CCR6	0.18	Chemokine receptor
CCR10	0.78	Chemokine receptor
CXCR5	0.59	Chemokine receptor
IL1A	0.34	Interleukin
IL1B	0.16	Interleukin
IL5	0.14	Interleukin
IL6	1.14	Interleukin
IL7	1.18	Interleukin
IL8	0.70	Interleukin
ILR1	1.19	Interleukin receptor
IL1RN	1.01	Interleukin receptor
IL6R	0.23	Interleukin receptor
IL10RB	1.86	Interleukin receptor
BMP2	1.99	Cytokine
SPP1	1.44	Cytokine
TNFRSF	1.12	Cytokine
TNFSF10	0.12	Cytokine
VEGFA	0.79	Cytokine
RPL13	1.00	Housekeeping gene

RESULTS

The optimal concentration of gabapentin to be used for cell treatment has been obtained using PrestoBlue™ cell viability test. Basing on this test the concentration used for the treatment was 1000 ng/ml. The gene expression profile of 29 genes belonging to the “Inflammatory Cytokines and Receptors” pathway was analyzed (Tab. I). Table II reported the significant deregulated genes and their Fold change (38-42).

Ten genes (CCL1, CCR1, CCR4, CCR5, CCR6, IL1A, IL1B, IL5, IL6R, TNFSF 10) were statistically significant. All but 1 gene resulted down-regulated after 24h of treatment with gabapentin. In fact, CCL1 was the only, although weakly, up-expressed genes. Significantly deregulated genes are those with a fold-change value superior to 2 (up-regulated) and less than 0.5 (down-regulated).

DISCUSSION

The relationship between drugs and gingival tissue is influenced by several factors, such as: age, genetic predisposition, presence of preexisting plaque, and gingival inflammation (43-50). There is a variable gingival response in patients taking drugs both in the variability in the extent and severity of the gingival changes (51-56).

In this article we wanted to study the effects of the gabapentin molecule on 29 genes belonging to “Inflammatory Cytokines and Receptors” pathway, present in human fibroblasts of healthy volunteers. Among the studied genes only 10 genes (CCL1, CCR1, CCR4, CCR5, CCR6, IL1A, IL1B, IL5, IL6R, TNFSF 10) were statistically significant. All but 1 gene resulted down-regulated after 24h of treatment with gabapentin. In fact, CCL1 was the only, although weakly, up-expressed genes. Probably we have not highlighted over-expression of the other inflammatory molecules because the study was performed on healthy people (57-60).

Gabapentin causes gingival hyperplasia in a limited number of patients by increasing expression of cytokines. This group of patients have probably a genetic background. Since our sample derived from healthy patients, we do not see an increase of cytokine

Table II. Significant gene expression levels after 24h treatment with gabapentin, as compared with untreated cells.

Gene	Fold change	SD (+/-)	Gene function
CCL1	2.20	0.59	Chemokine
CCR1	0.29	0.07	Chemokine receptor
CCR4	0.22	0.02	Chemokine receptor
CCR5	0.47	0.06	Chemokine receptor
CCR6	0.18	0.01	Chemokine receptor
IL1A	0.34	0.09	Interleukin
IL1B	0.16	0.01	Interleukin
IL5	0.14	0.01	Interleukin
IL6R	0.23	0.01	Interleukin receptor
TNFSF10	0.12	0.01	Cytokine

expression. Additional studies are needed to better understand the interaction between gabapentin and gingival hyperplasia (61-64).

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Salivary gland stones, systematic review, a better approach.

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The aim of this article is to analyze the different type of approach possible regarding to solve the symptomatology, focusing on the high risk of relapsing typical of this kind chronic gland inflammation. The minor salivary glands are important components of the oral cavity and are present in most of the oral cavity, their purpose is to moisten and lubricate the muscular and epithelial tissues of the oral cavity through their secretions and with the aim not least of ensuring a protection from bacterial activity, the minor salivary glands mainly play a defensive role within the oral cavity.

The minor salivary glands are important components of the oral cavity and are present in most of the oral cavity, their purpose is to moisten and lubricate the muscular and epithelial tissues of the oral cavity through their secretions and with the aim not least of ensuring a protection from bacterial activity, the minor salivary glands mainly play a defensive role within the oral cavity (1-5).

With the term calculosis we mean an obstruction of a duct in our salivary case whose pathogenic noxa can be multi-factorial, among the most common causes or contributing causes we find, in addition to salivary crystallization.

Abnormalities of the salivary ducts (stenosis or narrowing, acute angulations, “kinking”, ductal invaginations); thick mucus plugs (“mucus plugs”); polyps and granulation tissue (autoimmune diseases such as Sjogren’s s.); compressions from the outside (hypertrophy of nearby muscles such as masseter in bruxism or mylohyoid, inflamed lymph nodes); foreign bodies (vegetable fibers, pieces of toothpicks, food residues).

A mucosal retention cyst is a cystic cavity with a wall of epithelial lining. The etiopathogenesis is to be related to an obstructive phenomenon of the excretory duct of a salivary gland rather than to a phenomenon of rupture of the ductal wall (extravasation cyst) (6-10).

The differences between the two cystic formations, one often linked to obliterating phenomena (stones) the other to traumatic phenomena, another important component is the age in which the latter can occur, the mucosal retention cyst is it presents at a more advanced age after 30/40 years, unlike those from rupture (extravasation), it occurs at a less advanced age. The most frequent locations are the superficial lobe of the parotid in the major salivary glands and the accessory salivary glands of the palate. Other sites can be the cheek and the oral floor. The dimensions are usually between 0.5 and 1 cm in diameter and the consistency is stretched-elastic. The cysts located on the palate mimic the presence of a mucoepidermoid carcinoma (11-15).

Differential diagnosis is possible only through histological examination. An examination that

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remains fundamental for the differential diagnosis with Sjogren's syndrome or other pathologies that can affect the minor salivary glands (16-22).

The symptoms reported by patients are limited to a swelling, painful, with bruising, difficulty swallowing (depending on the area of expression), perception of an unpleasant / bitter taste while the onset is often before meals, due to an increase in salivation. (23-30)

Target

The aim of this article is to analyze the different type of approach possible regarding to solve the symptomatology, focusing on the high risk of relapsing typical of this kind chronic gland inflammation. Regarding salivary stones, there are different approaches to the problem; the approach is dictated by the extent of the training; salivary stones under 5 mm can be removed by sialoendoscopy under local anesthesia, using CO2 laser, operative fibroscopy.

In the case of stones larger than 5 mm, the first step is to break up the stone into smaller pieces, lithotripsy can be obtained both with extracorporeal shock wave devices and with endoscopically guided intracorporeal instruments such as lasers (such as also happens for kidney stones). The use of these new minimally invasive techniques allows, in most cases (about 95%), to remove the stone without having to remove the salivary gland (31-35).

MATERIALS AND METHODS

Fifty-three patients with salivary stones, 5 to 35 mm in diameter, located in Wharton's duct were treated. The treatment consisted of enucleation of the stones with or without sialodocoplasty, the results obtained were encouraging as the 48 patients experienced complete restitutio ad integrum of the gland and duct with no short- and long-term complications. Complete restoration of glandular function was achieved in all treated patients (36-41).

CONCLUSIONS

The previously reported study confirms that surgical treatment is still the most reliable method for the removal of salivary stones, with a predictability and

morbidity that other techniques (CO2 laser, operative fibroscopy) are currently unable to guarantee (42-56).

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Ozone therapy new protocols and innovations: a systematic review

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The objective of this experimental protocol is to evaluate the possible adjuvant effect of ozone therapy as a supportive therapy for the surgical approach in clinical cases of peri-implantitis. The usage of ozone therapy has bacteria and virus killer been hugely demonstrated thanks to the inner potential of ozone as immune stimulated effect direct against inflammatory cytokine in tissue and organs.

The usage of ozone therapy has bacteria and virus killer been hugely demonstrated thanks to the inner potential of ozone as immune stimulated effect direct against inflammatory cytokine in tissue and organs. The action mechanism of ozone therapy is powered by the active stimulation of the intrinsic Immunity system of the patient. The ozone therapy induces a increase of the percentage of oxygen in tissue with a radical decrease of the presence of free radicals which are the results of cellular oxidative stress (1-7).

The usage of ozone therapy in dentistry is especially in periodontology has been hugely demonstrated, the biologic benefits of ozone and its antimicrobial activity is a milestone: the oxidation of biomolecule precursors and microbial toxins involved in periodontal diseases and its healing and tissue regeneration properties make the use of ozone well indicated in all phases of gingival and periodontal diseases (8-15).

Ozone therapy has also been used to support Covid19 antiviral therapies. Thanks to the intrinsic potential of the latter on immunity (self) and as an effective antiviral, this therapy has been found to be useful in containing damage from ischemic reperfusion, thus suggesting a new method of immune therapy (16-20).

Target

The objective of this experimental protocol is to evaluate the possible adjuvant effect of ozone therapy as a supportive therapy for the surgical approach in clinical cases of peri-implantitis (21-27).

MATERIALS AND METHODS

To implement the following experimental protocol, a sterile 20v syringe with a 30 1/2 " (0.3x13mm) needle filled with ozone, an allotropic form of oxygen, was used. The ozone generator is calibrated to 5 µ. The administration protocol is as follows:

1st week: 2 administrations, each 48 hours apart
2nd week: 2 administrations, each 72 hours apart
3rd week: 1 single administration after 5 days of the previous one.

At the end of each session of administering ozonated water to enhance the beneficial effect, patients are also recommended to follow home rinses with ozonated olive oil 3.4 times a day for the entire duration of the therapeutic cycle, for the office a special tool *OZONE DTA which is an equipment capable of producing pure ozone in a focused area with a concentration from 10 to over 100 µg / ml was used. The ozone created then quickly transforms into

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oxygen, dispersing in the air. In a few seconds *OZONE DTA eliminates bacteria from the treated site without any discomfort or pain for the patient; promotes post-operative wound healing and the reduction of edema extremely quickly, thus allowing the patient to quickly return to his daily activities (28-30).

Prospective randomized studies performed with similar methods which involve the use of ozone therapy seem promising both in the prevention of peri-implantitis and in the control of the latter. The 12-month follow-up showed that in patients with peri-implants treated with ozone therapy, the plaque and gingival index values were significantly better than in the group of patients not treated with ozone therapy (ozone group $P < 0.05$). Probing depth decreased from $6.27 \pm 1.42\text{mm}$ and $5.73 \pm 1.11\text{mm}$ at baseline to $2.75 \pm 0.7\text{mm}$ and $3.34 \pm 0.85\text{mm}$ at the end of the observation period 12 months in the ozone and control groups, respectively. Similarly, clinical attachment level values changed from $6.39 \pm 1.23\text{mm}$ and $5.89 \pm 1.23\text{mm}$ at baseline to $3.23 \pm 1.24\text{mm}$ and $3.91 \pm 1.36\text{mm}$ at 12-month follow-up in the ozone and control groups, respectively. According to radiographic evidence, defect filling between baseline and 12 months postoperatively was $2.32 \pm 1.28\text{mm}$ in the ozone group and $1.17 \pm 0.77\text{mm}$ in the control group, a difference statistically significant between groups ($P < 0.05$) this important study has involved a total of 41 patients (22 males, 19 females; mean age, 53.55 ± 8.98 years) with moderate or advanced peri-implantitis were randomly allocated to the test group (ozone group) with the use of sterile saline with additional ozone therapy or the control group with sterile saline alone for decontamination of the implant surfaces in SRT of peri-implantitis. Clinical and radiographic outcomes were evaluated over a period of 12 months. (31-40).

CONCLUSIONS

The results of ozone therapy as an adjuvant approach to surgery in cases of peri-implantitis have been satisfactory, the effectiveness of ozone as a decontaminant substance, which can be used in various situations in dental practice and beyond, is undeniable. (41-46)

Another important aspect not to be underestimated in the use of ozone therapy is the ease of administration compared to other decontamination methods, the

latter in addition to being minimally invasive, avoids the presence of "Side effects" compared to other substances such as Chlorexidine. whose use allows to control bacterial proliferation in the first applications but is able to damage the implant surface if used in the long term or to favor bacterial resistance to treatment. (47-50)

Other Studies has also tested gaseous ozone therapy in vitro on titanium and zirconia disk, The results were encouraging, in fact Titanium (polished) and zirconia (acid etched and polished) had a lower colonization potential and may be suitable material for implant abutments. Gaseous ozone showed selective efficacy to reduce adherent bacteria on titanium and zirconia without affecting adhesion and proliferation of osteoblastic cells. This in vitro study may provide a solid basis for clinical studies on gaseous ozone treatment of peri-implantitis and revealed an essential base for sufficient tissue regeneration (51-54).

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Neof ormation of keratinized tissue in implant surgery, a new innovative procedure to avoid palatal tissue removal and grafts: the Tent Pole Technique

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The technique of the neo-generation of the keratinized tissue is based on the principle of the preservation of the clot through the creation of an empty space between the implant and the keratinized tissue.

In the dogma of implant surgery, the maintenance of a sufficient amount of bone to maintain the long-term survival of the implanted fixtures has always been recognized as such, according to recent studies even a correct amount of keratinized tissue is important in order to guarantee surgical success long-term (1-6).

In addition to the removal of tissue at the palatal level: a technique that involves a graft of keratinized tissue in the area of interest (7-10), a new technique has recently been developed that allows a neo-regeneration of keratinized tissue, this technique does not require samples from other anatomical sites, this means that it is possible to avoid subjecting the patient to a double operation, one in the area of the operation and in the sampling area, thus ensuring less discomfort and an easier post-surgical procedure (11-14).

MATERIALS AND METHODS

The technique of the neogeneration of the keratinized tissue is based on the principle of the preservation of the clot through the creation of an empty space between the implant and the keratinized tissue, the principle underlying also the GBR and the preservation of the post-extraction alveolus, the present technique is a reinterpretation of the Tent Pole Technique or Ringue techniques performed in the

GBR for the regeneration of deficient bone tissue (15-20).

This new technique was put in place by Dr. Tomas Linkevicius (Zero Bone Loss) and requires that after the positioning of the implant fixture it is placed in place of the cap screw with a healing screw with a height of 2 mm and at the same time a release is performed tissue possibly without relief cuts. The space thus created is filled by the blood clot which will stimulate the body in the formation of new keratinized tissue (21-24).

DISCUSSION

The surgical technique involves the detachment of a full thickness flap, with the release of the latter (25-28). The release is necessary in order to avoid tissue tension that could lead to dehiscence of the wound or unwanted exposures and fenestrations of the healing screw as well as an excessive adherence of the flap to the underlying bone tissue, this eventuality would nullify the curtain effect, term from which it derives the name of the technique by preventing the formation of keratinized neo tissue (29-33).

In the upper jaw the detachment and mobilization of the flap will be mainly vestibular as the palatal tissue cannot be mobilized due to its nature, while in the lower maxilla the detachment will be vestibular

Keywords: keratinized tissue, implant surgery, gbr

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and lingual, paying extreme attention to the lingual detachment which must initially be performed at full thickness. and subsequently in partial thickness joints at the level of the mylohyoid muscle with the help of a curette (34-39).

Two suturing techniques with two different types of sutures will have to be used successively. The base of the flap must be sutured with deep mattress sutures in the lingual-buccal direction, while the coronal part of the flap will be sutured with simple detached sutures (40-44). Mattress sutures should be done following the 5mm rule. This means 5mm from the edge of the flap, 5mm from the second perforation and 5mm before the next suture. The recommended suture material is a 4-0 poly-glycolic acid absorbable. For the remaining part to be sutured, coronal portion of the flap. a simple 6-0 non-absorbable polypropylene suture can be used (45-49). After two months of healing, the second surgical phase is performed, in this phase the 2 mm healing screws are removed to be replaced by 4 or 5 mm.

In the tent-pole technique, the healing screw lifts the tissues and prevents their collapse. In addition, it makes the second surgical phase (i.e. the uncovering of the implant) very simple. Only a small lunate incision is made without touching the bone; this reduces the trauma to the tissues and promotes fast healing.

To summarize, the tent-pole technique can be used when the vertical thickness of the tissues is thin (but not <1 mm) and there is an insufficient amount of bone for leveling or positioning under the implant crest. (50-51)

RESULTS

This new technique seems to have excellent results on patients eligible for its use, the advantages are innumerable as already explained above, there is no need for a sampling in another anatomical site, synthetic grafts or animal derivatives are avoided, if well performed it turns out to be a predictable and reliable technique.

CONCLUSION

Given the results obtained in the patient script analyzed by the cases of Dr. Tomas Linkevicius, it will

be our interest to investigate the results of this new technique in order to refute its effectiveness and the results obtainable from a sustainability perspective where the minimum invasiveness is (when applicable) one of the gold standards of modern dentistry (52-54).

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Clear aligners technique: patient's expectations and perceptions

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The use of aligners to resolve a malocclusion is very widespread today. Aligners present a lot of positive aspects such as comfort, esthetic, hygiene and gingival health. The aesthetic advantage has made it possible to use the technique even in adult patients. It appears that a major part of the sample analyzed shows a high level of satisfaction which sometimes, in a very little part of patients, can decrease when the communications and the information required is not fulfilled. A controversial topic is the effectiveness of the therapy that, according to some authors, is not ideal in some kinds of malocclusions. The aim of the present study is to evaluate through questionnaire how clear aligners therapy influence patients' quality of life, their comfort and oral health. The study wants to analyze the level of bruxism, TMJ pain, headache and mucosal lesions. Finally, it explores patients' satisfaction.

Clear aligner technology represents a revolutionary change in orthodontics, based on digital tools used during the full workflow, from diagnosis to planning of treatment. In this orthodontic technique, the time-tested principles of biomechanics intersect with the digital world of the 21st century that uses algorithms to design the displacement of each individual tooth. In this way clear aligners allow to product a custom-made orthodontic appliance where it is possible to adjust the rate of tooth movement according to the individual bone's morphology and the amount of root surface area.

The digital flow is very useful in the collection of orthodontic documentation, in the diagnosis, in the planning and in the orthodontic therapy. Therefore, we

can affirm that the digital flow has brought advantages both to the operator and to the patient. The advantages for the operator are access to digital documentation that can be consulted at any time and a type of therapy that requires less time in the chair with fewer emergencies (such as detached bracket in traditional orthodontics). The benefits to the patient are the lower impact on the aesthetics. The other advantages are that this treatment is more hygienic, and comfortable in comparison to fixed appliances (1-8).

Digital technique should not be considered a simpler solution that anyone can use, because additional orthodontic skills are required. Beyond its advantages and limitations, the digital revolution has been a positive development, especially during the

Key word: clear aligner, orthodontic technique, digital flow, survey, bruxism, TMJ pain, headache and mucosal lesions

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recent pandemic covid-19, as it allowed orthodontic treatments to continue even when physical contact between patient and clinicians was not possible.

