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MRI FINDINGS OF WORKING AND NON-WORKING TMJ DURING UNILATERAL MOLAR CLENCHING ON HARD BOLUS

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The aim of this study is to evaluate with MRI the TMJs behavior during one-side clenching on a hard bolus. The sample consisted of 13 patients. an MRI of TMJ using a 1.5 Tesla superconducting magnet and a dedicated surface coil was performed in all patients. Parasagittal scans of the TMJ (perpendicular to the long axis of the condyles) were made with the mouth closed, open and with a plastic thickness 10 mm high, 15 mm wide and 20 mm long always interposed between the molars of the left side; TSE T2W and DPW sequences were used. The working condyle is always positioned behind the non-working contralateral condyle and the morphology of the retrodiscal tissue upper lamina was curvilinear in all TMJs examined. This work confirmed that, during clenching on a unilateral hard bolus, the working condyle translates less than the balancing one and that the condyle-disc ratios are substantially the same in both sides. The study of the retrodiscal tissue imaging in the different functional phases, suggest that it does not exert any pulling force towards the disc.

The TMJ's movements during chewing have been studied over the years by many authors (1-3), also for the importance in prosthetic implant rehabilitation (36-41). The TMJs move in a reciprocal movement, acting alternatly as a working or balancing TMJ (4).

In the case of mainly one side chewing function, the working TMJ is subjected to a functional overload that leads to joint compensation or the onset of a dysfunctional symptomatology (5). In the literature it's know that the working condyle path is less than the controlateral one during chewing (6, 7).

Regarding the type of compressive forces acting on the TMJ, at closed mouth position, in the working TMJ, the strongest load is distributed on the posterior and medial part of the disc as highlighted by Miyawaki et al in a 2001 study (8, 51-58).

The association between habitual unilateral

chewing and TMD may suggest an excessive load on the working side TMJ, as described in the studies of Agerberg and Carlsson in 1975 (9), Tay et al in 1989 (10) and Kumay in 1993 (11). An occlusal inconsistency leads to periodontal problems (20-35, 61-64). The compressive forces were stronger on the contralateral side during one-side teeth clenching on a point of a molar as described by Faulkner et al in 1987 (12) and Koriath in 1997 (13).

On the contrary, the compressive forces were greater on the working side than the balancing one in a study carried out on a dynamic model by Langenbach and Hannam in 1999 (14).

Finally, as reported by Naeije e Hofman (15) in a 2003 study, the fact that the working TMJ is subjected to less compressive forces than tne balancing one may explain why patients with joint pain occasionally

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report less pain while chewing on the aching side. In the literature there isn't MRI study about chewing physiology. Therefore, the aim of this study is to evaluate with MRI the TMJs behavior during one-side clenching on a hard bolus.

MATERIALS AND METHODS

After a dental examination, a first sample consisting of 20 patients who had no signs and / or symptoms of TMD was selected (RDC / TMD). At the Dept. of Radiology of the Policlinico Umberto I of Rome an MRI of TMJ using a 1.5 Tesla superconducting magnet (Siemens - Erlangen, Germany) and a dedicated surface coil was performed in all patients. Seven of the 20 asymptomatic patients were excluded because they had at least one TMJ with reducible disc dislocation (RDD) and a moderate or severe degree of retrodiscal tissue fibrosis. Therefore, the sample consisted of 13 patients (4 males and 9 females) with an average age of 24 years, who presented only an initial fibrosis of the retrodiscal tissue.

Parasagittal scans of the TMJ (perpendicular to the long axis of the condyles) were made with the mouth closed, open and with a plastic thickness 10 mm high, 15 mm wide and 20 mm long always interposed between the molars of the left side; TSE T2W and DPW sequences were used (TR 2500-3000, TE 80-100 and 14, duration 2 '30', FOV 160, matrix 256 x 256, thickness 3mm, gap 0).

In the scans made with the plastic spacer tight between the molars, the anterior-posterior position of the condyle was assessed by measuring the distance, expressed in mm, of the most anterior point of the condyle in closed mouth position and with the spacer between the molars, compared to the highest point of the mandibular fossa. Only the median scan of the package perpendicular to the long axis of the condyle was considered in both the working and non-working side.

The position of the condyle under the disc was assessed anterior (A) if the condyle pushed on the front area of the disc, intermediate (I) if the condyle pushed on the intermediate area of the disc and posterior (P) if it pressed on the posterior band of the disc. The minimum distance between the mandibular and temporal condyles was measured using the Merge eFilm Workstation 2.00 software (Milwaukee, Wisconsin USA) which allows measurements with a millimeter scale.

The following parameters were therefore assessed in the MR scans during clenching:

- 1) The average of the measurements of the minimum distance between the mandibular condyle and the articular tubercle in the working and balancing side.
- 2) the average of the measurements of the translations of the working and non-working condyles and of their difference;
- 3) The position of the working and non-working condyle below the respective articular disc;
- 4) The morphology of the retrodiscal tissue (curvilinear or rectilinear).

Table I. Working (*left*) and non-working (*right*) condyle's traslation from closed mouth position (in mm) and difference between the values.

Non working side	Working side	Difference
8	6	2
14,5	4,54	9,96
10,1	3,3	6,8
5,16	3	2,16
12,8	5,71	7,09
8,8	3	5,8
7,7	4,44	3,26
7,5	6,66	0,84
5,5	1,5	4
7,69	6,92	0,77
7,14	2,85	4,29
3,33	3,07	0,26
11,25	6,25	5
2,5	1,87	0,63
10,1	2,5	7,6
9,33	4,37	4,96
5,5	2	3,5
4,37	3,12	1,25
8,46	8,33	0,13
8,75	4,37	4,38
8,75	5,71	3,04
7,85	4,28	3,57
8,33	5,38	2,95
7,65	4,38	3,27
8,12	4,98	3,14
7,03	5,82	1,21

RESULTS

In the cases examined, the working condyle is always positioned behind the non-working contralateral condyle. The average translation of the non-working condyles was 7,92 mm while that of the working condyles was 4,39 mm and the average of their difference was 3,53 mm (Tab – 1). Regarding the condyles' position respect the discs there was no substantial differences between the two sides: the working condyle was positioned 3 times under the posterior band of the disc and 10 in the intermediate part while the non-working condyle was positioned 2 times under the posterior part of the disk and 11 in the intermediate zone.



Fig. 1. Interdental spacer of 10x15x20 mm used in scans during clenching.

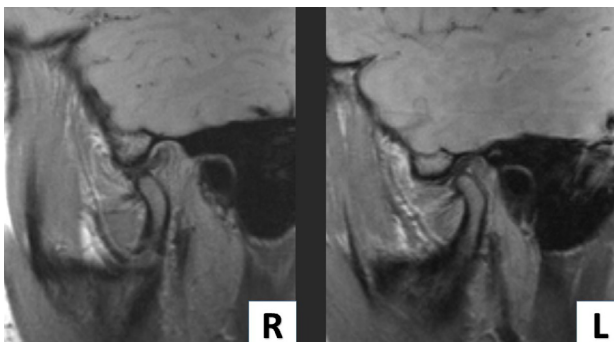


Fig. 2. TSE DPW parasagittal scans. Right and left TMJ with spacer.

The average of the measurements of the distance between the condyle and the tubercle on the working side was 2.1 mm while in the balancing side it was 1.8 mm. Finally, the morphology of the retrodiscal tissue was curvilinear in all TMJs examined.

DISCUSSION

The analysis of the anterior translation of the condyles confirms the data present in the literature (6, 7): the working condyle translates much less than the balancing condyle (just over half). The relationship between the position of the condyle and the respective disc highlights the fact that the condyle is almost always positioned below the intermediate area (I) of the disc and only in 15-23% of cases (respectively in the balancing and working side) is positioned under the posterior area (P) of the disc.

However it should be underlined that the upper belly of the MPL comes into tension in particular at closed mouth position and in the eventual posterior dislocation of the condyle inside the mandibular fossa (16), and especially during clenching on a hard bolus as reported in this study. To properly evaluate the type of insertion of the upper belly of the MPL it would be useful to evaluate it with the condyle positioned below the articular tubercle and with a parasagittal scan plane inclined medially and close to the medial pole of the condyle, but to make a more reliable measurement of the condylar movement, a point located halfway along the length of the long axis of the condyle was taken with a scan plane perpendicular to this, where the upper belly of the MPL is not always clearly visible (17). The distance evaluation between the condyle and the tubercle highlights a slightly greater amplitude of the joint space in the working side, this influences any conservatived and endodontic treatments (42-50, 59, 60).

The most significant datum is certainly the one concerning the retrodiscal tissue that shows always a curvilinear morphology (18), proving that it does not exert any type of pulling force on the disc in both working and non-working side, unlike what is often stated in the literature about a possible pulling action exerted on the disc by the upper lamina (19). However, these results are

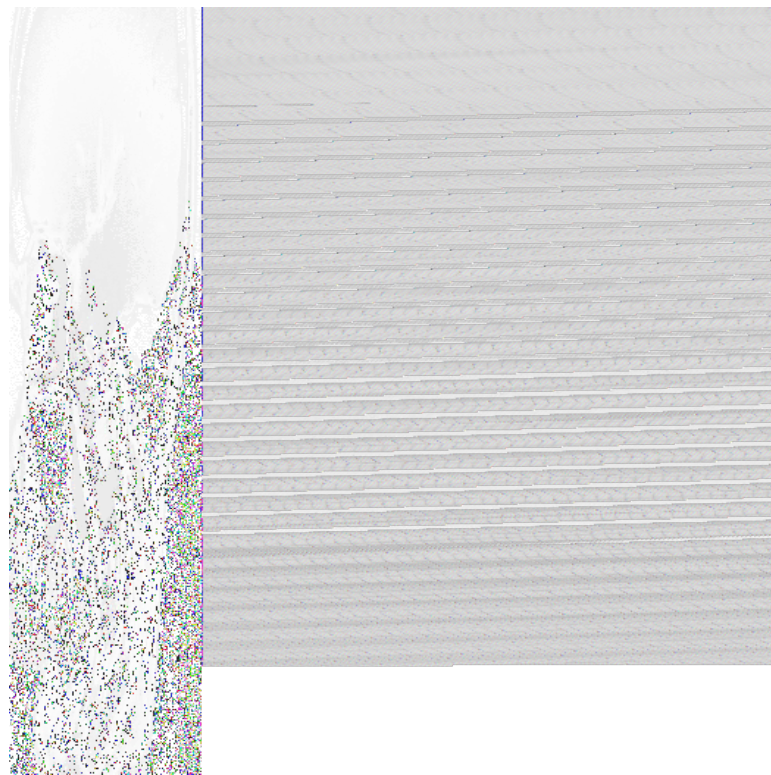


Fig. 3. *Spacer (white arrow) in the left side used in the scans during clenching.*

influenced by the small sample analyzed and also by the software used for intracicular measurements. Only the sagittal scan plane was used because it was the one that allowed the measurement of the translation of the condyles in the most reliable way.

To better evaluate the action of the upper belly of the MPL it would be necessary to carry out a study with a thinner thickness, a medially inclined scan plane and a larger study sample considering its insertion variability on the anterior wall of the capsule and condylar neck. .

This work confirmed that, during clenching on a unilateral hard bolus, the working condyle translates less than the balancing one and that the condyle-disc ratios are substantially the same in both sides.

The study of the retrodiscal tissue imaging in the different functional phases, in particular the conservation of its curvilinear morphology, suggest that it does not exert any pulling force towards

the disc. Finally, the average distance between the condyle and the tubercle is slightly less in the working joint.

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COMPUTER - GUIDED IMPLANT SURGERY: ANALYSIS OF DYNAMIC NAVIGATION SYSTEMS AND DIGITAL ACCURACY

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After the static computer-guided implant surgery, borns the concept of “navigated implantology” (NI) that offers significant advantages in the treatment planning and help clinicians to perform successful implant rehabilitation. NI allows an optimal final location, to avoid the risk of damaging the noble anatomical structures near the site and allows the achievement of satisfactory aesthetics. The aim of this study is analyze three different systems: the RoboDent® system (Berlin, Germany, 2001), today not for sale, was the first implant navigation system introduced on the market and made the history of dynamic surgery; X-Guide (Nobel Biocare, 2017), a dynamic computer assisted system that uses the principles of stereoscopic triangulation by optical video cameras. And last, the Navident® dynamic navigation system (Toronto, Canada, 2015), produced by the Canadian company ClaroNav, evolved from the Navient brand used in orthopaedic surgery, neurosurgery and otolaryngology, sharing the motion tracking technology. Dynamic computer assisted surgery systems allow more accurate implant placement when based on an accurate 3D CT-based image data and an implant planning software which minimizes errors and simplifies the surgical technique.

Dynamic (or “virtual”) navigation surgery is common in various branches of medicine, such as craniomaxillofacial surgery, neurosurgery and orthopaedic and vertebral surgery. Satellite navigation systems are based on motion tracking technology, commonly identified with a micro camera used as a tracker, also for orthodontic treatment and endodontic and conservative procedures (1-19). The transition from computer guided planning to the operative phase using navigation and frameless localization techniques therefore allows the surgeon to see in real time the position of the instrument on the reconstructed set of three-dimensional images of the patient.

The first attempt was a “mechanical navigation

system”, the Viewing Wand-System (ISG Technologies, Mississauga, Ontario, Canada), introduced in 1993. Systems driven by arms had specific disadvantages; therefore, frameless methods that didn't work with the mechanical connection, but based on the principle of satellite navigation, for example, through ultrasound or light emission, were increasingly developed (20). Now this technology is also available in dentistry for management of oral disease (21-39).

Starting from the concept of prosthetically guided implantology, with the advent of new imaging technologies applicable to the dental field, such as Cone Beam Computed Tomography (Cone Beam), in addition the use of dedicated software

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has developed the concept of “computer guided implantology”. Conventional treatment methods have therefore been transformed in order to simplify the pre-surgical planning of the treatment, which still maintains the principle of prosthetically guided planning, but allow virtual simulation of the surgery. Implant insertion can be performed in a simplified way with the creation of surgical templates, which can also be used in the diagnostic phase (40-42).

It was Fortin et al., in the mid-90s, who described the concept of navigation in oral surgery and dynamic guide during the surgical step. In dentistry, for example, its application can be used especially in the case of insertion of immediate post-extraction implants or extensive and complex implant-prosthetic rehabilitations, reducing the timing and increasing the accuracy of the surgical result (43-46) (Fig. 1).

MATERIALS AND METHODS

GPS device used in the cars’ driving system, is tracked by a satellite and guided along a predefined route planned on a map and a similar system is used in navigation systems, in which implant handpiece, with light-emitting diodes or passive reflective tracking elements, is tracked by an optical camera stereoscopic and guided along

an implant plane predefined on the patient’s computed tomography data (50-51). We studied and compared three different systems used in oral implantology and we focused on accuracy in dynamic computer guided dental implant surgery, in comparison with the conventional freehand method and static computer guided system.

RoboDent® passive system

The RoboDent® system (Berlin, Germany, 2001) was the first implant navigation system introduced on the market. Today it isn’t for sale, because it was based on the use of infrared light that hit pieces of quartz positioned both on the handpiece and on the patient’s arch which, reflecting the light, but didn’t allow a correct localization and perception of anatomical reality. In addition, the RoboDent® system used traditional CT, not the modern TC Cone Beam.

It was able to respond to all types of clinical situations that may happen in the implant site. The highly precise planning and navigation offered by RoboDent® made it possible to create the temporary prosthesis during the pre-surgical step and to carry out flapless technique, also allowing to modify the already planned surgical treatment in real time. The surgical planning with the RoboDent® software included three steps:

- in the phase one, six titanium indexes were found and marked in the navigation arch of the radiological

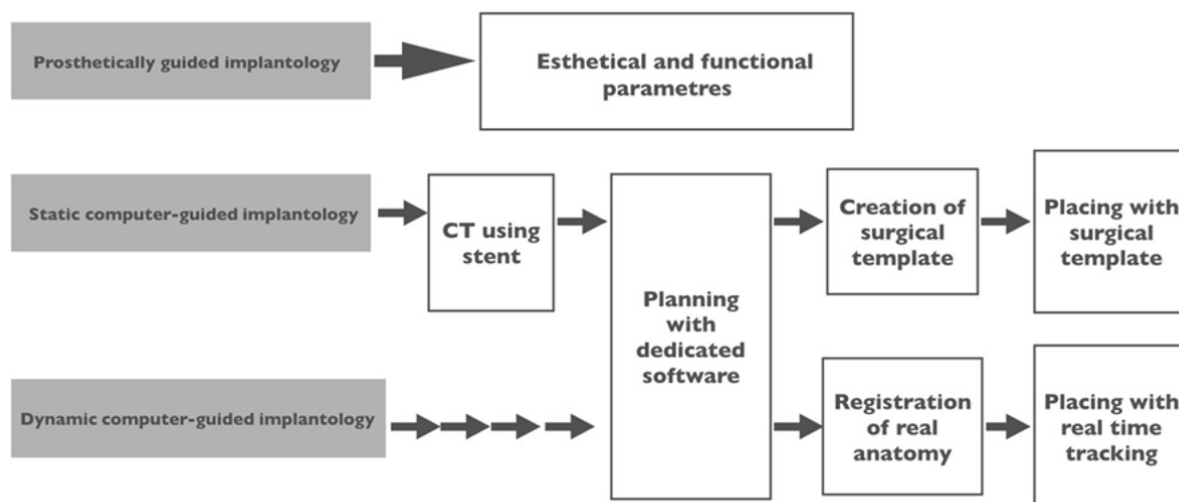


Fig. 1. development of three different guided techniques.



Fig. 2.

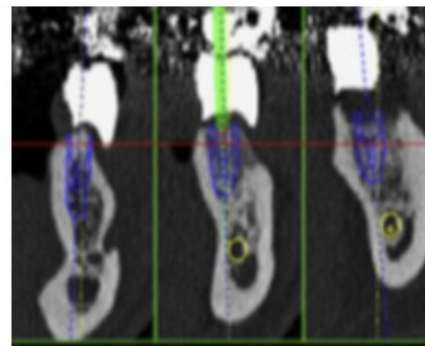


Fig. 3.

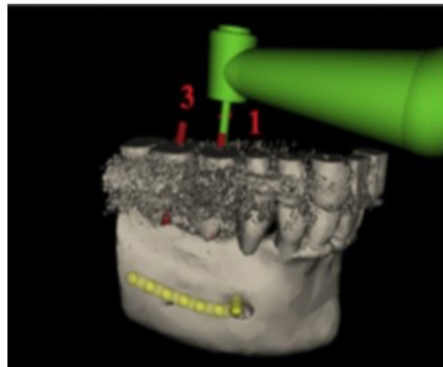


Fig. 4.

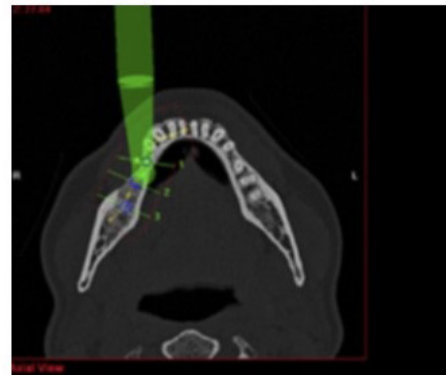


Fig. 5.

Fig. 2, 3, 4, 5. monitor images of three-dimensional positioning and 3D simulation during surgical step.

template coming from axial sections of CBTC, which, without artifacts given by the metal structures, were automatically detected by the software.

- The second step was represented by the determination of important anatomical structures, such as the lower alveolar canal and the mental foramen, the sinuses, the nasal cavities and the incisal foramen and finally, the implant positioning was virtually simulated considering the axial, panoramic and coronal sections and the 3D anatomical reconstruction. Since this three-dimensional positioning was performed with the chosen implant system, it was possible to view the axis of the fixture and the different distances between the elements involved (Fig.1).
- The third step was the surgical procedure using the dynamic navigation system RoboDent® (Fig. 2-5) (52-54).

X-Guide (Nobel Biocare®)

X-Guide is a dynamic computer assisted system based on the principles of stereoscopic triangulation by optical video cameras. During surgery, it dynamically tracks the movement of two dynamic reference frames (DRF), one rigidly fixed to the patient's surgical anatomy and one fixed to the surgical handpiece used by the implantologist. In totally edentulous, the system has to use mini screws like anatomic landmarks before the CBTC.

The DICOM file of the patient's CBTC is imported into the DTX Studio Implant system software that today supports a new operating flow, which allows not to use static surgical guides during the operating phase and to finalize the oral surgery with 3D navigation. During the surgery step, it's necessary to calibrate the DRF fixed to the surgical handpiece using the other DFR fixed in the oral cavity named E-Clip (Fig. 6).



Fig. 6. DFR fixed in oral cavity and surgical handpiece

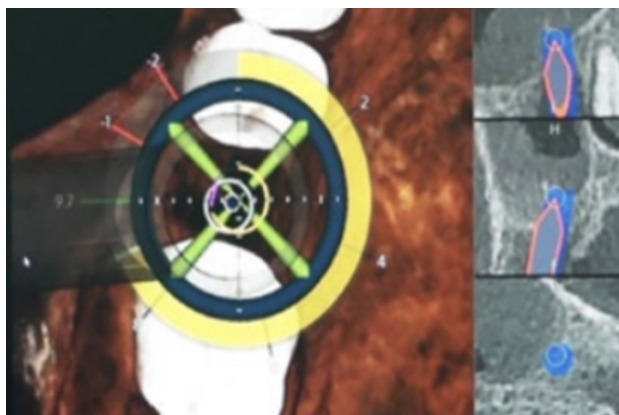


Fig. 7. Stage of calibration with color tracking

After stage of calibration, it's necessary to complete the last phase calibrating each drill used for implant site preparation as they are used and, during the surgical step, the X-Guide system offers color tracking of the preparation depth (Fig. 7) (55-57).

Navident® System (Toronto, Canada, 2015)

The Navident® dynamic navigation system (Toronto, Canada, 2015), produced by the Canadian company ClaroNav, offers an easy, very accurate, portable and economical way to plan the positioning of the implant and the final prosthetic restoration in a virtual way.

This innovative navigation system is composed by:

- a stereoscopic camera (MicronTracker);
- MacBook Apple® with operating Windows system that support the dedicated software (EvalNav);
- a CT- Marker;
- a thermoplastic stent (Navistent) or a contact touch probe (Tracer), due to the different protocol chosen;
- Drilltag (fixed on the surgical handpiece) and Jawtag (fixed in the patient's oral cavity);
- support for the Drilltag.

The workflow includes four phases: Stent or Trace, Scan, Plan and Place. (Fig.8)

RESULTS

Angle and depth of the implant fixture are most important values to have an optimal result for the functional rehabilitation, the healing of soft tissues and the osseointegration (58). Kramer et al. compared the precision limits of free hand technique vs. dynamic computer-guided systems like Jorba-Garcia et al. The result is that there is an improved precision of insertion surgery regarding the position,



Fig. 8 Navident workflow

angulation and depth of an implant, but we have higher precision with dynamic navigation technique, except for surgical time and vertical deviation of apical depth; however, if used by an expert surgeon, the navigation system only improves the angular deviation of the implant (59-60).

Analysing the precision of static computer-guided oral surgery vs. dynamic computer guided systems, it resulted that the movements of the surgical stent used in the static technique could cause differences in deviation during the preparation of the implant site and the support structures have a significant impact on the accuracy in static guided surgery (61). Surgical templates supported by mini-implants provide high accuracy in the positioning of implants and a lesser statistically significant precision has been demonstrated for bone-supported guides compared to other types of support (62).

Accuracy errors of computer-guided static implantology are reported in the range of about 0.3-1.6 mm or from 3° to 5°, usually exemplified in the following 3 different aspects of the final position implant: coronal deviation, apical deviation, angular deviation (63). Other clinical studies have recorded many important data in favor of implant insertion performed during navigation, reporting a deviation between the planned position and the real position superimposable or less than that obtained with the static technique (64).

Guided surgery seems to be more accurate and more precise in different high risk anatomic zone (65). The literature also describes an average error of navigation systems < 0.73 mm (66-67), overall accuracy of navigation systems is 0.96 ± 0.72 (68). Better results are also obtained in flapless surgery (69).

DISCUSSION

Today the progress of technologies, dental implantology, as well as other specialties of dentistry, can use increasingly innovative digital methods such as computer assisted design and production (CAD / CAM) used as auxiliary instruments in implantology and prosthetics, intraoral scanners for simplified taking of impression and 3D printers used to perform techniques in periodontal bone regeneration,

for the increase of the alveolar crest and/or for the conservation of post-extractive sites socket preservation (70). This digital flow, which also included modern computer-guided dynamic surgical technique, can improve accuracy by reducing the conventional errors that may be associated with traditional methods.

The RoboDent® system transferred the license of light infrared to Nobel Company and is used now in X-Guide Protocol. More updated is the Navident® by Claronav, it does not use infrared light but two micro cameras and has in common with Navient® the motion tracking technology.

This type of dynamic guide allows for implant surgery to change the surgical treatment plan in real time, while maintaining always both direct and indirect clinical vision on the structure monitor anatomical zone on where you are working. It can also be evaluated in real time mode the deviation from the preliminary planning, limiting any secondary prosthetic problems.

This modern technology has an important positive aspect in the medico-legal field given by the possibility of memorizing all useful data of each surgical case performed, starting from the CT DICOM file of the patient indicating an element indicative of the diagnostic phase, up to planning of the intervention carried out by means of the software and complete execution of the surgical act that are recorded through the micro cameras by the navigation itself.

In conclusion, 3D dynamic guide systems can require longer period for virtual planning, higher cost and the clinicians have to increase their digital skills. However, it clearly improves the implant surgery and the aesthetic results.

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APPEARANCE OF NORMAL MRI ANATOMY OF THE LINGUAL NERVE USING STEADY-STATE FREE PRECESSION SEQUENCES AT 3-T

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The aim of this study is to assess the value of SSFP MRI sequence in depicting the normal anatomy of the lingual nerve (LN), particularly in the molar region, in order to help the periodontists, dentists and oral surgeons in their daily practice. The study group included 24 patients who were to undergo MR study for a reason unrelated to our purpose. All imaging was performed by using a 3.0T system with a head and neck multiarray coil. The evaluation criteria included image quality factors such as the identification of the LN, its demarcation and its contrast to surrounding tissues on a five-point scale. The LN is clearly visible throughout its course from its origin from the mandibular nerve (MN) to the mylohyoid muscle. In edentulous patients, the LN could be damaged during surgical procedures specially it during the dissection and retraction of a lingual flap and, above all, during the suture due to a direct trauma caused by the needle or indirectly during tying the knot.

The knowledge of the course of the lingual nerve (LN) is important for preventing and reducing the incidence of traumas during oral surgery or anesthesia.

LN lesion may occur during periodontal and implant surgery (1), mandibular third molar region surgery (2) and specially in an atrophic mandibular crest (3-5), or following the use of laryngeal mask (6) and in relation to the local anaesthesia in mandibular injections (7).

LN injury may result in unilateral lingual anaesthesia and atrophy of fungiform papillae (8) and its damage is a common complication during oral surgery procedures in the third molar region.

Its course is variable, therefore the precise knowledge of anatomy of this nerve is important for decreasing the damage risk. Until now, the LN has been studied only on cadavers (9), and in vivo only with ultrasound (10).

A steady-state free precession (SSFP) sequence is any gradient-echo sequence in which a nonzero steady state develops between pulse repetitions for both the longitudinal and transverse relaxation values of the interrogated tissues. A small flip angle and short relaxation time are required for this to occur (11).

The clinical utility of an SSFP sequence lies in its ability to generate a strong signal in tissues that have a high T2/T1 ratio, such as cerebrospinal fluid (CSF) and fat. SSFP sequences are particularly useful for visualizing the cisternal segments of cranial nerves because they provide excellent contrast resolution between CSF and nerves, as well as high spatial resolution with sub millimetric section thicknesses (12, 13).

In the recent years, the intracranial segment of trigeminal nerve has been study with MRI with contrast-enhanced 3D constructive interference in

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the steady state (CISS) sequences, contrast-enhanced 3D time-of-flight (TOF) MR angiographic (MRA) sequences, 3D reversed fast imaging with steady-state precession (FISP) with diffusion-weighted MR sequences, 3D fast imaging employing steady-state acquisition (FIESTA) sequences and diffusion-based tractography by many authors (14-17).

On the contrary, the extracranial segment of mandibular nerve (MN) between the foramen ovale and the mandible is shown in only a few works and with a poor image quality (15, 18).

The use of new technologies has provided significant improvements in all dental procedures from radiological (21-23) and clinical (24-39) diagnostics to surgical therapies (40-49), to orthodontics (50-55) and finally to dental materials (56-70).

In the literature they are no studies that describe the MRI anatomy of the LN. Therefore, the aim of this study is to assess the value of SSFP MRI sequence in depicting the normal anatomy of the LN, particularly in the molar region, in order to help the periodontists, dentists and oral surgeons in their daily practice.

MATERIALS AND METHODS

Patients

The study group included 24 patients (9 men, 15 women; age range, 18 – 84 years; mean age, 49.5 years) who were to undergo MR study for a reason unrelated to our purpose. The patients gave their informed consent to undergo imaging with extra sequence. None of the patients reported a history of diseases related to cranial nerves and maxillofacial region, nor it had fixed dental prosthesis.

MR Imaging Procedures

All imaging was performed by using a 3.0T system (Discovery 750 GE, Milwaukee, WI, USA) with a head and neck multiarray coil. Parameters of FIESTA sequence were as follows: TR/TE = 4.9 ms/6 ms, flip angle = 35°, matrix = 288 X 288, FoV = 220 X 165 mm, slice thickness = 0,6 mm, number of 3D-partitions = 206, acquisition time = 2 minutes and 16 seconds.

Image Analysis

All images were analyzed and valuated collaboratively

by two authors (D.M., L.B.), who had 23 and 35 years of experience in oral-maxillofacial radiology, according to the criteria set in an evaluating form.

The evaluation criteria included image quality factors such as the identification of the LN, its demarcation and its contrast to surrounding tissues on a five-point scale (difficult to recognize 1, blurred 2, sufficient 3, good 4, excellent 5) on axial plane at the origin of the LN, medially to the ascending branch at the level of mandibular foramen and medially to the mandibular lingual cortex at the level of third molar, and on a frontal plane, only in this last site.

RESULTS

The LN is clearly visible throughout its course from its origin from the MN to the mylohyoid muscle. The LN runs parallel and in slightly anterior position to the MN until the mandible where it rotates forward and downwards in contact of the medial surface of the ramus above the insertion of the medial pterygoid muscle and then turns downwards and medially, passing below the superior constrictor muscle of pharynx, then, at the level of the third molar, he moves forward below the floor of the mouth, in contact with the medial surface of the mylohyoid muscle about 2 mm from alveolar bone.

The image quality at the level of the origin of LN (fig. 1) is excellent in the 87,5% (21/24) of cases examined and good in the 12,5% (3/24) of cases; at the mandibular foramen (fig. 2) the image quality is excellent in all the cases examined, while in the third molar site, on axial plane (fig. 3) the image quality is good in the 83,3% (20/24) of cases, sufficient in the 12,5% (3/24) of cases and blurred in only one case (4,2%) and on a frontal plane (fig. 4) is good in the 75% (18/24) of cases, in the 16,7% (4/24) of cases and blurred in the 8,3% (2/24) of cases.

DISCUSSION

With the SSFP sequence used in this study, the LN is clearly visible in the infratemporal fossa, thanks to the natural contrast between his hypointense signal within the hyperintense fat tissue and the very thin slices used; furthermore in third molar region too it's possible well identify the course of the LN to

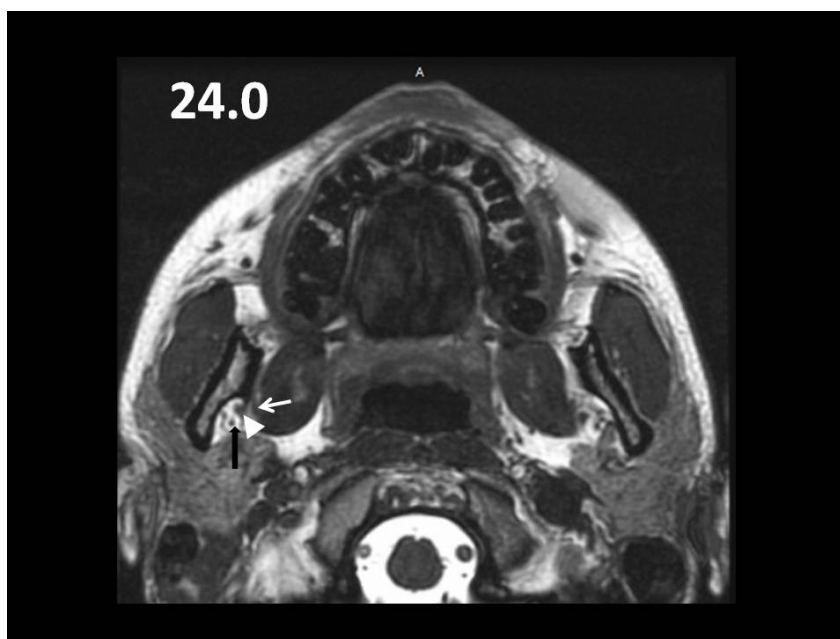


Fig. 1. FIESTA sequence on axial plane 10.2 mm below the foramen ovale. The short arrow shows the LN while the point arrow shows the MN.

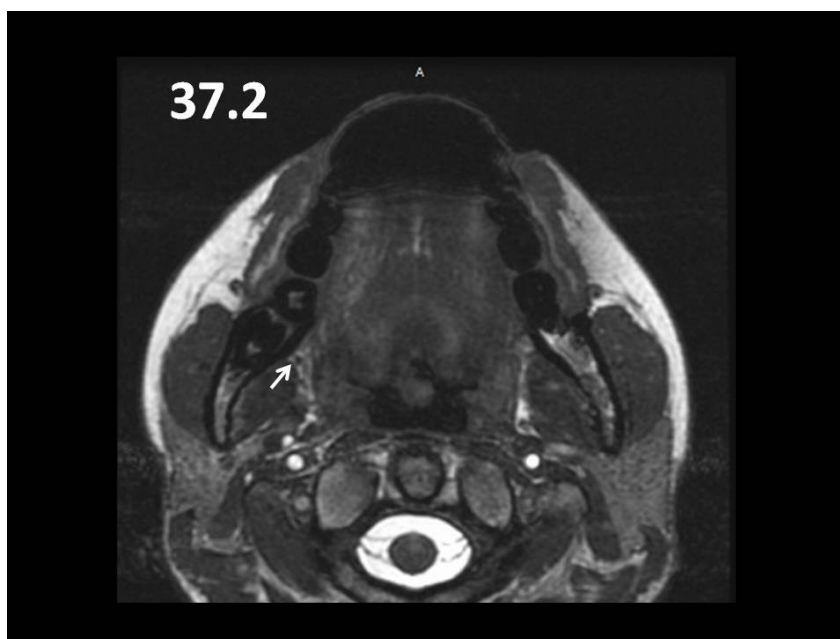


Fig. 2. FIESTA sequence on axial plane 24 mm below the foramen ovale. The white short arrow shows the LN, the point arrow shows the MN at the mandibular foramen while the black arrow shows the mylohyoid nerve.