The capability to use an aesthetic and more comfortable technique has pushed many adult patients to undergo a treatment with aligners. The possibility to use less noticeable orthodontic solutions rather than the traditional vestibular metal braces persuaded many adult patients to begin a new orthodontic treatment because it meets the desire of adult patients for aesthetic and comfortable alternatives to conventional fixed appliances.

The use of removable appliances improves oral hygiene procedures reducing the risk of gingivitis. Proper oral hygiene maintenance during the orthodontic treatment is an important issue for both the clinician and patients. The choice of removable clear aligners should be guided by the patient's oral hygiene condition which in adults might be worse than in teens.

Research in a cross-sectional study including teen and adult patients, showed a significant improvement in the number of times and duration of daily teeth brushing, and in the using of the dental floss, so patients' oral health-related behavior seems to improve during and after orthodontic treatment (9-14). In a prospective randomized clinical trial among teen patients, authors found no evidence of differences in oral hygiene levels among patients wearing clear aligners, self-ligated brackets or conventional elastomeric ligated brackets (15-17). However, Albhaisi et al. (18-22) highlighted the risk of enamel demineralization in people wearing an orthodontic appliance, either fixed or removable, such as a clear aligner. On the other hand, in literature there aren't any information about relationship between clear aligners therapy and salivation, but the presence of oral appliances usually increases the quantity of saliva (23-28). The amount of saliva influences oral health, in particular oral hygiene: it is known the lack or low level of saliva favors the presence of plaque and tartar and increases the risk of cavities.

Oral health influences the physical, social and psychological well-being. It is undeniable that dental aesthetics affects how people are perceived by society and how they perceive themselves. According to one

study, (29-33) there is a strict connection between oral health and life quality in adults. This study highlights that poor dental esthetics can lead to feelings of shame and it negatively affects the person's image. So, dental esthetics influences the psychosocial aspects of people, and a good dental appearance can lead to a better social function (34-39).

Literature reveals that patients receiving clear aligners therapy experience less pain than those using fixed appliances. Forces produced by fixed appliances may vary among therapeutic strategies. On the contrary, clear aligner technique allow to design each movement in a more accurate way with appropriate forces and well determined. About mucosal health, literature affirms that fixed appliances increase prevalence of mucosal ulcerations, because of metal brackets, bands, wires, compared to thermoplastic removable appliances. Patients' compliance represents an important aspect to reach the aim of the therapy. In fact, clear aligners require a use of at least 22h a day. According to literature, compliance depends on the single patient, and it appears that male patients are more compliant than females (40-47).

The aim of the present study is to evaluate how clear aligners therapy influence patients' quality of life, their comfort and oral health. In particular the study wants to analyze the level of bruxism, TMJ pain, headache and mucosal lesions. Finally, it explores patients' satisfaction.

MATERIALS AND METHODS

An anonymized questionnaire available in two languages (Italian, English), was diffused between patients who experienced clear aligner treatment. 175 adult patients, over 18 years old, who underwent orthodontic treatment with clear aligner technique were included in the present study and their questionnaire has been analyzed. Patients were asked to complete the questionnaire between January and February 2021.

All participants provided the informed consent and accepted the privacy policy for the protection of personal data before compiling the survey. No personal information that identifies the individuals was collected and the data was analyzed only in aggregate form. All responses were collected on an anonymous basis using the Google Form

service. The resulting data file used for data analysis was free of any identifiers, including mail and IP addresses or other electronic identifiers. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Consensus

All the orthodontic patients aged 18 were included in the study and those surveys lacking an answer to all the compulsory questions were excluded.

The questions asked to patients are the following:

How much are you satisfied of your orthodontic treatment with aligners? (1-10)

What kind of aligner are you using?

Were attachments cemented on your teeth?

Have you ever suffered of dry mouth during the treatment?

Have you ever had an excess of saliva (sialorrhea) during the treatment?

Have you clenched or bruxed more during the orthodontic therapy?

Did you have oral lesions because of the orthodontic devices?

How much pain have you felt during the aligners therapy?

Have you suffered of TMJ pain during the therapy?

Have you had more frequent headaches during the therapy?

Have you ever lost an aligner?

Have you ever stopped the orthodontic therapy because of the loss of a tray?

If it happened, how much time did you need to have a substitute one?

How much comfortable were the trays?

Have you ever felt embarrassed because of the aligners?

Have the trays ever got damaged or ruined?

Do you smoke?

Did you reduce smoking during the therapy?

How many hours did you use the aligners per day?

Was it easier to use at home or outside the home and at work?

Were you more steady during the holidays and the weekend or during the week?

Did you use them more during winter or summer?

Have you ever had doubts about the result of the treatment?

Once finished the treatment did you keep the trays?

If not how did you get rid of them?

Did they tell you how to get rid of them?

Have you ever needed more information about the treatment, more than the ones given by the orthodontist?

Have you ever consulted the sites of companies that produce trays?

Do you know what is a retainer?

Do you know how much time you need to use it after the end of the orthodontic treatment?

Did you express a preference between removable or fixed retainer?

All data was collected exporting from google forms to Excel (Microsoft corporation One Microsoft Way, Redmond, WA, USA) and statistical analysis were performed via SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Mann Whitney test was used to confront nonparametric data and Fisher exact test to assess correlations for dichotomic data.

Results in the consensus

Present treatment	
Satisfaction	10 (30.9%)
	9 (22.3%)
	8 (29.7%)
	7 (11.4%)
	6 (3.4%)
	5 (1.1%)
	4 (0.6%)
	3 (0%)
	2 (0%)
	1 (0.6%)
	0 (0%)
Comfort	10 (10.3%)
	9 (8%)
	8 (35.4%)
	7 (17.1%)
	6 (12.6%)
	5 (9.7%)
	4 (3.4%)
	3 (1.7%)
	2 (0.6%)
	1 (1.1%)

Embarrassment	Yes (24.6%) No (75.4%)
Kind of aligners	Invisalign (95.4%) Another brand (4.6%)
Use of attachments	Attachments (76%) No attachments (24%)
Dry mouth	Yes (33%) No (67%)
Sialorrhea	Yes (45.7%) No (54.3%)
Bruxism/Clenching	Yes (50.3%) No (49.7%)
Mucosal lesions	Yes (45.7%) No (54.3%)
Pain	10 (0.6%) 9 (0.6%) 8 (4.6%) 7 (12.6%) 6 (13.1%) 5 (14.9%) 4 (13.7%) 3 (20%) 2 (9.1%) 1 (10.9%)
TMJ pain	Yes (24%) No (76%)
Headache	Yes (26.9%) No (73.1%)
Aligners missed	Yes (14.3%) No (85.7%)
Aligners damaged	Yes (27.4%) No (72.6%)
Damaging time	1 day (4.1%) 2/3 days (2%) 4/5 days (24.5%) 6/7 days (22.4%) More than a week (46.9%)
Washing frequency	More than once a day (62.3%) Once a day (26.3%) More than once a week (8%) Less than once a week (2.3%) Never (1.1%)
Smoke	Yes (22.9%) No (77.1%)

Reduced smoke during the treatment	Yes (17.4%) No (82.6%)
Hours of aligners' using	More than 22h (14.3%) 22h (28%) 21h (23.4%) 20h (13.7%) 19h (4.6%) 18h or less (16%)
Easier at home/at work	At home (82.9%) At work (17.1%)
More regular during holidays or weekend/ during the week	During holidays or weekend (14.3%) During the week (85.7%)
More regular during winter/summer	During winter (91.4%) During summer (8.6%)
Any doubts about the results	Yes (33.1%) No (66.9%)
Other information need (more than the ones given by orthodontist)	Yes (32.6%) No (67.4%)
Aligners' web site company consulted	Yes (36%) No (64%)

RESULTS

It resulted that 82.9% of the patients had a grade of satisfaction over 8 (in a scale between 1 and 10), while the 53.7% found a level of comfort with aligners higher than 8 (in a scale between 1 and 10); in addition, only the 24.6% felt embarrassed. The 33% of sample suffered of dry mouth and the 45.7% had sialorrhea.

Half of patients had bruxism or clenching during the treatment (50.3%), while the 45.7% suffered of mucosal lesions. Moreover, only the 5.8% had a significant level of pain (over 8, in a scale between 1 and 10), the 24% experienced TMJ pain and the 26.9% had headache.

The most prevalent company of aligners was Invisalign® (95.4%) and the 76% of patients had attachments. Only the 14.3% of patients missed at least one aligner during the treatment; the 27.4% had an aligner damaged and the most frequent time of damaging was over 6/7 days (69.3%). About aligners'

hygiene, a major part of patients washes aligners at least once a day (88.6%).

Concerning patients' compliance, the 79.4% of the sample wears aligners 20h/day or more. Mostly at home than outside (82.9% vs 17.1%), during the week than holidays/weekend (85.7% vs 14.3%) and during winter than summer (91.4% vs 8.6%).

The questionnaire also asked patients about smoking habits: only 22.9% referred to smoke while the 17.4% referred to have reduced this habit during the orthodontic treatment. In addition, it was asked if patients have ever had any doubts about the treatment's result and the 66.9% didn't refer any doubts, while the 36% consulted aligners' company web site to have more information. In fact, the 32.6% declared the need of more information about therapy.

We confronted usage time and found that patients who wear aligners for longer times have a significantly higher probability of complain excessive salivation ($p < 0.05$) and bruxism, but not headache, oral lesions or dry mouth.

Headache was associated to bruxism ($p < 0.05$) but not to any other complain while social embarrassment was significantly correlated only to mucosal lesions ($p < 0.01$) and counterintuitively not to sialorrhea. Moreover, patients embarrassed by the treatments tend to state lesser satisfaction levels (7.81 ± 1.8 VS 8.8 ± 1.1 $p < 0.01$) on a scale from 0 to 10 and tend to be more dubious about treatment outcome ($p < 0.01$). Lower level of satisfaction does not seem to significantly increase need for information by the clinical or manufacturer website.

Performing a logistic regression on the question about consulting aligners' web site, the most prominent reason was obtaining more information than those provided by the clinician ($p < 0.01$ exp 3.44) while pain, discomfort, missing aligners or other complains proved to not significantly increase site traffic.

DISCUSSION

In this study, a high level of satisfaction by the most part of patients was found. Orthodontic treatment changes patients' quality of life, especially in adults, because it improves eating functions and chewing, smile and aesthetics. Even if usually orthodontic

treatments aren't characterized by comfort, in this study it appeared a grade of comfort significantly medium-high. This is probably explainable with the use of clear aligners that are less invasive, removable and easy to wash. In addition, this study shows that the 75.4% of the sample didn't feel embarrassed. This result is in accordance with literature that evidences how patients treated with clear aligners show higher level of acceptance in terms of confidence in public and smiling (48-51).

Concerning mucosal health, the study confirms the possible increase of mucosal lesions that were reported by almost half of the patients. Literature affirms that fixed appliances increase prevalence of mucosal ulcerations, because of metal brackets, bands, wires, compared to thermoplastic removable appliances; this complain seems to not be increasing with a more extensive use of the device.

The 33% of sample suffered of dry mouth and the 45.7% had sialorrhea. This can lead to the idea that in the sample sialorrhea is more present than dry mouth, even if it appears that some people suffered of dry mouth and sialorrhea in different moments of the therapy. It isn't possible to affirm with certainty that this kind of therapy determines sialorrhea or dry mouth because probably it depends on the single patient and there is a statistical correlation between the increased time of usage of oral trays and sialorrhea (52-55). Moreover, it is undeniable that aligners respect gingival health more than fixed appliances. This aspect is linked to a better oral hygiene due to the absence of brackets. These favor the food packing between teeth more than oral trays.

In regard to bruxism or clenching, from literature it emerges that usually aligners don't improve or worsen these habits. In this study, half of patients had bruxism or clenching during the treatment (50.3%) but it isn't known the parafunction's level before the start of treatment.

Even though patients have demonstrated high level of satisfaction with the treatment outcome, it seems that embarrassment can undermine satisfaction and perception of therapy's effectiveness. Negative aspects still are present, but with a low prevalence. In fact, results indicate that the 5.8% has a level of pain over 8. During the orthodontic therapy there are

periods of higher and lower pain that depend on the forces applied and the stage of the cure. It seems that aligners determine less pain than fixed appliances thanks to steady pressure. In addition, it seems that pain is felt more by females than males. This aspect is confirmed by the literature, which highlights that gender influences pain perception and tolerance. According to Shaefer et al. (7) there are hormonal factors as well that favor a higher perception of orofacial pain in women than in men. This aspect should be taken in consideration by the clinician in choosing the device and even the pressure applied to the teeth. The 24% have suffered of TMJ pain, although it is not clear which was the level before the treatment.

CONCLUSION

The study shows that clear aligners are an innovative technique which responds to the increased request of adults to improve their smile and oral functions while maintaining a high level of satisfaction and comfort. In this way they present more advantages than fixed appliances such as aesthetics (only the 24.6% of the patients felt embarrassed during the therapy). In fact, clear aligners can be considered the less visible orthodontic cure. The only other therapy as invisible as trays is lingual technique which isn't as comfortable as aligners. In general, most patients considered the clear aligners technique a comfortable therapy (56-60).

Furthermore, other positive aspects are hygiene and gingival health. It is evident that oral trays permit a higher and more careful attention to the daily hygiene preserving dental and gingival health. They do not favor plaque's accumulation and food packing between the teeth.

In addition, it resulted that aligners do not determine a high level of dental pain and, certainly, less than fixed braces because they apply a steady and soft pressure: only the 5.8% had a significant level of pain (over 8, in a scale between 1 and 10). Moreover, aligners don't cause more mucosal lesions than fixed devices. Patients, who suffered oral lesions, show a significant level of embarrassment more than the rest of the sample (61-63).

Even if there isn't enough research about bruxism, in general and in relation to clear aligners, from this study and the few articles in literature, it emerges that oral trays do not improve parafunctional habits.

Giving complete information about maintenance and use of aligners, it is crucial to improve patients' satisfaction and therapy adherence. Nevertheless, it seems that unsatisfied or complaining patients do not seek further information, suggesting that the clinician should seek feedback about satisfaction, complains and possible unnoticed information need.

However, aligners have still controversial aspects such as the efficacy in some kinds of malocclusions and the precision of orthodontic movement in comparison with conventional orthodontic therapy. According to few article, braces can be more effective in controlling teeth torque and transverse expansion and in achieving great improvement than aligners.

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The different perceptions of the gummy smile among patients and clinicians

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INTRODUCTION: The aesthetics of the smile has historically gained importance on the part of the patient: today there are even more standards imposed by the society in which there is a strong association between the concept of beauty with physical and mental well-being. The harmony of the smile derives from the balance of the three fundamental components which are the teeth, lips and gums. If the amount of gum exposed to the smile is excessive, the so-called gummy smile is configured, which although not a pathological condition itself, certainly represents an important aesthetic alteration, both for specialists in the dental sector and for ordinary people.

MATERIALS AND METHODS: An online questionnaire of 12 questions was diffused. It had the aim, through the proposal of photos of various types of smiles, to analyze the negative influence of excessive gingival exposure on perception and evaluation of the smile both by specialists and by people outside the dental area.

RESULTS: The questionnaire, filled in by 314 people (dentists and not) with an average age of 37.9 years, found a strong subjectivity in the evaluation of the aesthetics of the smile. However, the smile that has met with greater preference is the one in which the amount of exposed gingiva is between 0 and 2 mm, confirming previous studies in the literature. Tolerance towards this aspect is greater among ordinary people, given that the statistical analysis found a significant difference in the opinions of specialists in the sector compared to people not in the field. There was also an overlapping predisposition to orthodontic treatment for aesthetic purposes on the part of male and female individuals.

Since ancient times, the human being has reserved importance to the aesthetics of the smile. The definition and application of the golden proportion to the smile are concrete evidence of this. The WHO defines health not as a simple absence of disease but as a state of complete physical and mental well-being. In this scenario, the care of one's image and the investment

in economic terms and time in this sense are growing more and more, as the number of patients who turn to dentists for purely aesthetic needs grows.

Aesthetic taste is highly subjective, individual and is affected by the ethnicity of the subject both in clinical and cultural aspects. The somatic characteristics of the smile vary in different places in the world and it is

Key words: gingival smile, smile aesthetics, smile perception, smile evaluation, survey

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reasonable to conclude that each person is attracted to what the culture of his people proposes as beautiful through social media and the means of communication (1-6). Furthermore, although the smile is a universal gesture that unites people even very distant from each other, the function and meaning is different based on the reference culture.

According to a study conducted at the University of Glasgow and published in the *Proceedings journal of the National Academy of Science*, it emerged that while Westerners would express the emotion felt more clearly, for Easterners the interpretation of the feeling that triggers the smile would be less obvious. Furthermore, a different mimicry of the facial muscles was highlighted, because Westerners activate all the muscles in smiling but above all, the ones of the mouth, the contraction of the eye muscles would be more relevant and significant for communicative purposes.

The harmony of the smile is affected by various factors: the shape and size of the teeth, the proportion of the teeth to each other, the arch of the smile, the volume and length of the lips, gum margins, buccal corridors and finally the amount of gum exposed to the smile (7-11). The latter variable is affected by the sex and age of the subject, in fact typically women show the teeth and the gum more than men, young subjects more than the elderly.

When the exposure of the gum in proportion to the smile is excessive, there is the definition of gum smile which, according to what Kokich stated in 1996, does not represent a pathological condition properly called, but an aesthetic alteration both for specialists in the sector and for ordinary people (12-16).

Crucial in determining the amount of exposed gum is the smile line, that is, the imaginary line that the upper lip draws with its lower profile when smiling on the maxillary gum, thus determining the amount of exposed gum (17-21).

Tjan, conducting a study of 454 people, defined a high, medium or low smile line relative to the amelo-cementitious junction of the tooth. An average smile line is one that allows you to uncover 75 to 100% of the tooth crown and 1mm of interproximal gingiva, resulting in an ideal smile (22-26).

There is no universally shared and adopted classification of the gingival smile: with a good

approximation it can be said that when a quantity of gingiva on the smile greater than 2 mm is shown apical to the amelo-cementitious junction, a gummy smile is diagnosed (27-33).

There are several factors that can determine the genesis of this imperfection, thus recognizing a multifactorial etiology, in which the causes are often co-present, resulting in varied clinical pictures, in which the contribution of multiple specialists is essential for the purposes of therapy.