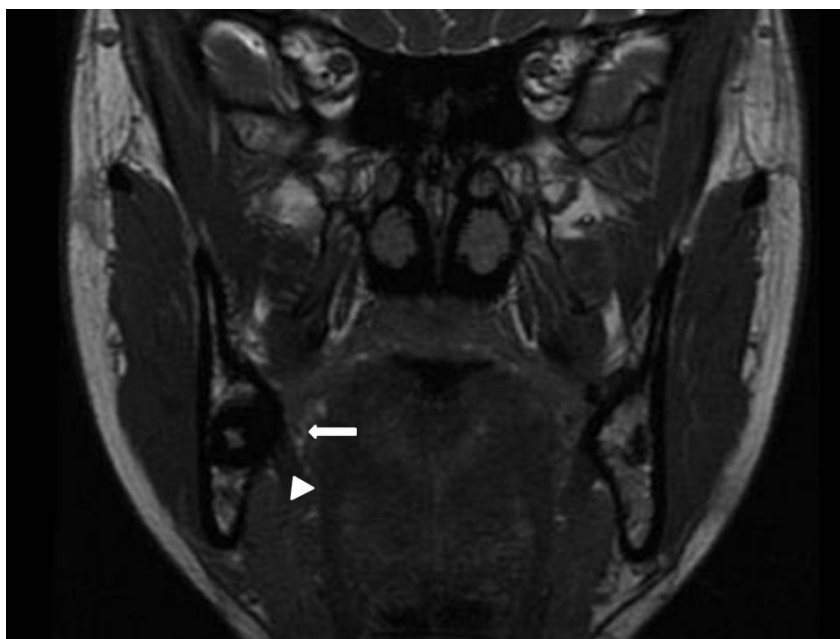


Fig. 3. FIESTA sequence on axial plane at the level of the third molar. The arrow shows the LN beside the mandibular lingual cortex.

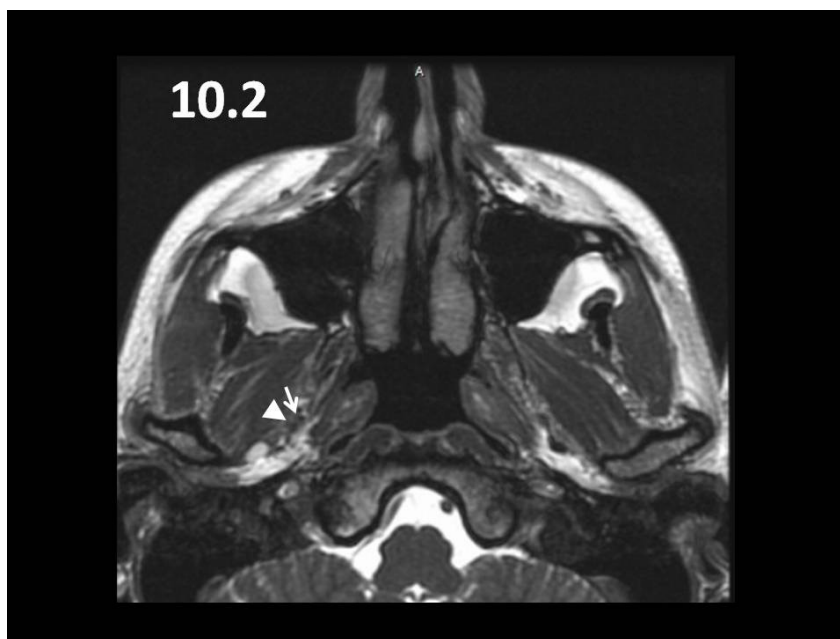


Fig. 4. FIESTA sequence on frontal plane at the level of the third molar. The short arrow shows the LN while the point arrow shows the mylohyoid muscle.

prevent nerve damage following surgical procedures, especially in edentulous patients in the posterior quadrants of the mandible.

Our sequence has always shown high-quality images with high signal-to-noise ratio (SNR) and excellent contrast between mandibular nerve and other soft tissues. The vessels show high signal intensity and this allows to highlight even small arteries like middle meningeal artery into the foramen spinosum. The others anatomical structures show the same signal intensity as in the turbo spin echo (TSE) T2-weighted sequences.

A shortened examination time may go hand in hand with increased patient comfort, while the resulting cost may be decreased (16). Our SSFP sequence, is faster than the sequences proposed by Zhang (15) for the cranial nerves and by Qin (19) for the study of the intraparotid tract of the facial nerve.

Furthermore, the total acquisition time with SSFP sequences is shorter than that with traditional MR imaging pulse sequences, helping to reduce CSF pulsation artifact (20).

Whereas traditional MR imaging sequences provide excellent soft-tissue resolution, they may lack the spatial resolution necessary to define smaller structures such as cranial nerves. SSFP sequences allow much higher spatial resolution and clearer depiction of tiny intracranial structures (13).

The contrast-enhanced 3D CISS sequences appeared to be the most appropriate sequence for evaluating the trigeminal ganglion, whereas contrast-enhanced 3D TOF sequences were most appropriate for depicting the cranial and foraminal segments of trigeminal nerve as reported by Yousry et al. in their study (14).

Instead Zhang Z. et al report that with the 3D-PSIF with diffusion-weighted imaging method, it was possible to clearly capture the full course of the cranial nerves from its origin; this technique appears to be suited for depiction of the portions of the cranial nerves lying outside the cranial compartment (15).

With the SSFP sequence utilized in our study can be obtained similar results without intravenous paramagnetic contrast injection. Tractography is an interesting method of imaging of the cranial nerves (17) but it is primarily applicable to large nerves in

the CSF and not in the extracranial segments.

The course of LN in the third molar region is very close to the alveolar bone, against which it can be crushed during insertion of a laryngoscope for general anesthesia. Also it can be damaged during the dissection and retraction of a lingual flap and, above all, during the suture due to a direct trauma caused by the needle or indirectly during tying the knot. The SSFP sequence used in this study shows the LN in its course at the level of the lower molars, where, especially in edentulous patients, it runs near the alveolar crest and it could be damaged during surgical procedures.

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THE EFFECTIVENESS OF SUBGINGIVAL IRRIGANT OZONE-BASED AS ADJUVANT FOR NON-SURGICAL PERIODONTAL THERAPY IN THE TREATMENT OF CHRONIC PERIODONTITIS: A REVIEW

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The aim of this review was to establish the efficacy of Ozone therapy (OT) in Periodontal disease's (PD) treatment. An electronic search of publications was established from two electronic databases: PubMed and Web of Science. The search strategy used a combination of controlled vocabulary and free-text words. Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were: all studies published in English language; studies in vivo on humans; studies analyzing the correlation between PD and OT. The search resulted in 102 titles. Only 9 articles were included in the quantitative analysis. By the analyzed studies a strictly connection is still not evincible. More clinical trials are recommended to investigate the role of ozone, in its various types of topical administration, as a valid subgingival irrigant to achieve the goal of periodontal tissue health.

Periodontitis is a multi-factorial disease of which bacterial plaque is recognized as the main etiologic agent in the initiation and progression of the disease (1). Inflammation can also be determined by endodontic and incongruous conservative procedure (54-62).

Periodontal infection is initiated by specific invasive oral pathogens that colonize dental plaque biofilms on tooth surface, and host immune response to inflammation plays a central role in disease pathogenesis (2). Among the diverse organisms known to be collated with the clinical progression of the disease, "red complex" which encompass *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Aggregatibacter actinomycetemcomitans* (Aa) and *Treponema denticola* (Td) are the most important pathogens of chronic periodontitis (3).

The host immune response to inflammation

may lead to destruction of connective tissue periodontal ligament, and alveolar bone. In clinical terms, Periodontal disease (PD) is characterized by periodontal pockets, gingival inflammation, and loss of attachment to and in the alveolar bone, which can eventually lead to tooth loss (4).

The mainstay of periodontal treatment, although various treatment modalities have been suggested, is the mechanical removal of microbial plaque by scaling and root planning (SRP) (5).

Studies related to root planning have shown that complete removal of calculus is usually difficult; incomplete removal of subgingival plaque has been stated to be equal to no plaque control at all (6). For these reason, systemic and topical chemotherapeutic agents have been used as adjunctive methods for the treatment of periodontitis.

Key words: periodontitis, chronic periodontitis, periodontal disease, subgingival irrigation, ozone

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However, the systemic antibacterial agents achieve a low concentration in the periodontal pockets and have certain unpleasant effects (7), whereas local antimicrobial agents, usually in the form of topical subgingival irrigants, achieve a higher concentration in the periodontal site at very low concentration (8).

Studies from the past two decades have reported improvements with the adjunct use of subgingival irrigation agents like povidone iodine, stannous fluoride, boric acid, chlorhexidine, etc. (9).

The bisbiguanide salt, chlorhexidine gluconate (CHX) considered the gold standard has a broad antimicrobial spectrum (9) conceptually, used as supra- and sub-gingival irrigation by clinicians and patients to help suppress bacterial etiologic agents (8). An alternative approach to inhibit the subgingival bacteria is to reduce their growth by changing the subgingival environment which is highly anaerobic with low oxygen tension (8). Various treatment modalities such as hyperbaric oxygenation, molecular oxygen, and hydrogen peroxide have been applied with the concept of oxygenating the periodontal tissues.

Recently, Ozone therapy (OT) has gained popularity in dentistry. The use of ozone has been proposed due to its disinfectant, antimicrobial and healing properties. Periodontal application of ozone is usually done in gaseous, aqueous and oil forms (9).

The antibacterial action of ozone is related to its reacting capacity with lipid double bonds, thus leading to bacterial wall lysis and bacterial cell content extravasation. By entering into the cell, ozone promotes oxidation of nucleic and amino acids; and cell lysis depends on the extent of these reactions (6). It also inactivates viruses by diffusing through the protein coat into the nucleic acid core, resulting in damage of the viral nucleic acid. The antimicrobial action of ozone is non specific and selective to microbial cells; it does not damage human body cells because of their major antioxidative ability (9). Periodontal disease is also related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. (10-16). Periodontal disease diagnosis and therapy has been increased a great interest in the last years both in basic (17-26)

and advanced research (27-34). Besides the treatment of periodontal disease requires a multidisciplinary approach (35-44).

The purpose of this review is to evaluate the effectiveness of ozone-based treatments as adjuvants for non-surgical periodontal therapy and, to assess if it may represent a further treatment options for the clinicians.

MATERIALS AND METHODS

Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were: all studies published in English language; studies in vivo on humans; studies analyzing the correlation between PD and OT. The exclusion criteria were: in vitro and in vivo on animals analysis; publications that reported the same data as later publications by the same authors; commentaries and letters to the editor; case reports; case series; reviews and works that correlated OT to systemic syndromes or other dental pathologies.

Types of interventions

All the studies examined the efficacy of ozone as an adjunct to SRP in the treatment of PD. In addition, recent studies examined microbiological efficacy of Ozone on bacterial species involved on PD.

Outcome measures

Each study evaluated the clinical efficacy of Ozone on clinical parameters Plaque index (PI), Gingival index (GI), Probing depth (PPD) and Clinical attachment loss (CAL) on the day 0 and one month or more after the treatment.

Research Question

Pico question. We have formulated a specific question based on the PICO format where P stands for participants, I for intervention, C for comparison and O for the objective. The question was: "Does exist an improvement of clinical parameters (O) using OT (I) as an adjunct to non-surgical periodontal therapy (C) in the treatment of patients (P) affected by chronic periodontitis?". An electronic search of publications was established from three electronic databases: Cochrane, PubMed, Cochrane and Web of Science. The search strategy used a combination of

controlled vocabulary and free-text words. The detailed search design and strategies, including keywords, developed for each database are presented below.

Search strategy

The search strategy involved searching electronic databases (MEDLINE, EMBASE, and COCHRANE LIBRARY) and was supplemented by cross-checking the bibliographies of relevant review articles. The following combination of words was used:

(subgingival[All Fields] AND (“therapeutic irrigation”[MeSH Terms] OR (“therapeutic”[All Fields] AND “irrigation”[All Fields]) OR “therapeutic irrigation”[All Fields] OR “irrigation”[All Fields])) AND (“chronic periodontitis”[MeSH Terms] OR (“chronic”[All Fields] AND “periodontitis”[All Fields]) OR “chronic periodontitis”[All Fields]) NOT (((“surgical procedures, operative”[MeSH Terms] OR (“surgical”[All Fields] AND “procedures”[All Fields] AND “operative”[All Fields]) OR “operative surgical procedures”[All Fields] OR “surgical”[All Fields]) AND periodontal[All Fields] AND (“therapy”[Subheading] OR “therapy”[All Fields] OR “treatment”[All Fields] OR “therapeutics”[MeSH Terms] OR “therapeutics”[All Fields])) OR (open[All Fields] AND (“surgical flaps”[MeSH Terms] OR (“surgical”[All Fields] AND “flaps”[All Fields]) OR “surgical flaps”[All Fields] OR “flap”[All Fields]) AND (“debridement”[MeSH Terms] OR “debridement”[All Fields]))) OR (“ozone”[MeSH Terms] OR “ozone”[All Fields]) AND (“periodontitis”[MeSH Terms] OR “periodontitis”[All Fields]) AND (“2015/03/16”[PDat] : “2020/03/13”[PDat])

RESULTS

Identified articles

The search resulted in 102 titles. Following the first stage of screening, after the records identification through database manual searching and set the filter at last 5 years, 30 potentially relevant studies were identified. After the second stage screening, 16 further articles were eliminated reading the abstract. Afterwards, after the full text reading of the 14 articles eligible, only the randomized clinical trial was selected. At the end, only 9 articles were included in the quantitative analysis.

DISCUSSION

This review assessed to research a possible effectiveness of ozone therapy, as an adjuvant to SRP, in the treatment of PD. There are several studies that investigated about OT, but the results are controversial.

Issac et al. in 2015 (9) evaluated the use of ozonized water subgingival irrigation as an adjunct to SRP in patients with chronic generalized periodontitis, compared with SRP only. The randomized clinical and microbiological trail highlighted how GI, PPD, CAL showed statistically significant reduction after 4 weeks in both test and control sites. However, compared to control site, test site showed more significant improvement. They concluded that ozonized water subgingival irrigation is effective in improving oral hygiene, reducing GI, decreasing PPD and increasing CAL when used as an adjunct to SRP in patients with chronic periodontitis. Furthermore, subgingival application of ozonized water along with SRP significantly reduces the anaerobic bacterial count in periodontal pockets and prevents their recolonization.

In 2016 Pandya DJ. et al. (45) have compared the efficiency of ozonated water with the 0.2% “gold standard” CHX gluconate in treatment of periodontitis. A split-mouth randomized design was employed. Among the samples, the treatments included was full-mouth SRP along with subgingival irrigation using: CHX gluconate solution, ozonated water and 0.9N saline solution. Among the different subgingival irrigants used, 0.2% CHX gluconate is most effective followed by ozonated water, whereas saline was found to be ineffective when compared to the other two subgingival irrigants.

Otherwise in the 2018 Seydanur Dengizek E. et al. (46) have carried out a randomized, parallel controlled, and double-blinded trial to evaluate the clinical effects of the use of gaseous ozone in addition to SRP in periodontal treatment. The participants, with chronic periodontitis, was divided in two groups: the experimental group received SRP plus 3 Watts gaseous ozone, whereas the control group received SRP plus placebo. Results showed that the application of gaseous ozone was not associated

with significant changes in periodontal recovery.

According to this, Bignardello-Petersen R. in 2019 (47) enrolled 40 participants with generalized chronic periodontitis and assigned them to undergo SRP with ozone or SRP with a placebo. They reported, one month after treatment, that there were no statistical differences between SRP with or without ozone regarding PI, GI, PPD, or CAL.

These results are confirmed by the following studies: Uraz A. et al. in 2019 (48) reported, in their randomized clinical trial, that both SRP-alone and SRP-ozone therapy treatment modalities resulted in significant improvements in all clinical parameters. It is noteworthy that there was no significant difference between the treatment methods after the follow up period with respect to the periodontal parameters. Although the antibacterial effect of ozone therapy was very strong and long lasting, the pocket depth reduction was similar to that in the SRP-alone quadrants; Tasdemir Z. et al. (49) showed, in their randomized split-mouth clinical trial, that ozone therapy did not have any additional effect on periodontal parameters (PI; GI; CAL and PPD) compared to SRP-alone treatment. In this study the effect of ozone therapy on the biochemical parameters was also evaluated and all cytokines were reduced after periodontal therapy. Only PTX-3 levels were significantly lower at ozone sites compared to those at the control sites.

Another randomized clinical trial by Ghandi et al. in 2019 (6), assessed and compared the clinical efficacy of subgingival irrigation with CHX and ozonated olive oil in adjunct to the SRP, on clinical parameters: PI, GI, PD and CAL. They also evaluated the effect of CHX and ozonated olive oil on Pg and Aa counts by using bacterial culture method. The adjunctive use of ozonated olive oil in the treatment of chronic periodontitis significantly improves clinical and micro- biological results and is equally effective as chlorhexidine, and at the same time is free from adverse effects.

In the same way Kaur A. et al in 2019 (8) compared the efficacy of subgingival irrigation with ozonated water to 0.2% CHX as adjuncts to SRP in chronic periodontitis. The study was divided into two groups with 20 sites each. The group I received

CHX irrigation and group II received ozonated water irrigation. Despite the substantivity of CHX, ozonated water shows slightly better results. Therefore, the use of ozone is highly recommended as a strong atraumatic, antimicrobial agent in-home care and professional practice to treat periodontal disease non-surgically.

At last, Vasthavi et al. in 2020 (50), in their randomized clinical-microbial study, evaluated of ozone as an adjunct to SRP in the treatment of chronic periodontitis. The participants from the Group A received subgingival irrigation with ozonated water; the participants from Group B received subgingival irrigation with distilled water. The clinical parameters assessed were PI, GI, and PPD, recorded at baseline and at day 14, day 21, and 2 months (51-53). In the study, statistically significant difference in clinical and microbiological parameters was reported in the study group as well as in the control group from the baseline to 2 months. However, no statistically significant difference was observed in clinical and microbiological parameters between the groups.

The effectiveness of OT as an adjuvant to non-surgical periodontal therapy is not fully understood. Although some studies do not report any improvement in periodontal clinical parameters compared to SRP treatment alone, other studies justify its use because of the hopeful results. In addition, OT proves to be a valid support against the survival and recolonization on dental surfaces by the anaerobic bacteria of the "red complex". More clinical trials are recommended to investigate the role of ozone, in its various types of topical administration, as a valid subgingival irrigant to achieve the goal of periodontal tissue health (54-66).

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REHABILITATION OF A SEVERE MANDIBULAR ATROPHY WITH FOUR-MILLIMETER EXTRA-SHORT IMPLANT AND GUIDED BONE REGENERATION (GBR): CASE REPORT WITH 7-YEARS FOLLOW-UP

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The aim of this study was to report the use of extra-short and short implants in combination with Guided Bone Regeneration (GBR) to rehabilitate a case of severe mandibular reabsorption. A 55-year-old female patient asked for a fixed implant supported rehabilitation in 4.5 – 4.6 zone which showed severe atrophy. It was decided to use an extra-short 4 mm length and 4.1 mm Ø, Roxolid and SLActive implant (Straumann Standard Plus Regular Neck, Institut Straumann AG) in 4.5 position and a short 6 mm length and 4.8 mm Ø, Roxolid and SLActive implant (Straumann Standard Plus Wide Neck, Institut Straumann AG) in 4.6 position. The implants supported cemented fixed prosthesis. Examinations were performed at the day of the surgery and up to 7-years in function to evaluate implant stability and periapical radiography. One extra-short implant and one short implant were placed. After 7 years in function no biological or prosthetic complication were recorded. Within the limitation of this case report, the use of short and extra-short implants to support fixed prosthesis seem to be a feasible treatment alternative in severe mandibular atrophy.

Dental implant rehabilitation is a safe and well-documented treatment. Nowadays, patients ask for an implant therapy that is as much as it can be minimally invasive and minimally morbid. Expectations are increasing but nevertheless many patients are reluctant to accept an implant treatment and are especially apprehensive if they require a bone augmentation procedure (1). The rehabilitation of posterior region of mandible is always a hard-complexity case because of the presence of the inferior alveolar nerve (2).

In most cases, there is enough bone height to place a standard measure implant. Unfortunately, several alterations affect bone quality and quantity after tooth loss, and it is mandatory to avoid any nerve injury when performing implant surgery in

this zone. In this situation, several techniques have been proposed such as the lateralization of inferior alveolar nerve (3), vertical ridge augmentation (VRA) and guided bone regeneration (GBR) and the use of short or extra-short implants. However, these techniques are risky for the patients because they are technically demanding, require longer treatments, and incur higher morbidity and costs (4).

In the last years, use of short (>6mm and <10mm) and extra-short (≤6mm) implants has become more and more increased by clinicians and more and more investigated by several researches (5). Previous studies suggest that the lateralization of the inferior alveolar nerve could lead to a moderate postoperative morbidity. In addition, the combination between extra-short implants and GBR can result in a reduced

Keywords: short implants, extrashort implants, mandibular atrophy, short dental implants

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complexity of the surgery. Short dental implants present scientific evidence from several clinical studies that allow their reliable uses (6). The core made by titanium-zirconium alloy (Roxolid®) has higher tensile strength than pure titanium 2 and has demonstrated improved osseointegration properties (7). The surface is chemically modified, sand-blasted, large grit and, acid-etched (SLActive®) that provides higher treatment predictability in challenging protocols (8).

The aim of this study was to report the use of extra-short and short implants in combination with Guided Bone Regeneration (GBR) to rehabilitate a case of severe mandibular reabsorption providing survival after 7-year follow-up. In fact, many studies have widely demonstrated that implant-supported rehabilitation associated with a GBR can prevent the onset of peri-implantitis disease and allow for proper maintenance of oral health (9-15). Implant dentistry plus GBR has been increased a great interest in the last years both in basic (16-25) and advanced research (26-36), involving many other dental disciplines (37-52).

Clinical report

A 55-years-old female patient came at the dental clinic because she wanted to be able again to chew properly. The patient had an old fixed prosthesis in 4.5 - 4.6 - 4.7 position that was mobile and caused pain

when pressure was put on it. The x-ray examination showed root fracture on 4.7 and periapical radiotransparency on 4.5 (Fig. 1). The purpose was to rehabilitate the compromised mandibular zone by means of dental implants. The patient already rehabilitated 2.6, 3.6 and 3.7 with dental implant so it was favorable to deal with implant surgery. The X-ray examination showed severe bone resorption in 4.5 and 4.6 zone. The treatment options were explained to the patient, involving lateralization of the mandibular nerve or osteogenic distraction and bone graft or short implants in combination with simultaneous GBR. Carefully each clinical phase of each possible treatment option was explained. The patient chose the short implants treatment option because of low morbidity and shorter treatment time. Treatment plan was defined, and an impression was performed in order to realize diagnostic wax-up and a surgical template.

On the day of the surgery, the patient received antibiotic prophylactic therapy: 2 g of amoxicillin 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. The precise fit of surgical template was checked before the surgery. A mucoperiosteal flap was raised to expose mental foramen and 4.5 and 4.7 roots were extracted in as



Fig. 1. *Pre-operative X-Ray.*

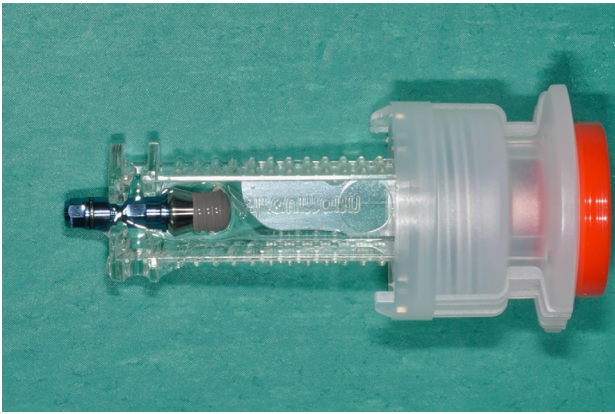


Fig. 2. *Extra-Short implant.*

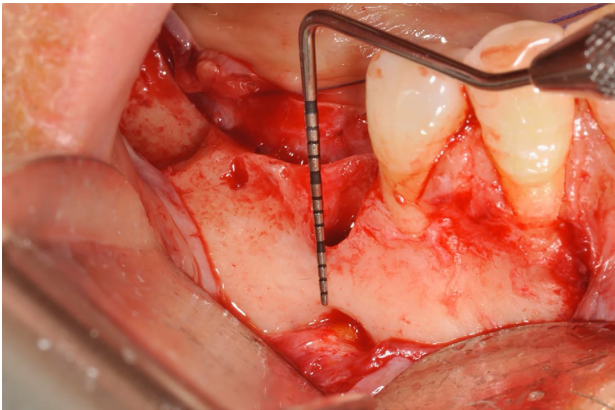


Fig. 3. *Distance from mental foramen.*

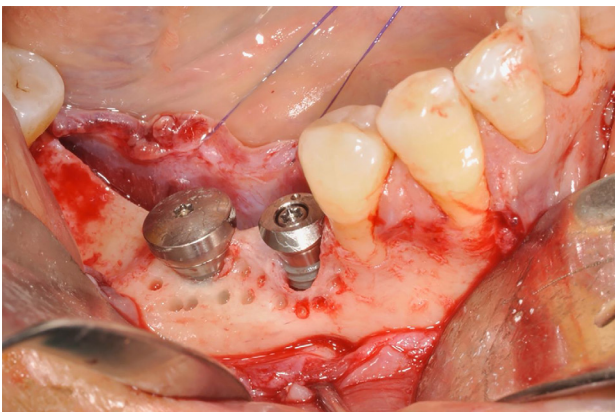


Fig. 4. *Implants placed and perforations performed.*

atraumatic as possible manner.

Care was taken during the flap opening to preserve the integrity of the periosteum. The distance that goes from 4.5 crestal bone to mental foramen was measured by means of a probe and was 5 mm (Fig. 3). The surgical template was positioned, and the

implants sites preparations the implants positioning were performed following carefully manufacturing instructions. Two titanium-zirconium (Roxolid®) (53) with chemically modified, sand-blasted, large grit and, acid-etched surface (SLActive®) (54) dental implants (Straumann Standard Plus Regular Neck and Straumann Standard Plus Wide Neck, Institut Straumann AG) were placed in 4.5 and 4.6 position.

After placement of implants cover screws, bone perforations (Fig. 4) were performed in order to facilitate the migration of osteoprogenitor cells from the bone marrow into the isolated space created. A mix of 50% of Deproteinized Bovine Bone Mineral (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) and 50% particulate autogenous bone (Fig. 5) was used as graft material and covered with resorbable collagen membranes (Bio-Guide®, Geistlich Pharma AG) (Fig. 6) according to the Guided Bone Regeneration (GBR) simultaneous approach criterion. An accurate adaptation of the flap margins by means of interrupted sutures was performed allowing primary wound closure with tension-free. Periapical x ray was performed at the end of the surgery (Fig. 7) to record MBL and repeated annually to check peri-implant bone status.

The patient was instructed to rinse with 0.2% chlorhexidine mouthwash for one minute twice a day for 15 days, to have a soft diet for one month and to avoid any trauma on the surgical sites. After healing period, an open-tray impression using polyvinylsiloxane was taken in order to realize fixed cemented prosthesis that can splint the implants.

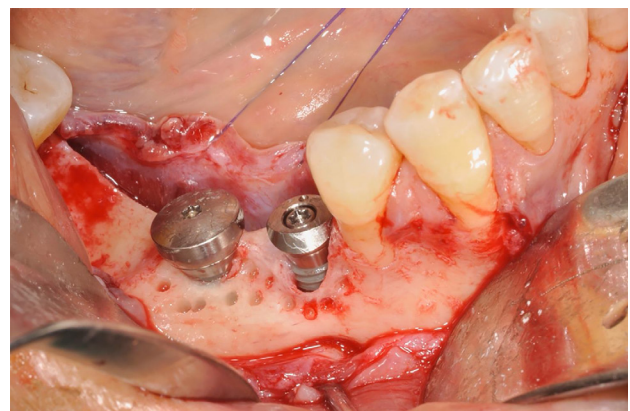


Fig. 5. *Bone apposition.*

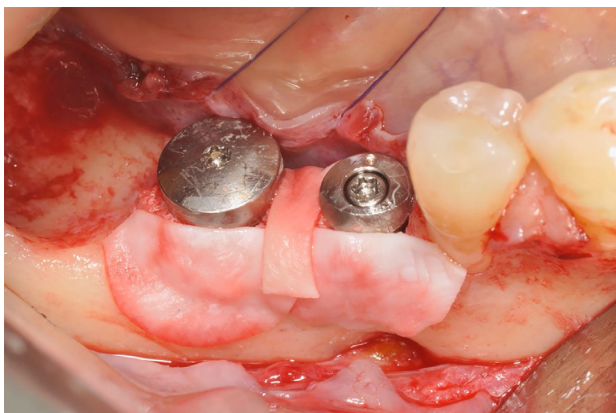


Fig. 6. Collagen membranes placed.

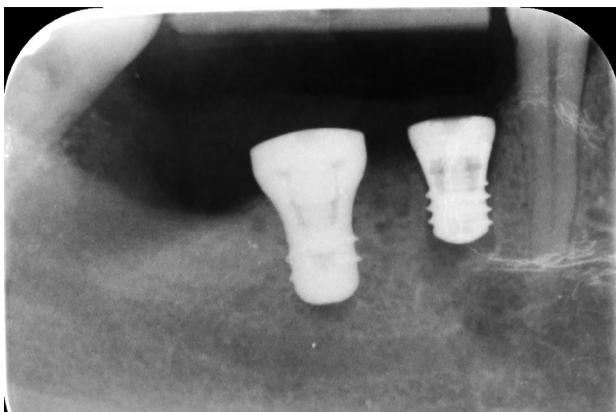


Fig. 7. Periapical X-Ray at the end of the surgery.



Fig. 8. Definitive prosthesis.

The abutments (RN and WN SynOcta 5.5 mm cementable abutment, Institut Straumann AG) were screwed directly on the dental implants and the

porcelain fused to metal definitive prostheses (Fig. 8) was cemented on them by means of temporary cement (Temp-Bond, Kerr, KaVo, Brea, CA, USA). Occlusion was checked and the patient was instructed to wear a nightguard and to receive professional hygiene every 6 months. After 7 years in function no implant or prosthetic or biologic complications during the entire follow-up.

DISCUSSION

This study aimed to report a case of rehabilitation of a severe mandibular atrophy in the posterior zone using extra-short implants associated with GBR providing survival after 7-year follow-up (Fig. 9, 10). In severe mandibular atrophy, the insertion of an extra-short implant is a treatment associated with lower morbidity and lower complications (55). In a recent systematic review, Ravidà et al. (5) demonstrated that short implants have a survival rate inferior to the standard-length implants but, when compared with bone augmentation procedures for using standard implants in atrophic zones, the survival rate at 5 years in function is 94.1% and justifies this alternative approach. In another 5-year retrospective study, Pieri et al (56) reported that clinical and radiographic study comparing outcomes of short (6mm) and standard-length implants (> 9mm) placed after vertical augmentation were similar for both groups (95.7% short implants and 95.5% standard implants). Slotte et al. in 2012 (57) placed hundreds of extra-shorts implants providing a survival rate of 95.7% and 92.3% after one and two-years of follow-up, respectively.

After the healing period, the definitive prosthesis was made in order to splint the implants. Some finite element analyses have demonstrated that occlusal load distribution on the splinted implants led to a reduction in forces generated onto the implant bodies (58-62). In addition, a systematic review by Theoharidou reported that splinted extra-short implants have a statistically significantly lower number of prosthetic complications and implant failure rate than non-splinted implants ($P < .01$) (63-67). The clinical case presented in this study have 7-years of follow-up and no implant or prosthetic

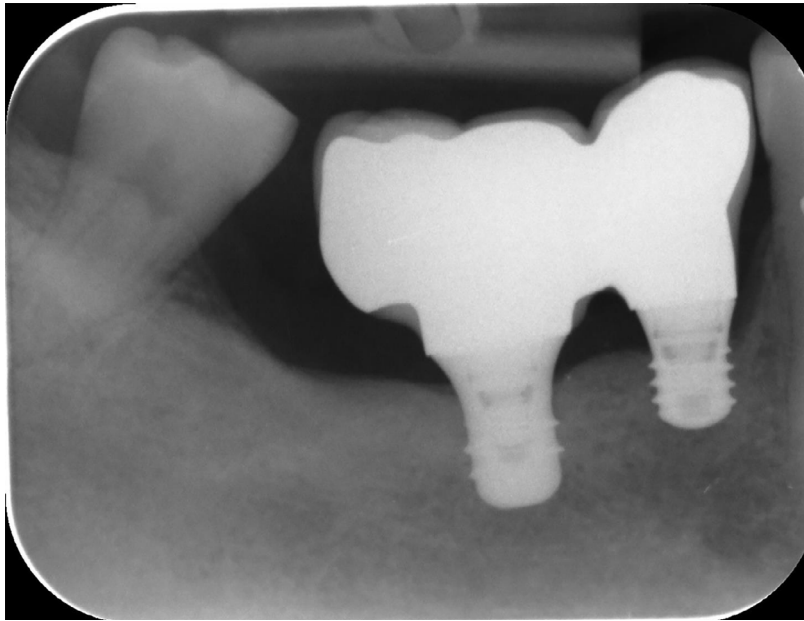


Fig. 9. *Periapical X-Ray after 7-years follow-up.*



Fig. 10. *Definitive prosthesis after 7-years follow-up.*

or biologic complications during the entire follow-up. It can be considered in accordance with the results of several studies. However, it is mandatory to remind that a single clinical case does not value as randomized clinical trials or prospective or

retrospective studies.

Within the limitation of the present case report the use of short and extra-short implants to support fixed prosthesis seem to be a feasible treatment alternative in severe mandibular atrophy.

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DIODE LASER AS AN ADJUNCT TO NONSURGICAL CHRONIC PERIODONTITIS THERAPY: A REVIEW

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The aim of this review was to investigate the additional effects of diode laser irradiation in combination with nonsurgical periodontal therapy. An electronic search of publications was established from three electronic databases: Cochrane, PubMed and Web of Science. The search strategy used a combination of controlled vocabulary and free-text words. Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were all studies published in English language, randomized controlled trials, based on human subjects. All trials were designed to evaluate the efficacy of the diode laser therapy as an adjunct to nonsurgical periodontal therapy in the treatment of chronic periodontitis patients. All studies analyzed are not older than five years. The search resulted in 188 titles. After the analysis of the abstracts, an article was excluded because it was not relevant for the study and two because it treated aggressive periodontitis. At the end only 6 articles were included in the qualitative analysis. The potential role in chronic periodontal disease treatment of diode laser is still not demonstrated. Several studies regard the diode laser therapy, but there is still heterogeneity for what it concerns research and clinical protocols. As a result, several randomized controlled trials are needed to obtain certain data.