In the dentogingival type, the patient typically has short clinical crowns due to the presence of excessively represented gingival tissue. This condition, known as gingival hyperplasia, may be due to the patient's poor oral hygiene and therefore to the stagnation of bacterial plaque or the long-term intake of certain categories of drugs (immunosuppressants, calcium channel blockers, anticonvulsants and antidepressants). There is also excess gingival tissue during a phenomenon known as altered passive eruption: in this case the gingival tissue during the eruption process of the tooth to reach the occlusal plane, does not migrate as it should in the apical direction but remains in a more coronal position (34-40).

In the dentoalveolar type of gingival smile there is a hyper eruption in the vertical and often anterior direction of the dentoalveolar complex, therefore also the gingival tissues follow the tooth in its eruptive process downwards showing an excessive amount of gingiva on the smile. Often the patient's upper incisors are also tilted back and a deep bite pattern occurs. It may also occur that there is a compensatory eruption of the upper incisors following mechanical wear of the antagonists: in an attempt to reach occlusal contact, the upper incisors extrude (41-43).

In the muscle type, the muscles of the upper lip are hyperactive, therefore during the smile, the excursion of the upper lip upwards, normally by 6-8 mm, is increased.

In the short upper lip type, the cause of gingival exposure to smiling and also often at rest, is excessive because the upper lip has a reduced length. The upper lip is defined as short when its length to the smile is less than 18 mm, measured from the stomion to the subnasal point. Then, when a person smiles, the length of the upper lip is reduced by another 3 mm.

However, in a study conducted by Roe the amount of gingival exposure to smile did not differ statistically significantly between individuals with short or normal upper lips, thus concluding that the cause is not the length of the upper lip itself, but the presence of other contingent factors present in the same patient to determine the gingival smile picture.

Finally, in the skeletal type, the cause is found in an excess of vertical or sagittal growth of the upper jaw. In vertical excess, the maxilla grows more than it should downwards, resulting in a clockwise rotation of the mandible and an increased lower height of the face. Open bite may or may not be present depending on whether the upper incisors are extruded or not. If they are, the gum smile will be evident in all its severity. In the case of excessive sagittal growth, there is a protrusion of the entire middle area of the face, with an increase in the value of the SNA and ANB angles and a class 2 clinical picture.

If this type of problem occurs in the age of growth, the orthodontist can play an important role by being able

to count on equipment capable of redirecting growth in a harmonious way, if instead the skeletal excess occurs in adulthood, the gold standard in terms of therapy is represented by orthognathic surgery (44-48).

Taking into account the varied etiology, and the changes that gingival exposure to the smile undergoes over the years, it is imperative to make an appropriate and complete diagnosis in order to satisfy the aesthetic request of the patient in a long-term treatment.

MATERIALS AND METHODS

The work involved the formulation of an online questionnaire of 12 questions (*Fig.1*). The questionnaire was shared on the most commonly used social platforms or sent electronically in private form. It was divided into three parts: a first socio-demographic section with questions on age, sex, work done by the interviewee (to distinguish between professionals in the dental field and not) and dental treatments received. The second section with questions aimed to probe the importance attributed today to the smile

ANONYMOUS SMILE SATISFACTION QUESTIONNAIRE

We ask you to kindly answer the following questions; the purpose of this questionnaire is to be able to do a survey on our smiles. Thanks for collaboration!

1. SEX ☐ F ☐ M
2. AGE
3. YOU JOB
4. WHAT DID YOU GO TOT HE DENTIST FOR?
 - a. Oral hygiene
 - b. Caries
 - c. Endodontics
 - d. Implants
 - e. Prosthesis
 - f. Orthodontics
5. HOW IMPORTANT IS THE SMILE FOR YOU?

nothing

a little

quite

so much

6. WOULD YOU WEAR BRACES TO IMPROVE YOUR SMILE? YES ☐ NO ☐
7. WOULD YOU WEAR BRACES ONLY FOR AESTHETICS? YES ☐ NO ☐
8. WHICH AMONG THESE 3 SMILES DO YOU LIKE MOST?





A ☐
B ☐
C ☐

9. DO YOU THINK THIS GIRL IS MORE BEAUTIFUL IN PHOTO A OR IN PHOTO B?



10. WOULD YOU WEAR BRACES TO REACH THE SMILE IN PHOTO B STARTING FROM THE ONE SHOWN IN PHOTO A? YES ☐ NO ☐



11. CHOOSE THE BEST SMILE IN YOUR OPINION



12. DO YOU THINK TO HAVE A MALOCCLUSION? IF YES, WHICH ONE?

A) crowding B) gummy smile C) third class malocclusion D) open bite



Fig. 1. Questionnaire

and the predisposition by the interviewees to undergo an orthodontic treatment to improve it. Finally, a specific part relating to the gum smile, in which through photos of different smiles, it was asked which was the most attractive. The smiles shown in the photos differed from each other in the amount of gum shown and therefore in the position of the smile line or it was shown the same patient diagnosed with a gum smile before and after treatment.

All participants, informed of the purposes that the questionnaire was intended, voluntarily consented to the compilation and publication of the results anonymously for the protection of personal data regarding the privacy policy. The study type did not foresee any risk for the participants in the study.

Statistical analysis

The data obtained from the questionnaire were subjected to descriptive statistical analysis in order to analyze the sample object of the study and the answers given to some of the questions proposed. A statistical analysis of an analytical type using Fisher's test was instead carried out to test the significance of the answers given regarding some selected questions to assess the level of satisfaction of the gingival smile.

RESULTS

The questionnaire was completed by 314 people between the ages of 14 and 80 with an average age of 37.9 years (Q1) (Fig. 1). 32.4% of the test participants were male, and the remaining 67.6% were female (Q2). Collecting and cataloguing the answers with respect to the work carried out, it emerged that 36 people, who took the test, were employed in the dental area (dentists, orthodontic specialists, dental technicians, dental assistants), and the other 264 were non-specialists in the field (Q3). Of the interviewees, 72.5% said they had gone to the dentist for oral hygiene procedures, 48.9% for caries, 28.5% for root canals, 14.4% for implants, 3.9% for prostheses and 32.5% for orthodontics (Q4).

82.5% of the participants declared the smile to be very important (Q5) and 90% would in fact be willing to undergo orthodontic therapy to improve it (Q6). 77.3% also stated that they would like to potentially undertake orthodontic treatment for cosmetic reasons only (Q7).

Question 8 of the questionnaire offered three photos of three different smiles based on the height

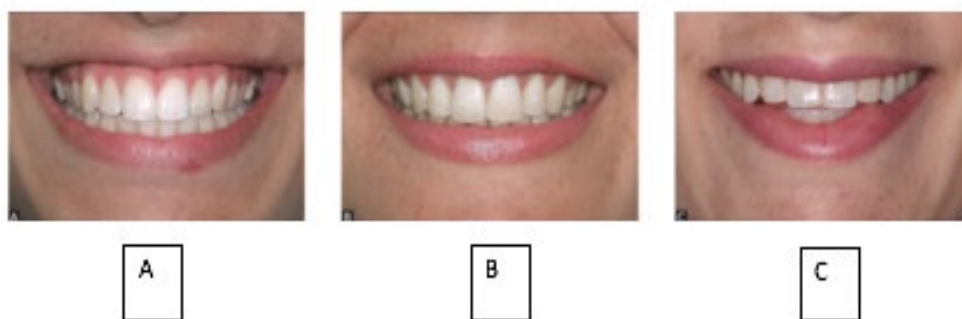


Fig. 2. Question 8 of the questionnaire: smile preference (enlarged photo)

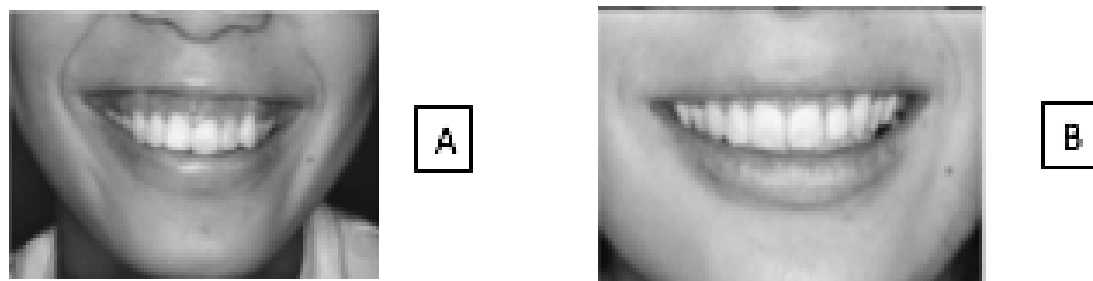


Fig. 3. Question 10 of the questionnaire (enlarged photo)

of the smile line (high, medium, low) asking the interviewee for the smile that is most pleasant for him (Fig. 2). Most of the votes were obtained for option b (smile line such as to show 75/100% of the crown of the teeth and 1 mm of interproximal gingiva), but the analytical statistical analysis conducted by Fisher's test found a statistically significant difference between the percentage of specialists in the sector and that

of ordinary people in the choice of this option. The p-value of 0.002 (<0.05) shows that proportionally, more specialists in the sector have chosen option b as the most pleasant smile.

Statistical analysis also tested the variability with respect to sex regarding the willingness to undergo orthodontic treatment to obtain the result of photo B starting from photo A (question 10 of the questionnaire) representing respectively a patient with a gum smile after and before the orthodontic therapy (Fig.3). The result was statistically insignificant with a p-value of 0.11 (> 0.05).

Finally, the Fisher test was performed for the results of question 11 (Table I). The question asked the interviewee to choose the most beautiful smile from 4 photos of smiles in which the amount of exposed gum differed (2,4,3, 11 mm compared to the amelo-cementitious junction of the tooth) (Fig. 4). The smiles that received the most preference were those shown in photos D and A.

Option A (smile such as to expose 2 mm of gingiva apically to the crown of the dental elements) received preferably overlapping percentages from specialists in the sector and not with a p-value equal to 0.71 (> 0.05).

On the other hand, option D (smile such as to show 1 mm of interproximal gum) has been proportionately more successful among specialists in the sector than among ordinary people with a p-value equal to 0.02 (<0.05).

DISCUSSION

In contemporary society, aesthetics plays a fundamental role: the media exert an imposing

Table I. Answers to questions 8, 10 and 11

Question 8		
	Dentists	Laypersons
Answer B	26	120
Answer not B	10	148
Question 10		
	Women	Man
Yes	200	83
No	19	15
Question 11		
	Dentists	Laypersons
Answer D	12	123
Answer not D	24	145
Answer A	11	92
Answer not A	25	176

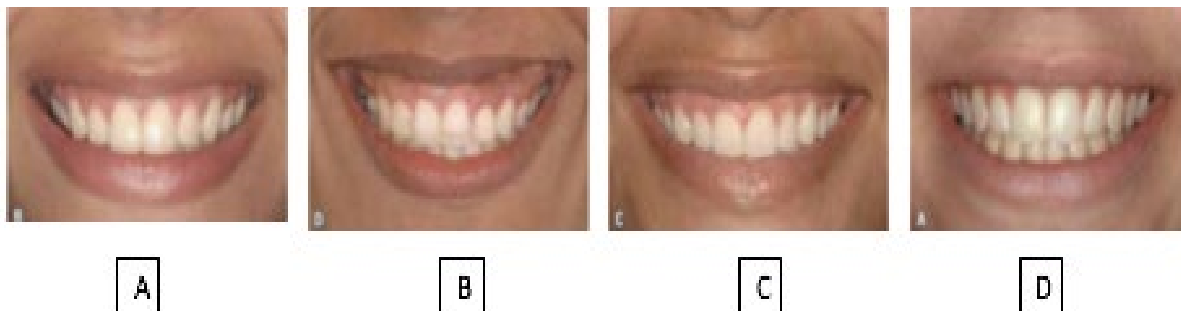


Fig.4. Question 11 of the questionnaire (enlarged photo)

influence on the public in this sense. The amount of gum tissue exposed to the smile is one of the elements that contributes to its harmony. If this quantity is excessive, the smile is negatively influenced and in this the results of our questionnaire are in line with what is reported in the literature.

Smiles of only female faces have been deliberately inserted in order to eliminate a potential bias effect that could orient the preference of the interviewees, beyond the fact that the gummy smile is a substantially female characteristic with a female-male incidence ratio of 2 to 1.

The most successful type of smile from both specialists in the sector and non-professionals is the one where the amount of exposed gum is about 1 mm; however, up to 2 mm of gingival exposure there is still tolerance and indeed some people consider

this smile even more attractive, probably because it is synonymous with femininity (women typically discover more than men) and youth. (9,

The degree of tolerance is greater among non-specialists in the field according to the studies of Kokich and Alhaija: in fact, a high percentage of specialists, greater than ordinary people, have chosen the smile 1 mm of interproximal gingiva as the most attractive (49-53). In this, the result is probably spoiled by the dentist's cultural background, who in the choice is not guided by the mere feeling of preference but by what can be defined as "ideal".

The studies found in the literature that share the topic of the research and the investigation method with this work are 2, as shown in the proposed table.

The study by Geron and Atalia was conducted in 2005 in Israel and included 100 total respondents,

Table II. *Comparison of studies*

AUTHORS OF THE ARTICLES	YEAR OF THE ARTICLE	METHOD	COUNTRY	SEX OF THE EVALUATORS	SEX OF THE PROBAND	NUMBER OF THE EVALUATORS	PROPOSED PHOYO	TYPE OF EVALUATORS	TOPIC OF THE INTERVIEW	RESULT ABOUT MOST ATTRACTIVE SMILE (GINGIVAL EXPOSURE)	RESULTS ABOUT GENDER
GERON AND ATALIA	2005	SURVEY	ISRAEL	M,F	M,F	100	ONLY SMILE	LAY PERSON	EXPOSURE OF LOWER AND UPPER INCISORS AND INCISOR PLANE INCLINATION	0-2 MM	WOMEN ARE MORE TOLERANT ABOUT GUMMY SMILE; HIGHER ORAL AND DENTAL ATTRACTIVENESS IS EXPECTED FROM WOMEN TO MEN
PAUCH AND KATSOUKIS	2017	SURVEY	GERMANY	M,F	M,F	42	FULL FACE	STUDENT OF DENTAL MEDICINE	EXPOSURE OF UPPER INCISORS AND GINGIVAL TISSUES	0-2 MM	OWN GENDER BEING RATED AS LESS ATTRACTIVE AND OPPOSITE GENDER AS MORE ATTRACTIVE. ORAL AND DENTAL IS EXPECTED FROM BOTH WOMEN AND MEN
OUR INTERVIEW	2021	SURVEY	ITALY	M,F	F	314	ONLY SMILE	DENTIST AND LAYPERSON RANDOMLY RECRUITED	UPPER GINGIVAL EXPOSURE AND WILLINGNESS TO UNDERGO ORTHODONTIC TREATMENT FOR GUMMY SMILE	0-2 MM	DENTAL ATTRACTIVENESS IS EXPECTED FROM BOTH WOMEN AND MEN WITH NO STATISTICALLY SIGNIFICANT DIFFERENCE IN WILLINGNESS TO UNDERGO ORTHODONTIC TREATMENT TO SOLVE/IMPROVE GUMMY SMILE

outside the dental field. The photos proposed concerned male and female smiles and the results showed a greater preference for a gingival exposure between 0 and 2 mm. The results also showed greater tolerance of women towards gum smile and greater esthetic expectation towards female sex by both male and female evaluators.

The study by Pauch and Katsoulis conducted in Germany in 2017, involved 42 subjects from the faculty of dentistry as interviewed people and the photos proposed were full faces of both male and female subjects. Also in this study, the smiles that met greater preference were those with an amount of exposed gingiva between 0 and 2 mm and an aesthetic expectation comparable between men and women (54-58).

Our study, in addition to being conducted on a sample with a higher number (314), included both non-dental professionals and specialists among the interviewees, thus being in our opinion more representative of the population. In fact, the students of the same faculty could express standardized opinions that are against a certain smile, due to the common didactic training received. Like the study of Geron and Atalia, the photos proposed represented only smiles and not whole faces, as the proposal of a face distracts the observer from what is included in the lip frame as there are more confounding factors. The smiles proposed were purposely of female subjects only as the gummy smile is a problem typically reserved for women, affecting twice as many women as men. Our results also predicted the smile with gingival exposure between 0 and 2 mm as the most pleasant smile: in this therefore all 3 studies are perfectly superimposable.

Unlike the other 2 studies compared, ours probed the willingness and propensity to undergo orthodontic treatment to improve one's gum smile was equally distributed between men and women. This explains a need and an equal aesthetic expectation between men and women, which appears in line with what emerged from the 2017 study by Pauch and Katsoulis, allowing us to reasonably conclude that the aesthetic standards are different today from those of years ago and therefore the conclusions of the 2005 study are now not well representative of contemporary society(59-60).

The studies are summarized in Table II.

In light of the results obtained, it is reasonably to conclude that the questionnaire revealed a particular attention of the interviewees to smiling (for 90% of the interviewees, the smile is very important). The aesthetic satisfaction of the patient is the factor that guides and motivates the use of specialized dental treatments, placing potential functional needs in the background (77.3% would undergo orthodontic treatment even for aesthetic needs only) (61-62). This is true for both female and male individuals, proving the fact that today males reserve the same importance historically reserved by females to aesthetics and the care of their image. However, the variability in the answers given regarding the preference of one smile rather than another highlights how beauty is actually a purely subjective concept.

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Is there any correlation between malocclusion, temporomandibular disorders, and systemic disease? The importance of differential diagnosis

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Temporomandibular Disorder (TMD) is the leading cause of pain in the orofacial region, including the head, face and related structures. The etiology and pathophysiology of TMD are poorly understood. It is generally accepted that the etiology is multifactorial. The study analyses the correlation between temporomandibular joint dysfunction and systemic diseases. One hundred and five adult patients were asked to complete a survey in which factors as the age and the gender were considered. The questions were about the typical symptoms of temporomandibular disorders, fibromyalgia and arthritis. The answers to our questionnaire revealed that the only statistically significant correlation was malocclusion, fibromyalgia and cervical pain, not arthritis.

TMDs (temporomandibular disorders) are multifactorial disease. They are determined by organic aspects (like occlusal features, systemic disease, osteoarthritis, muscular dysfunction, ligaments and tendons problems), genetic aspects that can bring to an abnormal development of the jaws and all the structures linked to TMJ or oral parafunctional habits and stress (1-3). The diagnostic criteria (DC), introduced by Dworkin, is considered the standard system to diagnose this disease in a specific way. It considered as well psychological aspects like stressful life, problems in the social relationships and dysfunctional aspects of the patient's character (4-7). Specific symptoms of temporomandibular disorders

are pain, clicks and limitation of oral aperture. In the last years, the possibility that orthodontic treatment could improve TMJ has been investigated (8-11).

Temporomandibular disorders are a widespread disease and the main reason for which people asked for therapy was the pain experienced. In general, TMDs have an annual incidence of more than 5% of the population (12-17) and, according to Lipton et al., about 6% to 12% of the population is affected by symptoms of TMD (18-21). A peak of incidence has been observed between individuals aged 20 and 40 years old (22-26). Sometimes TMDs and its symptoms are reported in some systemic disease, like fibromyalgia (FM), arthritis and cervical pain.

Keywords: TMJ symptoms, survey, systemic diseases, fibromyalgia, arthritis, neck pain

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Fibromyalgia is a systemic disorder characterized by widespread pain probably caused by an altered function of Central Nervous System (CNS). It is very common, even if the last years, as few articles affirm, there is an attitude to over diagnose it (27-30). In terms of prevalence, it is the third most common musculoskeletal disease, and the prevalence increases proportionally with age, peaking at 50–60 years old. Fibromyalgia (FM) is a very compound disease. It is characterized by a number of clinical symptoms: it affects mainly the Musculo-skeletal system but, in some cases, also the digestive system, the cognitive function, the skin and other aspects of patient's health. FM patients suffer, often, of chronic fatigue.

Fibromyalgia can have different sign and symptoms in the people affected. For that reason, it is not easy to detect it and diagnose it. FM etiopathogenesis, according to many scientists, is connected to physical or psychological trauma. In this way, for the clinician it is very significant the anamnesis and the physical record to recognize a possible event that can be the cause. Occasionally, when TMDs are suspected, anamnesis and physical examination can be very essential, because if the patients report a very high level of pain of TMJ and pathological aspects, are not enough to explain the sufferance, it is reasonable to hypothesize a systemic problem like FM. Moreover, the patients with FM during the anamnesis, report many symptoms and signs that are not typical of TMDs. For that reason, even if for orthodontist/dentist the FM is not a common disease and pathologic situation that he has to treat, it is very important to have an idea about it (31-36).

The patients with FM usually have a hypersensitivity to the occlusal defects, as the patients with idiopathic oro-facial pain. In this kind of patients, it is very important to reach the diagnosis as soon as possible, because every oral treatment would not be so effective. In this direction, it is very important the capability of the clinician to communicate in a clear way with the patient. In recent literature, it is said that fibromyalgia can be linked to a gut dysbiosis and some patients affirmed to have a significant improvement of the syndrome

having a gluten free diet. The only recognized therapies for this pathological condition are pharmacological and alternative treatments. The first ones consist of antidepressants like amitriptyline and newer ones as duloxetine and citalopram and muscle relaxants, even if in some cases there is the use of label of antiepileptics; the alternative therapies can be acupuncture or massages (37-40).

Another condition that can be connected to TMDs is arthritis. It is considered all over the world and, in particular in the U.S.A., one of the leading causes of disability. Arthritis is a very widespread disease: between 1999 and 2014, nearly 25% of American adults reported arthritis, osteoarthritis (OA), rheumatoid arthritis (RA) or other types of arthritis and its prevalence is expected to increase. Arthritis affects mainly females (75% of people affected) probably due to hormonal changes. Arthritis is a chronic inflammation: osteoarthritis, the most widespread, typically knees, back and hips; rheumatoid arthritis is an autoimmune disease that causes joint pain, stiffness, swelling and decreased joint movement leading to disability (41-46).

As arthritis mainly affects the joints, also TMJ may be involved. Temporomandibular joint is one of the joints most frequently compromised in juvenile idiopathic arthritis (JIA) which is a form that is quite widespread in the young people and children. It can be classified in 7 subtypes: systemic arthritis, oligoarthritis, rheuma-factor positive polyarthritis, rheuma-factor negative polyarthritis, psoriatic arthritis, enthesitis-related arthritis, undifferentiated arthritis. The orofacial disorders caused by TMJ arthritis range from a asymptomatic presentation to a dentofacial pain, dysfunction and deformity, such as facial malformation and malocclusion asymmetry, with a negative impact on quality life. The dentofacial deformity is a multifactorial condition caused by growth retardation, degeneration, mechanical overloading, dysfunction and dentofacial compensation. So there is a close connection between arthritis and orofacial disorders, frequently followed by malocclusion (47-50).

In recent years, it has been suggested a relationship between occlusion and TMDs and cervical muscles and posture as well. In particular,

occlusal contacts has been found to affect the correct function of the neck and cervical muscles. Further, Karppinen et al. has proposed occlusal adjustment to relieve chronic head and shoulder pain [However, an overview about the associations between occlusion and posture showed that even if that association have been found in some studies, there is not enough scientific evidence to support a cause-effect relations due to bias such as inappropriate study design and unsuitable diagnostic tests.

The aim of this study is to analyze the connection between Temporomandibular disorders and fibromyalgia, arthritis and neck pain and to evaluate the association with malocclusion.

MATERIALS AND METHODS

We enrolled from 105 responders (99 females and 6 males) from 25 June 2021 to 19 July 2021 through an online survey containing items about demographic data, Temporomandibular joint pain and arthritis. The online survey was disseminated via social network, and they were encouraged to pass it on to others.

All participants provided an informed consent and accepted the privacy policy for the protection of personal data before compiling the survey. The questionnaire was administered by an online form service (Google Form service, Google LLC, 1600 Amphitheater Parkway, Mountain View, California, U.S.).

Descriptive statistics were calculated for sociodemographic characteristics and additional health information variables. Age and symptom onset were tested for normality using Kolmogorov Smirnov normality test, normally distributed so it was applied T Student test for paired samples to confront onset ages, significance was set at $p < 0.05$. For dichotomic variables was applied Chi2 test. All the statistical procedures were performed with the Statistical Package for the Social Sciences (SPSS 25.0, SPSS Inc., Chicago, USA)

RESULTS

Of 105 responders 99(94.3%) were females and 6(5.7%) were males. About education, only 1 (1%) participants completed only primary school,

8 (7.6%) secondary, 26 (24.8%) high school and 70(66.7%) achieved an university degree.

Gender	
Male	6 (5.7%)
Female	99 (94.3%)
Education	
Primary	1 (1%)
Secondary	8 (7.6%)
High School	26 (24.8%)
University	70 (66.7%)
	Mean±SD
Age	40.8±11.1

Aggregating education data between who completed university and who obtained an high school diploma or lower, there were no significant differences in malocclusion, fibromyalgia or TMJ pain or previous orthodontic treatment reporting.

Question	Yes	No
Do you have dental malocclusion?	75 (71%)	30 (29%)
Did you have an orthodontic treatment?	62 (59%)	43 (41%)
Do you have neck pain/cervical pain?	83 (79%)	22 (21%)
Do you have fibromyalgia?	30 (29%)	74 (71%)
Do you have any kind of arthritis?	88 (83%)	17 (17%)
Do you have TMJ pain?	93 (88%)	12 (12%)
	After	Before
Did arthritis appear before or after TMJ pain?	45 (52%)	42 (48%)
	Mean±SD	
At which age did they diagnose your arthritis?	34.2±10.3	
At which age did the TMJ pain start?	29.17±11.6	

Regarding malocclusion, the majority of patients who reported malocclusion underwent orthodontic treatment ($p < 0.05$). The malocclusion was related to neck pain ($p < 0.05$), Fibromyalgia ($p < 0.05$), but not to

arthritis. Orthodontic treatment did not show benefits for 87 (83%) of whom reported cervical pain, 84 (80%) of arthritis pain and 85 (81%) of those reporting back pain. There is a 8.6% of patients who reported worsening of back pain after treatment. Report of fibromyalgia confronted to arthritis was significant.

For arthritis patients TMJ onset was equally distributed as 45(51.7%) stated that it started before arthritis diagnosis and 42(48.5%) reported that it started after diagnosis. Medical therapy for arthritis was reported not improving TMJ symptoms in the majority of cases (55pt; 60.4%). Confronting Arthritis and TMJ pain onset age it is significantly lower for TMJ pain (28.7 ± 1.2 Vs 34.21 ± 1.1 , $p < 0.01$) with a mean difference of 5.5 ± 10.0 years.

DISCUSSION

In the present study, that considered 105 patients, malocclusion was a very diffuse disease: 75 patients (71%) reported a dental malocclusion and only 30 patients (29%) were not affected. Although in the sample examined the female population was greater than the male one (99 women and 6 men) and it's not possible to find a statistically significant difference, it is reasonable to say that the pathologies evaluated are very common in the female gender, probably due to hormonal factors. Many studies in the literature confirm the different distribution of these pathologies among the females (51-54).

Malocclusion resulted related to Fibromyalgia and to cervical pain but not to arthritis. However, due to the high spread of malocclusion (71% of our population), it can't be said with certainty that there is a close association between them. On the other hand, it is evident that malocclusion is more related to Fibromyalgia and neck pain than to arthritis. That can be explained by the fact that these two conditions are linked to the muscle-skeletal system which is known to be influenced by maxillo-mandibular relationship. When TMDs are present along with other diseases, they must be considered and treated as independent diseases. In fact, orthodontic treatment did not show benefits for 87 (83%) of whom reported cervical pain, 84 (80%) of arthritis pain and 85 (81%) of back pain. So, in the present study, orthodontic treatment did

not reveal to be the preferred treatment for systemic disease like this. Orthodontic treatment is useful to treat dental malocclusions, but it is not enough for fibromyalgia, arthritis and cervical pain. In addition, our study found that the 8.6% of patients with back pain suffered a worsening of their condition after orthodontic treatment, probably because the different occlusion changes the cervical posture and muscle function of neck and back (55-58).

As the temporomandibular joint is often a target of fibromyalgia and arthritis, dentists should be able to recognize characteristic symptoms and make a differential diagnosis. If the patient reports a severe pain of the orofacial structures and temporo-mandibular complex that is not explainable according to the analysis of the TMJ, the dentist should investigate the presence of a systemic disease like Fibromyalgia. Instead TMJ arthritis can cause a range of symptoms, from a dentofacial pain to a facial asymmetry and malocclusion. The differential diagnosis, as early as possible, allows you to undertake the best therapy for the patient.

Fibromyalgia and arthritis are systemic disease, more complex than temporomandibular disorders, and they need specific systemic treatment. In addition, the correct diagnosis reduces the risk of over diagnosis; in fact, as few articles affirm, there is an attitude to over diagnose it. Fibromyalgia, being a musculoskeletal disease, it can present symptoms similar to those of the muscular suffering typical of temporomandibular disorders but the etiopathogenesis and therapy are different (59-61). Furthermore, evaluating the timing of onset of the different disease, our study found that in the 51.7% of patients TMDs started before arthritis diagnosis and in the 48.5% after arthritis diagnosis. So, it is not possible to conclude that TMDs are risk factor or consequence of arthritis and the timing of onset is not dependent. Trying to treat TMJ symptoms through medical therapy for arthritis, there was no improvement in the 60.4% of patients. So, it is correct to treat arthritis and TMDs independently.

CONCLUSIONS

This study underlines the correlation between

malocclusion and systemic disease. Fibromyalgia and cervical pain, according to the results, are linked to malocclusion. So, in case the clinicians consider correcting the occlusions problems, it should be done in a very careful way, because, even if the literature reports consequent benefits, in some patients of this sample there was a worsening of the symptoms after the orthodontic treatment. It is very important to be capable to do a differential diagnosis because that permits to have successful treatments and avoid useless therapies.

It is required to analyze and understand in a deeper way the connection between malocclusion, fibromyalgia, and cervical pain. It is needed to explore which is the causal factor between these conditions to understand which factor should be treated firstly. In this way, it can be important to have a larger sample of patients.

In this study, it doesn't appear a consequent relationship between arthritis and malocclusion, but it is evident from the literature that TMJ arthritis can create many problems. It can determine TMJ pain and abnormal development of the jaws. That usually requires an earlier diagnosis and therapy.

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The role of myofunctional therapy in the treatment of malocclusions: A clinical case

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Background: myofunctional therapy consists of exercises, which aim to eliminate atypical motor patterns, to establish new neuromuscular patterns and to optimize orofacial functions. In some clinical situations, even therapeutic devices such as orthodontic appliances are used to help to reach this purpose. These exercises improve muscle tone of lips, tongue, facial and chewing muscles and soft palate. They increase the range of motion, promote automation of habitual and unconscious physiological swallowing.

Aim: the purpose of this study was to verify the advantages of myofunctional therapy through the description of a clinical case treated only with this kind of therapy.

Conclusion: in the case described, myofunctional therapy alone resolved an abnormal function (atypical swallowing) and improved malocclusion. Moreover, it helped to ensure the stability of the treatment.

The mechanics of swallowing change in different periods of life, going through different phases. The first transition is from fetal swallowing (already present during intrauterine life) to infantile or "visceral" swallowing, typical of the newborn and infant in the first months of life. In the infantile swallowing, tongue lies on the floor of the mouth and squeezes the breast against the hard palate, and lips are sealed around the breast to prevent milk leaking during sucking.

Teeth erupt almost when the head acquires an upright position, during weaning. In this phase, there should be a transition to an adult type swallowing characterized by teeth in contact, tongue pressed on the palate to allow the bolus to be swallowed, and no lip contraction. This transition does not occur in some individuals and an infantile swallowing is maintained

even in adulthood, causing a pathological condition called atypical swallowing (1-5).

There are many reasons why this step does not occur and they may overlap: prolonged use of the pacifier, artificial rather than maternal breastfeeding, oral breathing, finger sucking, chronic tonsillitis, macroglossia, premature loss of deciduous anterior teeth, sleep disordered breathing, persistent acquired behavioral pattern, or nerve dysfunction (6-11).

A person with atypical swallowing will have dental alterations, as well as muscular and skeletal problems (12-16). Dentally, an anterior or lateral open bite can be observed, due to an anterior lingual thrust or sucking of the tongue. The most common alterations are diastemas between incisors, protrusion of the upper incisors and maxillary contraction.

Keywords: atypical swallowing, myofunctional therapy, open bite

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At a muscular level, an abnormal contraction of the perioral muscles (especially the chin) may be present trying to keep the lips closed in rest position and during swallowing to prevent the bolus leaking. Skeletally, atypical swallowing can be associated with several postural dysfunctions such as straightening of the cervical spine due to a forward head position in relation to the neck, shoulder blades rotated forward and shoulders rotated inward, a “belly out” posture with increased lordosis and often low back pain and flat feet (17-22).

Atypical swallowing can also influence correct diet: the neuromuscular pattern of swallowing is coordinated with the patterns of nasal breathing and of chewing. When chewing and nasal breathing are not properly coordinated, the bolus is not adequately triturated and requires the contraction of additional muscles to manage it, creating an atypical pattern of swallowing. Often, patients with this dis-coordination have digestive problems, due to the compromised salivation, shorter chewing because of competition with breathing. Furthermore, patients tend to chew with their mouths open swallowing air. It is not uncommon that they prefer soft food that they can easily swallow.

In this article the authors describe a clinical case of a young patient who had an improvement in orofacial functions and malocclusion with myofunctional treatment alone (23-29).

Case report

The patient, a 9-year-old female, came in for a visit without any previous orthodontic check. Her medical history reported celiac disease, sometimes bilateral

headaches localized at orbital area.

The functional medical history reported use of pacifier until 4 years old and artificial feeding. The physical examination reported anterior open bite (overbite -2mm), reduced maxillary diameter (however, no dental crossbite is present), hypoperturbation of elements 1.2-2.2-3.2-4.2, lower incisors lingualization (Fig.1).

The functional analysis reported oral breathing (during the Rosenthal test the child opened her mouth before the end of the 20 nasal breaths), atypical swallowing with anterior lingual thrust, restrictive upper and lower labial frenula, normal lingual frenulum. Child's mother referred snoring episodes during the girl's sleep. Feeding problems were reported: the girl was not able to eat hard consistency foods (e.g., crusty bread, raw vegetables), as she had difficulties chewing the bolus which is often spat out. Sometimes the patient had digestive problems. It was decided not to start an orthodontic treatment but to enroll the patient into a cycle of myofunctional therapy.

The protocol included check-ups on a weekly basis for 3.5 months, which represented the intensive phase of the therapy. After this period the patient should have acquired a correct swallowing. Subsequently, the patient was checked twice a week for another 2 months. Thereafter, the patient was checked once a month to ensure that the correct swallowing muscle pattern was maintained. The patient was provided with a kit for performing myofunctional exercises at home.

During the intensive phase of therapy every week, the child had to perform exercises that stimulate

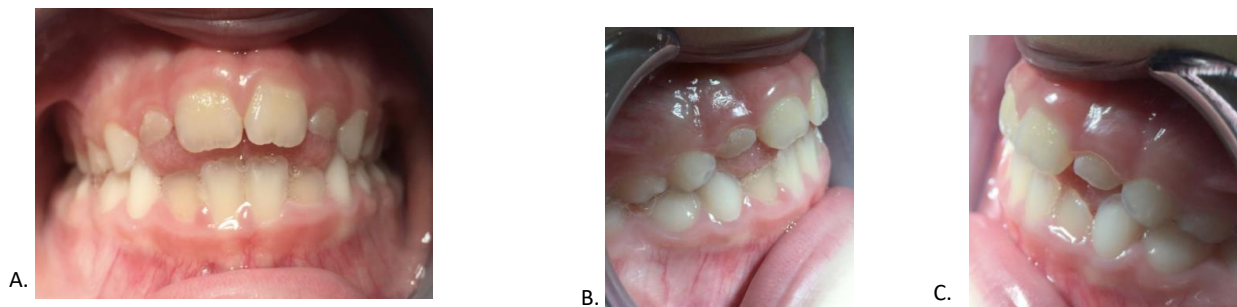


Fig. 1. A): Initial frontal view; B): Right overbite; C): Left overbite.

different muscles of the tongue, exercises for the lingual position at rest, for lip tone and nasal breathing. Quantitatively, each set of exercises required half an hour 3 times a day. The exercises (8 per week) proposed had different objectives.

The first one was teaching the child the correct lingual position at rest. Then, by stimulating the front, middle and rear part of the tongue, the exercises instructed the child to position the tip of the tongue on the incisal papilla and the remaining portion of the lingual body against the palate. The typical exercise consisted of holding a rubber band with the tip of the tongue on the retroincisal papilla. The exercise was carried out the first week for 10 minutes a day, with teeth not in contact and opened lips.

During the following weeks the time was progressively increased (15 min, 20, 30 etc.) and the exercise was carried out with teeth in contact at rest and closed lips. After the first few weeks the child was asked to perform the exercise while doing other activities, such as drawing or watching TV, to perform it without voluntary control.