Periodontitis is a chronic inflammatory status in the oral cavity associated with anaerobic, Gram-negative bacteria that leads to a progressive loss of the supportive tissues of teeth and induces and amplifies low-grade systemic inflammation resulting from the relevant bacteria and their components entering the body through the systemic circulation (1). The dental plaque is a biofilm that forms on the teeth and in subgingival spaces and it results in chronic inflammation on periodontal tissues. This inflammatory status may lead to periodontal pocket development, attachment loss, bone destruction and tooth loss (2). The first phase of periodontal treatment involves the elimination of bacterial deposits and niches by the removal of supra- and subgingival

biofilms. Traditionally, supra- and subgingival non-surgical mechanical instrumentation is performed by hand or/and in combination with power-driven instruments and results in improved clinical outcomes such as reduced bleeding on probing (BoP) and decreased probing pocket depth (PPD). Less microbial load and less bacterial metabolic products leads to a reduced inflammatory response and to better tissue healing (3).

However, it is not always easy to remove deposits from the root surface in moderate or deep periodontal pockets. In addition, access to furcation, cavities, grooves and distal region of molars is limited sometimes. To overcome these difficulties, flap surgery may provide better access

Keywords: diode laser, periodontal disease, chronic periodontitis, laser therapy

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and visual. Nonetheless, this method leads to severe postoperative morbidity and may cause gingival recession (4). To avoid the flap surgery, high-power output laser irradiation has been proposed in addition to the traditional scaling and root planning technique (4). In addition, low-power output lasers are employed for therapeutic cellular stimulation and the activation of antimicrobial agents after scaling and root planning. Both laser applications can be considered as a minimally invasive approaches to chronic periodontitis treatment. There are several type of lasers such as diode, carbon dioxide (CO₂), neodymium-doped yttrium aluminium garnet (Nd:YAG), erbium-doped yttrium aluminium garnet (Er:YAG) and erbium, chromium-doped: yttrium, scandium, gallium, garnet (Er,Cr:YSGG). All these wavelengths with moderate power output can be used in adjunct for initial periodontal therapy.

The diode laser is mostly used for soft tissue treatment because it can induce tissue ablation, vaporization, hemostasis and sterilization (5). The aims of laser irradiation are not only to debride connective tissue and epithelium within periodontal pockets, but also to inactivate bacteria that invade the periodontal tissues (6). The laser power is carried out through fine tips that help the clinician to perform precise and small procedures without damaging the around areas. The laser has a thermal effect on dental soft tissues and a resulting rising of tissues temperature. At 50°C most of periodontal bacteria can be deactivated (7). At 60°C coagulation and hemostasis are achieved. As a result, the goals of nonsurgical periodontal therapy such as reduced number of bacteria in the biofilm and coagulation of blood can be performed.

An additional application known as antimicrobial photodynamic therapy (aPDT) has been more and more investigated as an adjunctive approach to non-surgical mechanical instrumentation. The (aPDT) aims to destroy bacterial cells by means of production of highly reactive oxygen radicals in periodontal pockets. This reaction is possible because of the combination between low-laser light and photosensitizer. (8) The whole procedure is expected to reduce the bacteria load and the inflammatory status in periodontal tissues. In addition, it provides

a biostimulatory effect on damaged tissues (9). The aim of this review was to investigate the additional effects of diode laser irradiation in combination with nonsurgical periodontal therapy.

MATERIALS AND METHODS

Inclusion and exclusion criteria were defined by the authors, before the start of the study. The inclusion criteria were all studies published in English language, randomized controlled trials, based on human subjects. All trials were designed to evaluate the efficacy of the diode laser therapy as an adjunct to nonsurgical periodontal therapy in the treatment of chronic periodontitis patients. All studies analyzed are not older than five years.

The exclusion criteria were publications that reported the same data as later publications by the same authors and systematic reviews, commentaries and letters to the editor, case report, in vitro studies, studies in animal models and case series were not considered.

Types of interventions

The analyzed studies were randomized controlled clinical trials or split-mouth randomized controlled clinical trials. The test group was treated with scaling and root planing in combination with diode laser therapy and the control group with scaling and root planing only.

Outcome measures

Clinical parameters such as pocket probing depth (PPD), clinical attachment level (CAL), bleeding on probing (BoP) and plaque index (PI) were measured before intervention and following different follow-up protocols. Even though not all the studies adopted the same success criteria, they were always well specified in the publications included.

Research Question

We have formulated a specific question according to the Participants, Interventions, Control, and Outcomes (PICO) format. The question was: "Does exist an improvement of clinical parameters (O) using photodynamic therapy (I) as an adjunct to nonsurgical periodontal therapy (C) in the treatment of patients (P) affected by chronic periodontitis?". An electronic search of publications was established from three electronic databases: Cochrane, PubMed and Web

of Science. The search strategy used a combination of controlled vocabulary and free-text words. The detailed search design and strategies are presented below.

Search strategy

The search strategy involved searching electronic databases (MEDLINE, EMBASE, and COCHRANE LIBRARY) and was supplemented by cross-checking the bibliographies of relevant review articles. The following combination of words was used: (“lasers, semiconductor”[MeSH Terms] OR (“lasers”[All Fields] AND “semiconductor”[All Fields]) OR “semiconductor lasers”[All Fields] OR (“diode”[All Fields] AND “laser”[All Fields]) OR “diode laser”[All Fields]) AND (“periodontitis”[MeSH Terms] OR “periodontitis”[All Fields]) NOT (((“surgical procedures, operative”[MeSH Terms] OR (“surgical”[All Fields] AND “procedures”[All Fields] AND “operative”[All Fields]) OR “operative surgical procedures”[All Fields] OR “surgical”[All Fields] AND periodontal[All Fields] AND (“therapy”[Subheading] OR “therapy”[All Fields] OR “treatment”[All Fields] OR “therapeutics”[MeSH Terms] OR “therapeutics”[All Fields])) OR (open[All Fields] AND (“surgical flaps”[MeSH Terms] OR (“surgical”[All Fields] AND “flaps”[All Fields]) OR “surgical flaps”[All Fields] OR “flap”[All Fields]) AND (“debridement”[MeSH Terms] OR “debridement”[All Fields])))) OR (“lasers, semiconductor”[MeSH Terms] OR (“lasers”[All Fields] AND “semiconductor”[All Fields]) OR “semiconductor lasers”[All Fields] OR (“diode”[All Fields] AND “laser”[All Fields]) OR “diode laser”[All Fields]) AND (“periodontal diseases”[MeSH Terms] OR (“periodontal”[All Fields] AND “diseases”[All Fields]) OR “periodontal diseases”[All Fields] OR (“periodontal”[All Fields] AND “disease”[All Fields]) OR “periodontal disease”[All Fields]) NOT (((“surgical procedures, operative”[MeSH Terms] OR (“surgical”[All Fields] AND “procedures”[All Fields] AND “operative”[All Fields]) OR “operative surgical procedures”[All Fields] OR “surgical”[All Fields] AND periodontal[All Fields] AND (“therapy”[Subheading] OR “therapy”[All Fields] OR “treatment”[All Fields] OR “therapeutics”[MeSH Terms] OR “therapeutics”[All Fields])) OR (open[All Fields] AND (“surgical flaps”[MeSH Terms] OR (“surgical”[All Fields] AND “flaps”[All Fields]) OR “surgical flaps”[All Fields] OR

“flap”[All Fields]) AND (“debridement”[MeSH Terms] OR “debridement”[All Fields]))))

Selection criteria and data extraction

The search resulted in a great number of published studies about the topic, so a three-stage screening process was performed by two independent reviewers (P.C. and C.L.) and disagreement between the two reviewers was resolved after additional discussion.

All the titles were screened to eliminate irrelevant publications, review articles and animal studies. All abstracts of publications selected during the first screening were analyzed, excluding studies included in the exclusion criteria. Through analysis of the whole selected full texts, the study eligibility was based on the predetermined inclusion and exclusion criteria. A table with data from all the included studies was created and the results were discussed.

RESULTS

Identified articles

The search resulted in 188 titles. Following the first stage of screening, after the randomized controlled trials identification through database manual searching, 52 potentially relevant studies were identified. After the second stage screening we selected studies no older than 5 years, 18 full text publications were obtained and analyzed. Two studies were excluded because treated diabetic patients and 6 studies were excluded because not relevant to the purpose of the review.

After the analysis of the abstracts, an article was excluded because it was not relevant for the study and two because it treated aggressive periodontitis. At the end only 6 articles were included in the qualitative analysis (Table I).

DISCUSSION

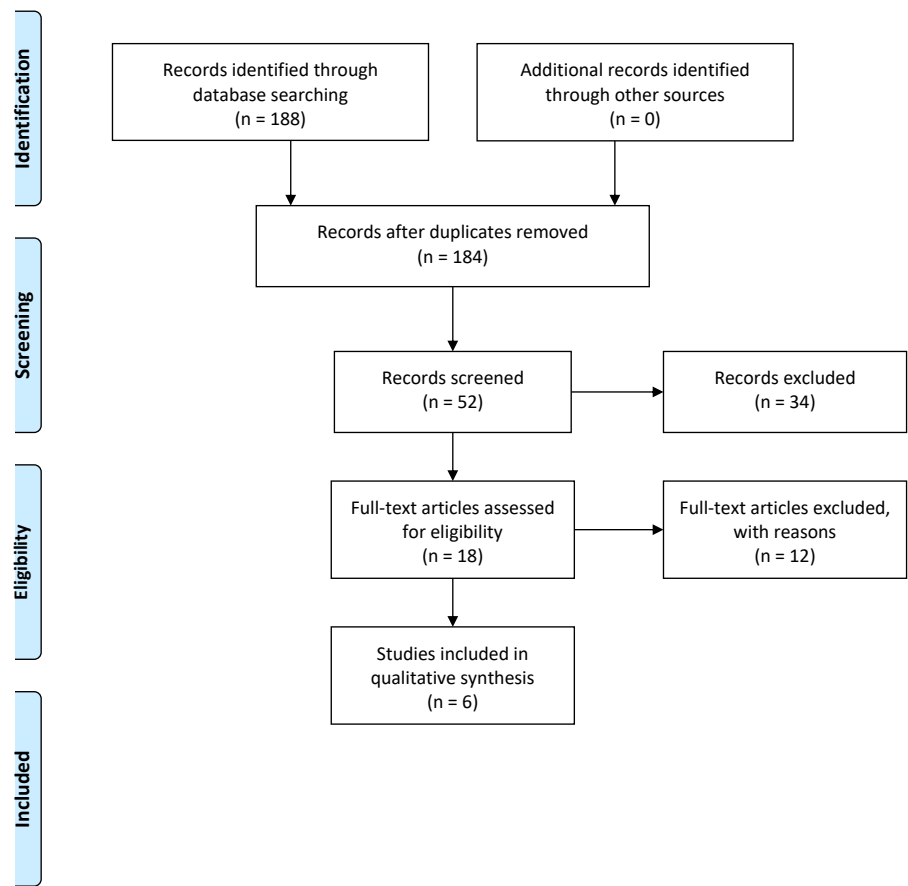
The aim of this review was to investigate the additional effects of diode laser irradiation in combination with nonsurgical periodontal therapy. The heterogeneous methods and follow-up of these studies is the main limitation to superimpose all the six studies and to perform a meta-analysis.

However, the selected studies investigated the same periodontal parameters such as CAL, PPD, BoP, PI with or without diode laser therapy and it is possible to perform a qualitative analysis because of the highly heterogeneous set of variables in literature such as type of laser, tip diameter, wavelength, photosensitizer, mode of periodontal treatment, number of treated sites, population and several possible combinations of these parameters the research strategy involved only 6 studies. (Table II and III). All six studies were randomized controlled clinical trials and they all treated chronic periodontal diseases with non-surgical therapy in the control group and with non-surgical therapy and diode laser in the test group. The results of the present review are in accordance with those presented in a recent systematic review by Salvi et al in 2019 (8) that reported highly heterogeneous literature data and the

difficulty to carry out any scientific evidence about laser contribution in the periodontal disease therapy. The combination between SRP and diode laser lead to a complete resolution of the inflammation and a significant reduction of the periodontal pockets. However, Monzavi et al (10) in 2016 reported that the application of this protocol did not have any additional advantages in terms of clinical parameters. The same results were reported by Carvalho et al (11) in 2015, Srikanth et al (12) and Giannelli et al (5) as there were no statistically differences in clinical parameters between traditional SRP and SRP with diode laser therapy.

On the other side, Shingnapurkar et al (13) and Alzoman et al (14) reported a statistically difference in clinical parameters after the combination of SRP and the application of diode laser. These results may be relevant for the future development of a new

Table I. Study selection process



treatment protocol. Nevertheless, it is mandatory to report that these studies have both too-short follow-up period to assert that the adjunct of diode laser is clinically and statistically significant.

The follow-up period is the main limitation of several studies about the diode laser and its application in periodontology. Giannelli et al (5) reported data from 1 year and 4 years of follow-up. At one year there were no statistically and clinically differences between the two groups of treatment. However, at 4 years of follow-up the SRP group showed a slightly to moderate worsening of the clinical parameters at the 1-year follow-up. Obviously, it is binding to report that these data only come from one study and there is the need of new several studies with at least the same protocol and the same follow-up to compare them. In fact, many studies have widely demonstrated that laser therapy can prevent the onset of periodontal and peri-implant disease and allow for proper maintenance of oral health (15-20). Laser

therapy has been increased a great interest in the last years both in basic (21-35) and advanced research (36-47), involving many other dental disciplines (47-65).

Srikanth et al (12) in their pilot study reported how indocyanine green may be used in the treatment of chronic periodontitis as an adjunct in combination with SRP and diode laser. Indocyanine green is a photosensitizer dye and when activated by a wavelength of 810 nm of a diode laser seems to be efficient against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas Gingivalis* as reported in an in vitro study. The combination of different factors against periodontal bacteria may lead to a more efficiency treatment of chronic periodontal disease. Of course, there is still the need of several randomized controlled trials to assert that this combination is more effective than SRP alone.

The potential role in chronic periodontal disease

Table II. Characteristics of included studies

	Article type	Test group A	Test group B	Control group	N. patients	Patients dropped out	Patients at least included in study	Follow-up period	Evaluated sites	Age (years)	Follow-up
Carvalho et al.	Randomized controlled parallel-group clinical trial	Photodynamic therapy in the treatment of residual pockets of chronic periodontitis patients.	-	Sham procedure.	38	4	34 Test group = 18 Control group = 16	12 months	-	Test group = 48.29 Control group = 51.98	3, 6, 12 months
Srikanth et al.	Split-mouth (premolar/molar sites), double-blind, randomized controlled clinical trial. Three sites from three different quadrants were selected and were randomly treated.	Scaling and root planning with application of 810 nm diode laser.	Scaling and root planning with application of 810 nm diode laser and indocyanine green at a concentration of 5 mg/mL.	Scaling and root planning.	30	3	27	6 months 4	-	30-55	1 week, 3, 6 months
Giannelli et al.	Split-mouth randomized controlled clinical trial. Maxillary left or right quadrants were randomly assigned to multiple photodynamic cycles laser treatment and scaling and root planning.	Multiple photodynamic cycles: 1) photoablative laser treatment phase, 2) photodynamic laser treatment.	-	Sham treatment and scaling and root planning.	26	2	24	4 years	-	46.1	1, 2 years
Shingnapak et al.	Comparative split-mouth randomized clinical trial. Split-mouth design where the test and control sites were randomly divided with a flip of coin.	Scaling and root planning + photodynamic therapy	-	Scaling and root planning.	-	-	-	3 months	30 sites for each group	25-55	1, 3 months
Alzoman et al.	Randomized, controlled, parallel design trial	Scaling, root planning and laser irradiation	-	Scaling and root planning.	32	2	30	2 months	-	48.8	-
Monzavi et al.	Randomized clinical trial	Antimicrobial photodynamic therapy + scaling and root planning.	-	Scaling and root planning.	50	-	-	3 months	-	35-55	1, 3 months

Table III. *Main findings of included studies*

		Baseline	1 week	1 month	2 months	3 months	6 months	12 months	4 years
Carvalho et al.	CAL	-Test group 5.56 +/- 0.83. -Control group 5.87 +/- 1.39. p values = 0.46.	###	###	###	-Test group 4.61 +/- 1.13. -Control group 4.78 +/- 1.44 p values = 0.68	-Test group 4.44 +/- 1.06. -Control group 4.78 +/- 1.44. p values = 0.92	-Test group 4.60 +/- 1.11. -Control group 4.33 +/- 1.21. p values = 0.79	###
	PPD	-Test group 4.79 +/- 0.47. -Control group 4.87 +/- 0.74. p values = 0.75.	###	###	###	-Test group 3.70 +/- 0.85. -Control group 3.39 +/- 0.76. p values = 0.76.	-Test group 3.41 +/- 0.93. -Control group 3.48 +/- 0.70. p values = 0.8.	-Test group 3.55 +/- 0.92. -Control group 3.23 +/- 0.77. p values = 0.75.	###
	BoP	-Test group 58.33 +/- 29.70. Control group 42.18 +/- 19.83. p values = 0.06.	###	###	###	-Test group 19.44 +/- 21.95. Control group 17.18 +/- 23.66. p values = 0.78.	-Test group 31.94 +/- 29.46. Control group 23.43 +/- 19.29. p values = 0.55	-Test group 23.61 +/- 21.82. Control group 15.63 +/- 22.13. p values = 0.86.	###
	PI	-Test group 18.05 +/- 23.95. Control group 18.75 +/- 29.58. p values = 0.92.	###	###	###	-Test group 12.50 +/- 22.08. Control group 10.93 +/- 18.18. p values = 0.97.	-Test group 16.67 +/- 21.00. Control group 09.33 +/- 12.50. p values = 0.85.	-Test group 12.50 +/- 17.68. Control group 6.25 +/- 19.36. p values = 0.82.	###
Srikanth et al.	CAL	-test group A 4.57 +/- 0.57. -test group B 5.00 +/- 0.80. -Control group 4.50 +/- 0.50. p values = .000	###	###	###	-test group A 2.29 +/- 0.44. -test group B 2.56 +/- 0.80. -Control group 3.00 +/- 0.01. p values = .000	-test group A 2.96 +/- 0.44. -test group B 2.53 +/- 0.68. -Control group 3.10 +/- 0.01. p values = .000		###
	PPD	-test group A 5.34 +/- 0.34. -test group B 5.81 +/- 0.89. -Control group 5.07 +/- 0.27. p values = .000	###	###	###	-test group A 3.11 +/- 0.43. -test group B 2.90 +/- 0.79. -Control group 3.00 +/- 0.01. p values = .000	-test group A 3.15 +/- 0.43. -test group B 3.07 +/- 0.68. -Control group 3.01 +/- 0.21. p values = .000	###	###
	PI	-test group A 2.02 +/- 0.23. -test group B 1.96 +/- 0.18. -Control group 2.02 +/- 0.34. p values = .000	-test group A 0.95 +/- 0.29. -test group B 0.77 +/- 0.22. -Control group 0.95 +/- 0.21. p values = .000	###	###	-test group A 1.24 +/- 0.28. -test group B 1.04 +/- 0.18. -Control group 1.23 +/- 0.21. p values = .000	###	###	###

treatment of diode laser is still not demonstrated. Several studies regard the diode laser therapy, but there is still not heterogeneity for what it concerns research and clinical protocols. As a result, several randomized controlled trials are needed to obtain certain data.

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AESTHETIC CORRECTION OF DOUBLE TOOTH: SIMPLIFIED TECHNIQUE

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The aim of this study is to report aesthetic conservative treatment of double tooth anomaly. Case report: A 7.5-year-old patient presents an anomaly of the maxillary right upper central incisor with a bifid crown and an increased mesio-distal width and a central incisal notch, probably amenable to a gemination. Single tooth anesthesia and conservative enameloplasty procedure was performed, with universal adhesive and a single shade universal composite restorative material. Adhesive aesthetic restorations allow to resolve dental anomalies quickly, cheaply and easily for pediatric dentistry.

Dental anomalies in number and shape can occur both in primary and permanent dentitions (1). Many authors often used the term “double tooth” to describe both fusion, gemination and concrescence (2-4).

Fusion is defined as the union of two or more separate tooth buds at the crown level (enamel and/or dentin), yielding a single large tooth during odontogenesis, with a single enlarged clinical crown not yet mineralized; the degree of fusion depends on the stage of tooth development (5). Radiographically two root canals and one or two roots may be evident.

Gemination is a malformation of a single tooth bud, and results in a large bifid tooth crown that radiographically has a single root and a single root canal. It is described as the attempt by a single tooth germ to divide itself in two (5). Concrecence is similar to fusion, but it is the union of two or more tooth buds at radicular/cementum level (6).

Double teeth are more prevalent in primary dentition than in permanent, with no sex predilection. The prevalence rate for double tooth in primary dentition varies from 0.5 to 4.1% while it has been reported to be 0.3-0.42% in the permanent dentition (4). Double teeth were reported to be 75% of dental

anomalies in primary dentition with 94% of fusions and 6% of geminations (4).

Clinically it is possible to distinguish fusion and gemination because in the first, counting as single the affected double tooth, the result is one tooth less than normal; while in gemination the result is a normal number of teeth (in this case the double tooth derives from fusion with a supernumerary tooth or actual gemination) (5, 7).

In literature, Hagman in 1988 reported that fusion in primary dentition was followed by hypodontia in permanent dentition in 75% of cases, instead Marinelli in 2012 (4) reported a higher incidence (88,7% of the subjects). In particular, fusion of the upper central and lateral incisor was followed by hypodontia of the lateral permanent incisor in 6 cases out of 7 (4). Gemination in primary dentition was followed by supernumerary teeth in the permanent dentition in 45% of cases (4) in agreement with the results of Nik-Hussein and Abdul Majid (2) (59%) and Skrinjaric and Barac-Furtinovic (1991) (8) (61%). Primary fused teeth are often unilateral (but also there were bilateral and triple teeth) (7) mainly affecting the lower lateral incisors/canines, instead gemination occurs more

Key words: double tooth, single tooth anesthesia, universal adhesive, one-shade aesthetic composite

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frequently to the upper lateral incisors. If hypodontia should occur, it will be necessary manage the dental arch space with orthodontic procedures (9-16) combined with periodontal evaluations (17-31), and possibly in adulthood to program implant-prosthetic treatment (32-37).

The etiology is still unknown, but Shafer 1974 (38) sustained that the influence of pressure or physical forces producing close contact between two developing teeth was a possible cause of fusion. Also, genetic predisposition (autosomal dominant trait with a reduced penetrance) (7) and racial differences were two possible cofactors. Other authors believe that fusion was the result of physical forces that put developing teeth in contact, producing necrosis of the epithelial tissue that separates them (39, 40) or that fusion was the result of embryological persistence of the interdental lamina between two tooth buds. Gemination was the result of a developmental aberration of both the ectoderm and the mesoderm (6). This was due to local metabolic interferences during morphodifferentiation of the tooth bud. Shafer et al. 1974 (38) suggested that gemination had also a hereditary tendency. In gemination, the epithelium and mesenchymal cells showed a complete

differentiation, with the result that ameloblast and odontoblast produced enamel and dentin in a normal way. Presently the epigenetic factors that cause the single-tooth germs split are still unknown (6). In 1999 Aguiló et al. (3) classified the primary double tooth (PDT) in 4 types:

- Type I: bifid crown and a single root that involved only the maxillary lateral incisor; it could be the result of a total fusion between a normal tooth with a supernumerary tooth bud;
- Type II: large crown and large root (one root canal) that involved either the central and lateral incisors or lateral incisor and canine;
- Type III: two fused crowns and double conical root (two pulp chambers, one large pulp canal in the middle third and two apical pulp canals) that involved either the central and lateral incisors or lateral incisor and canine;
- Type IV: two fused crowns and two fused root (two separate root canals) that involved either the central or the lateral incisor.

Case report

A 7.5-year-old boy was visited for routine dental check-up. Clinical intraoral examination evidenced

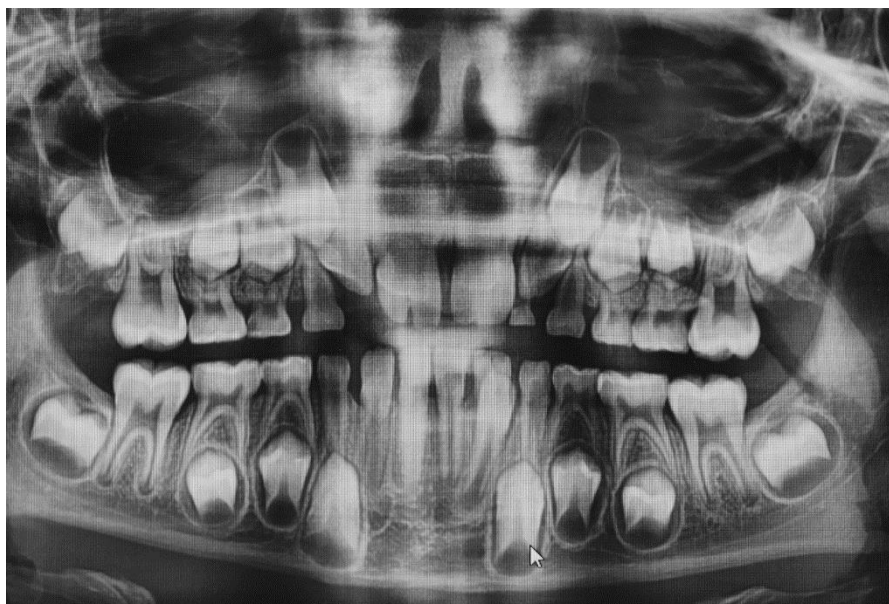


Fig. 1. Preoperative panoramic x-ray of the patient.

no anomalies of dental structures with the exception of the maxillary right upper central incisor. This tooth presents a bifid crown with an increased mesio-distal width and a central incisal notch, probably amenable to a gemination (Fig 1, 2, 3).

Radiographically, this tooth presents a double incisal and middle third coronal profile and a single root with a single root large canal.

According to the requests of the patient's parents, it was decided to proceed with a conservative restorative procedure, with direct composite restoration of the vestibular and palatal dental surfaces. A waxup was made on precision casts of the upper and lower arches of the patient, in order to make a rigid silicon palatal index to reshape the palatal wall and the incisal edge.

After single tooth anesthesia (41-43) and rubber



Fig. 2. *Preoperative image of the upper central incisor needing the aesthetic rehabilitation.*



Fig. 3. *Intraoral view of the lower left primary incisor presenting same condition of double tooth.*



Fig. 4. *Final view of the restored tooth.*

dam insulation, a thorough cleaning of tooth surface with mounted brushes was performed and the enamel surface to receive resin bonding was roughened with only an Arkansas stone bur. After rinsing and drying the enamel surface, a one-step Universal Adhesive (Tokuyama Universal Bond, Tokuyama Dental, Japan) was applied with a microbrush and then gently thinned and dried with air spray for 10", according to manufacturer instructions (44). The restorative procedures started using the palatal index to produce a layer of single-shade aesthetic universal composite (Omnichroma, Tokuyama Dental, Japan) (45). This new single-shade resin-based mimetic composite is a universal composite that matches all the 16 VITA classical shades, from A1 to D4 (45).

Composite resin was cured for 20" each time. After removal of the palatal index the operator completed the vestibular restoration with few more composite increments (46, 47). To obtain the final shine of the restored tooth, polishing of surface was performed with pointed sharp steel bur (Komet), three steps silicon rubber burs (Komet) and a final buffing with Aluminum Oxide paste (Fig.4) (48-61).

The use of modern anesthetic techniques and mimetic adhesive aesthetic materials allows to perform fast and minimally invasive restorations, that required almost no preparation and therefore no

sacrifice of healthy dental tissue. Furthermore, the quality of adhesion obtainable with new generation adhesives ensures a long-lasting success of the restoration. The ease and speed of execution and the cost-effective therapy make this approach very useful in pediatric dentistry.

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EPIDEMIOLOGICAL STUDY ON RADIOGRAPHIC DENTAL HEALTH IN ALBANIA

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The aim of this epidemiological study is to assess the dental health status of the Albanian population and the most frequently performed dental treatments, also highlighting the differences between sex, geographic area and educational level. The study was performed by analyzing digital orthopantomographic radiographs of 739 Albanian patients, male and female, divided into 5 age groups. In addition, the patients were divided according to regions of origin in North, Centre and South and classified according to the level of education. The parameters examined are as follows: Missed Teeth (MT), Filled teeth (FT), Endodontically treated teeth (TT), Prosthetic restorations (PR). There are no statistically significant results regarding the distribution by area. Subjects with at least one missing tooth (MT) decreases with increasing education level. The percentage of individuals with Missed Teeth in the population increases with increasing age groups. Educational background affects health. This study highlights the need to introduce a national oral health system in Albania that would extend interventions to a wider segment of the population.

Despite WHO oral health campaigns over the years, oral disease is still a widespread problem in developing countries (1). Data from various epidemiological studies in developing countries show that caries rates are increasing (2). Even in industrialized countries, the prevalence of caries in the population is as high as 95% in some areas (3-4). Therefore, the entire population must be considered at risk. A careful clinical and anamnestic evaluation must be carried out in order to assess also how risk factors, different lifestyles and socio-economic conditions can influence the onset of diseases affecting the hard tissues of the oral cavity and what their interactions with acute and chronic diseases of other organs and apparatuses can be (5).

Albania is an Eastern European country that from 1944 to 1990 was under the communist political regime and the dental care service was entirely

public, financed by the government and focused more on treatment than prevention (6). Since the early 2000s, the Albanian Government has been adopting legislative reforms aimed at prevention and maintenance of oral health, in line with the international guidelines for oral health promotion drawn up by the WHO.

In 2016 Hysi et al demonstrated that the caries experience of 5-year-old Albanian children (dmft) was lower in western and central Albania compared to other southern regions and these differences were statistically significant (7).

In Albania there is still no national caries preventive program and oral health services are provided mainly by private dental practitioners. In 1993 the dental care system went through a privatization process. Currently, private practice accounts for 95% of the provision of oral health care, while the dental

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public service only accounts for 5%. Most dentists are practicing in the capital, Tirana. Also, according to Locker and Ford, the socio-economic level of the area where people live has an impact on their health status beyond that expected by their personal and family situation (8). The introduction of orthopantomographic radiography has improved the effectiveness of early diagnosis of oral diseases (9).

Dental panoramic radiography was developed by Y.V. Paatero in 1945 and remains the most widely used radiographic examination for the diagnosis of oral diseases (10). The American Dental Association published in 2012 the guidelines for X-ray examinations in dentistry (11). Given the low radiation doses, panoramic radiography is the most suitable X-ray examination for the screening of oral diseases such as periodontitis (12-26), dental caries, bone loss and temporomandibular joint disorder (11, 27-32).

The American Dental Association today recommends periodic dental check-ups to assess the health of your mouth. In addition, it is recommended to undergo radiographic examinations such as orthopantomographic radiographies if necessary. Every dental radiographic examination is considered valid if it is carried out between 18 and 36 months from the day the patient undergoes the dental examination (11).

Shin et al. (33) reported that panoramic radiographs increased the rates of early detection of dental caries and periodontal disease by 23.1% and 31.9% respectively (34-44). In conclusion, panoramic radiographs may provide indications of periodontal status, carious root lesions, orthodontic status, periodontal fork lesions that are difficult to diagnose through objective examination alone (45-52).

The aim of this epidemiological study is to assess the dental health status of the Albanian population and the most frequently performed dental treatments, also highlighting the differences between sex, area and educational level.

In this study only recent orthopantomographic radiographs (within the last 8 months) already in the patient's possession were used to avoid further x-ray exposure.

MATERIALS AND METHODS

Sample selection took into account the population

density in different districts of the country, their geographical distribution, social economic status, education level and accessibility to examination sites. In Albania, the main economic activities are located in the west and central region followed by southern and northern regions (INSTAT 2014). The sample was drawn up and aligned to the Albanian population (ALSTAT 2019).

The study was performed by analyzing digital orthopantomographic radiographs of 739 Albanian patients, male and female, divided into 5 age groups (20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years) (Table I). In addition, the patients were divided (Fig. 1) according to regions of origin in North (Peshkopi, Kukes, Shkoder, Lezha, Dibra), Centre (Durrës, Elbasan, Tirane) and South (Fier, Gjirokaster, Pogdarec, Vlora) and classified according to the level of education in: elementary, high school, university.

The panoramic radiographs were all observed by the same operator calibrated having analysed 20 panoramic radiographs and the data obtained were controlled by two experienced operators before the study. The recorded parameters are as follows: Missed Teeth (MT), Filled teeth (FT), Endodontically treated teeth (TT), Prosthetic restorations (PR).

Statistical analysis

All data were initially entered into an EXCEL database

Table I. Population distribution by age and sex group.

AGE GROUP	North	Centre	South	Total Sum
FEMALE 20-65y	79	190	96	365
f 20-29	24	63	46	133
f 30-39	20	53	29	102
f 40-49	16	34	9	59
f 50-59	12	27	10	49
f 60-65	7	13	2	22
MALE 20-65y	90	191	93	374
m 20-29	28	62	36	126
m 30-39	19	54	35	108
m 40-49	18	46	14	78
m 50-59	12	21	4	37
m 60-65	13	8	4	25

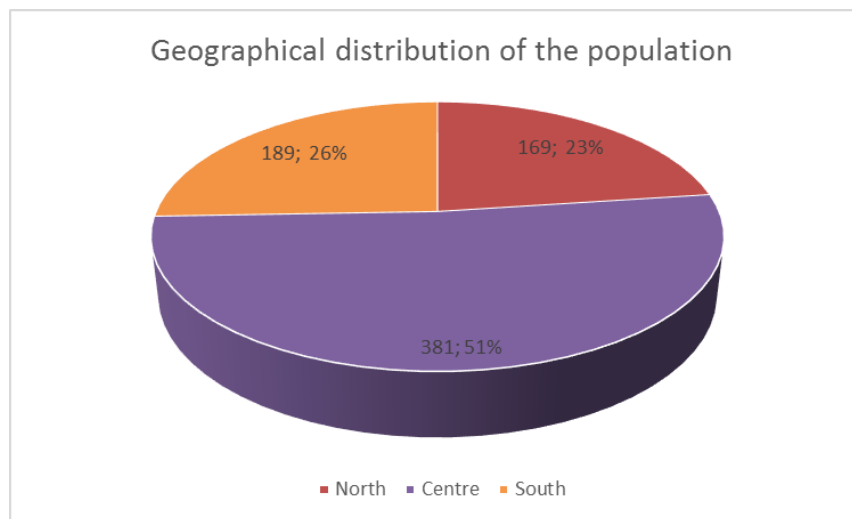


Fig. 1. *Geographical distribution of the Population.*

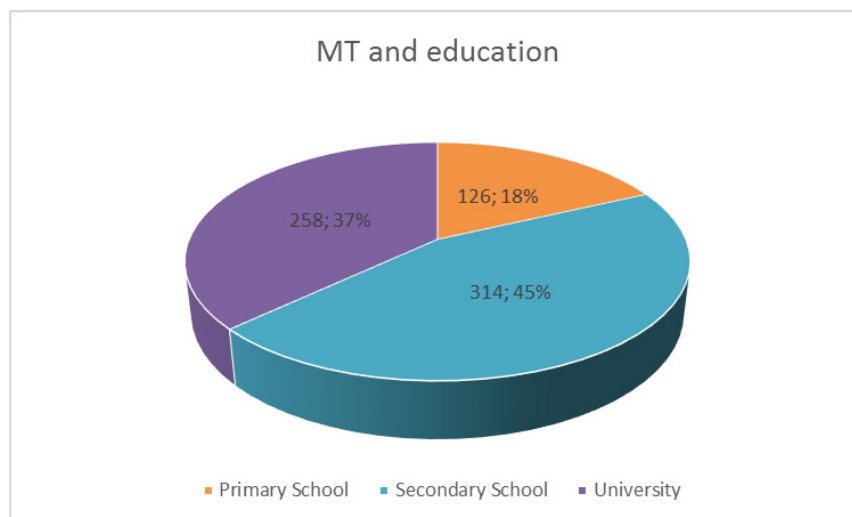


Fig. 2. *Geographical distribution of the Population.*

(Microsoft, Redmond, Washington, United States) and the analysis was performed using the Statistical Package for the Social Sciences Windows, version 15.0 (SPSS, Chicago, Illinois, USA). Descriptive statistics consisted of the mean $\pm$ standard deviation for parameter with normal distributions (after confirmation with histograms and the Kolgomorov-Smirnov test), median and range (min/max) for variables with non-normal distributions. Comparison among groups was performed with the one-way or univariate ANOVA with multiple comparisons (LSD) for normal-parametric variables, Kruskal-Wallis (groups>2) or Mann-Whitney (groups=2) for non-parametric variables and the Chi-Square test or Fisher's

exact test (if cells<5) for categorical frequency. A p value of <0.05 was considered statistically significant.

RESULTS

Sevenhundredandthirtynine subjects, equally distributed by sex, were analyzed for all the groups: Missed Teeth (MT), Filled teeth (FT), Endodontically treated teeth (TT), Prosthetic restorations (PR). There are no statistically significant results regarding the distribution by area, although it is good to point out that the area of the center shows the highest percentage of interventions. This is probably due to

migration flows to the capital Tirana located in the central area.

Referring to the correlation of the group level of education with the group Missed Teeth, there is a significant significance in the decrease of cases in subjects with university education, while in the other groups it seems that the level of education is not influential. The graph 2 highlights how the number of subjects with at least one missing tooth (MT) decreases with increasing education (33-52)

The Missed Teeth group shows an increase in the percentage of numbers as the age group increases

with a p-value of 0.000. This value increases between 20 and 40 years of age.

The percentage of individuals with missed teeth in the population increases with increasing age groups. (i.e. 100% of individuals between 50 and 69 years of age have at least one missed element). (Fig. 3).

On the other hand, filled teeth see a significant difference as the number of interventions decreases from 77% to 18% in women and 64%-48% in men as the number of extracted teeth increases with increasing age. (Fig. 4). The number of interventions in the group of endodontically treated teeth also

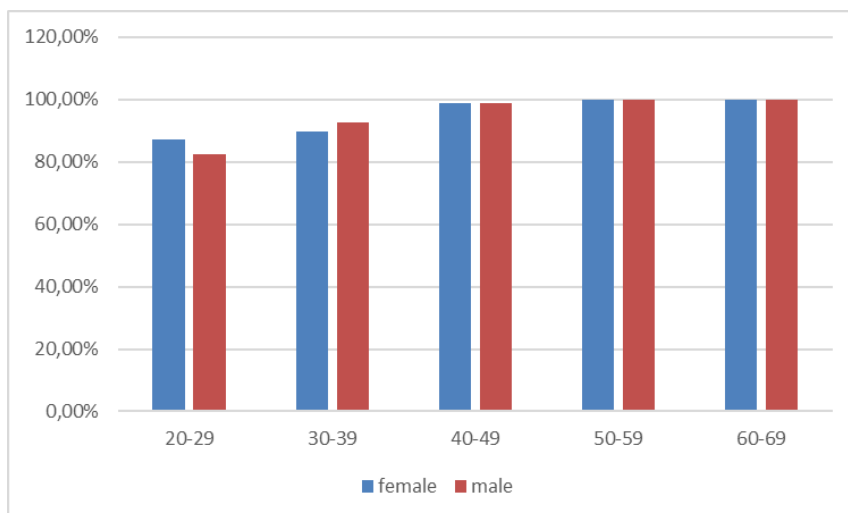


Fig. 3. *Distribution of MT, age and gender.*

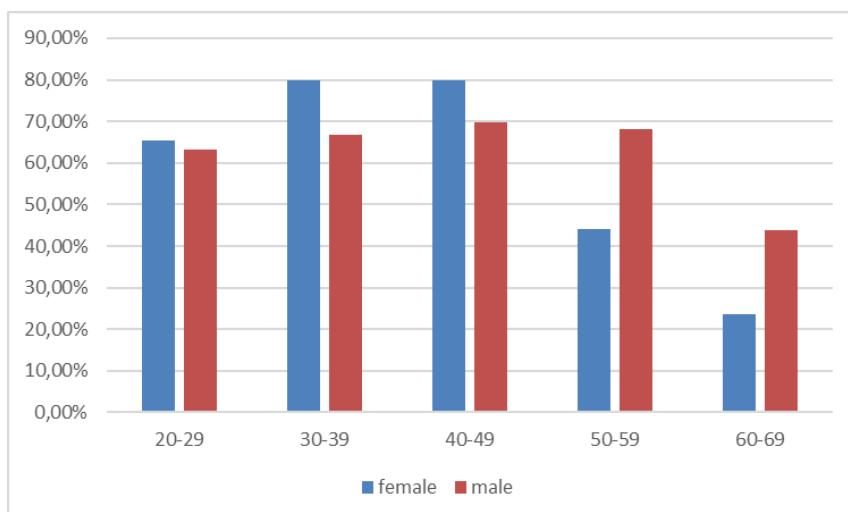


Fig. 4. *Distribution of FT, age and gender.*

decreases significantly with increasing age (79%-36% in women; 71%-40% in men). (Fig. 5).

From the results of the Mann-Whitney test we can see how the behavior of this decrease in the number of interventions of both FT and TT groups changes according to gender; in particular, females have a very high initial value between 20 and 29 years of age that gradually decreases to 60 years of age, while men decrease less quickly but significantly between 40 and 60 years of age. The presence of prosthetic restorations (RP GROUP) increases significantly as the age group increases, equally distributed between

males and females with values ranging from 7.3% in the younger age group to 33.35% in the older class (over 60) (Fig. 6).

DISCUSSION

Epidemiological studies are widely used in dentistry to establish the oral health level of the population and consequently to plan health interventions by establishing the real treatment needs. In Albania there is no national preventive program and oral health services are provided mainly

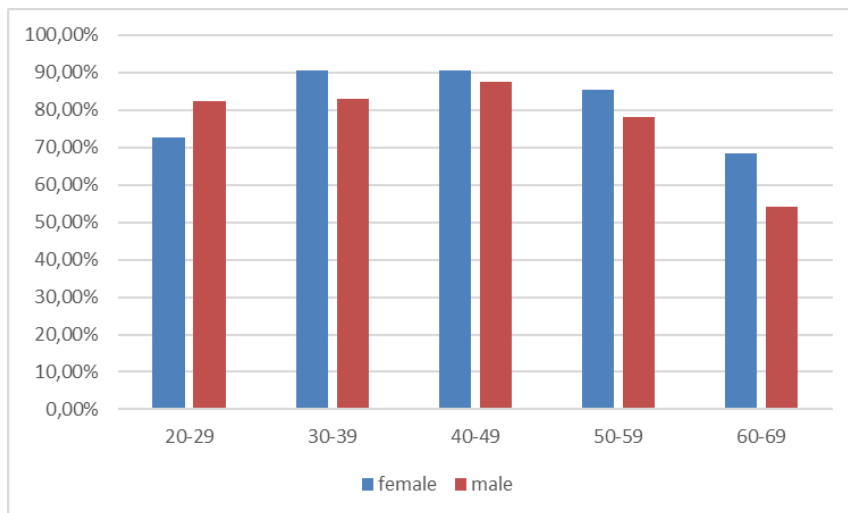


Fig. 5. Distribution of TT, age and gender.

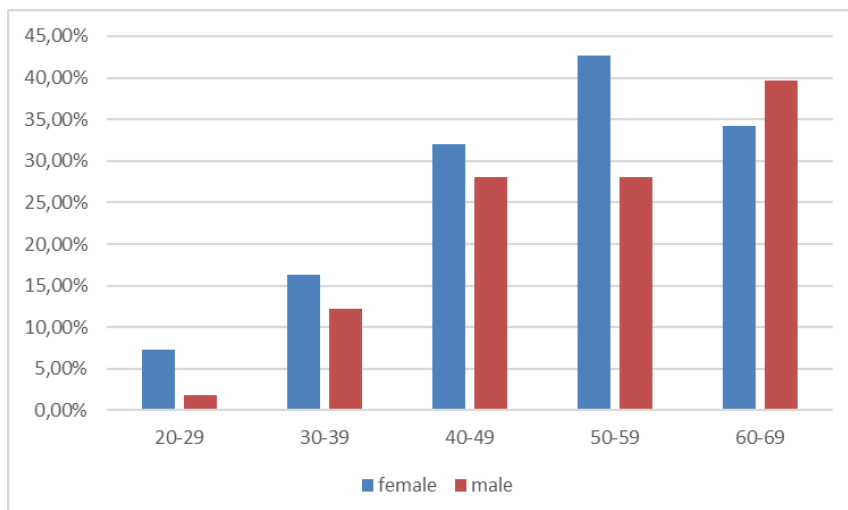


Fig. 6. Distribution of PR, age and gender.

by private dental practitioners. Oral care remains more focused on treatment than on prevention.

There are no recent data reporting national dental oral Health status in Albania. Also, no national study concerning oral health status in Albania has been published in the international literature. Most of the previous studies were performed locally and did not include areas with low socio-economic status and lack of oral health care.

Educational background also affects health and behavior, including dietary and tooth cleaning habits, or health service utilization frequency and patterns. Social position is further known to determine social participation, support, stability, and cohesion, which positively affect health and oral health (53-60).

Tooth loss is a good way to see familiarity with oral health system and in correlation to the age parameter, it represents the photograph of primary and secondary prevention habits in dentistry. In a developing Country, education, social position, and the sort of area you live in have a unique impact on oral health. There is evidence that all these may generate oral health inequalities.

In conclusion, this study highlights the need to introduce a national oral health system in Albania that would extend interventions to an increasing segment of the population.

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ANTIBACTERIAL ACTIVITY OF FIRST AND LATEST GENERATION BIO CERAMIC SEALERS ON THE ELIMINATION OF ENTEROCOCCUS FAECALIS: AN *IN VITRO* STUDY

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The main concern of endodontics are bacteria: the microorganisms and the toxins they release determine tissue destruction and inflammatory changes that trigger the pulpal and periapical pathology. Enterococcus faecalis is a particularly hostile bacterium; it is strongly associated with refractory periapical pathology. In this study 3 materials were tested: Aureoseal (OGNA, Italy) (Aur) (first generation bioceramic sealer); Endo Sequence BC sealer (Brasseler USA, Savannah, GA, USA) (EsBC) (latest generation bioceramic sealer); Endodontic (OGNA, Italy) (Endx) (calcium hydroxide material). The antimicrobial activity of these materials against Enterococcus faecalis was evaluated using agar diffusion test (ADT). The results confirm the antibacterial activity of bioceramic sealers, which however do not show significant differences in the obtained values; it can therefore be concluded that there has been no increase in the antibacterial efficacy of the latest generation bioceramic sealers compared to those of the first generation.

The main concern of endodontics are bacteria: the microorganisms and the toxins they release determine tissue destruction and inflammatory changes that trigger the pulpal and periapical pathology (1-5). To heal periapical tissues and preserve the affected tooth, an endodontic treatment which disinfects and seals the root canal is required (6-10).

Due to the complexity of the endodontic anatomy, it is not always possible to remove all microorganisms by chemo-mechanical preparation; bacteria can in fact protect themselves in endodontic ravines where neither tools nor irrigants can be effective. In these areas, bacteria can organize into a biofilm that prolongs their survival. Despite endodontic obturation, these microorganisms may be able to resist nutrient deprivation and remain latent even for a long time (11-16). When nutrients are made available again by infiltration of the filling,

these bacteria can exit their latency and resume their metabolism multiplying and establishing a new pathological process. This infection, as sustained by a biofilm of bacteria already strongly selected, often proves to be difficult to eradicate, and in some cases may require extraction of the tooth (17-24). In this perspective the antibacterial abilities of endodontic sealers can be useful in increasing the proportion of microorganisms eliminated and thus contributing to the success of the therapy.

Enterococcus faecalis is a particularly hostile bacterium because it is able to persist *ex vivo* for more than 12 months in the root canal and it is resistant to nutrient deprivation and intermediate endodontic medications; it is also able to penetrate the dentinal tubules, aggregate in biofilm and compete with different microorganisms (25-30). For these reasons Enterococcus faecalis is reported

Key words: bacteria, bioceramic sealer, antimicrobial activity

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to be strongly associated with refractory periapical pathology (31,32).

EndoSequence BC sealer is a latest generation bioceramic sealer based on calcium silicate (33-35). The composition includes tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, calcium hydroxide, zirconium oxide (radiopacifier), and water-free thickening vehicles to make it in a paste form. According to its manufacturer, EndoSequence BC sealer uses the moisture that remains within the dentinal tubules after canal irrigation for its setting reaction. Its antibacterial activity derives from the ability to raise the pH above 12 (36); this material has also been promoted as biocompatible and non-toxic.

The agar diffusion test (ADT) used to be the most commonly applied method to assess the antimicrobial activity of endodontic sealers (37, 38).

The objective of the present study was to compare the antibacterial efficacy of EndoSequence BC against *Enterococcus faecalis* using ADT, and to verify a higher antimicrobial effect of the latest generation bioceramic sealers compared to those of the first generation.

MATERIALS AND METHODS

In this study 3 materials were tested: Aureoseal (OGNA, Italy) (Aur) (first generation bioceramic sealer); Endo Sequence BC sealer (Brasseler USA, Savannah, GA, USA) (EsBC) (latest generation bioceramic sealer); Endodrox (OGNA, Italy) (Endx) (calcium hydroxide material). The antimicrobial activity of these materials against *Enterococcus faecalis* was evaluated using ADT.

Each sealer was tested in 12 plates and a total of 36 Biomeraux plates were used for the study. Each plate received 20 mL of Mueller Hinton agar medium with a pipette to form a flat 4mm deep layer. The plates were then inoculated with *E. faecalis* suspensions adequate to the 0.5 McFarland standard.

In each plate three equally spaced cavities were made, each measuring 4mm in depth and 7mm in diameter. Of these cavities: 1 was filled with the material to be tested (mixed according to the manufacturer's instructions); 1 was filled with chlorhexidine, designated as a positive control; 1 was left empty and designated as a negative

control. The plates were pre-incubated for 1 hour at room temperature for the pre-diffusion of the material and subsequently incubated at 37 degrees for 72 hours.

After 72 hours the antimicrobial activity of the materials was studied with ADT: the diameters of the zones of inhibition of bacterial growth around each cavity were measured. The recorded values have been entered in an excel database.

Statistic analysis

All data were entered into an Excel spreadsheet (Microsoft, Redmond, Washington - United States) and the analysis was performed using the Windows social sciences statistical package, version 15.0 (SPSS, Chicago, Illinois, USA). The description of the Normal parameters (after confirmation with the histograms and the Kolgomorov-Smirnov test) consisted in calculating the mean and standard deviation while for the non-Normal distribution parameters the median and the interval (minimum; maximum) was calculated. The comparison for normal variables was performed with Bonferroni's Anova oneway + post-hoc multiple test. A "p value" of <0.05 was considered statistically significant. All the charts have been produced with Excel (Microsoft, Redmond, Washington - United States).

RESULTS

In our study, chlorhexidine designated as a positive control achieved the highest values, with a mean inhibition zone of 19.43mm. Slightly lower results were recorded for Endodrox, with an average inhibition zone of 18.87mm.

The bioceramic sealers Aureoseal and EndoSequence BC have obtained the lowest values, respectively of 15.23mm and 15.48mm (Table I) (Fig. 1). The mean difference between these two sealers was not statistically significant (Table II).

DISCUSSION

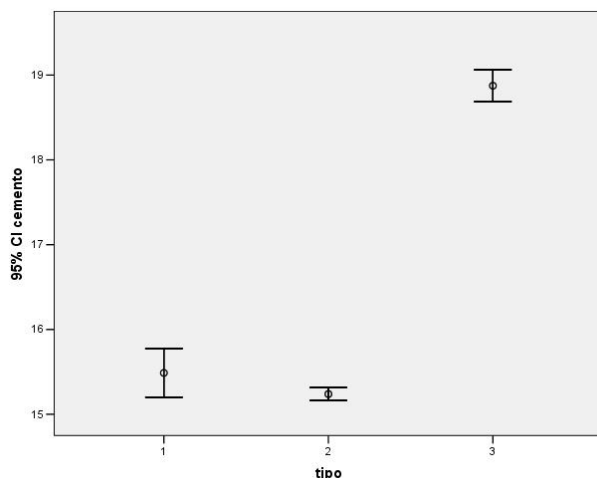
In this study the antibacterial activity of 3 endodontic materials (Aureoseal, EndoSequence BC and Endodrox) against *E. faecalis* was evaluated. In the present study, Endodrox obtained values comparable to those of chlorhexidine designated

Table I. Descriptives statistic.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Sealer	Endosequence BC	12	15.4875	.45303	.13078	15.1997	15.7753	14.86	16.50
	Aureoseal	12	15.2383	.12044	.03477	15.1618	15.3149	14.94	15.46
	Endoidrox	12	18.8742	.29614	.08549	18.6860	19.0623	18.23	19.13
	Total	36	16.5333	1.71035	.28506	15.9546	17.1120	14.86	19.13

Table II. Multiple comparison between sealer (Bonferroni), * The mean difference is significant at the .05 level.

Dependent Variable	(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Sealer	EsBC	Aur	.24917	.13069	.196	-.0805	.5788
		EndX	-3.38667(*)	.13069	.000	-3.7163	-3.0570
	Aur	EsBC	-.24917	.13069	.196	-.5788	.0805
		EndX	-3.63583(*)	.13069	.000	-3.9655	-3.3062
	EndX	EsBC	3.38667(*)	.13069	.000	3.0570	3.7163
		Aur	3.63583(*)	.13069	.000	3.3062	3.9655

**Fig. 1.** Values and Std deviation of three sealers.

as a positive control. Although today the intent is always to carry out the endodontic filling in the first endodontic session, in cases where this is not possible (for example due to draining periapical

lesions) calcium hydroxide or chlorhexidine can be useful as intermediate dressings thanks to their high antibacterial capacity, as confirmed by the present study and previous studies (39-42). Aureoseal and EndoSequence BC are both bioceramic sealers. Aureoseal is a first generation bioceramic sealer based on aluminum silicate (43), mainly used for the repair of endodontic perforations.

EndoSequence BC is a latest-generation bioceramic sealer based on calcium silicate, used as bioactive endodontic cement; it also has biocompatibility similar to that of MTA, but less toxicity. The antibacterial activity of these two bioceramic sealers derives from their ability to form, in contact with water, hydroxide ions able to alkalize the pH (44-49).

Previous studies have already shown the presence of antibacterial activity in bioceramic sealers. Zhang et al. in 2009 showed that freshly-mixed bioceramic sealer samples were able to kill the enterococcus

faecalis in 2 minutes, and that their antibacterial capacity was the highest compared to other sealers even for samples aged 1 day and 3 days (50). In 2014 Nirupama et al. confirmed the greater antibacterial activity towards the enterococcus faecalis compared to the other sealers under study, and observed a modest antimicrobial power also against *C. albicans* and *S. aureus* (51, 54-60). In 2016 Candeiro et al. compared bioceramic sealers and epoxy resin-based sealers with regard to antimicrobial capacity against enterococcus faecalis, cytotoxicity and genotoxicity. The bioceramic sealers showed an antibacterial power similar to the others, but less cytotoxicity and genotoxicity (52). In 2018 AlShwaimi et al. in their systematic review reported moderate scientific evidence regarding the antimicrobial capacity of bioceramic sealers, due to the small number of studies conducted on them and their low to moderate quality (53).

In our study, both sealers confirmed that they possess antibacterial capacity, obtaining results that are practically overlapping. The limitations of ADT method are nowadays well-recognized. The results obtained are not likely to reflect the true antimicrobial potential of the various sealers or disinfecting agents; therefore, ADT method should have to be supported by more specific microbiological tests such as direct contact test (DCT).

The aim of the present study was to confirm the antimicrobial activity of bioceramic sealers and to assess any increase in efficacy of those of the latest generation (EndoSequence BC) compared to those of the first generation (Aureoseal).

The results confirm the antibacterial activity of bioceramic sealers, which however do not show significant differences in the obtained values; it can therefore be concluded that there has been no increase in the antibacterial efficacy of the latest generation bioceramic sealers compared to those of the first generation.

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PREVENTIVE CONTAMINATION WITH *LACTOBACILLUS REUTERI* OF THE IMPLANT-ABUTMENT CONNECTION: AN *IN VIVO* STUDY

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The aim of this study is to preventively contaminate the abutment-fixture connection (AFC) with *Lactobacillus reuteri* (*L. reuteri*), to evaluate the presence of micro leaks in different types of implant-abutment connections, by measuring the concentration of *L. reuteri* in the sulcular fluid over time. This microorganism produces the Reuterine, an antibiotic which counteracts the development of other microbial species. Fourteen biphasic implants were placed on 10 patients (3 women; 7 men; mean age 55.9±16.54y). Eight implants had a flat top connection (internal Hex) while six implants had a tapered connection (conical plus octagonal). At the time of prosthetic finalization, before the healing screw was removed, the sulcular fluid was harvested, on each implant, by means of three sterile paper cones placed into the gingival sulcus and left in place for 20sec before to be transferred to a sterile tube. Once the healing screw has been removed, first the implant connection has been gently dried with air jet for 10sec and then completely filled with *L. reuteri* DSM 17938 (Reuflor, Italchimici, Italy). Immediately after the prosthetic finalization the sulcular fluid was then harvested again, with the same procedure described before, and repeated at 1 week and 1 month of follow up. The samples were then sent for subsequent DNA extraction and real-time PCR. Our results demonstrate that the concentration of *L. reuteri*, in the sulcular fluid, does not persist over time in case of preventive contamination of the AFC, demonstrating no significative differences between flat top and tapered connections. Therefore, the use of the latter does not lead to a lower risk of Peri Implant Disease (PID). Long-term studies, involving a larger number of samples, are advisable to confirm these findings.

Modern dentistry has witnessed an increased demand for implant-prosthetic rehabilitations as well as the increasing incidence of Peri Implant Disease (PID) (i.e. Mucositis and Peri-Implantitis) (1-3). Although the etiology of PID, probably multifactorial, has not been completely outlined, scientific evidence shows that the oral microbiome has a fundamental influence in its pathogenesis (1-3), also as in all dental specialties (65-78).

PID seems to be more associated with biphasic rather than monophasic implants and the cause seems to be due to the possibility of microbial contamination of the abutment-fixture connection (AFC) that characterizes the biphasic implants. Bacterial leakage is in fact favored by the presence of micro-gaps at the AFC level, allowing microorganisms to penetrate and colonize the inner part of the implant, leading to biofilm accumulation (4-7).

Key words: implant-abutment connection, Lactobacillus reuteri, preventive contamination, implant-prosthetic rehabilitations, Peri Implant Disease, PID

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The microbial contamination of the AFC would then create an inflammatory process at the tissue/implant interface, thus leading to PID development (4-7). The microbial contamination of the AFC is mainly due to the exposure of the AFC to the oral environment. In some cases it could be associated with a previous contamination, that sometimes occurs during the laboratory phases of preparation of the prosthetic device (i.e. contamination with *Streptococcus faecalis*) that can be prevented by previous sterilization of both the abutment and the prosthesis before their positioning in the mouth (4).

It has been proven that the type of AFC (ie., Flat top or Tapered) influences the effectiveness of microbial seal (8). Tapered connections feature a conometric coupling of the components, offering a higher seal and greater stability of the AFC, especially in relation to extra-axial loads, both static and dynamic, minimizing the micromovements, which facilitates the oral fluids transition inside the connection (9).

However, although Tapered connections are more resistant to microbial contamination than Flat

Top connections, they are not exempt from micro-leakage (9, 10).

The aim of this study is to preventively contaminate AFC with *Lactobacillus reuteri* (*L. reuteri*), to evaluate the presence of micro leaks in different types of implant-abutment connections, by measuring the concentration of *L. reuteri* in the sulcular fluid over time. This microorganism is a probiotic which produces the Reuterine, an antibiotic which counteracts the development of other microbial species.

MATERIALS AND METHODS

Patients Selection

Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program. The exclusion criteria were as follows: presence of bruxism, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood or kidney

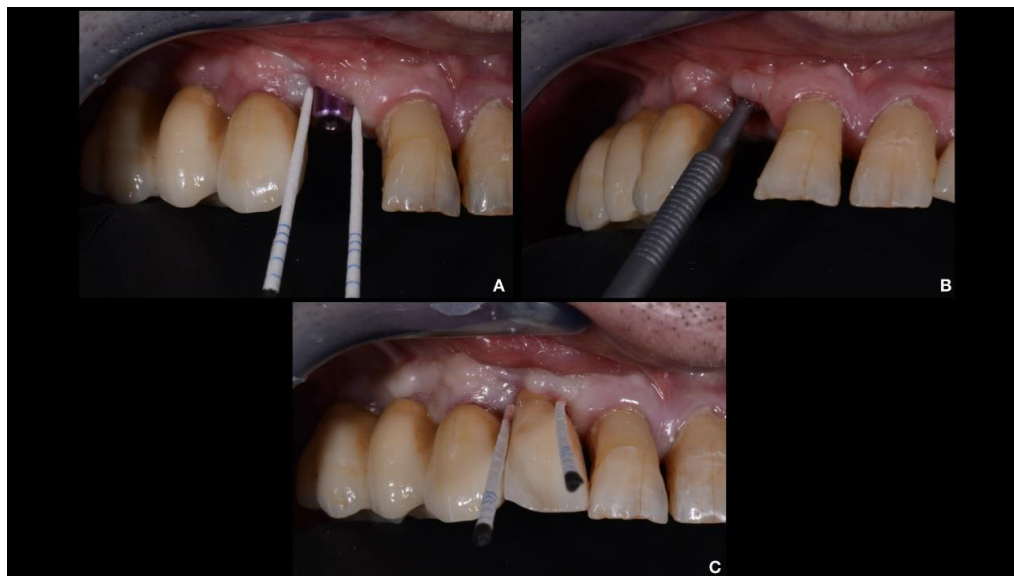


Fig. 1. A): before the healing screw was removed, the sulcular fluid was harvested, on each implant, by means two sterile paper cones (ISO 60), left in place for 20sec; B) once the healing screw has been removed, the implant connection has been prior gently dried with air jet for 10sec and then completely filled with *L. reuteri* DSM 17938, in watery environment, carried with an applicator; C) immediately after the prosthesis placement the sulcular fluid was then harvested again, with the same procedure described in figure 1A.

diseases, immune-suppressed patients, patients taking corticosteroids, pregnancy, inflammatory and autoimmune diseases of the oral cavity.

Variables

In addition to the concentration of *L. reuteri* and the total microbial load recorded in the sulcular liquid, of the implants tested, several other variables were investigated: demographic (age, gender); AFC type (flat top, tapered); patient's related pathologies (diabetes, smoking, hypertension); type of prosthesis (screw retained crown, cemented crown, overdenture); prosthetic material (monolithic zirconia, acrylic resin plus Cr/Cb alloy).

Implant Systems

Fourteen biphasic implants (FMD, Italy) were placed on 10 patients (3 women; 7 men; mean age 55.9 ± 16.54 y). Eight implants had a flat top connection (internal Hex) while six implants had a tapered connection (conical plus octagonal).

Samples Preparation

At the time of prosthetic finalization, before the healing screw was removed, the sulcular fluid was harvested, on each implant, by means two sterile paper cones (ISO 60, DiaDent, Almere, The Netherlands) placed into the gingival sulcus and left in place for 20sec before to be transferred to a sterile tube (fig. 1A). Once the healing screw has been

removed, the implant connection has been prior gently dried with air jet for 10sec and then completely filled with *L. Reuteri* DSM 17938, in watery environment (Reuflor gocce, Italchimici, Italy), carried with an applicator (Microbrush X, Microbrush International, Germany) (Fig. 1B). Subsequently the abutment and the abutment-screw, previously autoclaved at 134°C , were then mounted.

Each screw was subsequently screwed and unscrewed for 10 times, by hand, to favor its settling and hence tightened at 32Ncm using a torque wrench (11). For just 2 implants, one-piece abutments were used (ball abutment) to retain a removable prosthesis while, all the remaining implants, were finalized with a monolithic zirconia fixed prosthesis (5 cemented; 7 screwed). The sulcular fluid was then harvested again, with the same procedure described before (Fig. 1C) and repeated at 1 week and 1 month after prosthetic finalization.

Quantification of *L. reuteri* and total microbial load (TML)

The procedure was performed targeting genomic DNA molecules by real-time PCR, which directly relates to the number of bacteria in the specimens. Specimens were processed to disrupt bacterial envelopes with two consecutive incubations with lysozyme and proteinase K, that were effective on both Gram-positive and -negative species. Genomic DNA was then purified by QIAxtractor instrument using the dedicated QIAcube HT purification kit (Qiagen, Milan, Italy).

Table I. Contamination.

Patients	LR1	LR2	LR3	LR4	TML1	TML2	TML3	TML4	Age	Gender	Smoke	Hypertension	Diabetes	AFC	Type of Prosthesis	Prosthetic Material
1	0	7937	0	0	83196	53271	430852	34553	70	2	0	0	0	1	1	1
1	0	22440	166	0	90813	113674	183816	96127		2	0	0	0	1	1	1
2	0	3520	0	0	455450	55770	25532	50197	39	2	0	0	0	2	2	1
3	0	2897	0	0	415408	24460	101857	130758		1	0	1	0	1	1	1
3	0	546	16	0	67615	71087	7305375	108324	71	1	0	1	0	1	1	1
4	0	51541	0	17	91124	104390	82362	173999	39	1	0	0	0	2	2	1
4	0	16844	0	0	109596	32561	85483	27279		1	0	0	0	2	2	1
5	0	11776	184	0	78436	39081	93065	145120	42	1	0	0	0	1	2	1
6	0	784	14	60	66389	25101	63012	130898	65	1	1	0	0	2	1	1
7	0	2155	13	0	68675	14569	152224	98382	66	1	0	0	0	1	3	2
7	0	1790	0	0	83096	83045	75009	91231		1	0	0	0	1	3	2
8	0	276	0	0	119323	5922	323251	78890	66	1	1	1	0	1	2	1
9	0	14080	0	0	264756	465484	55824	171448	29	2	1	0	0	2	2	1
10	0	2258	0	38	39210	15441	18808	1033523	72	1	0	0	0	2	2	1

LR1 = quantification of *L. reuteri* prior to AFC contamination; LR2 = quantification of *L. reuteri* immediately after AFC contamination; LR3 = quantification of *L. reuteri* at 1 week follow up; LR4 = quantification of *L. reuteri* at 1 month follow up; TML1 = total microbial load prior to AFC contamination; TML2 = total microbial load immediately after AFC contamination; TML3 = total microbial load at 1 week follow up; TML4 = total microbial load at 1 month follow up; Age: expressed in years; Gender: Male = 1; Female = 2; Smoke: no smoker = 0; smoker = 1; Hypertension: absent = 0; present = 1; Diabetes: absent = 0; present = 1; AFC (abutment-fixture connection): flat top connection = 1; tapered connection = 2; Type of Prosthesis: fixed cemented = 1; fixed screwed = 2; overdenture = 3; Prosthetic Material: monolithic zirconia = 1; acrylic resin + Cr-Cb alloy = 2.

Quantitative real-time PCR was performed with the hydrolysis probes method to evaluate the TML and to quantify the amount of *L. reuteri*. Primers and probes oligonucleotides were designed on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1). All the sequences were aligned in order to find both *L. reuteri* specific sequences and consensus sequence for the evaluation of TML by degenerate primers. PCR conditions were optimized to ensure sensitivity, specificity, and no inhibitions in case of unbalanced target amounts.

Absolute quantification assays were performed using the ViiA7 PCR thermal cycler (Applied Biosystems, Monza, Italy). The amplification profile included a 10' incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15'' at 95°C and 60'' at 57°C, for a total of 40 cycles. Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany), in serial dilutions ranging from 101 to 107 copies, were used to draw a standard curve for the quantitative analysis. All the experiments were performed including non-template controls to exclude reagents contamination (12-14).

Statistical Analysis

The results, concerning the concentration of *L. reuteri* and the TML, were compared by Paired Samples t-Test while the correlation among the over-mentioned values and the other variables were investigated by means Wilcoxon signed-rank test. SPSS software was used to perform statistical analyses. A 5% level of significance and 95% confidence interval were used for all the tests.

RESULTS

The results and the variables recorded are resumed in the Table I. No significant differences were found between the detected *L. reuteri* concentrations and the TML except for the comparison between LR1-LR2 and LR2-LR3 pairs ($p = 0.019$ for both), demonstrating that the concentration of *L. reuteri* in the sulcular fluid does not persist over time. Not even the variables analyzed seem to have a statistically significant effect both on the concentration of *L. reuteri* and on the total microbial load.

DISCUSSION

Presence of gap at the implant-abutment interface, leads to microleakage and accumulation of bacteria which can affect the success of dental implants (15). Both mucositis and periimplantitis represent serious problems, given the increasing demand for oral implant rehabilitation (1-4).

A recent meta-analysis study indicates a reduced occurrence of peri-implantitis and bone loss at the abutment/implant level associated with tapered connections and a reduced-diameter platform switching abutments (8). Although tapered connections are superior, in term of mechanical stability and sealing, in comparison to flat top connection, the problem of micro-leakage persists (15). *L. reuteri* is a probiotic which create a biofilm and protect the oral tissues against the action of periodontal pathogenic bacteria (16).

The *L. reuteri* has already been used in the pediatric field for colonization of the digestive tract in cases of intestinal dysmicrobism (17, 18), as well as adjuvant in the treatment of periodontitis (19-21). Different studies in literature show that the application of *L. reuteri* in patients with implants presenting mucositis and peri-implantitis could be helpful both for treatment of PID and for its prevention (16, 22, 23).

Mucositis and peri-implantitis is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. also (24-36). Mucositis and peri-implantitis diagnosis and therapy has been increased a great interest in the last years both in basic (37-48) and advanced research (49-55). Besides the treatment of mucositis and peri-implantitis requires a multidisciplinary approach (56-58, 62-64).

A recent study though evaluated if the application of *L. reuteri*, after mechanical debridement and administration of 0.12% chlorhexidine, was effective in reducing mucositis, but the administration of *L. reuteri* did not seem to provide an additional clinical or microbiological benefit (59). In the same way a recent *in vivo* study indicates that the use of the *L. reuteri* did not influence in a statistically significant way the peri-implant microbiota in case of mucositis and periimplantitis, despite the fact that there was a reduction in the number of periodontal and peri-

implant species. This phenomenon was explained either by the small sample population or by the short evaluation period, together with the different biological characteristics of the implant-gingival interface with respect to the periodontal sulcus (60). Similar results have come from a previous study (61).