Other exercises were specific for promoting lip seal at rest. The child was taught to swallow with dental arches in contact and with her mouth closed, without any contraction of the peri-oral muscles. To achieve peri-oral muscles coordination, the exercise used a button attached to a string. The button was inserted into the girl's mouth between the dental arches and the lips. The button had to be held between the lips and not between the teeth. During in-office sessions the clinician pulled the string while the child resisted the release of the button, then at home the child pulled the string by herself.

In atypical swallowing there is an incorrect function and tone of the facial and masticatory muscles. Often the patients present with muscle hypotonia, which can also have an aesthetic impact as the face appears elongated, flaccid, and inexpressive. The patient was asked to inflate the cheeks with air. The air is then "passed" from cheek to cheek to stimulate both buccinator muscles squeezing the cheek, to promote lip seal preventing the air loss anteriorly, and to promote the elevation of the back of the tongue so the air does not escape posteriorly.

Commonly, patients affected by atypical

swallowing show also mouth breathing. Through a series of playful activities, the therapist promoted nasal breathing in which the child must keep her mouth closed and breathe. The child needed to hold a rubber band on the posterior incisal papilla with the tip of the tongue while holding a cotton roll between the lips. The cotton fell off when the child stopped breathing through the nose and opened her mouth. Eventually, this exercise was performed while doing other activities (drawing, watching TV, etc.) to stimulate nasal breathing without voluntary control.

Patients with poor nasal breathing and atypical swallowing, show other compromised orofacial functions, such as chewing. They prefer soft, refined foods that can easily be soaked with saliva and swallowed. Food must be well shredded and comminuted between the teeth and soaked up with saliva, so that the semiliquid bolus can be "received" in the anterior portion of the tongue, pushed towards the palate and swallowed. In order to do this, the tongue cannot lie on the floor of mouth or be pushed between the anterior teeth. At the 8th week of myofunctional therapy the patient was taught to swallow food correctly starting from swallowing water, then swallowing crackers (semi-solid foods) up to swallowing more consistent and chewable foods such as meat. The patient was taught that chewing needs to be bilateral and that food needs to be finely comminuted (at least 20 chewing acts were required for each meat bolus).

RESULTS

After the first week of therapy, the girl's overbite seems to be improved, the area of the premaxilla looked less contracted and the lingual interposition in the anterior gap was no longer noticeable (Fig. 2). After 3 weeks of myofunctional therapy, the child's mother pointed out that snoring had progressively decreased.

At the 9th week of follow-up, the improvements in therapy were clinically evident (Fig. 3), the snoring disappeared, and the eating difficulties have also been reduced.

After 15 weeks of myofunctional therapy (Fig. 4) the child became aware of correct swallowing. It was

important that the newly acquired swallowing pattern was maintained regardless of voluntary control.

The patient did not return to the controls at the 5 months follow up, but she returned to our observation after about a year from the first visit. The results of myofunctional therapy were clearly evident (fig. 5a, 5b): despite the fact that she was no longer checked, the girl acquired correct lips and tongue rest position and correct swallowing after 6 months of therapy. Open



Fig. 2. Frontal view one week after OMT.



Fig. 3. Frontal view after 9 weeks of OMT.



Fig. 4. Frontal view after 15 weeks of OMT.

bite was significantly improved (overbite 0.5mm), the upper dental arch appeared expanded while the upper and lower lateral incisors were further erupted.

DISCUSSION

In this case, myofunctional therapy allowed correction of lips and tongue resting position, atypical swallowing, and other orofacial functions. It is known that neuromuscular pattern plays an important role in morphogenesis. Neuromuscular pattern can be defined abnormal when they are absent or exaggerated, so they can be linked to deviant orofacial morphogenesis (30-35).

Myofunctional therapy is a non-invasive, exercise-based therapy used to correct an improper muscular pattern, in order to avoid the development of malocclusion or to fix it. It is important for the myofunctional therapist to explain to the patient each exercise in detail and to monitor how they perform them. For each exercise, the exact time to be performed at home must be specified. The follow-up must be weekly, to support and motivate the patient and to check the correct execution of the exercise. Undoubtedly, in order to obtain a good result in therapy, the patient must be cooperative: it is important that the patients and the parents, in the case of young patients, are sufficiently motivated for the treatment to be compliant (36-42).

In the case reported, the patients, through no tooth brace therapy, showed a visible improvement of malocclusion and swallowing function, important results were also achieved in sleep disorders breathing and nutrition. The correct swallowing function was maintained until last follow up, 1 year after the first visit (43-46).

The patient was a habitual snorer, probably the low position of the tongue during the night, that creates an obstacle to the air flow at the pharyngeal level, worsened by her supine position, due to the posterior collapse of the tongue towards the airways. The patient was also an habitual mouth breather: at night the open position of the mouth resulted in mandibular posterior rotation which further reduced the pharyngeal lumen. The correct swallowing act allows not only the entry of food into the pharynx but also it allows the proper and



Fig. 5. *A): Frontal view after 1 year of OMT; B): Right overbite after 1 year of OMT; C): Left overbite after 1 year of OMT; D): Occlusal view of lower arch after 1 year of OMT.*

safe opening of the Eustachian tubes that connect to the middle ear by sealing off the nasal cavities (47-51).

Finally, the finer chewing of food has meant that the little girl was able to eat more age appropriate and consistent foods with positive implications on her nutritional level.

CONCLUSIONS

According to the results obtained, we can conclude that in the diagnosis of a patient suffering from malocclusion, a careful functional analysis should be performed as complementary to the orthodontic evaluation (52-56). Since “The function creates the organ” if the dental or skeletal position are dependent from incorrect functions, we must first correct the function, unless the form is so impaired that that needs to be optimized first to then accommodate the proper function. Furthermore, a correct function allows stable results (57-79).

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Diagnosis, treatment planning and orthodontic treatment in patients with ameloblastoma.

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AbstractAim: The aim of the study is basically to underline the advantages of the digital approach for the diagnosis, the treatment plan and the surgical and orthodontic treatment in the patients affected by ameloblastoma.

Materials and methods: A patient affected by ameloblastoma of the upper maxilla underwent to a digital approach for the exact diagnosis and the virtual planning of the post-surgery orthodontic treatment using aligners. A 3D computer tomography was used to a precise detection of the anatomical limits of the neoformation. A concomitant myofunctional therapy was performed with a complete occlusal and functional restoration.

Results: A complete alignment of the residual dentition was achieved. The functional and esthetic outcome was completed using a temporary removable prosthesis waiting for a bone grafting and implant supported fixed restoration.

Conclusion: In patients with ameloblastoma the digital approach for a correct diagnosis and treatment planning with aligner can be extremely useful for a detailed control of the orthodontic movements together with an accurate virtual and clinical control of all the aspect that can interfere with the occlusion.

An ameloblastoma is a benign odontogenic tumor generally present in the jawbone and the tumor originates from the residual epithelium of the tooth germ, epithelium of odontogenic cysts stratified squamous epithelium and epithelium of the enamel organ and it represents approximately 1% of oral tumors. Ameloblastoma clinically appears as an

aggressive odontogenic tumor, often asymptomatic and slow growing, with no evidence of swelling.

The first person who identified the ameloblastoma was Cusack in 1827, while Broca explained it in 1868, and Falkson gave a detailed description in 1879. In 1853 Wedl (gave the first histopatological description and Wagstaffe the first histological drawing in 1871. In

Keywords: digital dentistry; ameloblastoma; orthodontic treatment; computerized tomography; dental scan; Invisalign; myofunctional therapy

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1885 Malassez used the term «adamantine epithelioma» and in 1890 Derujinsky the term «adamantinoma». The term «ameloblastoma» was traditionally attributed to Ivy and Churchill, but Emura described a case of «metastatic ameloblastoma» in 1923.

Sciubba defined the ameloblastoma as an aggressive odontogenic tumor that forms from odontogenic epithelium within a mature fibrous stroma devoid of odontogenic ectomesenchyme and Bassey et al. classified it as a benign tumor. According to Fregnani et al. (1-6), ameloblastoma represents between the 13% and the 58% of the odontogenic tumors in the world. According to Effiom et al. (7-10), it has a multifactorial etiology that involves different cellular pathways and molecular mechanisms: several dysregulations are related to sonic hedgehog, WNT/ β -catenin and mitogen-activated protein kinase signaling pathways.

The 2017 World Health Organization (WHO) classification of odontogenic lesions individuates four types of ameloblastoma: the conventional type, the unicystic type and the extraosseus / peripheral type and the metastasizing ameloblastoma; while ameloblastomas can show a predominant histological subtype (as desmoplastic, follicular, plexiform, achantomatous, granular and basalioid), all of them have non clinical significance. Ameloblast carcinoma is classified as a malignant tumor, while odontoameloblastomas are classified as mixed tumors.

MATERIALS AND METHODS

A 32-year-old female, the patient did not have any malformations or short lingual frenum (11-17) presenting an extended open bite, a loss of teeth 24, 25 and 26 and an atypical swallowing underwent to our observation asking to optimize the esthetic and the occlusion after a surgical treatment for an ameloblastoma. The first 2d radiological examination detected a severe bone loss of the left portion of the maxilla, as the result of the surgery.

A computer tomography without contrast, analyzed with a Dentascan software in axial slice (Fig. 1) was used to clearly detect the limits of the osteolytic area which was extended from the vestibular to the palatal margins of the left upper maxilla.

The coronal slices (Fig. 2) of the computerized tomography

completed the analysis of the defect showing hypodense solid tissue penetrating the maxillary sinus and a wide root resorption of the 2.4, 2.5 and 2.6. A minor resorption was also detected at the root level of the 1.4, 1.5 and 1.6. (Fig. 3).



Fig. 1. Axial slice of the Computerized Tomography.



Fig. 2. Coronal slice of the computerized tomography.



Fig. 3. D Virtual Reality imaging from the Computerized Tomography.

The 3D Virtual Reality imaging (Fig. 4) generated from the Computerized Tomography gave a clear vision of the maxillary bone defect. Digital photos were taken to collect the patient's occlusion (Fig. 5-7). A malocclusion and an interdental tongue thrust, which goes worse, during swallowing is clearly visible.

The orthodontic treatment options were then studied using ClinCheck®, a dedicated planning software (Fig. 8-13).



Fig. 4. 3D Virtual Reality imaging from the Computerized Tomography



Fig. 5. Frontal view of the patient after surgery.



Fig. 6. Left-side view of the patient after surgery.



Fig. 7. Right-side view of the patient after surgery.

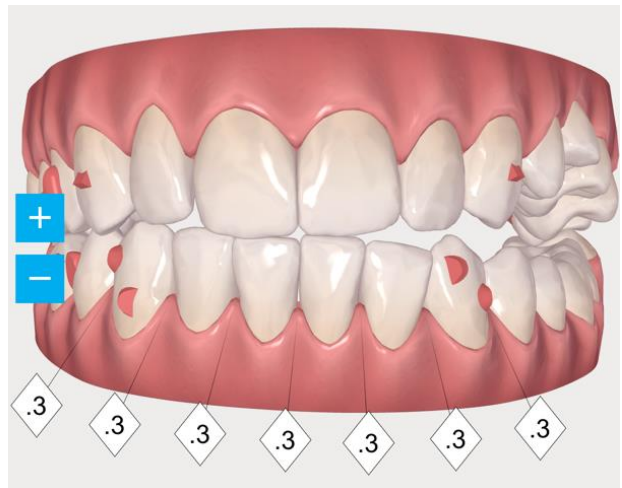


Fig. 8. Frontal view of ClinCheck®.

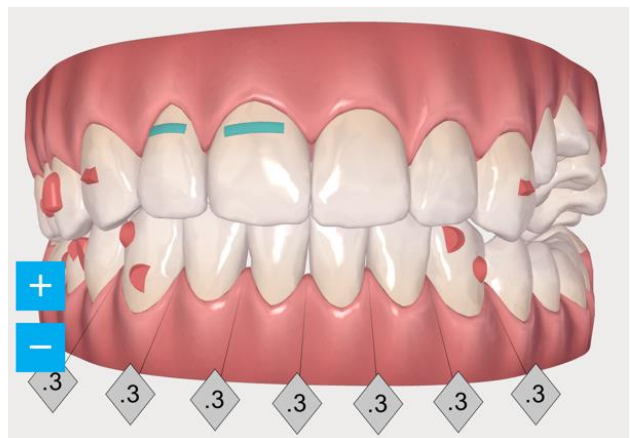


Fig. 9. Frontal view of ClinCheck®

According to the patient's desires, the use of aligners was chosen for the therapy while a myofunctional treatment was performed before starting. The basic aims of the myofunctional therapy were to correct the rest position of the tongue and to eliminate the parafunctions and the atypical swallowing.

The myofunctional therapy lasted 6 months. In this treatment period, the patient was seen on a weekly base for 15 weeks. At each appointment exercises to promote tongue posture at rest, create a labial seal, promote the nasal breathing, correct swallowing for liquids and solid food were assigned by the myofunctional therapist to the patient. The patient had to make all the homework, every day, three times a day (18-22).

After the first 15 weeks a second therapy sequence

of 3 more months was assessed checking the patient on a 3 weeks basis. When the myofunctional therapy was completed, the patient was ready to start the orthodontic treatment by the use of aligners according to the virtual treatment planning.

RESULTS

The myofunctional therapy produced a clear



Fig. 10. Right-side view of ClinCheck®

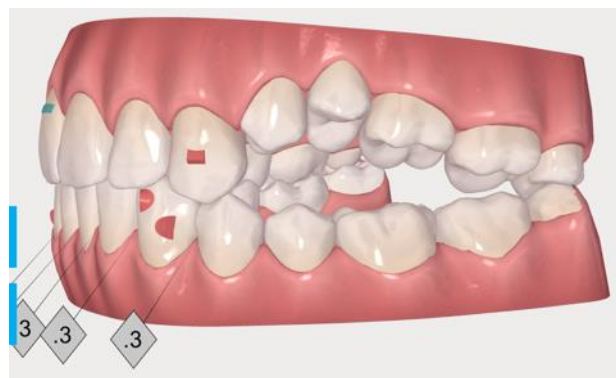


Fig. 13. Left-side view of ClinCheck®



Fig. 11. Right-side view of ClinCheck®



Fig. 14. Frontal view of the patient after myofunctional therapy.



Fig. 12. Left-side view of ClinCheck®



Fig. 15. Left-side view of the patient after myofunctional therapy.

reduction of the left posterior open bite and of the mandible protrusion (Fig. 14-16). The parafunctions were also corrected and this result represented a better starting for the orthodontic treatment. The patient was encouraged to continue the home exercises also after the end of the active myofunctional treatment. A total of 33 aligning trays were used to correct the



Fig. 16. *Right-side view of the patient after myofunctional therapy*



Fig. 17. *Frontal view of the patient with prosthesis*



Fig. 18. *Left-side view of the patient with prosthesis.*



Fig. 19. *Right-side view of the patient with prosthesis*

malocclusion [Align Technology Inc., Via G.B. Cassinis, 7, 20139 Milan, Italy].

Particular attention was made during the treatment for not creating any tensions to the left side maxillary teeth and the relative bone. A bone compression might have led to an extra bone resorption in the osteotomies area. The total active time treatment was about eighteen months after which a stabilizers stents was asked to be worn by the patient. The orthodontic treatment achieved the alignment of the dentition as virtually planned on the software. The functional and esthetic outcome was complete by the use of a temporary removable prosthesis (Fig. 17-19). The patient was addressed for a major bone graft procedure and implant supported fixed restoration.

DISCUSSION

Into the articles stored in PubMed there are only nine cases of patients with ameloblastoma who underwent an orthodontic treatment: the first is dated 1996, while the most recent was published in 2019 (23-30).

As shown in Table I, it was observed that most of them are female patients (88.9% of the cases), minors (66.7% of the cases), who had an ameloblastoma located in the mandible (77.8% of the cases) and who underwent a fixed orthodontic treatment (55.5% of the cases). Moreover, it was also noticed that the 44.4 % of the cases came from Asia, 33.3% from Europe and 22.2% from South America (31-38).

According to the most recent systematic review about the global incidence of the ameloblastoma, elaborated by Hendra et al. the sex distribution of the ameloblastoma is equal, because the differences that were found in the continents (male predominance in Africa, Asia and North America and female predominance in Europe and South America) are not statistically significant. According to a French study published by Lagorsse (39-44) the difference in need for orthodontic treatment between males and females isn't statistically significant, but there is a higher request of orthodontic treatment by females patients that is statistically significant.

According to the review of Hendra et al., there are differences statistically significant in age distribution, but within the second decade (representing 66.7% of this study's sample) ameloblastoma is not common,

although it's the decade in which most orthodontic treatments take place.

Besides, Hendra et al. revealed that the mandible is the location of the ameloblastoma in the 87,2% of the cases and this data is compatible with the high prevalence of the location in the mandible that we found in the nine orthodontic cases, above listed (45-49).

Finally, the distribution of the different orthodontic techniques is perfectly coherent with the different ages of the patients, so functional orthodontic treatments were applied when they were still growing and developing, while fixed braces and miniscrews were applied when the growth of the maxillary bones has already ended. However, to the best of our knowledge, there is no case reported in literature of using invisible trays and digital orthodontic technology in patients with ameloblastoma before the clinical case content in this article.

This case highlights how much it is important in these situations to have a very detailed and precise treatment. In particular, the aligners treatment permitted to predict in a very careful way the teeth movement and to reach what were the expectations (50-52). That probably could not be obtained, using the traditional devices which are in most cases very effective, but they cannot reach a high level of precision.

Moreover, in these cases where there is a very traumatic intervention with a great loss of bone support, it is very important to study the case meticulously to avoid mistake in the treatment plan. Furthermore, there is the risk to bring outside the bone the teeth causing iatrogenic damage to the patients who were already stressed by the very heavy health problem.

The patient that is the subject of the study, because of the surgery, lost a great quantity of bone and teeth that cause an open bite which was aggravated by her past and present atypical swallowing. For that reason, myofunctional therapy was a instrument that was used to help to solve a part of the problem (53-55).

CONCLUSIONS

Digital technologies appear to be extremely useful for a correct diagnosis and to explore all the possible options of the treatment planning of the malocclusion after the removal of the ameloblastoma. The whole orthodontic process can be evaluated in advance

and is able to be followed in every single step. An analogic accurate control of all the aspects that can interfere with the occlusion is also imperative so that a correct combination of analogic and digital approach is essential for a correct treatment.

This study points out how much it is very important in some cases to have a very detailed program of treatment which can predict in a very meticulous way the teeth movement. That can be particularly reached by aligners that permit a previous analysis of the oral space and simulate the final result. This kind of therapy can even help to solve apparent great problems, like open bite, which are caused by invasive surgeries. It also prepares the patient, when needed, to the prosthodontic treatment.