Our results demonstrate that the concentration of *L. reuteri*, in the sulcular fluid, does not persist over time, in case of preventive contamination of the AFC, demonstrating no significant differences between flat top and tapered connections. Therefore, the use of the latter does not lead to a lower risk of PID. However, more long-term studies, involving a larger number of samples, are advisable to confirm these findings.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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THE USE OF OZONE THERAPY FOR TREATMENT OF PERIODONTAL DISEASE: A SPLIT-MOUTH, RANDOMIZED, CONTROLLED CLINICAL TRIAL

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Periodontal treatment has the aim to reduce oral infection and prevent the progression of the disease. The potential benefits of new therapy with Ozonline® for periodontal treatment, include improved patient compliance and an easier access to periodontal pocket. The objective of this study was to explore the efficacy of Ozonline® in the treatment of chronic periodontitis in adult patients. A randomized controlled split-mouth study was carried out in ten patients (5 men and 5 women age 42-73 mean 55 ± 7) with a diagnosis of chronic periodontitis. None of these patients received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler with ozonated water (Ozonline®). 10 microbiological samples were collected from upper left quadrants and 10 from upper right quadrants from each patient. Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. 20 localized chronic periodontitis sites were selected (10 in left quadrants and 10 in right quadrants). After the treatment with Ozonline®, a remarkable decrease in bacteria amount, both for some species and for the total count was observed in the left quadrants respect to right ones. Specifically, *T. forsythia* and *T. denticola* were eradicated whereas Total Bacteria Loading and *Fusobacterium Nucleatum* showed a reduction of 38% and 55%, respect to right quadrants. Our study demonstrated the efficacy of the Ozonline® in the management of moderate to severe chronic periodontitis.

The term periodontal disease (PD) is generally used to describe diseases affecting the gums and tooth support tissues, causing damage to the connective tissue and alveolar bone (1). Specific bacteria of the biofilm formed by plaque cause PD. The bacteria leak into the periodontal ligament space, causing anaerobic infection creating a cascade of events, which end with the production of inflammatory mediators and bacterial metabolites (1). PD affects about the 50% of adults, or more, while the severe form of periodontitis affects around 10% (range: 5-20%) of adults and moderate periodontitis around

30%. In elderly people, the prevalence of PD is estimated about 70-90% of individual's aged 60-74 years (1). Periodontal therapy is important to reduce local inflammation and bacteremia. Recently it has been stated that periodontal disease appears to increase the risk of cardiovascular disease, pulmonary disease, preterm and low birth weight (2).

The oral microbiota is constituted by many bacteria species that form a biofilm. The biofilm includes both saprophytes and potentially pathogenic species. Periodontal treatment has the aim to reduce oral infection and prevent the progression of the

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disease. Periodontal non-surgical therapy with scaling and root planning associated with a good level of domestic oral hygiene can prevent the onset of the disease and allow for proper maintenance of oral health (3, 4).

Good oral hygiene has the aim to control bacterial plaque, but when the patient's attention to oral hygiene decreases, it is possible to experience a recurrence of the disease. In addition, periodontal disease presents a characteristic trend with periods of remission and exacerbation of the disease. Scaling and root planning are efficacy, but a better microbiological control of oral microbiota can be reached by the administration of ozonated water in association.

The aim of this study was to investigate the efficacy of ultrasonic scaling with ozonated water, acting as bactericidal in the management of moderate to severe chronic periodontitis.

Ozon therapy

The main use of ozone in dentistry relies on its antimicrobial properties. It is proved to be effective against both Gram-positive and Gram-negative bacteria, viruses and fungi (5). Nagayoshi et al (5) examined the effect of ozonated water on oral microorganisms and dental plaque. Dental plaque samples were treated with 4mL of ozonated water for 10 seconds. They observed that ozonated water was effective for killing gram-positive and gram-negative oral microorganisms and oral *Candida albicans* in pure culture as well as bacteria in plaque biofilm and therefore might be useful to control oral infectious microorganisms in dental plaque.

MATERIALS AND METHODS

Ten patients (5 men and 5 women) were randomly selected with a diagnosis of chronic periodontitis. Patients who qualified for the study were in the age group of age 42-73 (mean 55 ± 7) and did not receive any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they met any of the following criteria: pregnancy; a history of taking antibiotics or using anti-bacterial mouth rinses for the past 6 months; has teeth with furcation involvement; patients with a history

of smoking, and drug or alcohol abuse. The subjects that volunteered to participate in the study, signed consent forms and followed a detailed verbal description of the procedure. This study was conducted in accordance with the Declaration of Helsinki and independently approved and reviewed by the local Institution's Ethical Committee.

Clinical methods

Twenty sites (distal pockets of the left and right first upper molar) were selected from the 10 patients. Each selected site was subjected to microbial analysis. At the first visit, after recording the clinical parameters at each site in selected patients at the baseline, microbiological samples were collected from pre-selected sites. The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler plus ozonated water (4 mg/ml in distilled water) (Ozonline®). Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. For the collection of subgingival samples, the sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of sub-gingival samples and were immediately transferred to microbiological laboratory for processing. The microorganisms processed were the three bacterial species more involved in periodontitis that constitute the red complex group: *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*.

Several methods have been used for microbiological testing in periodontitis (7). However, many techniques have not been fully accepted due to low sensitivity or specificity, moreover sometimes they are slow, expensive and laborious. In this study we have used the (LAB-test®) developed by the LAB corporation (Ferrara, Italy), which is rapid and sensitive, allowing to detect and quantify the three bacterial species more involved in periodontitis that constituted the red complex group: *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*. Both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction. They appear closely 'linked' topologically in the developing biofilm and are considered the first pathogens involved in the clinical destruction of periodontal tissues.

Real-Time Polymerase Chain Reaction

Primers and probes oligonucleotides have been

designed based on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequences. For each sample, two real-time polymerase chain reaction (PCR) runs were performed. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe, matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria in a multiplex PCR. The reaction included a total of six primers and three probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed by using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15s at 95°C and 60 s at 57°C for 40 cycles. All the experiments were performed including non-template controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as a standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same number of plasmids, in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions. The copy numbers for individual plasmid preparations was estimated using a Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of the relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

Statistical analysis

Descriptive statistics was registered using Microsoft Excel spreadsheets. Paired T-test from Spss program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

RESULTS

After the treatment with ultrasonic scaler plus ozonated water with Ozonline®, a remarkable decrease in total bacteria amount and in the individual microbic species was observed in the left periodontal pockets respect to right pockets. Specifically, *T. forsythia* and *T. denticola* were eradicated whereas the total bacteria loading and *Porphyromonas gingivalis* showed a percentage reduction of 38% and 55%, respect to right pockets. Ozonated water with Ozonline® did not show any side effects and was not observed to cause discomfort or to produce adverse reactions in time. No patient reported pain, burning, tingling sensation or numbness. Despite the spread of the data, preventing a correct statistical evaluation, the results obtained clearly demonstrated a reduction of microbial concentration after treatment with Ozonline®.

DISCUSSION

The oral microbiota is constituted by many bacteria species that form a biofilm. The biofilm includes both saprophytes and potentially pathogenic species. It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic microorganisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health. The oral cavity is suitable for invasion of many microorganisms. Mechanical debridement is effective in both breaking the biofilm and reducing the bacterial load.

However, the effects of mechanical instrumentation may be improved with the association of Ozonline®, providing an additional benefit in controlling the progress of disease. Nagayoshi et al (5) tested the efficacy of three different concentrations of ozone water (0.5, 2, and 4 mg/ml in distilled water) on the time-dependent inactivation of cariogenic, periodontopathogenic and endodontopathogenic microbes (*Streptococcus*, *Porphyromonas gingivalis* and *endodontalis*, *Actinomyces actinomycetemcomitans*, *Candida albicans*) in culture and in biofilms. They confirm

that ozonated water was highly effective in killing of both gram positive and gram-negative microorganisms.

Depending on the dosage, the oral microbes were inactivated after 10 seconds. Gram negative anaerobes, such as *Porphyromonas endodontalis* and *Porphyromonas gingivalis* were substantially more sensitive to ozonated water than gram positive oral streptococci and *Candida albicans* in pure culture. Furthermore, ozonated water had strong bactericidal activity against bacteria in plaque biofilm. In addition, ozonated water inhibited the accumulation of experimental dental plaque *in vitro*.

Ramzy et al (6) irrigated the periodontal pockets by ozonized water in 22 patients suffering from aggressive periodontitis (age range from 13 to 25 years). Periodontal pockets were irrigated with 150 ml of ozonated water over 5 to 10 minutes once weekly, for a clinical four weeks study, using a blunt tipped sterile plastic syringe. High significant improvement regarding pocket depth, plaque index, gingival index and bacterial count was recorded related to quadrants treated by scaling and root planning together with ozone application. They also reported significant reduction in bacterial count in sites treated with ozonized water.

In the study by Karapetian et al, (8) peri-implantitis treatment with conventional, surgical and ozone therapy methods was investigated, and it was found that the most effective bacteria reduction was in the ozone-treated patient group. The authors concluded that the main challenge seems to be the decontamination of the implant surface, its surrounding tissue and the prevention of recolonization with periodontal pathogenic bacteria.

Kshitish and Laxman (9) conducted a randomized, double-blind, crossover split-mouth study on 16 patients suffering from generalized chronic periodontitis. The study period of 18 days was divided into two time-intervals, i.e. baseline (0 days) to 7th day, with a washout period of 4 days followed by a second time interval of 7 days. Subgingival irrigation of each half of the mouth with either ozone or chlorhexidine was done at different time intervals. They observed a higher percentage of reduction in plaque index (12%), gingival index (29%) and

bleeding index (26%) using ozone irrigation as compared to chlorhexidine. The percentile reduction of Aa (25%) using ozone was appreciable as compared to no change in Aa occurrence using chlorhexidine. The antifungal effect of ozone from baseline (37%) to 7th day (12.5%) was pronounced during the study period, unlike chlorhexidine did not demonstrate any antifungal effect. No antiviral property of ozone was observed. The antiviral efficacy of chlorhexidine was better than that of ozone. They concluded that despite the substantivity of chlorhexidine, the single irrigation of ozone is quite effective to inactivate microorganisms.

Periodontal disease and peri-implantitis is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. also (9-16, 51-53). Periodontal disease and peri-implantitis diagnosis and therapy has been increased a great interest in the last years both in basic (17-28) and advanced research (29-35). Besides the treatment of periodontal disease and peri-implantitis requires a multidisciplinary approach (36-50, 54-70).

Our study evaluated the efficacy of ozonated water Ozonline® in the management of moderate to severe chronic periodontitis. The results of this investigation demonstrated an overall decreasing in bacterial loading. A new dimension was added to this investigation by microbiological examination of bacterial morphotypes using periodontal tests. It was thought appropriate to evaluate the effect of Ozonline® on subgingival microbial population, the primary etiological factor for periodontitis. The results demonstrated a marked reduction in total number of organisms in each patient from baseline to 7 days.

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MESENCHYMAL STEM CELLS IN ORAL MEDICINE: AN OVERVIEW

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This review aims to address the procedures and indications, required for the application of the mesenchymal stem cells treatment strategy of regenerative dentistry. Mesenchymal stem cells have rarely been used in this field; conversely, experience from other clinical fields and basic research seems to recommend the suitability of this scope of application. We reviewed 31 out of 206 articles on Web of Science, Scopus, PubMed, etc. The main purpose of this paper is a short review of the literature evaluating the application of stem cells in regenerative dentistry, their ability to get a multilineage differentiation and to introduce a safe and useful alternative way of harvesting and selection. The most common derivation of stem cells used for regenerative dentistry is from the adipose tissue. There are conditions in which the levy adipose cannot be easily achieved, or where large amounts of injectables are not needed. For this purpose, the possibility of selecting stromal stem cells directly from the lax subcutaneous connective tissue preferably of the head region would allow a technical simplification and a greater homology in tissues.

This review aims to address the procedures and indications, which are required for the application of the mesenchymal stem cells (MSCs) for regenerative dentistry. MSCs have rarely been used in dentistry; conversely, experience from other clinical fields and basic research seems to recommend the suitability of this scope of application.

With this objective, in the preliminary phase, we consulted the scientific literature which appeared after the introduction of Coleman's procedure into clinical practice in 1995. We took into consideration the key term regenerative dentistry. We considered 206 bibliographic references, from which we eliminated all those which were not in English, all case reports, all observational studies, and all the basic research studies which repeated findings already published

with the aim of producing a review which is as up-to-date as possible and which succinctly sets out an overview of the rationale and the technical methods of the use of MSCs for regenerative dentistry. We limited our review to 31 articles.

The most common derivation of stem cells used in oral tissues regeneration is from the adipose tissue. Investigators have postulated several non-exclusive mechanisms through which adipose derived stem cells MSCs can be used to repair and regenerate tissues. First, MSCs delivered into an injured or diseased tissue may secrete cytokines and growth factors that stimulate recovery in a paracrine manner. The MSCs would modulate the "stem cell niche" of the host by stimulating the recruitment of endogenous stem cells to the site and promoting their

Key words: Mesenchymal stem cells, dentistry, biostimulation, regenerative dentistry, oral medicine, stem niches

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differentiation along the required lineage pathway. In a related manner, MSCs might provide antioxidants chemicals, free radical scavengers, and chaperone/heat shock proteins at an ischemic site. As a result, toxic substances released into the local environment would be removed, thereby promoting recovery of the surviving cells. Exciting studies have suggested that transplanted bone marrow derived MSCs can deliver new mitochondria to damaged cells, thereby rescuing aerobic metabolism (1).

Human adipose tissue obtained by liposuction can be processed to obtain a fibroblast-like population of cells in the processed lipoaspirate (PLA). These cells can be maintained *in vitro* for long periods showing low levels of senescence. The majority of PLA cells are of mesodermal or mesenchymal origin with contaminating pericytes, endothelial cells, and smooth muscle cells, as demonstrated by immunofluorescence and flow cytometry. PLA cells can differentiate *in vitro* into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors. The surface antigen profile of MSCs isolated from human adipose tissue, changes *in vitro* as a function of time and/or passage in culture. After two or more passages *in vitro*, MSCs surface immunophenotype resembles that of MSCs, with a similarity greater than 90%. MSCs display stromal markers, such as CD44, CD90, CD73, CD166, and CD29 pericytic markers, such as CD140a and CD140b, and cytoskeletal markers, such as α -smooth muscle actin and vimentin. Nevertheless, some differences in surface protein expression have been described (2).

In the mid-90s Coleman noted that the positive effect of structural fat grafting was beyond the filling effect, in fact, the characteristics of texture, pigmentation and skin tropism suffered a visible improvement (3).

The use of more and more thin cannulas for harvesting and re-injecting adipose tissue has revealed that although the positive effect is always present, in nano fat, after the emulsification of the fat, there is a disappearance of vital adipocytes. Thus, the clinical improvement must be due to the mesenchymal stem cell component that has been typed in this method (4.)

The selection of mesenchymal stem cells is possible following a liposuction but these cells, once re-injected without cultural expansion, will undergo a differentiation primarily in adipocytes, and only in a small percentage into osteo-, chondro- and fibroblastic lineage. In fact, embryonic stem cells can differentiate into multiple lineages while adult stem cells are usually capable of differentiating only into lineage-specific cells. Some Authors tempted to clarify the mechanisms through which fat affects overlying skin and demonstrated in an animal model a possible trans-differentiation of MSCs toward epithelial skin cell lineages, identified by the p63 stem cell marker (5).

In the *in vitro* study, during cell culture, factors such as donor age, type (white or brown adipose tissue), and localization (subcutaneous or visceral) of the adipose tissue, exposure to plastic, plating density, and media formulations, might influence both proliferation rate and differentiation capacity of MSCs. In a murine system, both the cellular composition and the differentiation capacity of the stromal vascular cell fraction display heterogeneity according to the localization of the adipose tissue. In rabbits, the osteogenic potential of MSCs isolated from visceral adipose tissue was reported to be more effective compared with MSCs isolated from subcutaneous adipose tissue.

Different anatomical localizations of fat tissues have their own metabolic characteristics, such as lipolytic activity, fatty acid composition, and gene expression profile, the source of subcutaneous adipose tissue grafts (abdominal-subcutaneous vs. peripheral-subcutaneous) might influence the long-term characteristics of the fat grafts (6).

Moreover, recent studies on microRNA have shown that there are different qualities of stem cells from adipose tissue according to the donor site. Abdominal adipose-derived mesenchymal stem cells are suitable for reconstruction of breast tissue engineering. The expression profiles of microRNAs and mRNAs between these cells are in fact very similar. Conversely, abdominal mesenchymal stem cells might not be suitable for facial rejuvenation, since there are 38 highly expressed genes targeted by microRNAs in facial

adipose derived stem cells that are non-expressed in the abdominal adipose stem cells (7).

In recent years, stem cell application has been suggested as a possible novel therapy for regenerative medicine. This is due to their unlimited capacity for self-renewal as well as the ability to differentiate into multiple cell types under appropriate stimuli (8).

The clinical use needs procedures able to isolate the stem cells in closed systems and successively to expand them. However, almost all these methods require fetal bovine serum (FBS) for culture with some limitations associated with clinical application, especially prion and viral transmission or adverse immunological reactions against xenogenic components. Recently the use of autologous platelet rich plasma (aPRP) was proposed to replace FBS in culture of umbilical cord blood stem cells. In primary culture, medium containing 10% aPRP significantly stimulated MSCs proliferation. These observations demonstrated that aPRP contains all the essential components similar to those in FBS for support of cell attachment and proliferation. However, for the safety of the procedure it is admitted only an auto-transplantation of tissues potentially rich in mesenchymal stem cells without in vitro culture, expansion, or any kind of enzymatic treatment and in a closed system for the clinical use.

Cervelli et al. documented that PRP increases adipose stem cells proliferation and, when associated to insulin, enhance the cytoplasmic lipid content. Moreover, this association synergistically up-regulates fibroblast growth factor receptor and give rise to a clinical amelioration and fat grafting stability over time (9). Oral diseases are related with systemic diseases (10-23), such as diabetes, inflammatory diseases (24-35, 52, 53). Mesenchymal stem cells therapy may be effective in regenerative dentistry for treatment of periodontal disease and peri-implantitis (36-51, 54-68).

DISCUSSION

The most common derivation of stem cells used in regenerative medicine is from the adipose tissue. The procedures of fat harvesting, processing and reinjection are different in macro-, micro-, and nano

graft. The major difference is in cannulas diameter, thus, to reduce the cannula dimension (27 gauge in nano fat) is a way to separate cells on dimensional basis. Furthermore, there are conditions in which the levy adipose cannot be easily achieved, or where large amount of injectable fat is not needed, as in the case of bio stimulation and bio revitalization of the cleavage or in the scalp. For this purpose, the possibility of selecting stromal stem cells directly from the lax subcutaneous connective tissue, preferably of the head region, and to process them through mechanical disaggregation and filtration, would allow a greater homology in tissues.

The harvesting could be made during another surgical procedure, as a small plastic surgery intervention, or through an intra oral approach, taking care to remove the epithelial layer. The described selection procedure could represent a new way to perform a stem cells therapy simply, without manipulation, in a totally closed system. Further research and clinical trials are needed to perform a quantification of the amount of obtained stem cell and to investigate the possibility to use a PRP in addition to obtain a further proliferative potential similar to what happens in stem cell cultures directly in vivo.

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USE OF EXTRA-ORAL SCANNER FOR THE STUDY OF ARCH FORM IN A SAMPLE OF ITALIAN ADOLESCENTS WITH IDEAL OCCLUSION

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The aim of this study was to evaluate the shape and the average size of the maxillary and mandibular arch in an Italian adolescents sample with correct occlusion, using the digital technology. The study sample was composed, after the use of an extra-oral scanner and after the application of inclusion and exclusion criteria, by the digitized dental casts of 79 Italian adolescents (39 females and 40 males), aged 14±1 years, with correct occlusion. On each model, both upper and lower, the reference points of the dental arches (FA), of the alveolar bone (WALA ridges) and of the incisal edge of the central incisors were identified. With these points, using a software, fourteen parameters were evaluated for each cast: basal and dental intermolar and inter-canine width, basal and dental molar and canine depth, basal and dental molar and canine ratio, overjet, overbite. Finally, the shape of the arches was assessed, dividing it into ovoid, triangular or square. Chi-square test and Student's T-test for each parameter were adopted with a $p < 0.05$ significance level. The results showed that the ovoid form was the most frequent, followed by the triangular one for the upper arch and by the rectangular one for the lower arch. On the canine level, both upper and lower, both for dental and for basal references, the triangular shape showed the lowest width and ratio values and the highest depth values in comparison with the other two groups. The square one showed the opposite situation, and the ovoid one presented in the intermediate value. On the molar level the trend is quite similar to the canine one. The results obtained maybe suggest that on a significant percentage of the patients of the sample is expected to use a preformed ovoid arch wire, and the data found could be useful to study the adequacy of the arch wires currently on the market or to design new ones.

The evaluation of the dental arch shape in the orthodontic therapy is an important part in the treatment planning to get the most satisfying results. Choosing the right arch wire, which must be adapted in the best possible way to the patient's arch, can in fact increase the ability in moving teeth and the stability of the results over time (1). It is in fact known that a change in inter canine width during the treatment may represent a predictive factor for future recurrence (2). According to the literature, several authors, over the decades, have proposed geometric models to correctly identify the arch shape (3), and different shapes and sizes of the arches in various

ethnic groups can be described: there is no absolute unanimity regarding the forms proposed using the different methodologies, but most studies currently identify three main forms: ovoid, triangular and square. Among these the most represented seems to be the ovoid one, followed by the triangular one, with the square one less frequently observable (4).

The premise from which this work starts is that in literature we can find studies that suggest that the arch-wires currently on the market are not adequate for the real average measurements of the population. This is the reason why the evaluation of the arch shape and an accurate and precise measurement of the

Key words: Arch form; Italian; Extra-oral scanner; Ideal occlusion

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numerical values of the dimensions obtained through the identification of certain anatomical points can prove to be fundamental in identifying the correct geometry of the wires to be used or eventually to be designed.

As mentioned above, there are different ways to deal with this kind of study: to simplify, we can divide them into analogical and digital methodologies. Analogical studies start from the creation of plaster models or photographs and have been used by many authors over the years (5). Many studies have widely demonstrated that orthodontic therapy has been increased a great interest in the last years both in basic (6-25) and advanced research (26-37), involving many other dental disciplines (37-49).

Digital methods are becoming increasingly widespread in the dental and medical fields: the use of these systems is leading to an important evolution in the diagnostic, prognostic, therapeutic, educational and epidemiological fields.

The purpose of this study was to investigate, using an extra-oral scanner and a data analysis software, the shape and the average size of the dental arches of a homogeneous sample of Italian adolescents.

MATERIALS AND METHODS

Study group

In this retrospective study the plaster models of 380 Italian patients who have never received an orthodontic treatment, aged 14+1, were taken into consideration. A wax bite was also recorded for each patient to recreate the correct occlusion on the models.

Operating procedure

The models were subsequently digitized using an extra-oral scanner (3Shape D800). This scanner has innovative facility scanning capabilities and two 5 MP cameras with reduced angle. It has a three-axis movement system that tilts, rotates and jerks the model, facilitating scanning from any point of view, so as to accurately detect all the anatomical details of the model. The scanning process takes place through three steps: a first scan of the maxillary model, then a scan of the mandibular one and finally a scan of both in occlusion. The digitized renderings were subsequently analyzed using "Ortho analyzer" software.

All measurements were performed by the same operator between March and July 2018.

Once the articulated models were obtained on the monitor and the Cartesian reference and occlusal planes were recorded, for each model certain points were identified. For each tooth, from the first right molar to the first left molar, both at the mandibular and maxillary level, the FA and WALA ridge points were identified (Figure 1).

The FA point is defined as the midpoint of the facial axis of the clinical crown, which is the most prominent part of the central lobe on each crown's facial surface except for the molars. For the first molar, the facial axis of the clinical crown is represented by the mesio-buccal groove that separates the 2 large facial cusps. The WALA ridge point is defined as the most prominent point on the soft tissue ridge immediately occlusal to the mucogingival junction. It is located at or nearly the same vertical level as the horizontal centre of rotation of each tooth. WALA was measured directly below FA of each perpendicular to the occlusal plane.

Once these points were positioned by the operator, the software automatically calculated several values (Figure 2), both at maxillary and mandibular level:

- inter-canine dental width (the distance between the canine FA points) and basal width (using WALA ridge instead of FA points);
- canine dental depth (the shortest distance between a line connecting the FA points of the canines with an interincisive reference that is halfway between the FA points of the central incisors) and basal depth (using WALA ridge instead of FA points);
- canine dental ratio (the ratio between the dental intercanine width and the dental canine depth) and basal ratio (using WALA ridge instead of FA points);
- intermolar dental and basal width, molar dental and basal depth, molar dental and basal ratio were calculated in an analogous way (using molar FA and WALA ridge points instead of canine ones).

A point has been identified on the margin of each central incisor: this allowed the software to calculate overjet and overbite.

The lower interincisal point was then positioned and, measuring the distance of this point from the sagittal plane passing through the upper interincisal point, the program calculated the deviation of the lower midline from the upper one.

In the next phase, the last two points of this analysis were positioned: the first on the mesiobuccal cusp of the first upper molar, the second at the level of the buccal groove of the first lower molar. The distance between these two points is useful for evaluating the dental Angle class. Each measurement was taken to the nearest 0.1 mm.

Finally, using the FA points of the central incisors, of the canines and of the first molars, the software has traced a curve that represents the shape of the arch. This one was subsequently analyzed by cataloguing it in ovoid, triangular or square shape, in order to compare the results obtained with those present in the international literature.

Inclusion and exclusion criteria

After obtaining the dental and basal measurements of the models, some inclusion and exclusion criteria were applied. The inclusion criteria were as follows:

- permanent dentition with normal shape, number and size of teeth;
- molar and canine bilateral class I and absence of serious malocclusions;
- correctly developed and perfectly preserved plaster models.

The exclusion criteria were as follows:

- anomalies of eruption or tooth formation;
- presence of restorative artifacts extended to contact

areas, cusps or incisal margins;

- presence of mono or bilateral cross-bite;
- deviation of the interincisive midline > 2 mm;
- overjet > 4 mm or < 0 mm, overbite > 4 mm or < 0 mm;
- crowding > 2 mm;
- rotated dental elements.

After the application of these criteria, 79 cases were selected, 40 males and 39 females, therefore characterized by correct occlusion.

Statistical analysis

A statistical analysis was performed using the SPSS software for Windows. For each parameter found, mean and standard deviation were calculated.

An analysis was performed with the chi-square test with a significance level $p < 0.05$ to compare the prevalence of the three arch forms with each other.

In addition, the difference for each dental and basal parameter in the three groups divided by arch shape with a significance level $p < 0.05$ was calculated with the student's t test.

Furthermore, all the variables were measured twice, with a 4-weeks interval between the two registrations on 20 pairs of models randomly selected: intraobserver variability was calculated by using Cohen's Kappa coefficient and a high correlation was obtained: $k: 0.9$.

RESULTS

Table I. Prevalence of the different shapes of upper arch

	Ovoid	Triangular	Square
Number	55	22	2
Pct (%)	69,6%	27,9%	2,5%

Table II. Prevalence of the different shapes of lower arch

	Ovoid	Triangular	Square
Number	40	16	23
Pct (%)	50,6 %	20,3 %	29,1 %

An analysis of the arch shape, both upper and lower, of all cases was performed (Table 1 and 2), then the dimension values were analyzed based on the arch shape, first upper (Table 3 and 4), then lower (Table 5 and 6).

DISCUSSION

Based on some studies published in the literature (50-53), which question the appropriateness of the current wires on the market when compared with the most common arch forms in the samples examined, it is important to evaluate the average arch shapes and dimensions to be able to develop preformed arches with similar characteristics which could lead to an increase in the effectiveness and stability of orthodontic treatment. What emerges from this study

is that in the sample examined the prevalent form is the ovoid one.

This data is statistically very significant for both the upper and lower arches. At the maxillary level, the second most prevalent shape was triangular, while the frequency of square shapes was very low. At the mandibular level instead, the second form for prevalence was found to be the square, and third the triangular, with a percentage slightly lower than the square one.

The difference between the percentages that emerged from the different studies can be attributed to multiple factors. Certainly, the means on which the shape is analyzed must be considered: in some studies plaster models were used, in other photographs of the models, in still others digital reproductions using intra-oral or extra-oral scanners. Although there are

studies (54) that suggest that there are no significant differences between the values collected with the various methods (analogical or digital), this data must still be further verified, and so this can influence the analysis.

Another factor to consider is the degree of “subjectivity” of the evaluation: despite the fact that over the years mathematical functions have been sought that could best identify the arch forms, for example the TPS (thin-plate spline) to date there is no full consensus in preferring one method over another. For this reason, the identification of the arch shape is often left to the interpretation of the operator: also due to this, therefore, there may be a certain degree of discrepancy between the results of the different studies.

Finally, the sex, the age and the race of the

Table III. *Average dental values according to the shape of the upper arch*

	Inter canine width	Inter molar width	Canine depth	Molar depth	Canine ratio	Molar ratio
Ovoid (55)	37,1 mm ± 2,3 mm	56,1 mm ± 3 mm	8,9 mm ± 1,1 mm	33,7 mm ± 2,3 mm	4,2 ± 0,5	1,7 ± 0,1
Triangular (22)	35,5 mm ± 1,6 mm	55,9 mm ± 2,7 mm	9,8 mm ± 0,8 mm	34,1 mm ± 1,3 mm	3,7 ± 0,3	1,7 ± 0,1
Square (2)	38, 1 mm ± 2,1 mm	57,5 mm ± 5,9 mm	7,8 mm ± 0,3 mm	31,4 mm ± 1,3 mm	4,9 ± 0,1	1,8 ± 0,1
Whole sample (79)	36,7 mm ± 2,2 mm	56,1 mm ± 2,9 mm	9,1 mm ± 1,1 mm	33,7 mm ± 2,1 mm	4,1 ± 0,5	1,7 ± 0,1

Table IV. *Average basal values according to the shape of the upper arch*

	Inter canine width	Inter molar width	Canine depth	Molar depth	Canine ratio	Molar ratio
Ovoid (55)	37,1 mm ± 2,3 mm	58,6 mm ± 3 mm	7,6 mm ± 1 mm	29,1 mm ± 1,9 mm	5 ± 0,7	2 ± 0,2
Triangular (22)	35,6 mm ± 2 mm	58,4 mm ± 3,3 mm	8 mm ± 0,8 mm	29,4 mm ± 1,6 mm	4,5 ± 0,5	2 ± 0,2
Square (2)	38,4 mm ± 2,6 mm	59,4 mm ± 3,5 mm	6,1 mm ± 0,1 mm	27,5 mm ± 1,2 mm	6,3 ± 0,2	2,2 ± 0,1
Whole sample (79)	36,7 mm ± 2,3 mm	58,6 mm ± 3 mm	7,7 mm ± 1 mm	29,2 mm ± 1,9 mm	4,9 ± 0,7	2 ± 0,2

analyzed subjects must also be considered: the studies should focus on samples that have a high level of homogeneity, in order to better frame what is the frequency of a specific arch shape in that precise group of subjects, to then compare the results obtained with those ones from other groups (55-59).

The analysis of the dental values of the upper arch divided by shape showed that the most significant differences are found in the canine level in the triangular shape: the inter-canine width and ratio values in this shape are significantly lower than the ones found in the ovoid and square shapes, while the canine depth is significantly greater. At the molar level, the differences between the triangular shape and the other two groups are less marked, with only the molar dental depth which was significantly greater in the triangular shape than in the square one. The triangular shape is therefore the one that differs most from the others, especially in the anterior part of the arch.

From the comparison between the ovoid and square shapes it was found that the widths and ratios, both at the canine and molar level, are smaller in the ovoid forms than in the square ones, while the depths, both at the canine and molar level, are greater in the first group. Interestingly, no parameters analyzed at the dental level showed significant differences between the two groups. However, it must be taken into consideration a limit of this study: the sample with an upper square shape was poorly represented

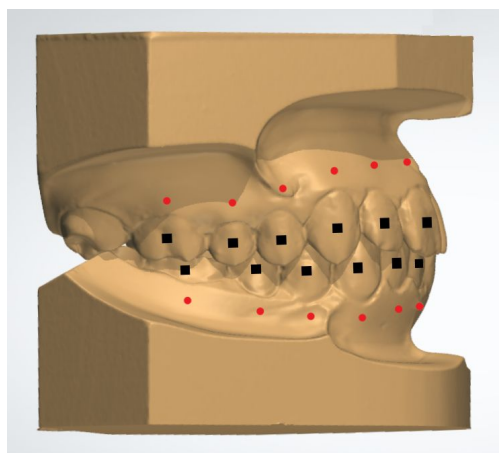


Fig 1. Position of WALA ridge (alveolar) and FA (dental) points

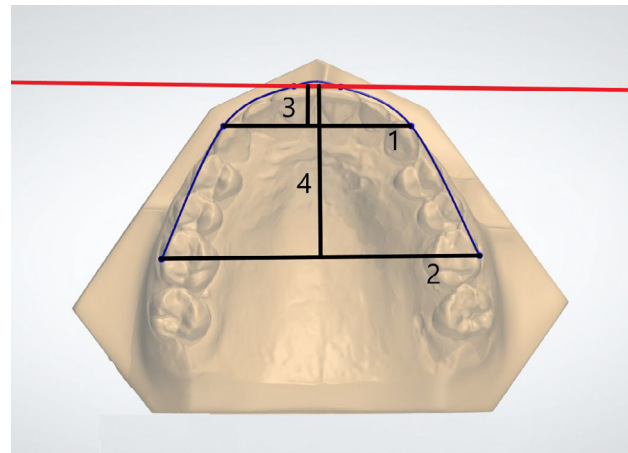


Fig 2. 1) Inter-canine dental width. 2) Intermolar dental width. 3) Canine dental depth. 4) Molar dental depth.

(only 2 cases), and therefore to evaluate precisely how significant the differences between the two groups are, other studies with a larger sample of square shapes would be necessary.

Also from the analysis of the dental values of the lower arch divided by shape, it was found that the most significant differences are found in the canine level in the triangular shape, confirming what is seen in the upper arch. In fact, even for the lower arch, the dental intercanine width and ratio in the triangular shape are significantly lower than the values found in the ovoid and square arches, while the canine depth is greater than in the other two groups (significantly compared to the square, not significant compared to the ovoid).

At the molar level, the differences between the triangular shape and the other two groups are less marked, with only the molar dental depth which was significantly greater in the triangular shape than in the square one.

From the comparison between the ovoid and square shapes, an aspect emerged that differs slightly from what is seen in the upper arch: in this case, both at the canine and molar level, the depths were significantly greater in the ovoid shapes compared to the square and the ratios were significantly smaller (in the upper these differences were not statistically significant). Looking at the upper and lower values as a whole, it is possible to conclude that at the

canine level the triangular shape is the narrowest and deepest; the square turns out to be the widest and the least deep; the ovoid appears to have intermediate values between the other two. Even at the molar level this pattern repeats itself, albeit with some slight differences.