Aligner therapy can be a tool to keep in consideration when facing extreme cases, even if have higher costs. Moreover, the patient can have all the benefits of this kind of therapy which are the aesthetics, less pain and less time in the dental office.

It is also important to study the oral situation in all its aspects, in particular the tongue position which affect teeth position. Myofunctional therapy is an answer to this request and can be helped by frenectomy, if needed. The analysis of the tongue action is very important, because it can ruin what the orthodontic therapy achieved.

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The relationship between TMJ symptoms and gut disease

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The aim of this study is to investigate the overlapping conditions in patients who suffer of temporomandibular disorders, (TMD), and to analyze the relationship between temporomandibular joint, (TMJ), symptoms and gut disease. One-hundred-sixty-nine patients are asked to complete a survey including questions about gut's diseases, dental malocclusions and TMJ symptoms, in particular about temporomandibular joint pain and headache. The answers to the questions showed that the only statically significance correlation exist between gut's disease, leaky gut, TMJ pain and between headache and TMJ pain. It's possible to suppose the influence of gastrointestinal pathologies and symptoms on TMJ and vice versa, but this hypothesis has to be confirmed by further studies.

Temporomandibular disorders are very common in the global populations and affect the TMJ and masticatory muscles; they are the main cause of not-dental facial pain. Patients who suffer from TMD usually refer at least one of these symptoms: click or smack, pain of facial muscles, around eyes and ears and difficulty to open the mouth without pain or fatigue (1-4).

The latest data from the literature report that the prevalence of the TMD in general population is between 12-60 %; the prevalence of the signs is between 33-86% and prevalence of the symptoms 16-59%. Furthermore, there is a difference between

male and female (1:4) that suffer much more of this disease. The most affected age group is from 25 to 40 years old (5-8).

The most common system of classification for TMD is the "Diagnostic Criteria for TMD", introduced by Dworkin. This divides TMD into three groups: muscular disorders, dislocations of the disk and Arthralgia-Arthritis-Arthrosis. On the other hand, the American Academy of Orofacial Pain, AAOP, divides TMD in two groups: disorders of the masticatory muscles and disorders of the joint (9-11).

TMD are often correlated with other systemic condition and several studies show the overlapping

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of TMJ symptoms, especially orofacial chronic pain, with pain in other regions of the body. Furthermore, the most frequent association of TMJ and systemic conditions occurs in chronic inflammatory diseases³ and, according to the literature, this relationship is very common for conditions such as cervicalgia, body pain, ear-nose-throat disorders, musculoskeletal disorders, diabetes, cardiopulmonary and gastrointestinal disease (12-16). At the same time, it is known that TMD could be associated with dental malocclusion, even if, nowadays, the possible relationship is in continuous studying.

The malocclusions are among the main buccal health problems all over the world, together with dental caries and periodontal disease. They can involve only the dental arches or the maxillary and mandibular basal bone as well (17-20). Malocclusion can affect the sagittal, vertical or transversal plane and can be classified according to many different systems:

On the sagittal plane, according to *Angle Classification*, there are I, II and III class

On the vertical plane there are deep bite or overbite, frequently related to hypodivergent and hyperdivergent biotype

A lot of studies have supposed a direct relationship between malocclusion and TMD disorders, but the literature is not unified about this hypothesis.

Amira Aboloanga et Al. demonstrated that dental malocclusion, (static and dynamic), should not be considered a certain predictor of TMD; in their sample they found a relationship between joint disorder with vertical craniofacial pattern. At the same time, a lot of studies supposed that deep bite and a reduced vertical dimension can be considered a casual factor of muscular hypertonia and muscular disorders. Thus, sagittal malocclusion can cause TMDs: for example the III class and anterior cross bite, without canine and incisive guide determine the overuse of the posterior sector and the flattening of the articular surfaces.

Despite what it was said before, the opinions of the researchers about this topic is very variable and a lot of studies refuse this relationship: Manfredini et al. analyze in their sample that there is no clinical influence of malocclusion on TMD as clicking or pain (21-26). On the other hand, among gastrointestinal disorders that could be related with TMD there are

gut diseases such as leaky gut and irritable bowel syndrome.

The leaky gut syndrome, LGS (syndrome of the runny bowel), is an inflammatory condition that affects the intestinal mucosa, and it is caused by several factors related with style life and alimentation. The LGS causes an increased permeability of the mucosa and absorption of substances not digested or partially digested. Consequently, the inflammation of the GALT (gut's associated lymphoid tissue) occurs and then a general stress condition of the body; the LGS is the main cause of food intolerances and the alteration of the intestinal flora.

Many studies reported that patients who suffer from leaky gut or other bowel inflammatory diseases often suffer from headaches and chronic fatigue. The increased permeability of the mucosa and the resulting inflammatory autoimmune process that occurs are probably responsible of the headache and other not specific symptoms; in fact, a great accumulation of toxins and waste substances occurs in the bowel and in the body

According to some researchers, there is a high percentage of patients with TMDs who suffer from chronic orofacial pain and show other comorbidity or stress-associated pathologies like irritable bowel syndrome, premenstrual syndrome, and interstitial cystitis (27-35).

The IBS is a very common chronic condition that affects about 10% of the global population mainly of female sex and a higher prevalence from 20 to 50 years old. This pathology is characterized by abdominal discomfort or pain associated with impaired bowel function and accompanied by swelling or distension. The etiology is multifactorial including psychological and biological factors and is very frequent to suffer from general symptoms such as headache, anxiety, depression, fibromyalgia and chronic fatigue and temporomandibular symptoms. As it could be found among the literature IBS is one of the many stress-condition that could be associated with TMD; S. Gallotta et Al. (36-42) evidenced that IBS patients had a more than three times greater risk of TMD compared to healthy people. IBS patients that also fulfilled criteria for TMD seem to share along with chronic oro-facial and abdominal pain a significant

co-occurrence with psychiatric disorders and female preponderance. Korszun et al. supposed that the clinical overlap between these conditions may reflect a shared underlying pathophysiologic basis involving dysregulation of the hypothalamic-pituitary-adrenal stress hormone axis in predisposed individuals.

Unfortunately, there is no scientific evidence of the direct association between bowel diseases and TMD; moreover, some studies affirm a significant relationship only with musculo-skeletal conditions. Gary D. Slade et All found 90 % of correlation for TMD and Fibromyalgia and a very weak association between TMD, headache and irritable bowel syndrome in their sample; while musculoskeletal conditions exhibited some features that could be explained by a single functional syndrome, headache and irritable bowel syndrome did not.

Considering all that, it's not simple to study and identify which kind of relationship exists between TMD symptoms, Leaky Gut and bowel diseases; so the purpose of this study is, even, to underly if there is an overlapping of symptoms for these disorders and suggest further researchers to explore a possible mechanism of the correlations.

The purpose of this article is to find out the possible relationship between TMDs, headache and gut's disease. As well, the study wants to determine the prevalence of malocclusion in the patients affected by these pathologies.

MATERIALS AND METHODS

We enrolled in our research 169 consecutive patients including male and female over 18 years old; there were no exclusion criteria and the only inclusion criteria was that patients suffer from TMD and/or bowel disease. To make an analysis as objective as possible we decided to submit a "yes or no question list" by an online form service (Google Form service, Google LLC, 1600 Amphitheatre Parkway, Mountain View, California, U.S.) and later collect the data anonymously.

All participants provided the informed consent and accepted the privacy policy for the protection of personal data before compiling the survey. No personal information that identifies the individuals was collected and the data was analyzed only in aggregate form. All responses were collected on an anonymous basis using the Google Form service.

The form, submitted in bilingual Italian / English, was composed of five questions:

Do you know what is a dental malocclusion? Sai che cos'è una malocclusione?

Do you suffer from leaky gut/ gut's disease? Soffri di sindrome dell'intestino gocciolante/ malattie intestinali?

Do you have a dental malocclusion? Hai una malocclusione dentale?

Do you suffer from temporomandibular joint pain? Soffri di dolore dell'articolazione temporomandibolare?

Do you suffer from headache? Soffri di mal di testa?

No homogeneity test was performed neither for age and sex and after collecting the answers an inferential statistical analysis was done. A T-student test was performed considering the association between two variables (only two questions answered by every patient) statistically significant for p value < 0.01 .

RESULTS

All the participants answered the survey, and the results show statistically significant associations (Table I):

71 patients suffer from leaky gut's/ gut disease and TMJ pain ($p < 0.01$)

81 patients suffer from leaky gut's/gut disease and headache ($p < 0.01$)

93 patients suffer from headache and TMJ pain ($p < 0.01$)

DISCUSSION

TMD are multifactorial conditions that can be influenced by many factors, but at the same time they can involve the health of the whole body. It is quite known that TMD can influence the psychological state of the patients affected. In the same way gut's disease can influence the whole health state of the patient, with many negative effects on quality of life, lifestyle, and emotive status. Therefore, it's possible to define TMD and some gut's disease as systemic pathological disorders and because of that nature, they can condition emotional sphere, cognitive capacities and psychological features. In fact, according to many studies it is reported that people who suffer from TMD, leaky gut, IBS and other gut disease frequently refer

Table I. *Results of the survey.*

		Do you know what is a dental malocclusion?	
		Yes	NO
Do you suffer from leaky gut/ gut's disease?	Yes	83	30
	NO	23	35
Significance	P<0.01		
O.R.	11.1		
		Do you have a dental malocclusion?	
		Yes	NO
Do you suffer from leaky gut/ gut's disease?	Yes	20	33
	NO	51	65
Significance	N.S		
		Do you suffer from temporomandibular joint pain?	
		Yes	NO
Do you suffer from leaky gut/ gut's disease?	Yes	71	6
	NO	47	47
Significance	P<0.01		
O.R.	15.6		
		Do you suffer from headache?	
		Yes	NO
Do you suffer from leaky gut/ gut's disease?	Yes	81	37
	NO	41	11
Significance	N.S		
		Do you know what is a dental malocclusion?	
		Yes	NO
Do you suffer from headache?	Yes	69	54
	NO	37	11
Significance	P<0.01		
O.R.	6.7		
		Do you have a dental malocclusion?	
		Yes	NO
Do you suffer from headache?	Yes	72	49
	NO	26	22
Significance	N.S		
		Do you suffer from temporomandibular joint pain?	
		Yes	NO
Do you suffer from headache?	Yes	93	23
	NO	25	30
Significance	P<0.01		
O.R.	8.9		

irritability, anxiety, depression e difficulty to focus. In our sample it is evidenced the overlapping between TMJ pain, headache and gut disease with a statistically significant value; considering that, it's possible to suppose that there is a common pathophysiological alteration between these disorders, but no scientific study demonstrated that. So, the hypothesis is that the cause of the coincidence of clinical symptoms of TMD and gut's disease in the sample could be found into a psychological-emotive pattern (43-45).

In our sample there is not statistically significant association between malocclusion and guts disease; indeed, there aren't studies that sustain this hypothesis, but some research supposed a correlation with gastric system. Toagawa et Al. (5)a survey on a small sample found gastroesophageal reflux disease symptoms associated often in patients with skeletal Class III malocclusion more than in subjects with a normal occlusion. Moreover, gut's disease is usually associated with a musculoskeletal disease called Fibromyalgia which often affects TMJ and determines orofacial pain.

Fibromyalgia is a pathology with no defined causal factors; the recent literature individuates as cause of this disorder an alteration of the microbiota and that can be an explanation of the relationship between musculoskeletal symptoms and gut's disease (46-52).

CONCLUSION

The study, even if considering a little sample of patients, highlights a statistically significant correlation between a complex disorder as the temporomandibular and gut's disorders like leaky gut and irritable bowel syndrome. Moreover, it correlates these problems with a common daily issue as headache.

Despite headache can be linked to many other pathologies, including malocclusion, it is evident that headache can be one of the principal symptoms of gut's disorder and TMD (53-55). To confirm these conclusions, in a stronger way, more studies are required on larger sample.

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The histological behaviour of bone tissue and mucous membranes in Italian school immediately loaded “one-piece” implants

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Aim: to clarify that reparative osteogenesis “one-piece” implants of the Italian School evolves favourably towards osteoanchylosis (osseointegration) and that the behaviour of the soft tissues around these implants is also histologically similar to the mucous membrane surrounding healthy natural teeth.

Materials And Methods: Italian School bone-implant “block sections” and peri-implant mucosal sampling in humans and experimental animals.

Discussion And Conclusions: This further publication demonstrates that Italian School one-piece immediately loaded implants, similarly to biphasic implants, can be osseointegrated without the recession of the surrounding tissues. Following 2400 serial sections, the peri-implant mucosa appears histologically similar to the healthy mucosa of natural teeth.

This work aims to investigate the intimate relationship between host tissue (bone and mucosa) and Italian implantology school titanium endosseous implants. Modern implantology (Formigginì 1947) began with immediate prosthetic loading of post-extractive implants. In recent years, clinical practice in implant prosthetics has increasingly moved towards the adoption of immediate loading, even by the school which had dogmatically adopted the ‘philosophy’ of deferred loading. In 1962, university research by Ugo Pasqualini scientifically resolved the issues relating to the biocompatibility of implant materials, their osseointegrative potential, communication with the external environment and occlusal load. In 1963, after years of research, Stefano M. Tramonte developed his grade 2 titanium wide-spiral self-tapping screw - the first in the world to use titanium in implant prosthetics. In 1972, Dino Garbaccio patented a self-tapping screw, also made of grade 2 titanium, introducing the

concept of bicorticalism in screw implantology, taken from Scialom’s needles (1963). The Italian School was the first to have and use Mondani’s intra-oral welding machine since 1978, a tool that is capable of welding titanium directly in the mouth and making immediate functionalisation of retainers possible and predictable (1).

At present, several international authors have also presented one-piece implants similar to those of the Italian School (2-4). Analysing the results of some of the various histological studies carried out on these implant types, we have a clear picture of the excellent behaviour of the bone tissue and peri-implant mucous membranes.

MATERIALS AND METHODS

Histological research used “block sections” of post-mortem examined implants, implants removed from living

Keywords: histology, Italian implantology school, one-piece implants, immediately loaded osteoanchylosis

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humans for traumatic fractures after years of function and sections taken from experimental animals.

The preparation methods for studying the behaviour of the contact zone between alloplastic structures and host tissues used in this research were: the abrasion technique to obtain histological preparations comprising bone and metal in well-coloured sections just a few microns thick, which allows simultaneous analysis of the alloplastic material and the hard tissues surrounding it, a method improved by Prof. Karl Donath of Hamburg University in the 1980s (5, 6).

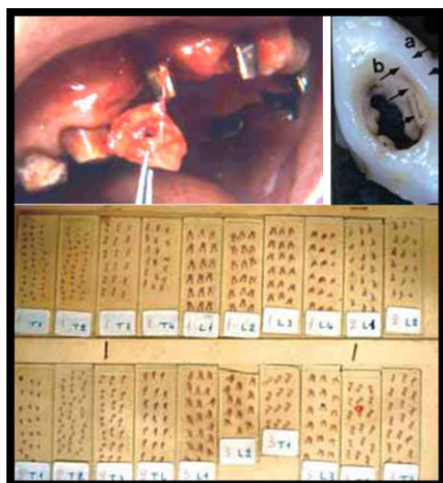


Fig. 1. Biopsy sampling at the neck of an Italian School one-piece screw. Progression of histologically observed sections in the architecture of the outer mucosa (a) compared with that of the inner mucosa (b). Some of the 2,400 histological preparations.

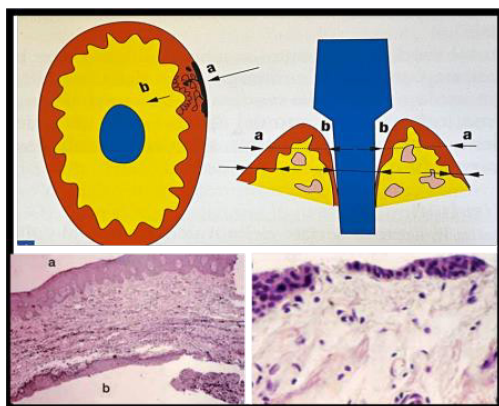


Fig. 2. Drawing that illustrates the scheme of orthogonal sections. All serial sections of the mucosa attached to the neck of the screws were histologically similar: the outer mucosa is always protected by an adherent keratin layer. The internal mucosa, facing the implant, lacks a keratin layer with progressive loss of cell layers until only the basal cells are monostratified.

Mucosal peri-implant samples were analysed using the classic method: fixed in 5% formalin and then embedded in paraffin, prepared in 4 mm serial sections and stained with haematoxylin-eosin at progressive magnifications up to a maximum of 400 x. Mucosal biopsy research was used to progressively assess and control the histological behaviour of the epithelium and submucosal corium at the level of the epithelial connective attachment. The samples were divided into two parts examined in the two typical cutting sections: orthogonal or parallel to the axis of the implant emergence. The total number of sections examined was 2,400 (7) (Fig. 1).

Histological research

Karl Donath, Professor at the University of Hamburg, made it possible to obtain sharp and precise ground sections of the metal inclusions and their surrounding tissue. These show that osseointegration does not require macroscopic retention since it is organised through a process of osteoanchoylosis even along the smooth surfaces of submerged titanium structures (8).

Piattelli and collaborators of the University of Chieti,

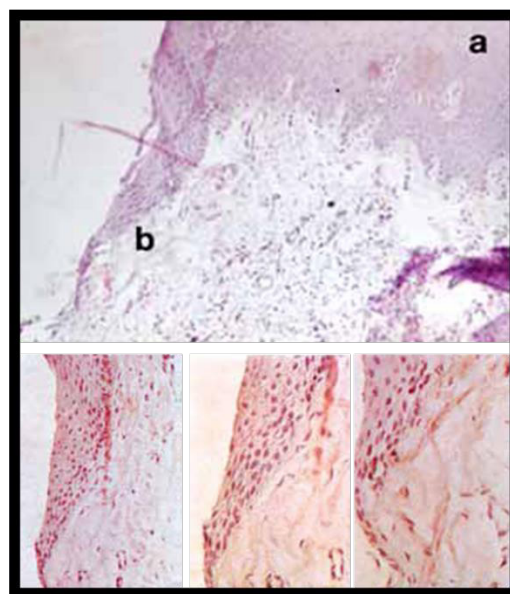


Fig. 3. Cytomorphology of periodontal tissues around the implant neck. The inner mucosa (a) thins out and progressively loses layers until it becomes monolayered with a normal underlying corium without any epithelial digitation. Note the regular reduction of the inner epithelium layers from the surface towards the area of the epithelial attachment. At maximum 400X magnification, only the cells of the basal layer are evident.

using both Donath's original technique and their own techniques, confirmed similar bone-implant interface results (9, 10). Vantaggiato, Iezzi and Piattelli described Donath's technique in demonstrating the long-term osseointegration of three one-piece screw implants (11).