From the analysis of the basal values, both at the upper and lower levels, these show a trend approximately similar to the dental ones. Also this result is in agreement with what is present in the literature, in particular with the study by Bayome et al. (55), in which the relationship between the dental and basal arches was evaluated using cone beam computed tomography (CBCT) and three-dimensional (3D) virtual models in a sample with

normal occlusion. These authors concluded that the dental and basal arches are strongly correlated in patients without malocclusion. In the present work, only teenagers aged 14 ± 1 years, Italian, were chosen to have a very homogeneous sample.

The adolescent age was chosen because most of the multibrackets treatments are performed in the stage where all the permanent teeth are erupted and because the canine and molar widths have been found to show minimal changes after the age of 14 (56). However, in order to have a broader view of the arch forms present in the general population, and the related dimensions, other studies are needed that include subjects of other ages and ethnicities, and subjects with malocclusion.

Table V. Average dental values according to the shape of the lower arch

	Inter canine width	Inter molar width	Canine depth	Molar depth	Canine ratio	Molar ratio
Ovoid (40)	28,9 mm $\pm 1,7$ mm	52,4 mm $\pm 2,6$ mm	5,5 mm $\pm 0,7$ mm	30,2 mm $\pm 1,8$ mm	5,4 $\pm 0,6$	1,7 $\pm 0,1$
Triangular (16)	27,9 mm $\pm 1,4$ mm	52,2 mm $\pm 2,6$ mm	5,7 mm $\pm 0,7$ mm	29,8 mm $\pm 1,7$ mm	5 $\pm 0,6$	1,8 $\pm 0,1$
Square (23)	29,1 mm $\pm 1,3$ mm	53 mm ± 3 mm	4,2 mm $\pm 0,5$ mm	28,6 mm $\pm 1,4$ mm	7 $\pm 0,9$	1,9 $\pm 0,2$
Whole sample (79)	28,7 mm $\pm 1,6$ mm	52,5 mm $\pm 2,7$ mm	5,2 mm $\pm 0,9$ mm	29,6 mm $\pm 1,8$ mm	5,7 $\pm 1,1$	1,8 $\pm 0,2$

Table VI. Average basal values according to the shape of the lower arch

	Inter canine width	Inter molar width	Canine depth	Molar depth	Canine ratio	Molar ratio
Ovoid (40)	30,2 mm ± 2 mm	56,7 mm $\pm 2,6$ mm	4,6 mm $\pm 0,8$ mm	27,5 mm $\pm 1,6$ mm	6,7 $\pm 1,1$	2,1 $\pm 0,1$
Triangular (16)	29,5 mm $\pm 1,5$ mm	56,6 mm ± 2 mm	4,9 mm $\pm 0,6$ mm	27,6 mm $\pm 1,7$ mm	6,1 $\pm 0,7$	2,1 $\pm 0,1$
Square (23)	30,3 mm $\pm 1,5$ mm	57,4 mm $\pm 2,9$ mm	3,6 mm $\pm 0,7$ mm	26,4 mm $\pm 1,5$ mm	8,8 $\pm 1,8$	2,2 $\pm 0,2$
Whole sample (79)	30,1 mm $\pm 1,7$ mm	56,9 mm $\pm 2,6$ mm	4,4 mm $\pm 0,9$ mm	27,2 mm $\pm 1,7$ mm	7,2 $\pm 1,7$	2,1 $\pm 0,2$

The present study carried out on Italian adolescents with ideal occlusion has shown that the most frequent arch shape is the ovoid one. At the maxillary level, the second form for frequency is the triangular one, while at the mandibular level the second is the square one.

Both at canine and molar level, both upper and lower, in the comparison between the three groups, the triangular shape showed the lowest width and ratio values, and the highest depth values. The square shape appeared to have the opposite situation, with the largest width and ratio values, and the lowest depth values. The ovoid shape showed intermediate values between the other two ones. The basal values found were similar, in terms of proportions between the measures in the different arch forms, to the dental ones (57-63).

The results of this study can be useful both for a comparison with further works performed with different methods and samples, and as a reference for future studies that will investigate the shapes and sizes of the wires on the market.

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FACIAL GROWTH AND MORPHOLOGY OF CLEFT LIP AND/OR PALATE PATIENTS AFTER CORRECTIVE SURGERY ACCORDING TO P.I.S.A. TECHNIQUE

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Cleft lip and/or palate patients (CLP) undergo corrective surgery that can affect facial growth. The aim of this study was to analyze facial growth and maxillary development of CLP subjects after surgery according to P.I.S.A. technique (Peri osteoplasty Improves Symmetry and Aesthetic). Cephalometric tracings of 55 patients were performed, thirty-three of which belonged to the test group, while the lasting twenty subjects were part of the control group. The test group was formed by cleft lip and/or palate patients after surgical repair according to P.I.S.A. technique. The control group included patients unaffected by this malformation, with an Angle's first class, selected from the Michigan Growth Study sample. Facial growth and upper maxilla development analysis was carried out by comparing the data obtained from the cephalometric traces of cleft patients who performed an early surgery, with the values of the same parameters measured in non-cleft subjects, providing the normal values. The results of this study showed, in the test group, a maxillary and mandibular bi-retrusion, a more negative facial convexity, the absence of a marked discrepancy in the skeletal relationships, a slight tendency towards hyper-divergence.

Cleft lip palate (CLP) is the most common congenital deformity of the craniofacial district and occurs following the failure to merge one or more anatomical structures during fetal development. Cleft lip and/or palate is estimated to occur in 1 in every 700 births, with a wide variability caused by both intrinsic and environmental factors (1). Patients suffering from this malformation undergo corrective surgery that can affect facial growth and upper jaw development. In literature there is conflicting evidence regarding the influence of corrective surgery and its timing on facial growth. Some studies report that unoperated patients have normal facial growth (2), while patients undergoing surgery exhibit a retrusion of the upper jaw (3-7).

Facial growth is a complex phenomenon, consistently governed by genetic factors and modified by environmental and evolutionary influences. The presence of craniofacial malformations, such as oral clefts, together with their surgical repair, contributes significantly to determining alterations affecting the individual facial growth. From the analysis of cleft patients' facial growth emerges the presence of an intrinsic baseline deficiency in the skeleton of the middle third of the face.

Leaving aside the surgery repair outcomes on the facial growth, it was found that the maxilla was not in a retruded position, but consistently shorter in length than what can be observed in non-cleft patients (about 3.5 mm shorter at age 17, using the

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anterior nasal spine as a landmark) (3).

From the comparative studies between cleft subjects and non-cleft population, more severe differences have emerged in skeletal morphology. These differences are attributable not exclusively to the pattern of skeletal alteration of cleft patients, but, above all, to the surgical repair of the palate. The corrective surgery of the palate results in a posterior displacement of the maxilla, in a further slight shortening of the basal length of the maxilla and in a marked retrusion of the alveolar process (3).

Patients who have undergone corrective surgery, due to the presence of this deformity, therefore often have a retrusion of the superior maxilla. Numerous controversies are still reported in Literature today on the cause to which this retrusion can be traced: in fact, there is no univocal scientific evidence on which intervention has the greatest influence, whether lip surgery or palate surgery. In this regard, some studies show that there are no significant differences in patients undergoing surgery, or between patients with cleft lip and patients with cleft lip and palate in terms of maxillary retrusion; while other studies state that maxillary retrusion is exacerbated by surgical repair of the cleft palate rather than the lip (3-7).

Furthermore, there is no unambiguous evidence in identifying the major responsible for this retrusion: whether it is a purely intrinsic and genetic nature connected to the presence of clefts, whether it depends on the operation on the lip or whether from the palate repair. A further complexity arises due to the presence at national and worldwide level of several surgical protocols for the correction of this malformation, which differ in an earlier or later approach. The early timing is considered by some Authors as responsible for the retrusion of the middle third (4). P.I.S.A. (Periosteoplasty Improves Symmetry and Aesthetic) technique is characterized by an early approach. An earlier intervention, especially at the alveolar level, leads to a better cleft repair and allows a greater production of bone through primary periostoplasty, which is carried out at 3 months of life.

This approach aims to maximize the formation of bone tissue, taking advantage of the maximum osteogenic potential that occurs around 4 months

of life (8). The surgical protocol includes initial interventions, which adapt to the individual clinical case, varying according to the type of cleft, its laterality (unilateral or bilateral) and its extension (greater or less than 7 mm, as a dimensional cut-off).

In unilateral and bilateral cases, periosteoplasty procedure is always performed during the cheiloplasty at 2.5-3 months of age, a previous lip adhesion is performed in severe maxillary disorders at 40 days of age; the palatal surgery is performed at 6 months of age.

Therefore, P.I.S.A. technique consists of:

- Lip Adhesion according to Randall-Graham around 40 days of life, in cases of bone cleft greater than 7 mm;
- Tennison cheiloplasty modified with Massei periostoplasty and Mulliken-based rhinoplasty in unilateral clefts, within 3 months of life;
- Mulliken's cheilorhinoplasty with bilateral periosteoplasty according to Massei in bilateral clefts at about 3 months of life;
- Palatoplasty at about 6 months to guarantee correct palatal musculature function as soon as possible for sucking, swallowing and future phonation;
- Eventual further bone cleft repair operations: secondary periostoplasty according to Massei at about 6 years of age; bone grafting at 8-11 years; rhinoseptoplasty and orthognathic surgery at the end of growth.

The aim of this study was to analyze facial growth and maxillary development of CLP subjects after surgery according to P.I.S.A. technique.

MATERIAL AND METHODS

The present study was conducted in the Cleft Lip and Palate Center, within the Plastic and Reconstructive Surgery Unit of the "Santa Chiara" Hospital in Pisa (Italy). It is a spontaneous monocentric retrospective non-interventional observational study, carried out in cleft lip and palate patients, treated by the medical-surgical team of the Pisa Center according to P.I.S.A. technique. All phases of the study were conducted according to the principles of the Declaration of Helsinki. Inclusion criteria were:

- a) single or bilateral cleft lip and alveolus;
- b) single or bilateral cleft lip and palate;
- c) surgical repair of the cleft according to Pisa Surgical Protocol (early approach);
- d) radiographic age between 5 and 10 years;
- e) digital lateral teleradiograph of the skull without any previous orthopedic and/or orthodontic treatment;
- f) absence of others oro-facial malformations or syndromes.

Exclusion criteria were unilateral and bilateral CL (cleft exclusively on the lip), or isolated CP (cleft exclusively on the palate, involving only the soft palate, only the hard palate or both) and submucosal cleft patients, absence of any recognized syndrome, absence of a previous or present orthodontic treatment. The group included 19 subjects (11 males and 10 females), aged between 5 and 10 years old, who had a recent lateral radiograph and orthopantomography. The group included: 33 patients (20

males and 13 females), of which:

- 27 patients with unilateral CLP (14 patients with left CLP and 13 with right CLP), of which: 8 patients with an initial bone cleft size less than 7 mm and 19 patients with an initial bone cleft size greater than 7 mm.
- 4 patients with bilateral CLP, of which: 2 patients with an initial bone cleft size greater than 7 mm and 2 patients with an initial bone cleft size less than 7 mm.
- 2 patients with left unilateral CLA (cleft lip and alveolus) (Fig. 1) (Table I).

Control group consists of lateral radiographs selected from the international database promoted by the Michigan Growth Study of the American Association of Orthodontists Foundation (AAOF) (9). The control group included lateral radiographs of 20 patients (12 males and 8 females), with a radiographic age between 5 and 7 years with an Angle I class and matched for age and sex to the test group. The cephalometric analysis was performed with cephalometric software (Delta-Dent software). All the tracings have been performed by a single blind operator. Moreover, two measurements were performed after 14 days to reduce the error of method.

The following landmarks were used: nasion (Na), midsella point (S), A point (A), B point (B), pogonion (Pg), menton (Me), anterior nasal spine (ANS), posterior nasal spine (PNS), basion (Ba), articulare (Ar), condylion (Co), porion (Po), gonion (Go), gnathion (Gn). The definitions of these landmarks correspond to those given by several Authors in Literature, such as Bjork, McNamara, Riolo et al. (10).

Table I. Test group divided for cleft type.

Cleft type	n°
Unilateral cleft lip and palate (UCLP)	27
Bilateral cleft lip and palate (BCLP)	4
Cleft lip and alveolus (CLA)	2

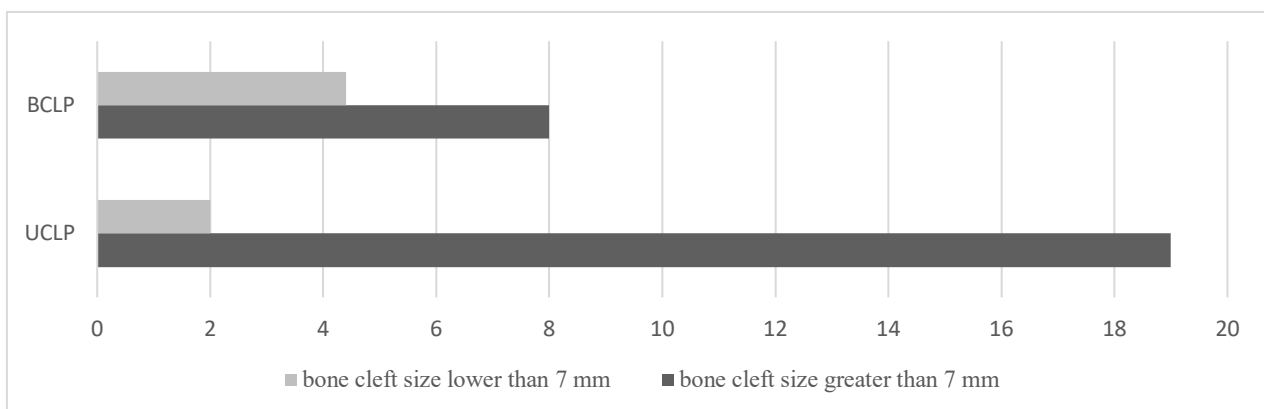


Fig. 1. Bone cleft size in the test group.

The following measurements were performed: 5 angular parameters and 8 linear parameters, for sagittal and vertical evaluation. Angular parameters were SNA ($^{\circ}$); SNB ($^{\circ}$); ANB ($^{\circ}$); cranial deflection angle ($^{\circ}$); Go-Gn $\wedge$ SN ($^{\circ}$). Linear parameters were WITS (mm); Co-Sna (mm); Co-A (mm); Co-Pog (mm); Co-Gn (mm); A-Na perp (mm); Pog-Na perp (mm); A-NPog (mm).

The straight line through the point Na (Nasion) is perpendicular to the Frankfurt plane (Fig. 2).

In order to assess the maxilla development there were analyzed SNA ($^{\circ}$), Co-Sna (mm), Co-A (mm), A-Na perp (mm), A-NPog (mm) values; while the mandibular development was assessed by the evaluation of SNB ($^{\circ}$), Co-Pog (mm), Co-Gn (mm), Pog-Na perp (mm) values. Cephalometric data obtained from the test group were compared to the cephalometric values of the control group.

A lateral cephalogram calibration was performed to assess the right scale. Student's "t" test for unpaired data was performed. A difference was considered significant if the "p" value was lower than 0.05; while the difference between two averages was defined as highly significant when the "p" value was lower than 0.01.

RESULTS

Upper jaw evaluation

The values of the SNA angle ($^{\circ}$) of the test group were significantly lower than the controls.

The difference between the two groups for the SNA parameter was statistically significant, ($p < 0.01$).

Linear values of Co-A and Co-Sna indicated the length of the upper jaw and its sagittal development. The difference between averages for this parameter was statistically significant ($p < 0.01$), meaning a highly significant reduction for these linear distances in patients belonging to the test group.

The facial convexity (A-Na perp) of the patients belonging to the test group was lower than controls. The difference between averages for this parameter was statistically significant ($p < 0.01$). Table II shows cephalometric values in both groups.

Mandibular evaluation

The values of SNB angle ($^{\circ}$) were reduced in the test group. The difference between the averages of the two groups for this parameter was significant ($p < 0.01$). The values of the linear parameters Co-Pog and Co-Gn were reduced in the patients belonging to the test group; the difference between averages of the two groups for these parameters was statistically significant ($p < 0.01$).

The linear Pog-Na perp value was lower in the test group patients than in the controls, however the difference between averages for this parameter was not statistically significant ($p > 0.05$).

The test group cranial deflection angle average can be considered normal.

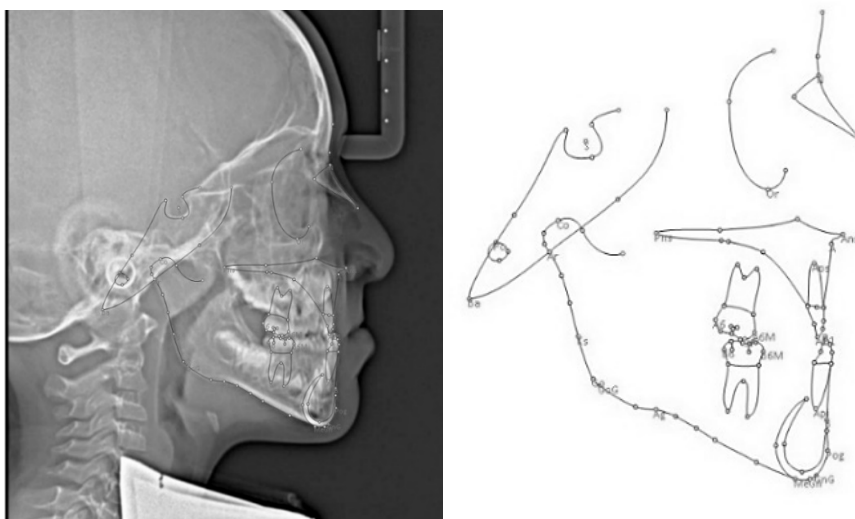


Fig. 2. CLP cephalometric tracing performed by Delt-Dent software.

Assessment of sagittality and skeletal class

This assessment was performed by analyzing the ANB (°) and WITS (mm) parameters.

For both variables, the test group seemed to settle in the norm, with the difference between averages for these parameters not statistically significant ($p > 0.05$).

Assessment of verticality

This evaluation was performed by analyzing the angular parameter $\text{GoGn}^\wedge \text{SN}$, for which there was no statistically significant difference between the test group and the control group, although the patients of the test group had slightly higher values than the control, but still within a range of “normality”.

Many studies have widely demonstrated that orthodontic corrective surgery has been increased a great interest in the last years both in basic (11-25) and advanced research (26-37), involving many other dental disciplines (37-53) (Fig. 3).

DISCUSSION

The present study, conducted in patients with cleft lip and palate who underwent corrective surgery according to the P.I.S.A. technique, found a significant retrusion of the upper jaw, alongside

a mandibular retrusion. In literature there are conflicting evidences regarding the analysis of facial growth of patients with cleft lip and palate. If an intrinsic basal deficit that affects facial growth is observed in patients with this malformation (3), on the other hand some studies show that patients with CLP who do not undergo corrective surgery have an almost normal facial growth (2).

Another long-debated topic in literature concerns the role of the interventions of corrective surgery in facial growth and, in particular, the role of early corrective surgery of the palate in a deficient growth of the upper jaw. Mars, as well as other authors, identifies the earliness of intervention on the palate as a cause of facial growth deficit, with resulting maxilla hypoplasia (1-3); while Skoog claims that there is no significant difference in maxillofacial complex growth among patients who underwent early gingivoplasty (at 3 months) and patients who have never undergone surgery (54).

A 1978 study, conducted by Hellquist and Pontén, analysed the correlation between infantile periostoplasty and facial growth development in patients with complete cleft lip and palate. From that study, it emerged that patients treated early and followed longitudinally, at the age of 5-8 years,

Table II. *Cephalometric parameters in both groups.*

<i>Parameters</i>	<i>Test group</i>	<i>Control group</i>	<i>"p" value</i>
SNA [°]	77.6	82.0	0.0000
SNB [°]	74.4	77.3	0.0004
ANB [°]	3.12	4.74	0.0684
cranial deflection angle [°]	20.4	21.7	0.2837
$\text{GoGn}^\wedge \text{SN}$ [°]	37.1	35.6	0.2784
WITS [mm]	-0.15	-0.24	0.9240
Co-Sna [mm]	69.3	89.2	0.0000
Co-A [mm]	67.4	86.5	0.0000
Co-Pog [mm]	84.7	104.3	0.0000
Co-Gn [mm]	85.6	106.4	0.0000
A-Na perp [mm]	-5.47	-1.98	0.0010
Pog-Na perp [mm]	-15.7	-12.1	0.0951
A-Npog [mm]	2.08	4.02	0.0064

showed a bi-maxillary retrusion and inversion of the anterior mating tendency within the limits reported by the analysis of the same parameters in patients suffering from the same type of cleft, at the same age, who had never undergone surgery (periostoplasty and/or bone graft). In addition, the Authors reported a lower tendency to upper jaw collapse following orthopedic expansion in patients with good bone formation within the cleft area, obtainable following an early periostoplasty intervention (55). P.I.S.A. technique involves an early intervention periostoplasty, defined periostoplasty according to Massei, conducted at 3 months of life, which guarantees a high production of bone in the cleft area, maximizing its effectiveness, as it exploits the maximum osteogenetic potential that it is around 4 months old for the young patient. On a prognostic level, this intervention allows in 70% of cases to obtain a complete bone production in the cleft, in

22% a partial production and only in 7-8% of cases bone production is absent or negligible (8).

From the analysis of the group included in the present study, in cleft lip and/or palate patients treated according to the Pisan Surgical Protocol, it emerges the presence of a maxillary and mandibular bi-retrusion, associated with a reduced sagittal development of the upper jaw and the jaw itself. The results of this study also show a more negative facial convexity, a tendency to normal mandibular growth and the absence of a marked discrepancy in the skeletal relationships of test group patients. There is also a slight tendency towards hyper-divergence and post-rotation mandibular growth.

From a surgical point of view, in addition to the influence of an early intervention timing on facial growth it is also necessary to consider the presence of scar tissue, as a result of the surgical correction intervention itself, which greatly limits

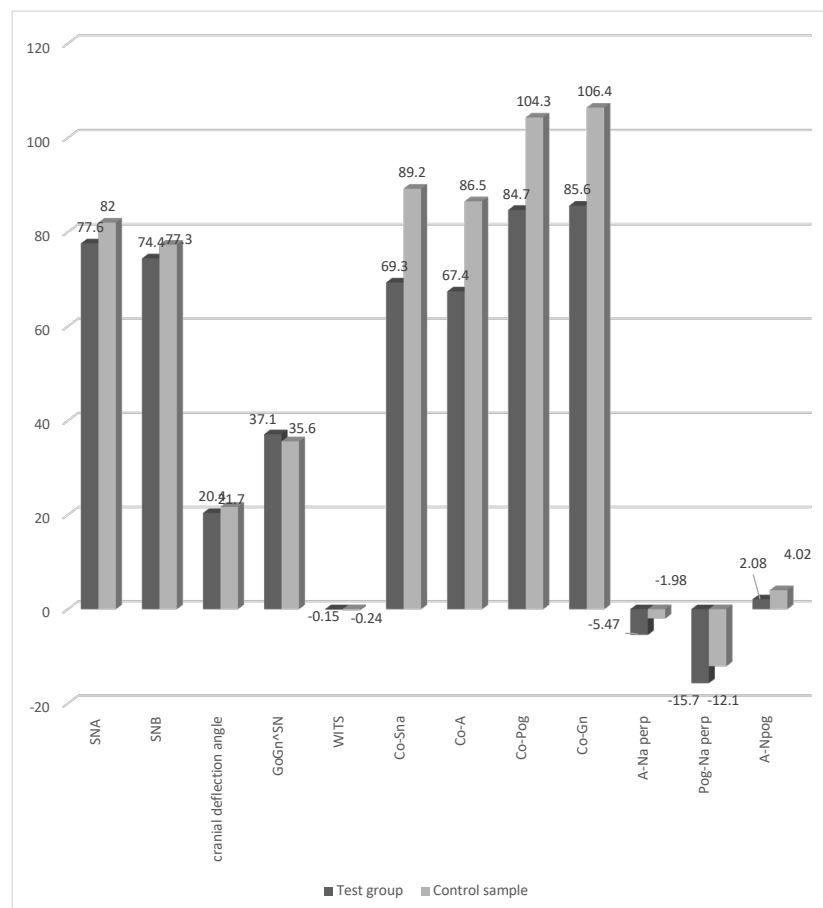


Fig. 3. Graphical representation of the results.

the maxillary bone development in the transverse and sagittal direction, by virtue of the scar retraction phenomenon.

By the way, there are also numerous studies in Literature to support early intervention. One of the main reasons, which justifies an early timing, concerns the phonatory development. Lohmander and Agerskov, in a 1998 study, showed that early intervention is associated with better speech development and better articulation of speech, compared to what was observed by speech therapy specialists in patients treated later (56). Regarding speech development, Ysunza et al. they argue that the repair of the palatine anatomy should be performed in the pre-linguistic stage of the child's development, i.e. before 12 months of age, in order to prevent the establishment of non-physiological sound articulation patterns, as compensatory activities in the presence of an anatomical defect, difficult to eradicate after (57).

Furthermore, in a 2001 study, Anastassov argues that an early periostiotomy intervention not only contributes to the normalization of the floor of the nasal cavities and to the support of the base of the wings of the nose, but also determines in 1/4 of patients a bone production sufficient for the eruption of the dental elements. In this article, Anastassov recalls the complex morpho-functional unit of which mimic muscles, periosteum and bone sutures are part, to underline the importance of restoring the lost balance between primary and secondary growth centres. Therefore, reactivating the myo-periosteum-sutural apparatus and closing the perioral and perinasal muscle rings are the fundamental steps to be taken as early as possible, to promote normal, proportionate and chronologically correct growth of the middle third (58).

The present cephalometric study was performed in patients who have never undergone orthopaedic or orthodontic corrective treatment, therefore, the possibility of obtaining an adequate facial growth with a congruous orthodontic treatment must be considered.

Some Authors defend the opportunity to overcome the problems related to growth, resulting from an early surgical repair, with a correct orthodontic treatment in childhood, allowing adequate development of the

upper jaw during adolescence (57-66).

The results of the present study showed a tendency to bi-maxillary retrusion of patients belonging to the test group, associated with a reduction in sagittal maxillary and mandibular development. In the interpretation of these data, it is necessary to consider the multifactorial nature of the causes that may have determined their onset. Therefore, it is necessary to consider an intrinsically genetically determined deficient component of maxillary bone development in patients suffering from this malformation, because of the complex systemic and genetic framework that accompanies the cleft presence itself.

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IMPACT OF SJÖGREN'S SYNDROME ON ORAL HEALTH AND QUALITY OF LIFE: AN OBSERVATIONAL CROSS-SECTIONAL STUDY

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Sjögren's Syndrome is a complex disease, due to an autoimmune physiopathology, that strongly impacts both patients' primary needs (nutrition and speaking), and patients' relationship life related factors (psychological health and quality of life). In Literature, few studies had investigated oral health status in Sjögren's syndrome and its impact on patients' quality of life, so the aim of this study was to analyse that issue. 30 patients were enrolled, within the Department of Rheumatology (University of Pisa), both first diagnosis patients' and both patients who had been diagnosed with Sjögren's syndrome in the past. For each patient, a medical record was filled out together with the compilation of the Oral Health Impact Profile questionnaire. Then, during a specialistic rheumatologic visit, Sjögren's Syndrome Disease Damage Index Score (SSDDI) was determined. Results showed a direct proportion between years from diagnosis and severity of oral health issues. It was found that these issues were related to soft tissue damage and an overall worse, reported quality of life and psychological health.

Sjögren's Syndrome is an autoimmune disease that affects salivary and lachrymal glands with a negative impact on fluid production (saliva, tears); patients are diagnosed between 40 and 60 years of age and is more common in women than in men (1). Primary Sjögren's Syndrome is a benign, low mortality disorder with a 16-19 times higher risk of developing Non-Hodgkin lymphoma (2). This syndrome, however, is highly disabling because of the permanent damage (both anatomical and functional) of salivary and tear glands (3).

Few studies investigated the impact of this syndrome on oral health and quality of life, highlighting issues such as the development of depression in these patients (4,5). Objective of this observational cross-sectional study was to report the impact of Sjögren's Syndrome on hard and

soft oral tissues. In order to do this, the Sjögren's Syndrome Disease Damage Index Score (SSDDI) (6) was used: a useful tool to investigate oral, ocular and systemic damage and determine the impact on oral health, with sub scores ranging from 0 to 2.

The oral sub score of the SSDDI has two items considered as irreversible consequences of the presence of this syndrome: one is the total or partial loss of teeth and the other is salivary flow impairment at values less or equal than 1.5ml/15min. Additionally, the Oral health Impact Profile (OHIP) (6) questionnaire was used in order to evaluate the impact of this pathology on patients' quality of life. Demographic, clinical biological and histological data were collected, focusing on the relationship between damage to oral health and systemic health issues.

Key words: Sjögren's syndrome, rheumatology, oral health, quality of life, autoimmune disease

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MATERIALS AND METHODS

For this observational cross-sectional study, 30 patients were enrolled (27 Females, 3 Males) in the Department of Rheumatology (University of Pisa) between January 2019 and March 2019. Of these patients, 21 (70%) had been diagnosed with Sjögren's Syndrome in the past, while 9/30 (30%) were first-diagnosis patients. Inclusion criteria were an age of 18 years or more and Primary Sjögren's Syndrome diagnosis based on the American-European Consensus Group (AECG) criteria (7): Upon enrolment, a medical record for each patient was filled out: personal data, general and oral health-specific anamnesis, and the Oral Health Impact Profile questions were asked to each patient. In a concomitant rheumatologic visit, bioumoral, clinical and histologic data were collected and the Sjögren's Syndrome Disease Damage Index Score (SSDDI) for each patient was determined.

The focus score was also calculated: it's the number of mononuclear cell infiltrates containing at least 50 inflammatory cells in a 4 mm² glandular section and it's

also one of the most important test results for the diagnosis of this syndrome (8). Sjögren's syndrome is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. (9-16). Sjögren's syndrome diagnosis and therapy has been increased a great interest in the last years both in basic (17-26) and advanced research (27-34). Besides, the treatment of Sjögren's syndrome requires a multidisciplinary approach (35-43). All data collected was treated following Italian Law, which ensures that it can be used solely for research in the healthcare field.

METHOD

Fisher's exact test was used to compare dichotomous nominal variables, while the continuous variables were compared using Student's t-test if they were parametric, or Mann-Whitney's test for non-parametric ones. Correlation between continuous variables was investigated using Spearman's rank correlation coefficient. A P value of 0.05 was considered for statistical significance.

Table I. Demographic, clinical and histological patient data.

Data		Results
Mean Age (years)		53±14
Mean years from first diagnosis (years)		5.4±7.6
Mean Focus Score (n/4mm ²)		2.3±2.5
Mean unstimulated salivary flow (mL/15min)		2.4±2.4
Positive to ENA/SSA test (n, %)		15/30 (50%)
Smoker (n, %)		1/30 (3.3%)
Comorbidities (n, %)		
	Cardiovascular diseases	4/30 (13.3%)
	Diabetes	1/30 (3.3%)
	Hypertension	7/30 (23.3%)
	Thyroid pathologies	10/30 (33.3%)
	Use of Estrogens	2/30 (6.7%)
	Use of antidepressants	7/30 (23.3%)
Fibromyalgia (n, %)		10/30 (33.3%)

RESULTS

Demographic, clinical and histological patient data can be found in Table I. The average age of the patients enrolled in this study was 53 ± 14 years old, and they were affected by Sjögren's Syndrome from 5.4 ± 7.6 years. The average focus score was 2.3 ± 2.5 over 4 mm^2 and the mean salivary flow registered without stimulation was $2.4 \pm 2.4 \text{ mL/15min}$, with positive Extractable Nuclear Antigen for Anti-Sjögren's-syndrome-related antigen A (ENA/SSA) in 15 patients (50%).

Only one patient was a smoker (3.3%) and 10 patients had Fibromyalgia (33.3%). Regarding the comorbidities, 4 patients had a Cardiovascular pathology (13.3%), only one had diabetes (3.3%), 7 had hypertension (23.3%), 10 had thyroid pathologies (33.3%), 2 made use of estrogens (6.7%) and 7 patients made use of anti-depressants (23.3%).

Oral health problems related to Sjögren's

Syndrome can be found in Table II. Subjective Xerostomia was reported by all 30 patients (100%) with 12 patients (40%) with a salivary flow less or equal than 1.5 mL/15min ; difficulty to ingest food was reported by 10 (33.3%) and parotid swelling was present in 8 patients (26.7%). Regarding general oral health, 26 patients (86.7%) had multiple direct composite restoration, a mean of 12 ± 11 missing teeth and 8 patients (26.7%) were edentulous or almost edentulous.

Regarding soft tissue, 20 patients (66.7%) had colour (15 patients, 50%) or tissue consistency (8 patients, 26.7%) alterations. Tongue alterations were present in 23 patients (76%) in the form of Fissures (18 patients, 60%), Dysgeusia (5 patients, 16.7%) and pain or burning sensation (18 patients, 60%); gingival bleeding was present in 12 patients (40%). Only 24 patients (80%) had fixed 19 patients, 79.2%) or removable (5 patients, 20.8%) Prostheses, and 3 of them (12.5%) reported problems with them.

Table II. *Oral health issues.*

Oral Health issues		Results
Subjective Xerostomia (n, %)		30/30 (100%)
Difficulty ingesting food (n, %)		10/30 (33.3%)
Parotideal swelling (n, %)		8/30 (26.7%)
Multiple obturations (n, %)		26/30 (86.7%)
Average missing teeth		12 ± 11
Complete or almost complete edentulism (n, %)		8/30 (26.7%)
Oral Prosthetic solutions (n, %)		24/30 (80%)
	Fixed	19/24 (79.2%)
	Removable	5/24 (20.8%)
Difficulties with prosthetic solution (n, %)		3/24 (12.5%)
Soft tissue alterations (n, %)		20/30 (66.7%)
	Color	15/30 (50%)
	Consistency	8/30 (26.7%)
Tongue alterations (n, %)		23/30 (76.7%)
	Fissured tongue	18/30 (60%)
	Dysgeusia	5/30 (16.7%)
	Pain/Burning sensation	18/30 (60%)
Gingival bleeding		12/30 (40%)
Salivar flow $\leq 1.5 \text{ mL/15min}$		12/30 (40%)

From the data gathered it can be observed that irreversible consequences of Sjögren's syndrome such as complete tooth loss or a salivary flow less or equal than 1.5ml/15min were present in 13 patients (43.3%). The mean SSDDI regarding oral health issues was 0.67 ± 0.85 , whilst the mean extra-glandular (systemic) SSDDI was 0.7 ± 1.6 . Statistically significant correlations between the number of missing teeth and salivary flow reduction ($r = -0.690$, $p = 0.0001$), between oral and systemic SSDDI ($r = 0.450$, $p = 0.01$) and between SSDDI and years from initial diagnosis ($r = 0.380$, $p = 0.04$) were found.