Using another "ground" technique, Vito Terribile, Wiel Marin, Piero Passi, and Antonino Miotti of the University of Padua documented a similar bone apposition on a Tramonte's screw that had been under load for years before fracturing (12). Andrea Bianchi of the San Raffaele University of Milan carried out extensive research into how peri-implant bone adapts in terms of density and architectural organisation in response to the functional

prosthetic stresses to which implants in living bone were subjected. Bianchi also considered the term "functional ankylosis" between the bone tissue and the enclosed osseointegrated implant more accurate and in line with the results of his scanning electron microscope (SEM) research (13). This definition was confirmed by the studies of Professor Carlo Zerosi of the University of Pavia, who

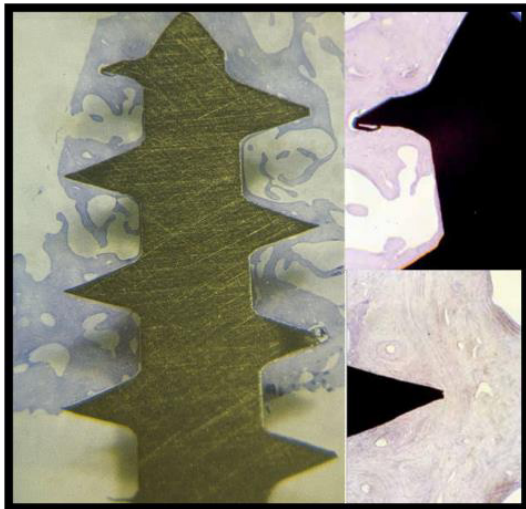


Fig. 4. Beautiful histology of a fractured implant after years of function, with the detail of the coils with no interruption between metal and bone tissue.



Fig. 5. Spongiosa compaction. The screw removed after 12 years of prosthetic loading shows a significant increase in bone density around it. Detail of a coil also with the SEM (lower right).

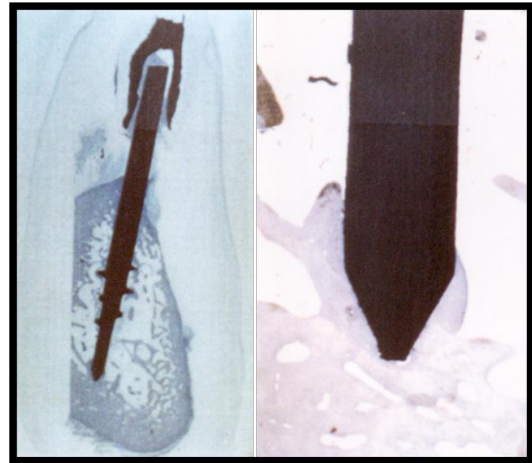


Fig. 6. "Block section" performed by Professor Donath of the University of Hamburg demonstrating the absence of connective tissue between bone and surface in Garbaccio bicortical screw implants.

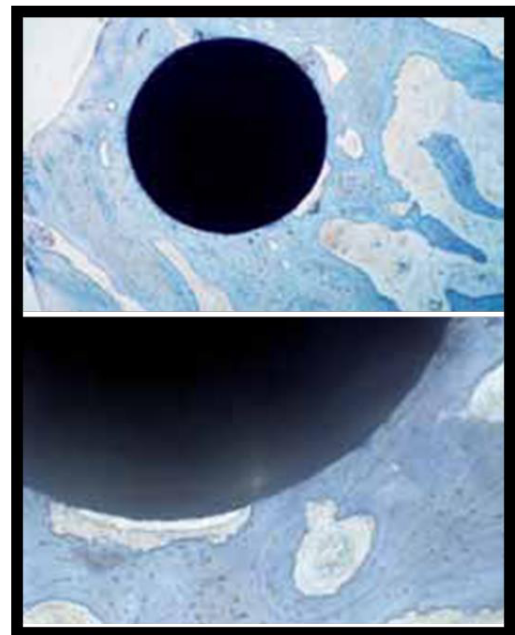


Fig. 7. Needles with a smooth metal surface also offer great resistance to load and traction, as confirmed by the study of block sections that provide the same histological picture of an ankylosis apposition of newly formed bone, tenaciously attached to the titanium wall (15X to 250X magnification).

defines ankylosis as the relationship between bone and implant, commonly referred to as osseointegration (14, 15).

To study the metal-including bone interface of a screw implant, Bianchi adds that *“between the peri-implant bone and the titanium screw surface there was an almost constant empty space of about one micron, where it was never possible to find fibrous tissue or other non-mineralised soft tissue, even at maximum magnification (SEM 250x)”* (16) (Fig 2-5).

Histological research on the behaviour of the so-called “epithelial attachment” marginal to the abutments of one-piece implants has shown that the epithelium around implants behaves in the same way as the epithelium around healthy natural teeth. The serial sections of the mucous membrane (2400 sections) attached to the neck of the screws are histologically similar: the external mucous membrane always appears protected by a keratin layer, while the internal mucous membrane, facing the metal, is always devoid of a keratin layer with progressive loss of the cellular layers up to the monostratification of the basal cells only. Underneath is a normal corium. The 1972 work by Camera and Pasqualini U of the University of Modena was republished after further scientific confirmation in 2009 (17).

Histological analyses prove that the supracrestal soft tissues around one-piece implants are similar to the gingival soft tissues surrounding healthy teeth (Fig. 6, 7).

CONCLUSIONS

The histological studies have proved the ability of Italian School single-phase one-piece immediately loaded implants with a thin emerging neck (abutment) and a reduced diameter compared to the body of the coils (fixture) to realise an adequate epithelial seal (Platform Switching) (18,19); a necessary condition for the bone to heal perfectly, achieving osseointegration.

The biopsy samples show that the soft tissue sections at the neck of the screws feature a physiological pocket with a depth of approximately 1.5 mm, similar to that observed around the collar of healthy human teeth. It has been also proved the perfect placement of healthy newly formed bone, both compact and spongy, around all the metal structures of the various morphologies of the one-piece implants without the interposition of collagen fibres.

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Figures 2 and 3 are Courtesy of Andrea Bianchi, taken from the book *Implantologia e Implantoprotesi*, Torino: UTET; 1999.

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Comparative evaluation of 4 root-end filling materials sealing ability in an *in-vitro* study

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The aim of this study is to evaluate and compare the marginal adaptation of four retrograde filling materials by micro-infiltration of basic dye and related analysis with stereomicroscope. Forty human teeth extracted with single-channel roots were selected for the study. The root canals were first instrumented and filled with gutta-percha and endodontic cement. Subsequently the root apices underwent apicectomy and 3 mm deep retrograde cavities were prepared. The teeth, divided into four groups, were filled with four different retrograde filling materials assigned to the specific group and subsequently treated with basic dye solution (Methylene blue) for the determination of micro-infiltrations. The apical portions of the study teeth were cut to 1 mm and 2 mm to obtain cross-sections to be analyzed with the stereomicroscope. The dye, infiltrated in the vacuum areas (gaps) between dentin and retrograde filling cement, shows the marginal filling materials adaptation with the dental surface in retrograde techniques. The statistical analysis showed, with statistically significant values, fewer internal voids, and therefore greater marginal adaptation with Biodentin and Aureoseal, compared to ProRoot MTA and MTA Flow.

Among the most frequent failure causes in endodontic treatment, certainly the apical seal lack with consequent root canal infiltration, is the main cause of failure and frequent indication for orthograde or retrograde retreatment (1). To seal the root canal three-dimensionally and prevent bacterial proliferation, a retrograde filling material should minimize the gaps between it and the teeth dentin and ensure proper three-dimensional stability over time, thus ensuring the microleakage absence necessary to promote and achieve the periapical tissues healing. Microleakage is defined as the passage of bacteria,

fluids and chemicals between teeth dentin and the filling material. In the literature many methods have been used in the various in-vitro studies to estimate the sealing capacity of endodontic cements.

The most used method to evaluate the presence or absence of 3-dimensional voids, has been the penetration technique of a basic dye along the endodontic cement (2), evaluated and microphotographed with a stereoscopic microscope. Usually, the tracers used in in-vitro microleakage studies, are Toluidine Blue (3), Rhodamine B (4, 5) and Methylene Blue. The main purpose of this in-

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vitro study is the evaluation and comparison of the marginal adaptation of 4 endodontic cements: MTA Flow (Ultradent), Biodentine (Septodont), ProRoot MTA (Dentsply) and Aureoseal (OGNA). The study will be conducted by penetration testing of the basic dye Methylene Blue and evaluation with stereoscopic microscope at 80x magnification.

MATERIALS AND METHODS

Criteria for sample selection

Forty extracted human teeth were collected and stored in saline solution (Sodium Chloride 0.9%). All samples were previously instrumented with manual Scaler to remove calcifications and tartar from the dental surface. Samples teeth were chosen based on inclusion and exclusion criteria; *inclusion criteria*: Single root tooth with single endodontic root canal, endodontic root canal pervious and with curvature $<5^\circ$ (6), root with mature apex (fully formed); *exclusion criteria*: root fractures and cracks, root caries, embryology anomalies, immature apex, multiple endodontic root canals, endodontic root canal calcification, endodontic root canal curvatures $>5^\circ$, previous endodontic root canal treatments.

To verify the presence of a single endodontic root canal and the absence of internal resorptions and calcifications, mesio-distal and vestibulo-lingual X-rays of each root teeth were performed using DF-58 intraoral radiographic units (Ultra-Speed, Carestream Dental), setting at 8 mA, 70 kV, exposure time 0.1s and developed with automatic

machine (Periomat Plus, DURR DENTAL). Subsequently, all samples were further examined using the surgical operating microscope (SOM) (M320, Leica) to exclude the presence of caries, root restorations, blasting apices, fractures or cracks.

Endodontic root canals preparation and filling

Not being influential for the purposes of the study, the crown of single tooth was removed at the amelo- cement junction (CEJ) by a diamond bur (Komet 856 314 012) mounted on a high-speed handpiece under continuous water spray (Fig. 1).

Once the endodontic root canal pervious was evaluated and the working length was determined through K-file #08 and K-file #10 (MICRO-MEGA), the channel was prepared with Ni-Ti instruments (MTwo, Sweden & Martina). The sequence of instruments used was: 10/04, 15/05, 20/06 and 25/06. The instrumentation was finalized with Ni-Ti apical instruments (MTwo Apical, Sweden & Martina) using A2 and A3 sequence. Irrigation was carried out after each sequence instruments, with 2 ml of 5% sodium hypochlorite (NICLOR 5, OGNA) followed by physiological solution. At the end of the preparation and to remove the smear layer, an additional irrigation was carried out for 1 minute with 5 ml of 17% ethylenediaminetetraacetic acid (EDTA, OGNA) followed by a final wash with 5 ml of 5 % sodium hypochlorite before and irrigation with physiological solution after. The canal was dried with paper cones to proceed with the orthograde filling technique chosen



Fig. 1. Sample teeth after crown removal.

and adapted a master cone of gutta-percha of 02 conicity (Gutta Percha points, Inline) and coated with endodontic cement (Argoseal, OGNA), the procedure was finalized by introducing plasticized gutta-percha according to the endodontic technique M-Seal (MicroHeat System, Sweden & Martina) until the root canal is completely filled. Once the apical seal was obtained, the coronal access of the single tooth was sealed with composite (Tetric EvoFlow A2, Vivadent Ivoclar) after mordant with 37% phosphoric acid and subsequent application and photopolymerization of the dual hardening adhesive (ExcITE F DSC, Vivadent Ivoclar).

Retrograde cavity preparation and filling

The root was dissected, perpendicular to the longitudinal axis of the tooth, 3 mm from the root apex with an angle of 0 degrees using a diamond bur (Komet, 856 012) mounted on a high-speed handpiece under a continuous water spray (Fig. 2).



Fig. 2. Sample tooth after orthograde filling and root sectioning 3 mm from the apex. SOM documented.



Fig. 3. Control of the 3 mm depth of the root retrograde preparation with periodontal probe. SOM documented.

Retrograde cavity was prepared with steel ultrasonic tip (CT-1-S Straight, Kerr Dental) mounted on piezoelectric ultrasonic unit (Newtron Booster, Acteon) at a 3 mm depth parallel to the root longitudinal axis under abundant water jet. The depth of the retrograde cavity was checked with a calibrated periodontal probe (Fig. 3) and properly dried with an air/water syringe.

Retrograde filling materials (MTA Flow, Ultradent; Biodentine, Septodont; ProRoot MTA, Dentsply; Aureoseal, Ogna) were handled and prepared according to the manufacturer's instructions. Then, we proceeded to the retrograde cavity filling helping, in the correct compaction of the material, with a microplugger (B&L Biotech). The procedures for preparing and filling the retrograde cavity were performed by a single operator using the surgical operating microscope (M320, Leica) adjusted to a medium magnification (8x-16x).

The study tooth samples were randomly divided into:

- Group A: MTA Flow (Ultradent) (Fig. 4 A);
- Group B: Biodentine (Septodont) (Fig. 4 B);
- Group C: ProRoot MTA (Dentsply) (Fig. 4 C);
- Group D: Aureoseal (OGNA) (Fig. 4 D).

In addition, samples tooth X-rays were performed at the end of the retrograde filling to verify the uniformity, density and quality of the seal (Fig. 5).

The roots were stored in a chamber at 100 % humidity for 72 hours to allow complete adaptation of the material. Then, the samples tooth was coated with two layers of insulating polish (Fig. 6) except for the last apical 3 mm and subsequently were immersed in a 2% basic dye Methylene Blue for 48 h (Fig. 7).

After removing the two polish layers with manual scaler (Hu-Friedy), they were rinsed under spray water to remove excess dye.

Assessing marginal adaptation method

The roots have been incorporated, in the first millimetres of the coronal part, in thermopolymerizing resin and on a hard plaster base to allow a correct positioning on the stereomicroscope and on the isoparallelometer. The root apexes, with retrograde filling, were examined at 80x magnification using a stereoscopic microscope (SZR-10, Optika) and microphotographed by a Digital Single-Lens Reflex (DSLR - D90, Nikon). Subsequently, the samples were dissected under abundant irrigation, obtaining transverse resections at 1 mm and 2 mm from the root

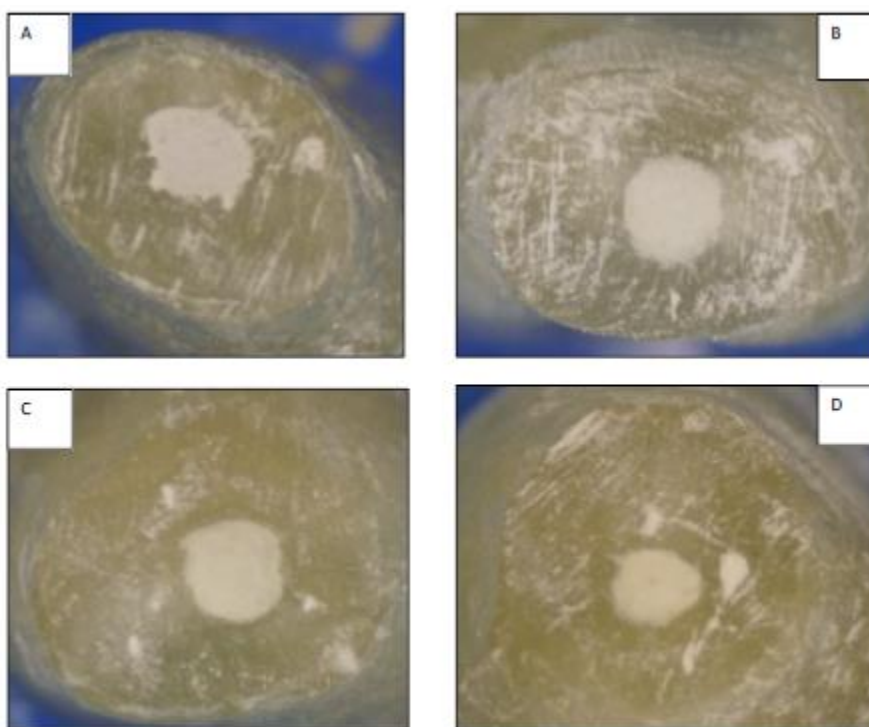


Fig. 4. SOM images of retrograde cavities sealed with retrograde filling materials. (A): MTAFlow, Ultradent; (B): Biodentine, Septodont; (C): ProRoot MTA, Dentsply; (D): Aureoseal, OGNA.

apex using separator discs (Dynex, Renfert) on a straight high-speed handpiece mounted on the isoparallelometer to obtain a precise and predictable cut on each sample tooth (Fig. 8). Also, the transverse resections obtained at 1 and 2 mm from the radicular apex were examined at an 80x magnification by stereoscopic microscope (SZR-10, Optika) and microphotographed using Digital Single-Lens Reflex (DSLR) (D90, Nikon).

The analysis of the marginal adaptation of root-end filling materials was carried out by analysing microphotographs with a computer program for digital image processing (ImageJ 1.53a, National Institute

of Health, USA). The retrograde cavities have been delimited and analyzed to highlight the coloured areas on the apical resections (0 mm) from methylene blue dye after calibration in mm². On the surfaces of the transverse resections, the overall areas of the gaps at 1 and 2 mm, highlighted by the presence of the dye and characterized

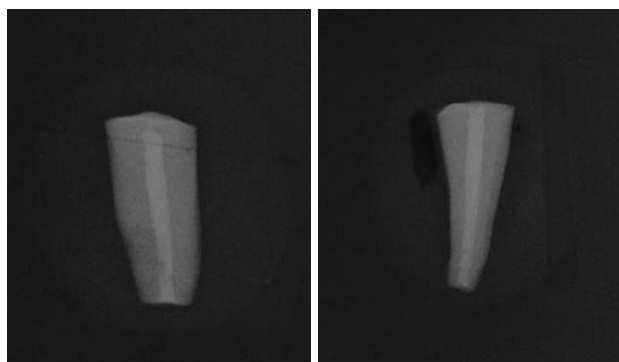


Fig. 5. Sample X-ray images after retrograde filling.



Fig. 6. Samples tooth coated with two layers of insulating polish.

by the internal voids and marginal gaps present in the dentine-material interface, were measured on the overall area of the third apical after calibration of the software to collect data in mm². Subsequently, the areas of internal voids and marginal gaps at 1 and 2 mm of apical resection were correctly delimited and analyzed in mm².

Statistical analysis

The data showed a normal distribution after performing

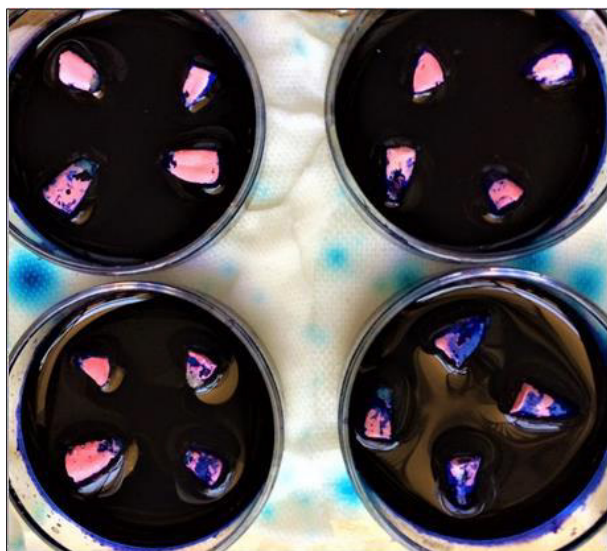


Fig. 7. Samples tooth immersed in a 2% basic dye Methylene Blue



Fig. 8. Study roots mounted on the isoparallelometer to obtain a precise and predictable cut.

Shapiro – Wilk tests and were therefore statistically analyzed through variance analysis (ANOVA) and Tukey's post-hoc test for multiple comparisons (Prism 7.0; GraphPad Software, Inc, La Jolla, CA) with a significance level of 5% ($P < 0.05$).

RESULTS

The results of root-end filling quality in terms of total gap area in 1 mm resections (Fig. 9, (Table I) reported a statistically significant difference in Group A (MTA Flow) compared to both Group C (ProRoot MTA) and Group B (Biodentine), with the latter characterized by a smaller gap area, while the upper gap area was recorded in Group A and Group C characterized by a total intermediate gap area between Group A and Group B. No statistically significant difference was found between Group B (Biodentine) and Group D (Aureoseal). The same trend was found regarding internal voids and marginal gaps at 1 mm, i.e., a statistically significant difference between Groups A and C with respect to Groups B and D and a statistically significant difference between Group A and Group C, with the presence of upper and lower marginal gaps and voids in Group A and lower in Group B and D, the latter with no statistically significant difference.

Slight differences in results were found in 2 mm resections (Fig. 10, Table II); in total gap areas and marginal gaps, no statistically significant difference was found both between Groups A and C and between Groups B and D and there was a statistically significant difference between Groups A and C and Groups B and D, the latter characterized by total areas and smaller marginal gaps. Regarding internal voids, the same trend is confirmed except for the presence of no statistically significant difference between Group B and Group C, with Group D characterized by fewer internal voids and Group A confirming itself as the worst group in terms of sealant capacity with greater internal voids.

Almost confirming the results on the gap areas in 1 mm resections, the areas coloured by methylene blue staining (MBS) on the apical resections (0 mm) (Fig. 11, Table III) reported a statistically significant difference of Group A (MTA Flow) with respect to both Group

C (ProRoot MTA) and Group B (Biodentine) and no statistically significant difference between Group B (Biodentine) and Group D (Aureoseal), characterized

by smaller colored areas than Group A (MTA Flow) and Group C (ProRoot MTA), the latter characterized by the largest colored area.

Table I. Averages and standard deviations (mm²) of gaps on 1 mm resection surfaces.

	Groups			
	A (MTA Flow)	B (Biodentine)	C (ProRoot MTA)	D (Aureoseal)
Total area of gaps and voids	0,308±0,2458 ^a	0,041±0,0226 ^b	0,079±0,0320 ^c	0,041±0,0202 ^b
Interna				
I	0,232±0,1894 ^a	0,004±0,0045 ^b	0,011±0,0135 ^c	0,006±0,0115 ^b
Voids				
Marginal				
Gaps	0,076±0,0615 ^a	0,036±0,0250 ^b	0,068±0,0334 ^c	0,034±0,0228 ^b

The different letters indicate a statistically significant difference ($P < 0.05$).

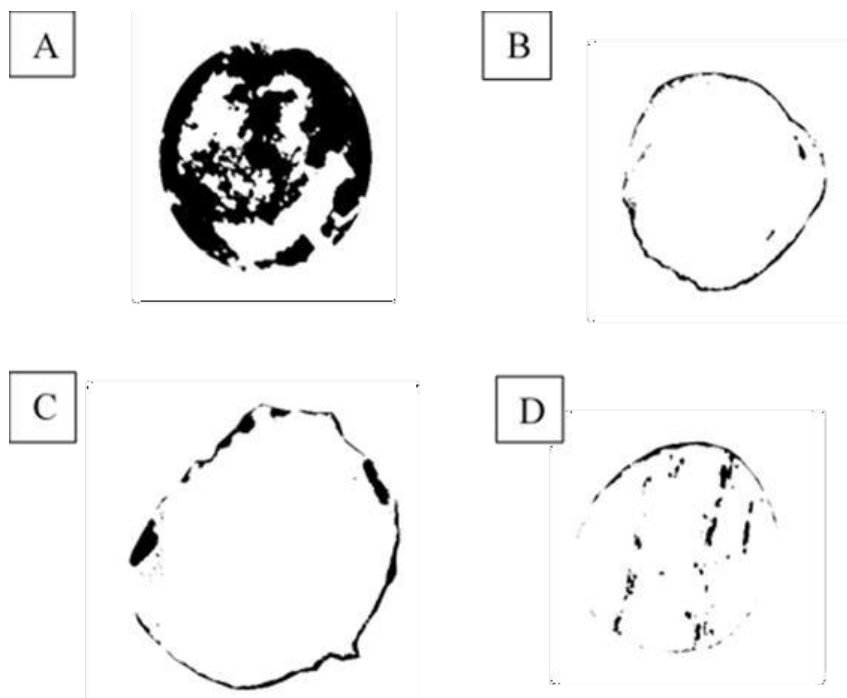


Fig. 9. Image of the total gap areas highlighted on the surfaces of 1 mm resections with ImageJ software. (A): MTA Flow, Ultradent; (B): Biodentine, Septodont; (C): ProRoot MTA, Dentsply; (D): Aureoseal, OGNA.

DISCUSSION

The main objective in surgical micro-endodontics (EMS) for achieving long-term therapeutic success is to realize, after a correct cleansing of the root canal

system, a three-dimensional filling that seals the endodontic space preventing the entry and proliferation of bacteria and bacterial toxins (7). The quality of the apical seal obtained from root-end filling materials has been evaluated by different methods, mainly by

Table II. Averages and standard deviations (mm²) of gaps on 2 mm resection surfaces.

	Groups			
	A (MTA Flow)	B (Biodentine)	C (ProRoot MTA)	D (Auroseal)
Total area of gaps and voids	0,144±0,1190 ^a	0,031±0,0218 ^b	0,103±0,0905 ^a	0,043±0,0134 ^b
Internal				
Voids	0,047±0,0634 ^a	0,019±0,0169 ^b	0,026±0,0216 ^b	0,008±0,0075 ^c
Margina				
I Gaps	0,097±0,1338 ^a	0,011±0,0062 ^b	0,077±0,1045 ^a	0,034±0,0180 ^b

The different letters indicate a statistically significant difference ($P < 0.05$).

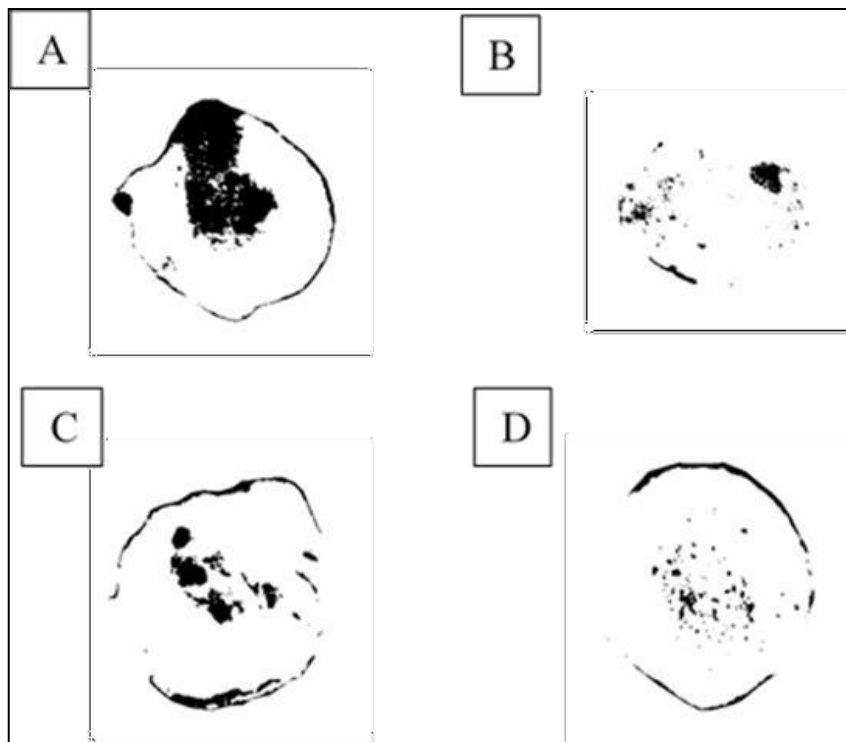


Fig. 10. Screenshot of total gap areas highlighted on 2mm resection surfaces with ImageJ software. (A): MTA Flow, Ultradent; (B): Biodentine, Septodont; (C): ProRoot MTA, Dentsply; (D): Aureoseal, OGNA.

techniques of colouring infiltration, fluid infiltration, protein infiltration and bacterial penetration. The dye penetration technique is the method most frequently used to evaluate the sealant capacity of dental materials and, of course, the blue methylene dye is widely chosen for convenience, easy availability and

for its reduced molecular weight that gives it a high degree of penetrability. In addition, methylene blue is very soluble in water, easily penetrates into the interstitial fluids of the tooth and does not absorb on apatite crystals. (2, 8).

Most in vitro studies using dye penetration

Table III. Average and standard deviations (mm²) of areas colored by methylene blue staining (MBS) on apical resections (0 mm).

	Groups			
	A (MTA Flow)	B (Biodentine)	C (ProRoot MTA)	D (Auroseal)
MBS colored area on apical resection (0 mm)	0,474±0,1973 ^a	0,320±0,1681 ^b	0,633±0,2108 ^c	0,363±0,1691 ^b

The different letters indicate a statistically significant difference ($P < 0.05$).

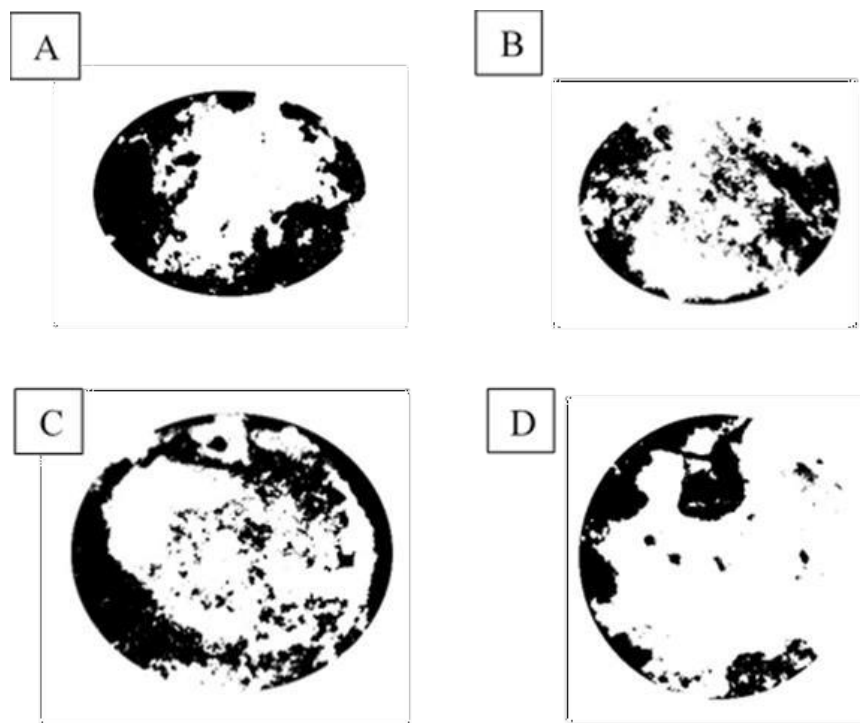


Fig. 11. Image of methylene blue colored areas on apical (0 mm) resections analyzed with ImageJ software. (A) MTA Flow, Ultradent. (B) Biodentine, Septodont. (C) ProRoot MTA, Dentsply. (D) Aureoseal, OGNA.

techniques evaluate samples after making a longitudinal cut along the root axis to measure the length of dye infiltration along the stereomicroscope retrograde filling material, but do not evaluate perimeter infiltration and marginal gaps in the endodontic dentine-cement interface; instead, the transverse resections at 1 and 2 mm, correctly performed with the help of the isoparallelometer, made it possible to evaluate the presence of the dye in the internal voids and marginal gaps to the material or to highlight the gaps even where the dye had not penetrated. (4, 9, 10).

ImageJ software was calibrated to the correct grayscale range (0-255) that highlighted the colored areas on endodontic concrete from methylene blue, the internal voids of root-end filling material, and the marginal gaps in the dentine-material interface, after converting images from 8-bit JPEG format and set to measure gaps in square millimeters (mm²) (11).

MTA Flow (Ultradent) is a calcium silicate cement introduced in 2017 composed of a powder of about 50% tricalcic silicate (Ca₃SiO₅) for about 30% bismuth oxide (Bi₂O₃) about 20% from bicalcium silicate or belite (Ca₂SiO₄) and about 5% from calcium sulfate (CaSO₄) and a water-based gel solution.

MTA Flow showed remarkable alkalizing capacity, low solubility, good radiopacity, ability to form calcium phosphate deposits after being immersed in a simulated body fluid and induced the formation of mineralized tissue after being implanted into subcutaneous rat tissues, showing bioactivity and biocompatibility values similar to ProRoot MTA, Biodentine and Angelus MTA. (12, 13). Biodentine (Septodont) is a calcium silicate cement, alternative to MTA, introduced in 2008 and available in the form of a capsule containing the ideal ratio of powder to liquid. The powder consists of tricalcic silicate (Ca₃SiO₅) for 80.1%, bicalcic silicate (Ca₂SiO₄), calcium carbonate (CaCO₂) 14.9% as a filler, zirconium oxide (ZrO₂) for 5% as radio-opacifying and iron oxide as a dye agent. The liquid contains water, calcium chloride (CaCl₂) (14) as an accelerant agent and water-soluble polymer as a water reducing agent.

Biodentine is distinguished by the mixing between the two components that takes place mechanically, through the help of a dental amalgamator, and, in addition, the setting time is only 10.1 minutes

due to the presence of calcium chloride in the liquid. Another feature of Biodentine is the interaction with dentine: a study with laser-scanning confocal electron microscopy revealed that Biodentine penetrated the dentine tubules forming tag-like structures. In addition, scanning electron microscopy revealed that dentinal tubules appeared with mineralization crystal caps just below the interface. These results may explain the micromechanical retention of the material on the one hand and marginal sealing on the other. The excellent results of hermeticity of this cement as a root-end filling material are confirmed by in vitro studies present in the literature when Biodentine is compared with MTA cements. (5, 11, 15).

ProRoot MTA (Dentsply) is a calcium silicate cement, introduced on the market in 1997 and composed of a powder 75% portland cement, i.e. tricalcic silicate, bicalcic silicate and tricalcic aluminate, 20% bismuth oxide and 5% sulfate calcium bihydrate or gypsum, as well as some trace elements. In-vitro studies on the ultrastructure of the MTA-dentine interface have shown the formation of a mineral-rich interface and a tag-like structure that extends into dentine tubules that could be responsible for the good marginal seal. Thus, it is amply demonstrated by numerous in vitro studies that ProRoot MTA has an excellent sealing capability compared to alternative root-end filling materials, such as amalgam, IRM and SuperEBA, but only when the correct setting time is reached, which usually occurs after 210-320 minutes and is influenced by the powder/liquid ratio, time that increases with the increase in water volume.

While salivary contamination during the placement of the ProRoot MTA also showed an increase in bacterial infiltration. Following the manufacturer's instructions, ProRoot MTA demonstrated the good quality of the three-dimensional seal obtained when used as a root-end filling material, supported by numerous in-vitro studies conducted with various sealant capacity assessment techniques. (2, 16-21)

Aureoseal (OGNA) is a calcium silicate cement, introduced in 2004, which differs in the presence in the formulation of apatite [Ca₅(PO₄)₃OH] and calcium tungstate (CaWO₄), a radiopacifying agent along with bismuth oxide (Bi₂O₃) present in a lower percentage, when compared to ProRoot

MTA. In addition, Aureoseal demonstrated the same biocompatibility as ProRoot MTA in an in-vivo study and showed high antimicrobial activity higher than ProRoot MTA and comparable to calcium hydroxide. The results of a case series characterized by a new surgical endodontic approach were promising using, as endodontic sealant cement, Aureoseal, as long-term clinical success was found (follow-up at 12 and 33 months). (22- 25). The results of the in-vitro study are probably due to the type, method of mixing, consistency and setting time of the materials.

These root-end filling materials are derived from Portland cement, therefore characterized mainly by the presence of calcium silicates in the composition but seem to differ in consistency after mixing the correct powder-liquid ratios recommended by the manufacturer, which can lead to different results in the compaction of the material, worse in the case of MTA Flow and better in the case of Biodentine and Aureoseal. In particular, Biodentine differs from other materials in the method of mixing powder-liquid components and in the setting time; MTA Flow, ProRoot MTA and Aureoseal are endodontic cements that mix manually on a glass plate and with the help of a spatula, while Biodentine is characterized by mechanical mixing inside a capsule with the correct powder-liquid ratio that is inserted into an amalgamator set to 4000 rpm for 30 seconds that facilitates and speeds mixing on the one hand and improves handling properties compared to manually mixed silicate cements on the other. In addition, the fastest setting time of Biodentine, which occurs in 10.1 minutes due to the presence of an accelerant agent such as calcium chloride (CaCl_2) in the liquid component, is an advantage over other root-end filling materials, since a longer setting time can increase the risk of partial infiltration of the material and alteration of the apical seal (2, 26).

Finally, the results include values of internal voids greater in 2 mm resections than 1 mm resections in samples filled with Biodentine, ProRoot MTA and Aureoseal that could be attributed to the different compaction difficulties of the respective materials.

CONCLUSION

Within the limits of this in-vitro study, the evaluation

and analysis of the average areas of internal voids and marginal gaps revealed that the apical sealing ability of Biodentine and Aureoseal proved superior to MTA Flow and ProRoot MTA when used as root-end filling materials in micro-surgical endodontics.

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