In particular, it has been observed that patients affected by Sjögren's syndrome for longer showed more oral health issues: they had an higher mean number of missing teeth (15 ± 12 compared to 6 ± 4 for recently diagnosed patients with $p = 0.047$), lower mean salivary flow without stimulation (1.8 ± 1.9 compared to 5 ± 3 for recently diagnosed patients with $p = 0.02$) and higher oral SSDDI with 0.9 ± 0.9 compared to 0.1 ± 0.3 for recently diagnosed

patients with $p = 0.01$. Patients affected by Sjögren's syndrome for longer also reported solid dysphagia more than patients with a recent diagnosis (10 versus 9, $p = 0.01$) and had higher incidence of tongue texture alterations (19 versus 4, $p = 0.01$).

A statistically significant higher oral SSDDI was reported in patients with major salivary glands swelling (1.4 ± 0.9 compared to 0.4 ± 0.6 compared to those without swelling, with $p = 0.004$). Patients with a positive ENA/SSA test also had a statistically significant higher oral SSDDI (1 ± 0.8) compared to those with a negative test (0.3 ± 0.7) with $p = 0.03$. No statistically significant correlation was found between the focus score and salivary flow and tooth loss. Comorbidities did not have an influence on the oral health issues (Table III).

The impact that Sjögren's syndrome had on the patients' quality of life was significant: only one patient (3.3%) needed a dental visit every two years: of the remaining patients, 16 (53.3%) had to request a dental visit every six months, while 13 (43.3%)

Table III. Statistically significant correlations.

Statistically significant correlations	R	P
Number of missing teeth/Salivary flow reduction	0.690	0.0001
Oral SSDDI subscore/Systemic SSDDI subscore	0.450	0.01
Oral SSDDI/N° years from first diagnosis	0.380	0.04
N° years from first diagnosis/number of missing teeth		0.047
N° years from first diagnosis/Salivary flow reduction		0.02
N° years from first diagnosis/Solid food dysphagia		0.01
N° years from first diagnosis/Tongue alterations		0.01
Oral SSDDI subscore/Major salivary glands swelling		0.004
Total SSDDI/Positive ENA-SSA test		0.03
		0.007

once a year. The most common questionnaire's items identified by the patients as negatively affected by Sjögren's syndrome were correct word pronunciation, social life issues, emotional distress, difficulty eating only a few reported a negative impact of this pathology on life itself or on their job (Table IV).

Patients have been asked if the complete or partial loss of teeth had an impact on word pronunciation: most patients who previously reported having difficulties with pronunciation agreed (7 out of 8, 87.5%) while between those without difficulties only 8 out of 22 (36.4%) agreed ($p=0.03$). The reported dysphagia and difficulty eating are significantly correlated to tongue damage and low salivary flow.

DISCUSSION

Few studies have investigated oral health status in Sjögren's syndrome and its impact on patients' quality of life. A paper by D. Isenberg et al. (44) analysed, through a retrospective study, the systemic damage caused by the syndrome but did not observe damage to hard and soft oral tissue. According to literature, the main factors determining the quality of life for a patient affected by Sjögren's syndrome are fatigue, subjective xerostomia and joint involvement (45-47): oral health issues are often overlooked.

This observational cross-sectional study describes the prevalence and the spectrum of oral anatomical and functional alterations in a cohort of unselected

patients with Sjögren's syndrome focusing on their impact on patients' quality of life. Our results clearly show that one third of the patients underwent partial or complete tooth loss and that in around the 40% of the subjects the unstimulated salivary flow was severely compromised. However, in addition to salivary flow and teeth structure impairment, several patients also present a wide spectrum of issues with their tongue and oral soft tissues, thus requiring frequent dental visits in the vast majority of the cases (47-63).

As expected, disease duration was the most important aspect correlating with poor oral health, thus highlighting the critical need for more effective strategies able to prevent oral damage accrual since the early stages of the disease. From this perspective it is not negligible the high impact of the oral damage on the patients' quality of life as emerged from the analysis of the OHIP questionnaire. Many aspects of a patient's life, such as social life, work life and everyday activities appeared as negatively affected by the issues caused by their oral health conditions; Word pronunciation, eating and relationship life were reported as the most compromised aspects of a patient's everyday life, together with psychological issues caused by being affected by a chronic debilitating pathology. Of course, this study has some limitations essentially related to the limited number of the patients enrolled. This aspect must be considered when analysing the association between patients' oral health and their comorbidities, and in

Table IV. *Answers to Quality of Life questions.*

Question	Positive answer (n, %)	Negative answer (n, %)
1	15 (50%)	15 (50%)
2	14 (46,7%)	16 (53.3%)
3	21 (70%)	9 (30%)
4	17 (56%)	13 (43.3%)
5	8 (26,7%)	22 (73.3%)
6	9 (30%)	21 (70%)
7	4 (13.3%)	26 (86,7%)

particular cardiovascular comorbidity. The possible impact of oral health status on cardiovascular risk in patients with Sjögren's syndrome indeed may deserve further investigations. However, this study highlights the negative impact of oral health status on patients' quality of life and the importance of a multidisciplinary approach in preventing damage accrual.

Sjögren's syndrome heavily impacts patients' primary needs (nutrition, speaking) but also their psychological health and quality of life by impairing their relational life. Statistically significant correlations between number of lost teeth and salivary flow reduction and between oral health issues and systemic health issues were found there is a direct proportion between years from diagnosis and severity of oral health issues.

It has been observed that these issues are also related to soft tissue damage and an overall worse reported quality of life and psychological health. The multi-disciplinary impact of this syndrome calls for the development of new health policies aimed at improving the management of patient's affected by Sjögren's syndrome.

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LASER IN PERIODONTAL POCKETS: IN VIVO AND IN VITRO STUDY

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The aim of this study was to verify the sterilizing effectiveness of the laser in the treatment of the periodontal pockets *in vivo*, with the recording of clinicians' parameters and microbiological analysis, and *in vitro* with particular attention to the presence of specific bacterial stocks. During our study, in particular, it has been used the diodes laser. In order to estimate the effectiveness of the treatment of the periodontal pockets with laser, it has been examined the microbial content of the pockets carrying out withdrawals of the sulcular material before, immediately after and twenty minutes from the radiation. The microbiological results of the studies assert that, although substantial qualitative discrepancies between the several colonies of pathogen do not exist, quantitative differences are taken place with respect to the lessening of number and dimension of the present colonie.

The term periodontal disease indicates a chronic inflammatory process of multifactorial etiology characterized by the gradual involvement of the periodontal tissues. This condition has been the object of increasing interest on the part of many investigators who have contributed to a better understanding of the etiology, created a more reliable hypothesis regarding the pathogenesis and have developed more effective and technologically advanced therapeutic techniques (10-14). Among the most recent advances has been the introduction of the laser as an adjunct to root planning (1-7). In such therapeutic field, the mainly used lasers are the laser CO₂, Diode Valve Semiconductor, and the laser Nd-YAG (Neodymium, Itrium-Alluminium-Granatum): these are the most experienced lasers in literature, the effectiveness of whom is tried in other pathologies of the oral cavity too. For every one of these it has been defined an adopted therapeutic protocol from the greater part of the operating ones (7-9).

During our study, in particular, it has been used the Deode laser, it is instead a semiconductor laser source whose active medium is a mix of semiconductor materials such as l'InGaAsP (Indium-Gallium arsenic-Phosphorus) which emits a band of wavelengths with a part in 1064 nm.

Its use in surgical field is based on the phenomenon of the photocoagulation and photo-ablation (2).

In periodontal field, its use is indicated in the cases in which:

- It appears the relapse of the lesions after an open surgery.
- It appears side phenomena as abscesses and fistulas.
- There are patients whom cannot or do not want to undergo an operation (for example coagulapatic patients or allergic to the anaesthetics, etc).

There is the certainty of a scaling and a rootplaning that have removed nearly all the tartar from the pocket. The radiation through the optical fibre allows

Key words: laser, periodontal disease, Porphyromonas gingivalis, microbiologic analysis, periodontal pocket

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us to touch the gingival area to the par of the cement area realizing a *courettage* thanks to the own effects of decontamination.

The clinical result must show an obvious symptomatic and radiographic improvement with a clear re-mineralization of the lesions and a drastic reduction of the probing that must be capacities to a level of maintenance within 4 millimetres.

The purpose of this study was to evaluate the effectiveness of the laser for sterilization of the periodontal pocket using both *in vivo* and *in vitro* methods of analysis.

MATERIALS AND METHODS

In vivo Study

The study involved 58 patients from the unit of Periodontology of the Dental Clinic of L'Aquila between the ages of 45 and 65 years. They were chosen based on the following criteria (26-31):

- depth of probing greater of 4 millimetres;
- absence of spontaneous bleeding;
- absence of adherent plaque;
- radiographic evidence of reduction of the bony support;
- negative anamnesis for antibiotic therapy in last the 60 days;
- negative anamnesis for systemic pathology (diabetes, ischemic heart disease, treatment with anticoagulants, connective tissue disease, renal insufficiency, etc.).

The patients must have had no periodontal therapy in at least the last 60 days to eliminate factors that could influence the nature of the bacterial flora and the absence of alterations from orthodontic treatment (41-48). Each patient had to sign an informed consent in which the methodology and the aim of the procedure was explained and fill out a questionnaire with anamnestic and clinical data regarding their general and oral health (32-40).

In order to estimate the effectiveness of treatment of periodontal pockets with the laser, analysis of the microbial content of the pockets was carried out, using a sterile paper cone (n°40), by withdrawing sulcular material before, immediately after and twenty minutes after exposure to the laser beam. It was then analyzed for *Porphyromonas* *Gingivalis*, *Actinobacillus* *Actinomycetemcomitans*,

Intermediate *Prevotella*, *Fusobacterium* *Nucleatum*, *Prevotella* *Buccae*, *Prevotella* *Oralis*, *Prevotella* *Denticola* and *Porphyromonas* *Endodontalis*, which have previously been used as indices of periodontal disease (15-25).

Procedure

After the periodontal probing had been carried out, the most suitable periodontal pocket for the study was selected on the basis of the parameters overexposed. A single operator then inserted a sterile paper cone (n. 40) in the deepest aspect of the pocket and the insertion point was marked with a pencil. Following this, laser therapy was performed. The laser device used was the SiroLaser Advance (Dentsply Sirona Italia). The laser treatment was carried out using the standard parameters reported in the literature (3):

1. Power of 1.5 Watt
2. Frequency of 10 Hertz
3. Duty cycle 50%
4. Time of radiation: 1 minute.

The laser beam was passed quickly over the walls of the pocket, avoiding excessive stopping on the same point. Placement of the sterile paper cone, followed by application of the laser beam, was repeated in a similar manner twenty minutes later (2,49,50). At the end of each withdrawal, the sterile cones were placed in a test-tube containing 5 millilitres of Tyoglycollatum broth. The test-tube was sealed hermetically and stored in a plastic bag (GEN BAG, bioMerieux), provided with a generator of CO₂ to maintain an anaerobic atmosphere.

The bag was then taken to the laboratory in a thermal bag at a bacteriostatic temperature (4°C). In laboratory, the specimens are centrifuged for 10 seconds and then seeded in a selective culture medium for the pathogenic species of interest (Bacto blood agar, Biolife, enriched with defibrinated sterile blood). Each Petri dish they were seeded with 0.1 millilitre of the specimen. Thereafter, the dishes were placed in a jar under anaerobic conditions and incubated at 35°C for 7 days. The bacterial isolation was carried out for every group of morphologically similar colonies. Finally, the biochemical identification of the various species was done using the galleries BEES and apparatus ATB expression (bioMerieux).

In vitro Study

The *in vitro* study was done using a bacterial stock

of *Porphyromonas Gingivalis* (MicroBiologics) (7). This is a Gram-negative rod, anaerobically obliged, not sporiferous, pertaining to group 6 second in the classification in Bergey's Manual. A pellet of *P. Gingivalis* was reconstituted in a test-tube containing 0, 5 millilitre of distilled sterile water. The bacteria in the suspension were then seeded on Schaedler Blood Agar (Biolife). The plates were then placed in a jar under anaerobic conditions (Oxoid) and incubated at 37°C for 3-6 days. (Bacterial colonies from the net margins have obtained themselves, of the equal diameter to 0.5mm, of colour brown, typical of the *P. Gingivalis*; the Gram coloration of the bacterial material has confirmed the own morphology of the stock in study. This material has been therefore placed in 4 tests-tube containing 5 millilitres of Tyoglycollatum broth (Oxoid) and incubated at 37°C for 72 hours in anaerobic conditions.

From the positive broths of cultivation, they are seeded, with the technique of the spilling in surface, twelve slabs containing Schaedler Blood Agar.) These were immediately placed in GenBag envelopes (bioMérieux) and transported to the dental operatory room for treatment with the laser. Before the treatment was done, a 1cm sq zone was marked on the external surface of the plate with an indelible pencil within which the laser beam was directed using the same technique and the same apparatus as in the *in vivo* phase. The operator was always the same. The optic fibre was maintained in constant contact with the surface of the material in the slab for of 60 seconds. Ten slabs were treated after which they were sealed anaerobically in envelopes and transported immediately to the laboratory where were incubated at 37°C for 48 hours. Observation was carried out after 48 hours using an optical microscope (Nikon) at 10x magnification (51-56).

RESULTS

In vivo Study

The microbiologic analysis done in this aspect of the study was qualitative because of the impossibility of exactly quantitating the number of bacteria in the periodontal pocket or on the paper cone. However, the standardization of the method and the adoption of precise criteria of withdrawal of the specimen has permitted two aspects to relate to the quantitative aspects: the degree of density of the bacterial colony

distribution on the surface of the selected area and the dimension of the colonies.

The identification of the species that was carried out showed the following species to be present: *Prevotella Intermediae*, *Prevotella Buccae*,

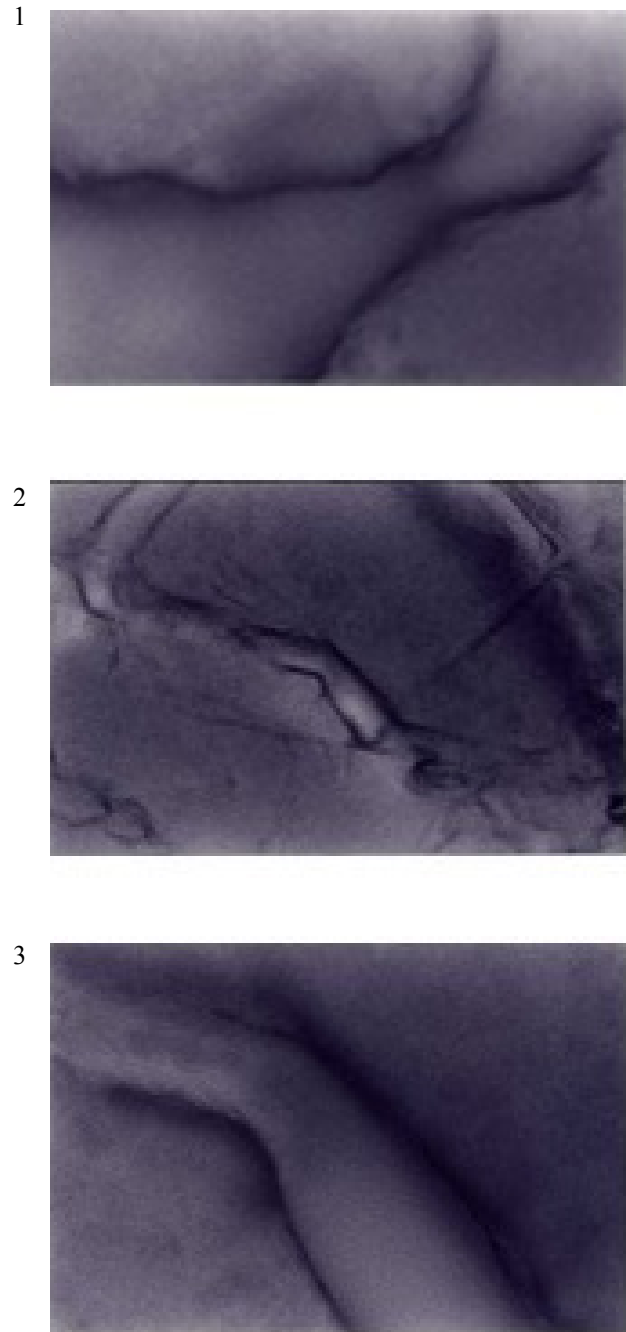


Fig. 1-3: Within this delimited area there are numerous very thin groves corresponding to the passage of the laser beam, where the bacterial presence is absent or extremely reduced.

Porphyromonas Endothelialis, Velionella SPP, Fusobacterium Nucleatum and Prevotella Oralis. Prevotella Intermediae species was present at all three study periods indicating that laser treatment does not seem be effective against this organism. On the other hand, the constant reduction of the diameter of the bacterial colonies (0.5-2 millimetres before treatment, 0.7-1.3 millimetres immediately after, and 0.5-1 millimetres after 20 minutes), and of the density of distribution of these colonies, appears to indicate that this may have been due to contamination just prior to the laser treatment. Some bacterial species, such as Prevotella Oralis, were isolated exclusively in the specimens taken twenty minutes after the treatment. Clinically, the eight treated patients all showed improvement as indicated by less bleeding based on the Probing Bleeding Index (PBI).

In vitro Study

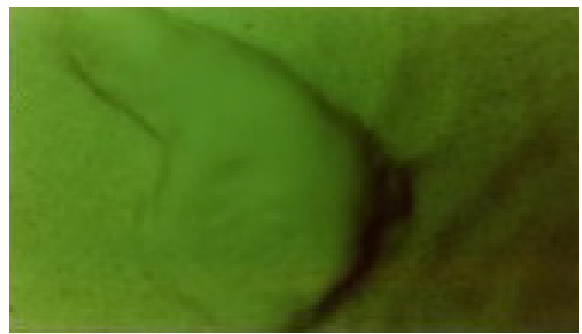
The analysis of the plates had evidenced a homogenous bacterial coating on the entire surface of the culture broth except in the signed area exposed to the laser beam. In that delimited area there were numerous thin furrows corresponding to the line of passage of the laser beam in which the bacteria were either absent or extremely reduced.

Observing to the optical microscope with enlargement 10X, it had been evidenced of the micro areas, clearly delimited, deprived of the bacterial patina; in vicinity of such zones completely decontaminated, it did not find the presence of any inhibition halo or decrease of bacterial replication but the bacterial presence turns out homogenous and similar with the other zones of the spilled on and colonized culture broth.

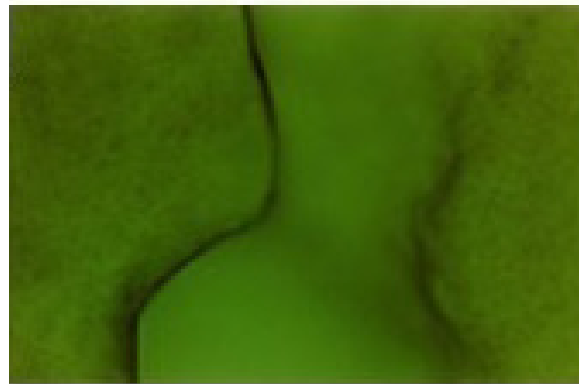
DISCUSSION

The results obtained in this study agree with the conclusions in the international literature (1-10). The microbiological results of the study show that, although it is not possible to show substantial qualitative differences between the several colonies of pathogens that were found, quantitative differences were present with respect to lessening of the number and dimension of the colonies.

4



5



6

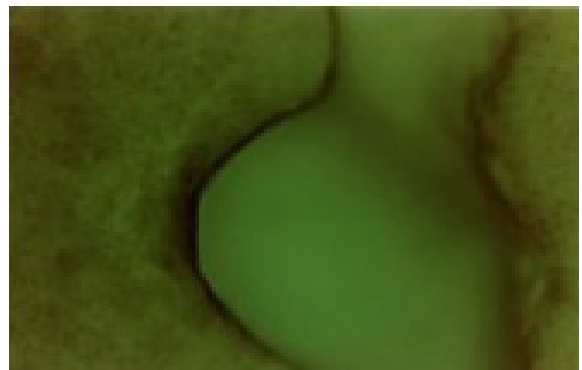


Fig. 4-6: Looking at the optical microscope with 10X magnification, micro-areas clearly delimited with respect to the surrounding soil, without bacterial patina are highlighted, in the vicinity of these completely decontaminated areas the bacterial presence is completely homogeneous and in compliance with the other areas of the seeded and colonised culture medium.

Some authors believe that the secondary liberation of oxygen or other molecules a few minutes after exposure to the laser beam can produce cellular lysis. If this is true, no pathogenic bacterial species should have been found on the cone twenty minutes after the laser was applied. However, the results of

this study do not support this hypothesis because the same species was present, even if in reduced numbers.

Although this may have been due to contamination in the twenty minute period, it also may have resulted from recolonization or a failure to eliminate all of the organisms with the initial laser treatment. The latter concept is supported by the *in vitro* study, which showed that the sterilization by the laser beam was limited to the zone of passage and not the adjacent areas.

It has been found that the lessening of the number of the colonies but not their disappearance, fact this explicable with the impossibility for the operator to irradiate all the surface of the pocket.

Although this study shows that it is not possible to completely sterilize the periodontal pocket with the laser, it can produce clinical improvement as indicated by a absence of soft tissue erythema and edema, and reduced bleeding as based on the probing bleeding index (PBI). Moreover, the fact that there is no pain associated with the procedure renders the use of a local anesthetic superfluous.

CONCLUSIONS

In the light of the results, we can draw the following conclusions. Treatment of the periodontal pocket with a laser does not eliminate all the bacteria that are the cause of the inflammatory process. This is probably due to the beam not reaching all the surfaces of the pocket and the tooth. Thus, sterilization is only accomplished in those areas contacted by the beam and that only reduces the bacterial load, as indicate in our *in vitro* study.

However, there are other advantages to the use of the laser. The reduced bleeding is an enormous advantage for the patient and the operator. The hemostatic properties of the laser provide the periodontologist with better visibility of the operating field, and it allows treatment of cardiac patients in whom there may be disturbed coagulation. The postoperative complications are also reduced due to the well-known anti-inflammatory and anti-edema actions and to the rapid healing.

The results of our experiments confirm the clinical evidence regarding the improvement patient's

periodontal health when treated with a laser (2, 3, 13, 18), but these effects do not appear to be due to sterilization of the pocket.

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IMPLANT-SAFE TEST IN PATIENTS WITH PERI-IMPLANTITIS

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Matrix metalloproteases (MMPs) are a family of zinc-dependent endopeptidases, produced by numerous cell types including fibroblasts, endothelial cells, osteoblasts, macrophages, lymphocytes and neutrophils, and capable of degrading different components of the extracellular matrix (ECM), but also cytokines, receptors and factors that regulate cell motility (1). MMPs represent the main proteolytic enzymes involved in the remodeling and degradation of the components of the extracellular matrix, in the modifications of interactions between cells, and those between cells and the ECM that regulate, for example, the processes of cell migration (2, 3). Due to these characteristics, the MMPs are involved in numerous physiological processes (angiogenesis, apoptosis, bone remodeling, wound repair, morphogenesis, inflammation, immune response) response to incongruous conservative and endodontic treatments (29-37, 46, 47) and pathological (periodontitis, arthritis, cancer, cardiovascular diseases, neurological diseases, osteoporosis etc.) (5). Metalloproteinase-8 (MMP-8) is an important indicator of tissue decomposition and is present in case of periodontitis in the gingiva and in the sulcular fluid. The concentration of MMP-8 in the sulcular fluid of patients with chronic or aggressive periodontitis is higher than that found in healthy patients (4, 6). MMP-8 was also significantly correlated with gingivitis index, plaque index, probing and clinical attack level. For this reason, the concentration of MMP-8 in the sulcular fluid could constitute a useful index to monitor periodontitis activity and be used to predict disease progression, also because of orthodontic treatments (38-45). Patients with periodontitis had elevated concentrations of MMP-8 salivary compared to patients with gingivitis and healthy tissues. Through this experimentation we wanted to demonstrate the real effectiveness of using this test as a means of preventing peri-implant pathology.

Targets

Analysis of the patient in peri-implantitis using traditional methodology of periodontal analysis (23-28) (periodontal and peri-implant indexes such as the survey of selected implants, the Full Mouth Plaque Score (FMPS) and Periodontal Screening and Recording (PSR) - Detect the concentration of

active metalloproteinase-8 (aMMP-8) of the peri-implant sulcular fluid in the case of peri-implantitis by using the ImplantSafe salivary test (Biomolecular Diagnostic S.r.l. Firenze, Italy). Selecting, using a statistical analysis of the data obtained, a value of the concentration of aMMP-8 able to discriminate peri-implantitis from other conditions.

Key words: Matrix metalloproteases, mmp, peri-implantitis, extracellular matrix, ecm

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MATERIALS AND METHODS

The transverse epidemiological study concerns 1 group of patients presenting at least 1 implant.

Study design - setting

The study is performed at the Dental Clinic of the University of L'Aquila. After explaining to the patients the nature, objectives and methods of the study, emphasizing the potential dangers and inconveniences that participation in it could have involved. Patients received a document explaining the nature and purpose of the study and signed an informed consent for participation.

The inclusion criteria are:

- age > 18 years
- presence of a titanium dental implant (for peri-implantitis study)

The exclusion criteria are:

- smoking (more than 7 cigarettes / day)
- pregnancy or breastfeeding
- use of drugs
- acute illnesses (for example: fever, sore throat, diarrhea)
- presence of inflammation of the oral mucosa (eg: presence of aphthae, lichen planus, oral wounds, leukoplakia, oral carcinomas and other oral cavity lesions).

Patients responded to a questionnaire followed by a medical, clinical and radiological survey. The examination of the oral cavity included the analysis of periodontal and peri-implant indices such as the survey of the selected implants, the Full Mouth Plaque Score (FMPS) and the Periodontal Screening and Recording (PSR). This phase is followed by the salivary swab and the collection of peri-implant sulcular fluid.

Operating procedure

Periodontal folder (values to be analyzed)

1. Dental mobility
2. Vestibular PPD (probing depth)
3. Lingual PPD (probing depth)
4. Furcation
5. Vestibular recessions
6. Palatal recessions
7. BOP (index of sanguinamento)
8. Plaque index (O'Leary)

Implantsafe

Diagnostic test for the prevention of peri-implantitis (Biomolecular Diagnostic S.r.l. Firenze, Italy). It is possible to carry out an early diagnosis of the degenerative processes of periodontal and peri-implant tissue through

Table I *Control group*

Control group	Periodontal probing	Bleeding/Suppuration	Appearance/color/consistency	Implantsafe
1	0	Absence	Physiological	Negative
2	1	Absence	Physiological	Negative
3	2	Absence	Physiological	Negative
4	0	Absence	Physiological	Negative
5	1,5	Absence	Physiological	Negative
6	1	Absence	Physiological	Negative
7	2	Absence	Physiological	Negative
8	2,5	Absence	Physiological	Negative
9	2	Absence	Physiological	Negative
10	1	Absence	Physiological	Negative
11	1,5	Absence	Physiological	Negative
12	0	Absence	Physiological	Negative
13	0	Absence	Physiological	Negative
14	1	Absence	Physiological	Negative
15	2	Absence	Physiological	Negative

the ImplantSafe test, which allows the detection of the presence of the AMMP-8, which reveals a principle of tissue degeneration long before even a minimal sign of inflammation appears, in advance of weeks or even months compared to traditional methods such as probing, bleeding index (BOP) or X-rays(7-22). Implant Safe is a safe, simple and reliable test, which ascertains the presence of this enzyme in the peri-implant sulcular fluid (PISF): if the value of aMMP-8 remains continuously high in the PISF, the degeneration of collagen can ultimately lead to a loss of soft and bone tissues.

In the case of peri-implantitis, the values of aMMP-8 can be 1000 times higher than the values found with healthy implants. A regular screening with ImplantSafe identifies implants at risk of peri-implantitis, simplifying any therapy or treatment.

Implantsafe

ImplantSafe protocol - Sample preparation and collection

1. Prepare the sampling point: clean the site where the system was placed with a light air spray

Table II *Test group*

Implant shape	Periodontal probing	Bleeding/Suppuration	Appearance/color/consistency	Implantsafe
Cylindrical	0	Absence	/	Negative
Cylindrical	2	Bleeding	Inf. Lieve	Positive
Cylindrical	1,5	Absence	/	Negative
Cylindrical	3	Bleeding/Suppuration	Inf. Grave	Positive
Cylindrical	3,5	Bleeding	Inf.Grave	Positive
Cylindrical	/	Absence	/	Negative
Cylindrical	/	Absence	/	Negative
Cylindrical	4	Bleeding	Inf. Grave	Positive
Cylindrical	4,5	Bleeding/Suppuration	Inf. Grave	Positive
Cylindrical	1	Absence	/	Negative
Cylindrical	/	Absence	/	Negative
Cylindrical	3,5	Bleeding/Suppuration	Inf. Grave	Positive
Cylindrical	2	Bleeding	Inf.Lieve	Positive
Cylindrical	2	Bleeding	/	Positive
Cylindrical	1	Absence	/	Negative
Conical	2,5	Bleeding	Inf.Lieve	Positive
Conical	1,5	Absence	/	Negative
Conical	/	Absence	/	Negative
Conical	3	Bleeding/Suppuration	Inf. Grave	Positive
Conical	1,5	Absence	/	Negative
Conical	1	Absence	/	Negative
Conical	/	Absence	/	Negative
Conical	2	Bleeding	Inf. Lieve	Positive
Conical	/	Absence	/	Negative
Conical	3,5	Bleeding/Suppuration	Inf. Grave	Positive
Conical	3	Bleeding/Suppuration	Inf. Grave	Positive
Conical	2	Bleeding	Inf. Lieve	Positive
Conical	1,5	Absence	/	Negative
Conical	1	Absence	/	Negative
Conical	4,5	Bleeding/Suppuration	Inf. Grave	Positive

2. Take a sample of the peri-implant fluid: insert the absorbent strip (collection strips) in the gingival sulcus, as deep as possible for 30 sec.
3. Elution: insert the absorbent strip in the appropriate container and close the lid.
4. Turn the container upside down 5 times without shaking it and wait 5 minutes (the strip must float in the elution liquid).
5. Turn upside down 5 times.

ImplantSafe protocol - Sample analysis and evaluation

1. Open the test tube and immerse the yellow part of the Dipstick test in the elution liquid until this liquid becomes visible in the reading window. Place the test on a flat surface.
 2. Read the result after no more than 5 minutes.
 3. Evaluation and interpretation of the result obtained
- The study was carried out based on the detection of:

- Poll
- Bleeding - Suppuration
- Appearance, color and consistency of peri-implant mucosal tissues
- Evaluation method using ImplanteSafe system

We carried out these measurements both on a control group consisting of 15 healthy, implant-carrying patients, who were included in the inclusion criteria mentioned above and who therefore do not have peri-implant pathology (tab I). Subsequently the same measurements were performed on a group of selected patients with different types of systems:

- 15 pz with cylindrical implants
- 15 pz with conical implants

RESULTS

The data collected both through the classical periodontal analysis method and through the Implant safe test, both in the control group and in the study group, led us to elaborate the following conclusions based on the correlation between the two methods:

- In the control group consisting of 15 healthy patients with implants we found a number of positive Implant Safe tests equal to zero as the healthy patient does not present any peri-implant tissue pathologies.
- In the test group, consisting of 30 patients (15 cylindrical implant carriers and 15 conical implant

patients) we obtained the following results:

- Of the 15 cylindrical implants observed, 8 implants showed no signs of inflammation, therefore they are negative for the Implant Safe test. N° 7 implants show signs of inflammation of the peri-implant tissues: N° 2 implants showed mild inflammation and N° 5 implants showed severe inflammation: these patients tested positive for the Implant Safe test. N° 5 patients experienced bleeding and 3 patients showed both bleeding and suppuration. The Implant Safe test is positive in these latter cases.

- Of the 15 conical implants observed, 8 implants showed no signs of inflammation, therefore they are negative for the Implant Safe test. N° 7 implants show signs of inflammation of the peri-implant tissues: N° 3 implants showed mild inflammation and N° 4 implants showed severe inflammation: these patients tested positive for the Implant Safe test. N° 3 patients experienced bleeding and N° 4 patients showed both bleeding and suppuration. The Implant Safe test is positive in these latter cases.

DISCUSSION

According to the data in the literature (48-52), we can state that the concentration of aMMP-8 in the peri-implant sulcular fluid is higher in the case of peri-implantitis than in the case of a healthy implant (10-25). Comparing the data in the literature on the sensitivity and specificity of the Implant Safe test with the data obtained from our study, we can state that the Implant-Safe test has a good reliability in identifying implants that could present tissue degeneration in the future long before even a minimal sign of inflammation appears, weeks or even months ahead of traditional methods such as probing, bleeding index (BOP) or X-rays.

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AUTOTRANSPLANTATION OF IMMATURE THIRD MOLARS AS SUBSTITUTES FOR CONGENITALLY MISSING SECOND PREMOLARS: AN ALTERNATIVE SOLUTION IN A YOUNG PATIENT WITH OLIGODONTIA

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Auto transplantation of immature donor teeth can be a strategic therapeutic solution in young patients. It is preferable to choose this approach instead of prosthetic restorations because it offers a unique and definitive solution. Orthodontic space closure is not always deemed desirable, especially in non-extraction cases (53,54). Successful auto transplantations allow alveolar growth through eruption of donor teeth together with the adjacent dentition when skeletal and dental development is not yet completed. Auto transplantation of third molars is less well-recognised and less documented. The available literature shows promising success rates. Immature donor teeth are reportedly associated with better outcomes than mature donor teeth. Aim of this case report was to analyse the short-term outcomes of auto transplantation of immature maxillary third molars to replace the missing mandibular second premolars in a 17-year-old healthy female with oligodontia. The surgical procedure was performed as a single step. Left and right lower second deciduous molars (7.5-8.5), close to exfoliation, were extracted. Donor upper third molars with developing root were extracted as traumatically as possible and immediately placed into the surgically modified recipient sites. They were stabilized by a sectional wire. One year after surgery, the survival of both transplanted teeth was achieved. They showed periodontal health, normal mobility and continuation of root development during the follow-up period. The upper left third molar responded to all success criteria, no signs of ankylosis, root resorption (infection or inflammatory), and pulp necrosis. The upper right third molar had long-standing evidence of not progressive cervical external inflammatory root resorption without any clinical signs. Further research is needed to determine their long-term survival and success rates.

In childhood or early adolescence, the congenital absence of one or more permanent teeth (agenesis) is the most common anomalies with a prevalence from 3% to 11% among European and Asian populations (1). Tooth agenesis in young patients represents a

challenge for clinicians because skeletal and dental development is not yet completed (43-50).

As a rule, the therapeutic solutions to the missing teeth consist of fixed or removable prosthetic restorations, osseo-integrated implants, or

Key words: tooth autotransplantation, tooth agenesis; hypodontia; oligodontia; congenitally missing mandibular second premolars, incomplete root formation, success rate, survival rate, systematic review, meta-analysis, deciduous second molar exfoliation

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orthodontic space closure (2).

An alternative treatment option is auto-transplantation. This surgical procedure involves the repositioning of an autogenously erupted, partially erupted or un-erupted tooth from a donor site to a recipient site in the same individual (3).

Successfully transplanted teeth, due to a full understanding of the applicable biological principles and proper clinical techniques (3), offer several advantages compared to osseo-integrated implants. Autotransplants ensure a viable periodontal ligament and a continuous eruption of their root along with the adjacent dentition during growth. Furthermore, these teeth preserve alveolar bone volume and the interdental papilla and they can be moved by orthodontic or physiological forces (4).

The management of bacterial inflammation is of fundamental importance (12-27). Osseo-integrated implants do not grow down with the developing dentition and behave like an ankylosed tooth, involving an infra-occlusion in growing patients (3,28-33).

Auto-transplantation is too often overlooked in patients with missing teeth. Especially, auto-transplantation of third molars is often not considered and perhaps less well-recognised and less documented. Third molars have been generally

transplanted to replace heavily damaged or missing molars. However, previous studies showed promising success rates (5,6).

The aim of the study was to analyse the short-term outcomes of auto transplantation of immature maxillary third molars in replacing missing lower second premolars.

MATERIALS AND METHODS

The patient was a 17-year-old healthy female with oligodontia. Probably she inherited this tooth agenesis through nonsyndromic autosomal-dominant paternal transmission. On clinical and radiograph examination, she showed permanent teeth and over-retained deciduous teeth associated with congenitally missing permanent successors (Fig. 1-2).

In order, she had agenesis of the upper right lateral incisor (1.2), the upper right canine (1.3), upper right first and second premolars (1.4-1.5), the upper left lateral incisors (2.2), upper left first and second premolars (2.4-2.5), the lower left second premolar (3.5), the lower left third molar (3.8), the lower right second premolar (4.5) and the lower right third molar (4.8) (Fig. 3).

After a careful diagnosis, the treatment planning consisted of the autotransplantation of immature upper right and left third molars in place of the missing lower



Fig. 1. Initial check-up of a 17-year-old girl with congenitally missing teeth. a) upper occlusal view, b) lower occlusal view, c) right lateral view, d) frontal view, e) left lateral view.

second premolars after extraction of over-retained second deciduous molars on the mandibular dental arch, and, orthodontic space closure and postponed dental implants until the end of skeletal and dentoalveolar growth on the maxillary dental arch (Fig. 2).

The patient's well facial balance, the absence of crowding and protrusion, and the oligodontia have been the factors why orthodontic space closure was not considered (7). Before surgical procedure, it was carefully analysed donor teeth and recipient sites.

Left and right upper third molars (1.8 and 2.8), as donor teeth, showed a root development from initial to one quarter, corresponding to stage one of Moorrees' classification (8).

Recipient sites included over-retained second deciduous molars showing high tooth mobility and progressive root resorption close to the exfoliation. They ensured the best space and adequate alveolar bone width and height to receive donor teeth. Therefore, autogenous bone graft or green-stick fracture was not necessary.

Systemic prophylactic antibiotic was prescribed preoperatively. Disinfection of the oral environment with 0.2% chlorhexidine gluconate, pre, during and after surgery on prescription (0.12%) was performed. Local anesthetic to the bilateral inferior alveolar nerves by block technique was delivered.

The surgical procedure was performed as a single step.

Left and right lower deciduous molars (7.5-8.5) were extracted. The size of socket sites was surgically modified with a low-speed round bur under abundant irrigation with sterile saline, removing intra-alveolar septum and debris to allow effective seating of donor teeth. After full-thickness flap retraction and subsequent osteotomy, donor third molars were extracted as atraumatically as possible to preserve the delicate periodontal ligament cells and immediately placed into the prepared sockets, slightly below the occlusal plane. Following surgery, they were splinted to the adjacent teeth using a .016x.022 passive nichel titanium sectional wire and composite resin. Bite blocks were performed on upper molars to avoid the possibility of premature contacts (Fig. 4).

The patient was followed-up at one, three, and six months, and at one year after autotransplantations (Fig. 5). The splint was removed after 6 months. Endodontic treatment of transplanted teeth was not necessarily due to the incomplete root formation. The patient was followed up at one, three, six months and one year after transplantations.

RESULTS

One year after surgery, the survival of both transplanted third molars was achieved. The transplants showed periodontal health and no sign

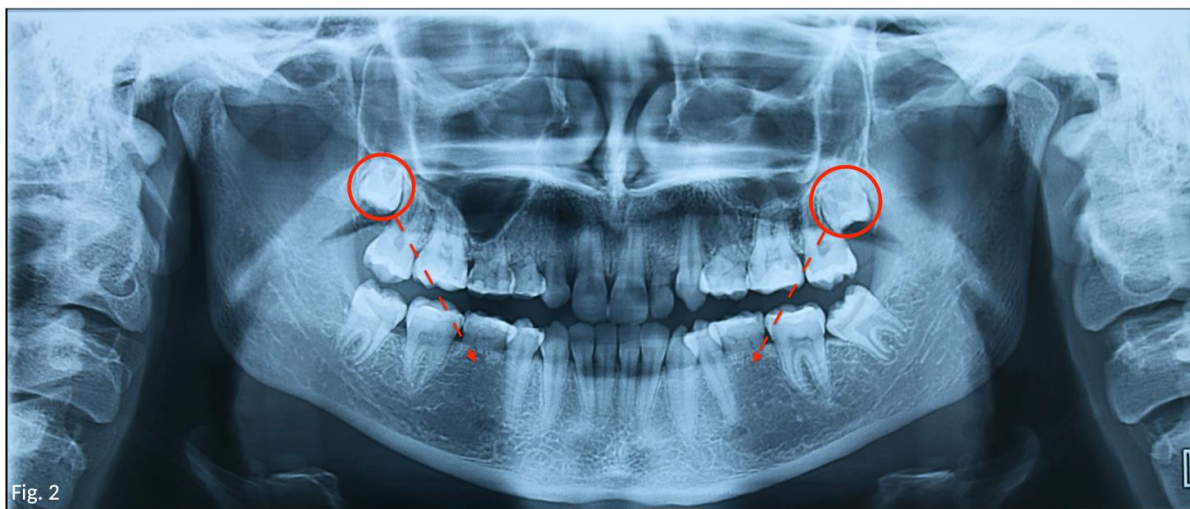


Fig. 2 Pre-treatment orthopantomogram (OPG) of a 17-year-old girl with congenitally missing teeth. The maxillary third molars were transplanted to the mandibular second premolar regions.

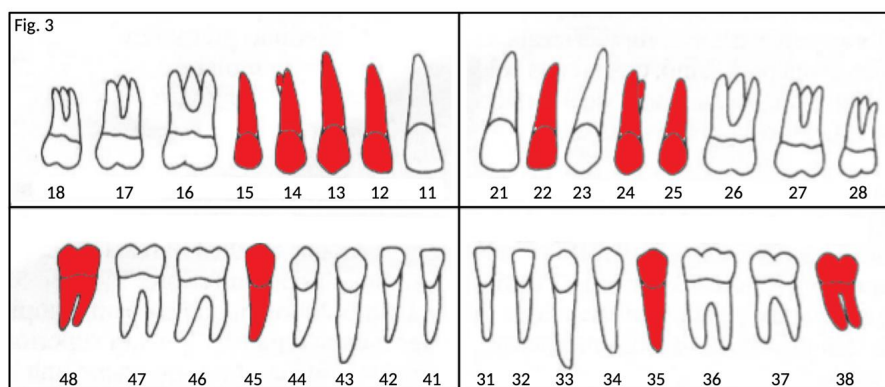


Fig. 3 Odontogram with agenesis of the following teeth (in red): agenesis of the upper right lateral incisor (1.2), the upper right canine (1.3), upper right first and second premolars (1.4-1.5), the upper left lateral incisor (2.2), upper left first and second premolars (2.4-2.5), the lower left second premolar (3.5), the lower left third molar (3.8), the lower right second premolar (4.5) and the lower right third molar (4.8).

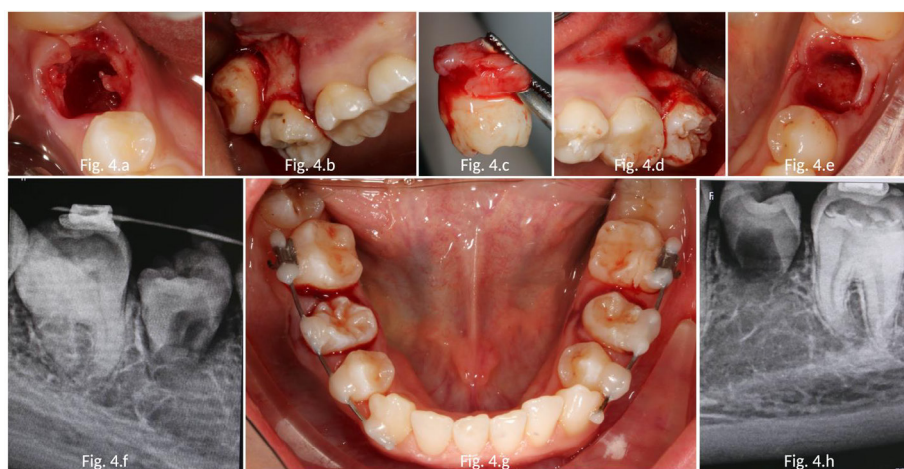


Fig. 4 Sequence of surgical procedure: Recipient sites: lower right second premolar region (4.a) and lower left second premolar region (4.e); Donor teeth: upper right third molar (2.8) (4.b-4.c) and upper left third molar (1.8) (4.d); Transplanted teeth stabilized using composite-wire splint (4.g); Post-operative periapical radiographs of transplanted teeth 1.8 (4.f) and 2.8 (4.h).



Fig. 5 Check-up at one year after transplantations: a) right lateral view, b) frontal view, c) left lateral view e) lower occlusal view; periapical radiographs of transplanted teeth 1.8 (5.d) and 2.8 (5.f) obtained at one year after surgery.

of marginal attachment loss or inflammation. They presented normal tooth mobility and function. They responded normally to percussion testing. Both third molars showed a continuation of root development. The upper right third molar responded to all success criteria, no signs of ankylosis, root resorption (infection or inflammatory), and pulp necrosis during the follow-up period and alveolar bone and lamina dura have successfully developed.

On the contrary, not progressive cervical inflammatory root resorption on the mesial root surfaces of the left upper molar has been reported in each follow-up. However, performed probing assessments along the tooth circumference (buccal, lingual, mesial, and distal) were of an average of 2-3 mm.

DISCUSSION

The present study has demonstrated that autotransplantation of immature third molars to replace missing mandibular premolars can be considered a viable treatment alternative. The well-established now approach to examining the outcome after autotransplantation of donor teeth is to present success rates by recording the occurrence of sequelae to the transplant (2). The longevity of transplants is usually expressed by the survival and success rates of autotransplanted teeth (4). Survival was defined as the percentage of transplanted teeth still present during the follow-up. Auto-transplantation can carry complications, which include :conservative problems (34-42), pulp necrosis and infection (51-58), loss of periodontal healing, that is, root resorption: the surface-, the inflammatory- and the replacement resorption (ankylosis) and lack of root development (9, 10). Success was defined as the percentage of transplanted teeth with no signs of these complications during the follow-up period (2, 4).

Rohof et al. reported that the survival rates after 1, 5, and 10 years were 97.4, 97.8, and 96.3%, respectively. They estimated an annual weighted 98.2% survival rate and a 96.6% success rate for overall donor teeth. For donor molars, it has been reported an annual weighted 97.2% survival and a 95.5% success rate, which were promising values even if slightly lower compared to those

for overall donor teeth. Mejàre et al. reported an 81.1% survival rate of 50 third molars over four years after transplantation. Yu et al. compared the success between donor third molars in a socket of an unrestorable tooth with third molars in a surgically prepared socket and they reported survival rates over 93% at 9.9 years for both. Yan et al. 94% showed a survival rate of third molar without infection, ankyloses and resorption of 94% (6).

Several factors would affect the prognosis of auto transplantation. Regarding patient factors, better results in younger patients, free of major systemic and metabolic problems or specific habits (e.g., smoking), with satisfactory oral hygiene and good compliance was obtained (3). The patient in this study was well-motivated in good health with a cooperative attitude and no specific habits.

Each donor tooth was required to meet several selection criteria: healthy tooth, a normal morphology to fit well into the recipient site; an immature root or half to three quarters of the normal root length according to the classification of Moorrees (8). Immature donor teeth are reportedly associated with better outcomes than mature donor teeth. Tsukiboshi reported an 82% success rate in a 6-year follow-up study of 250 cases. Lundberg and Isaksson reported a 94% success rate for donor teeth with incompletely formed roots and an 84% success rate for donor teeth with completely formed roots. Success rate was influenced by the type of donor tooth too.

It has been found that molars have a success rate lower than premolars, with higher percentages as regards the onset of complications (9,10). However, 75% of autotransplanted third molars have been successful over a long period of time (3). Each upper third molar of this study presented a conical, single immature root in stage one of Moorrees' classification (8), optimal to promote pulp revascularization and periodontal health. Rohof estimated the 100% survival rate for the teeth transplanted in stage one (4).

The recipient site would be affected by the following factors: location, local inflammation, alveolar bone volume, and quality. Rohof reported on the premolar region, that is, the recipient site of donor upper third molars, an annual weighted 98.6% survival rate and a 97.8% success rate per year. The

close to falling mandibular deciduous second molars were not affected by caries or pulp damage. Therefore, the surgically prepared sockets were healthy free from infection or local inflammation. Donor upper third molars showed a mesiodistal width similar to that deciduous second molars. Donor teeth' coronal anatomic shape looked like those of premolars.

Quality of the intervention, antibiotic use, experience of the surgeon, damage of the periodontal ligament, need for an autograft or osteotomy, storage method and extraoral time of the graft could affect the surgical procedure (4). Transplantations have been immediately performed after deciduous molars extractions and surgically prepared sockets. The less extraoral exposure preserved the vitality of the periodontal ligament of donor teeth (9,10).

The stabilization method consists generally of the use of sutures and/or wires. This split should provide an adequate and flexibly secure stabilization, of an average duration of 7-10 days (6). Nevertheless, conflicting results regarding the influence of stabilization technique on the success of auto transplantation has been reported (4). In this study, composite wire splints were removed six months after transplantation to provide greater stability. Bite blocks were performed because it is very important to remove the transplanted dental element from the postoperative traumatic occlusion (2, 11).

The annual weighted estimated complication rates for donor molars were higher compared to overall teeth in terms of ankylosis (2.2%), root resorption (5.0%), and pulp necrosis (2.5%) (4). There was no sign of complications on the upper right third molar transplanted, therefore it responded to all criteria of survival end success. The upper left third molar had long-standing evidence of cervical external inflammatory root resorption. Even if at the follow-up one year after surgery, it seemed lightly decreased.

However, the follow-up does not allow evaluation of the prognosis of the transplanted teeth beyond one year after transplantation (5). Transplants that have survived after one year indicate a favorable prognosis for a longer period of survival of up to 10 years (4). According to literature and the absence of complications, even if it is too early to predict the donor teeth survival and success rate, a favorable

prognosis could be expected. Due to the mesial inflammatory root resorption of the upper left molar, not predictable longevity of this tooth can be defined.

The auto-transplantation of available immature third molars as substitutes for congenitally missing second premolars can be a viable alternative therapeutic solution for a young patient, especially with oligodontia. As this approach is not well-known further research is needed to determine their long-term survival and success rates. Successful third molars transplanted could be replace effectively second premolar agenesis until the majority of vertical facial growth is completed, ensuring in the meantime biological, functional and aesthetic goals. Even if it can occur failure, these biological space maintainers could be replaced with an osteo-integrated implant at the end of the facial growth.

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BRUMA MAD: A NEW WAY OF TREATING OSAS PATIENTS

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The aim of this study was to introduce a new adjustable device in the treatment of obstructive sleep apnea syndrome (OSAS). The new appliance has a different way to regulate and move the mandible through the presence of two occlusal lifts, that can leave an open posterior space, and the complex system formed by the peduncle and the steel plate that can easily move forward the mandible. This system guarantees a posterior space for the tongue but mostly for the passage of air. The principal aim of Bruma MAD is to contrast the pharyngeal collapsing and to prevent hypopnea or apnea. Further clinical studies are needed to confirm and to evaluate the predictability of this new device.

Nowadays an increasing number of orthodontists are involved in the multidisciplinary management of patients with obstructive sleep apnoea syndrome (OSAS) and “socially disruptive snoring”. The OSAS is characterized by a multitude of signs and symptoms related to arterial oxygen desaturation and sleep fragmentation, caused by pharyngeal obstruction during sleep (1).

Sleep-disordered breathing (SDB) can have high prevalence within the population as shown by the HypnoLaus study, which found rates of 49.7% for men and 23% for women (2)-

The main problem in SDB/OSA patients is the pharyngeal collapse which can lead to hypopnea (reduction in ventilation) or apnoea (cessation of respiratory airflow).

SDB is also related to snoring, mood disorder, disruptive behaviour, and depletion of quality of life. Some oral anatomic features associated with SDB may include high arched or narrow hard palate and retrognathia (3).

“Adenoid facies” has been associated with SDB, and may lead to a reduced size of the upper airway((53-57), causing not just sleep breathing-related problems but also a constricted maxilla, usually causing posterior crossbites and crowded teeth.(4) Impaired growth, cardiovascular problems, and attentional, learning and behavioural problems are also linked to SDB, with increased periodontal problems(12-18) the management of bacterial inflammation is of fundamental importance (1927). These problems are critical and are related to craniofacial proportions (43-50). Often the dentist is the first person to recognize these issues, and indeed the approach most indicated to treat SDB in adults is by means of the oral appliance (OA). The OA are patients not indicated in implant prosthetic rehabilitation due to the consequent gingival inflammation (28-33), with conservative problems (34-42, 51, 52).

The OA can be categorized into two types: mandibular advancement device (MAD),(5) which

Key words: Bruma, Mandibular Advancement Device, sleep apnea, snoring, OSAS, MAD

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aims to protrude the mandible and associated soft tissues to open the airway and reduce the apnea-hypopnea index (AHI),(6); or the less frequently used tongue retaining device (TRD), where the objective is to maintain the tongue in a forward position, to avoid obstruction of the airway. The continuous positive airway pressure (CPAP) remains the gold standard treatment for OSA anyway (7).

According to a recent systematic review OA therapy is an effective first-line treatment for patients suffering from primary snoring and in whom mild to moderate OSA has been diagnosed. OA therapy is a treatment option for patients with severe OSA who are, for whatever reason, unable to adhere to CPAP therapy. Dentists should screen for SDB/OSA; however, only dentists with good knowledge of dental sleep medicine or dentists who regularly update their knowledge and training in dental sleep medicine should be part of the multidisciplinary team to treat patients with SDB/OSA. It is important to know that there are more causes that could affect sleep other than SDB of which the dentist should be aware of (8).

The aim of this study was to describe a new adjustable mandibular advancement device, the *Bruma MAD*, patented in 2005 (Patent number pg233489572) (Fig. 1).

Mandibular advancement devices (MADs) represent the main non-continuous positive airway pressure (non-CPAP) alternative for patients with

obstructive sleep apnoea (OSA). Custom-made MADs reduce apnoea/hypopnoea index (AHI) and daytime sleepiness compared with placebo devices. CPAP more effectively diminishes AHI, while increasing data suggest similar outcomes in relation to symptoms and cardiovascular health from these treatments. Patients often prefer MADs to CPAP. Milder cases and patients with a proven increase in upper airway size because of mandibular advancement are most likely to experience treatment success with MADs. A custom-made device titrated from an initial 50% of maximum mandibular advancement has been recommended. More research is needed to define the patients who will benefit from MAD treatment compared with CPAP, in terms of the effects on sleep-disordered breathing and on other diseases related to OSA.

The treatment must be followed up and the device adjusted or exchanged in relation to the outcome. (9) The therapy with MAD is also associated with several side effects: in the short term, articular and muscle impairments, gum irritation, and excessive salivation that can be managed or even prevented with a conservative behavioural therapy, whereas in the long-term, the main side effects reported are represented by dentoskeletal changes. The masticatory muscles attempt to restore the normal position of the mandible, thrust forward by the MAD that transmits the reciprocal forces, generated by the advancement of

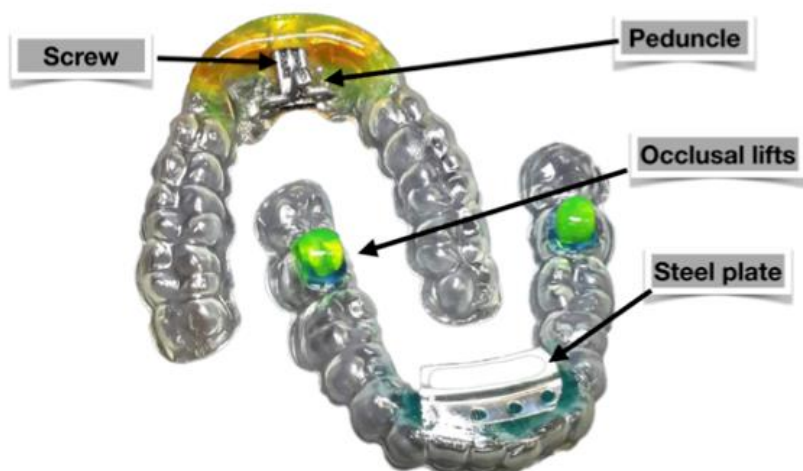


Fig. 1. The oral appliance with the peduncle, the screw, the steel plate, and the occlusal lifts

the lower jaw, to the hard tissues and to the teeth. The consequent occlusal changes that can occur during the treatment cannot be considered negligible, since the patients must wear the MAD every night for a lifetime and therefore, they will likely face this issue. The literature presents several studies that investigated the side effects occurring with a MAD therapy but the parameters considered to assess the dental and skeletal modifications are not always the same and it is not reported if these side effects are influenced by the duration of the therapy (10).

Mandibular advancement device features

Mandibular advancement devices carry out an anterior and inferior movement of the jaw, generating anatomical variations in the upper airway (UA) that enable an increase in the pharyngeal area. This action fixes the jaw and the hyoid bone, preventing the posterior rotation of these structures during the decubitus and the resulting airway obstruction. Although its main effect appears in the velopharyngeal area, it has repercussions for all the pharyngeal segments. There is initially an increase in rigidity and a swelling of the space between the anterior and posterior pharyngeal pillars. The soft palate is displaced ventrally, and this increases the thickness of the lateral walls of the velopharyngeal area which are factors that drastically reduce snoring. Functional mandibular advancement induces

changes in the position of the hyoid bone towards a more forward position. It creates a new position of balance of the suprahyoid musculature, which in turn, favours an increase in volume and permeability of the UA. The pharyngeal fat pads relocate laterally from the airway and the tongue base muscles move anteriorly. This leads to a reduction in pharyngeal collapsibility.

Mandibular positioning

The degree of mandibular advancement is an important modulator of the treatment outcome since there is a dose-dependent effect on nocturnal oxygenation and pharyngeal collapsibility. A small advancement produces a less noticeable effect, while a larger advancement produces more significant effect. It is generally assumed that more than 75% of maximum protrusion is needed for a satisfactory effect. An excessive advancement can, however, also produces some side-effects. Therefore, inappropriately large mandibular advancements should be prevented, since the long-term negative side-effects on occlusion and the temporomandibular joints are still unknown.

There is currently no agreement regarding the method for measuring and defining the degree of mandibular repositioning in the individual patient. An adjustment procedure millimetre by millimetre has, therefore, been recommended to achieve optimal



Fig. 2. Intraoral image showing the head of the screw and the device fitting

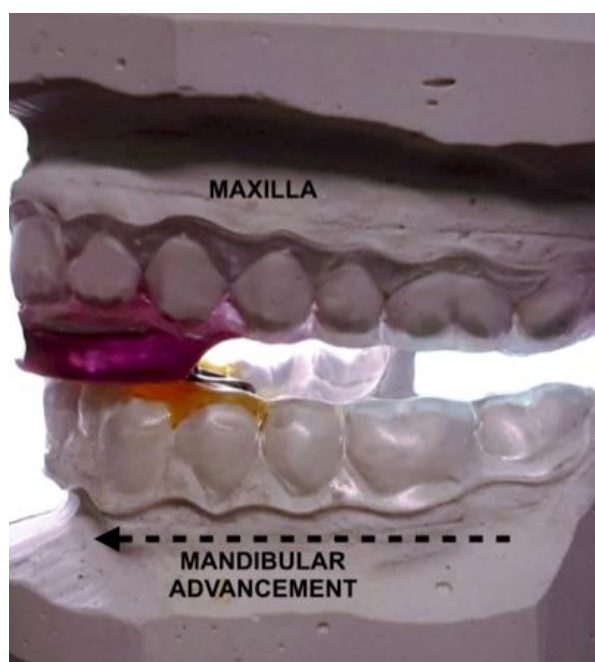


Fig. 3. Model view: showing the posterior open space and the mandibular advancement

results. A MAD is usually adjusted using a screw located in the mid-line, anteriorly or posteriorly, or laterally with arms of different lengths on single or both sides of the appliance. Some designs permit the opening of the mandible and some lateral movement, while others fixate the jaws more rigidly.

Bruma MAD is an innovative oral appliance that consists of a screw, located in the middle line among the incisors, from which starts a peduncle that slides on a special steel plate/guide located on the lower arch. Its unique design allows lateral and vertical movements of the jaw while preventing the release in the night hours (Fig. 2).

An advantage of the device is the no-use of acrylic on the occlusal areas: the upper and lower base do not get in contact in the posterior area, keeping free space for the accommodation of the tongue, the main cause of the UA collapse. The forward thrust is rear and parallel to the occlusion applying less pressure to the temporomandibular joint (TMJ). However, the most important part of the appliance is the screw. It is graduated and can be adjusted to the tenth of a millimetre, thus changing the position of the peduncle and its engagement with the lower guide.

The activation of the screw must be made exclusively by a competent clinician according to the direction of the arrow. Each activation consists of one quarter turn and is equal to 0,25 mm. Activating the screw the Bruma MAD tends to close, and then, slowly, the lower part moves forward (Fig. 3).

Bruma Mandibular Advancement Device has shown to be a useful device in the treatment of snoring and OSA. In normal subjects with simple snoring without any systemic illness, Bruma MAD can be quickly suggested by the dentist. Nevertheless, in the case of severe OSA, cardio-respiratory evaluation must be done before starting the treatment.

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THE DENTIST'S ROLE IN DIAGNOSIS AND TREATMENT OF OBSTRUCTIVE SLEEP APNEA SYNDROME: A LITERATURE REVIEW

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The purpose of this work was to present a review of the literature concerning obstructive sleep apnea syndrome, and the role of the dentist in this pathology, both to identify elements useful for a good diagnosis and to apply the available therapeutic strategies. In literature there is no unanimous opinion on the treatment of OSAS. The multidisciplinary approach is necessary, creating teams made up of dentists, otolaryngologists and medical experts in sleep disorders, in order to develop a cooperation-based treatment plan for the disease. In this review, the importance of early diagnosis, orthodontic therapy in order to restore normal function is underlined, since OSAS is linked to a high risk of hypertension, cardiovascular diseases, daytime sleepiness, domestic and work accidents, with consequent deterioration of the quality of life.

Obstructive Sleep Apnea Syndrome (OSAS) is a highly morbidity disease in western world with serious consequences on population health. In this review, we investigated dentist role in this pathology, both in identifying elements useful for a good diagnosis and in applying available therapeutic strategies. Being OSAS a multidisciplinary interest disease, this role will be studied in relation to other figures involved, according to existing guidelines indications.

DISCUSSION

OSAS is characterized by partial or complete episodes of upper airway obstruction during sleep. The phenomenon can be important enough to reduce blood oxygen saturation. Episodes of decrease flow of at least 50% are called hypopneas; if drop in flow is at least 90% (or total obstruction) they are

called apneas. Severity of the disorder is assessed by polysomnography, a sleep study usually performed overnight in a sleep laboratory monitoring electroencephalographic, electromyographic, electro-oculographic, oximetric and respiratory parameters (1). By data obtained, it is possible to calculate AHI (Apnea Hypopnea Index) useful for staging disease severity. Index is calculated by adding apneas and hypopneas episodes for each hour of sleep monitored during the exam (AHI 5-15 = mild OSAS, AHI 15-30 = moderate OSAS, AHI > 30 = severe OSAS). By an epidemiological point of view, this is a particularly widespread disorder, affecting 4% of males and 2% of females (2); rising with increasing age and reaching peaks (involving mild cases) of 67% in elderly men and 54% in elderly women (3). However, it is still difficult to determine OSAS incidence: night disorders are noticed more

Key words: obstructive sleep apnea syndrome, snoring, OSAS, MAD, mandibular advancement devices

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often by an eventual partner rather than by affected person and daytime symptoms are often mild.

Costs of the disease both in economic terms and human lives are particularly significant (4). Most important consequences include lack of adequate sleep and constant snoring, day-time symptoms are particularly relevant: ability to pay attention and memorize are reduced, with frequent episodes of sleepiness. These conditions are associated with serious psychological consequences, such as depression and anxiety (5). OSAS syndrome frequently leads to high blood pressure and correlates with an increased risk of cardio-circulatory events and strokes. Apneas or hypopneas force autonomic nervous system to change physiological responses to normalize oxygen tissue uptake. OSAS also modifies individual basal metabolic rate, in particular lipids usage, acting as a cofactor for the development of diabetes, also favored by obesity associated with the underlying disease (6). The frequent phases of hypoxia and re-oxygenation favor lipids oxidation and peroxidation and bring to a general inflammatory state. (7).

Continuous hypoxic events can damage most oxygen demanding cells, first those of the nervous system. Role of OSAS as a cofactor in neurodegenerative diseases is on evaluation (8). Over the years, several publications allowed to understand mechanisms underlying the obstruction and strategies to adopt to solve it. By a mechanical point of view, OSAS is caused by the collapse of the pharyngeal or immediately adjacent structures: soft palate, tongue, lateral walls and epiglottis. Reason of the collapse is the loss of nocturnal muscle tone that occurs in during REM phase. Contributing factors are obesity (in particular fat deposits in the neck) or enlarged glands and lymph nodes of buccal or pharyngeal apparatus. Even specific genetic conditions modifying anatomy of the skull and respiratory structures can determine OSAS onset or aggravate its status (9).

Specific evaluation of flows through the pharynx can allow to understand which anatomical structure is mainly involved in the disorder, permitting a better approach (10). Collapse of epiglottis generates the most relevant flow reductions and is associated

with an increase in frequency of apneas, while the involvement of other anatomical structures correlates with milder symptoms. Some studies have correlated craniofacial measurements with OSAS onset: distance between joid bone and mandibular plane as well as distance between the palate and pharyngeal wall or the Frankfurt plane can predict risks of pathology development (11). Mallampati scale, used to assess the ease of endotracheal intubation, is a predictor of OSAS (12).

OSAS treatment may initially begin with weight loss, increased physical activity (13), decreased daily stress factors as well as the elimination of alcohol and smoking (14). If patient leads a substantially healthy life but still suffers from this disorder -of medium entity or more- a strategy is to force respiratory resistance by applying a positive airway pressure (CPAP, continuous positive airway pressure) (15), with several devices evolved over time (16).

Use of a mask with a continuous positive air flow is the most common treatment, although patients report difficulty wearing this support at night. In severe cases, surgery can also be performed on the structures directly involved. Surgical therapy aim to remove obstructive and respiratory factors in the anatomical districts of interest. Through oropharyngeal surgery, stiffening and remodeling of jaw isthmus area are obtained, both at level of palatine tonsils and in the region of palatine veil.

Hypopharyngeal surgery has the purpose of expanding posterior airspace by reducing the volume of the tongue base. Particularly relevant for the treatment of OSAS is maxillofacial surgery for maxillo- mandibular advancement. In some patient's treatment may even lead to a permanent tracheostomy, with evident psycho-physical impact (17), but usually an attempt is made in order to reduce anatomical portion affecting air flow (18). In dental field, dentist has access to patient's mouth and, if properly trained during studies, can identify some morphological traits associated with OSAS (19). An emerging discipline is dental sleep medicine, connecting dentists with other clinical specialists. In United States, the need to integrate dentist training courses with these contents is

increasingly pressing and required directly by dental associations (20). Over the years number of dentists informed about OSAS is increasing (75%), although percentage of those able to diagnose this pathology is still lower (42%) (21). Based on these evidences, several guidelines are being developed. However, dentists only in one case out of two, while suspecting a sleep disorder, contact further specialists for a multidisciplinary assessment (22). Despite progress, many open points remain (23).

In the United States, this issue is particularly complex, occasionally making it necessary to ask opinion of a court in order to decide whether it is the dentist or the specialist who must decide for a treatment or not (24, 77-90). Interdisciplinary collaboration obviously represents the main strategy to treat this pathology: on the one hand the clinician has a complete picture of OSAS and its complications, through the possibility of integrating different physiological and laboratory parameters, from the another the dentist can decide whether or not an OAT (oral appliance therapy) is indicated rather than a surgery treatment. In dental field, some treatments can solve the disease and are generally well tolerated. OAT -wearing a removable oral appliance in mouth during sleep- represents a first-line treatment in those patients who refuse CPAP. It is not recommended if teeth and gums health is compromised and in presence of other jaw disorders. OAT choice must be conditioned by various factors such as: comfort, ease of use, modifiability of the mandibular advancement and ability of jaw movement. Term OA generically describes all different devices used in treatment. Some specific devices are MAD (mandibular advancement device), MRD (mandibular repositioning device) and MAA (mandibular advancement application). Devices are divided into custom or non-custom, and adjustable and non-adjustable. Custom devices, built on specific patient measures, are generally more effective in reducing symptoms, such as number of apneas, oxygen saturation, number of awakenings etc. Use of non-custom and non-adjustable devices is therefore not recommended (25). Reference OAs for OSAS are MAD since about 1980. Market has proposed many variants, but the ultimate goal is to move jaw slightly forward in order not to allow

relaxation of muscles during REM phase conducting jaw and tongue on air flow path. Attack, however, must take place on jaw too. Once the most suitable OA has been chosen, it is necessary to take arches impressions. Very important is the choice of the “construction bite” which determines, once the OA is applied, the therapeutic position of the jaw. Once the device has been delivered, it will be necessary to find the optimal mandibular advancement: it is important to obtain maximum condition of comfort for patient in which mandibular advancement is not associated with an algic symptomatology of the muscular component and of TMJ (temporomandibular joint). Most recommended MAD are made starting from the alginate mold or from digital reconstructions of patient's arches. Usually, registration is provided at approximately 50/75% of patient's maximum protrusion. (26, 27, 66-76). It is also necessary to obtain a slight rotation of the jaw. The OA must have an attack of at least six teeth on each arch and clearly must be biocompatible and last for at least 3 years regarding structural integrity (28). OA can be monobloc or bib lock and several studies have confirmed an equal effectiveness of different designs (29). Over time, dentist will check tolerance and functionality levels according to device allowed settings.

Several techniques are used to produce the advancement: from rubber bands to screws, from telescopic devices to connectors etc. To verify OA effectiveness, it is recommended that patient daily report snoring degree (helped by partner), possible presence of tiredness upon awakening and daytime sleepiness. A polysomnographic control study is then planned with application of the OA to establish its effectiveness. If pathology is not under control, patient should be sent to a specialist in treatment of sleep disorders. It is also not recommended to use OA in the most serious cases of OSAS: in case of failure patient's health can be significantly worsened. (30,31), Overall, positive outcomes of OATs even in serious cases are well documented. (32, 33, 34, 55-65). Use of OATs is associated with an improvement in overall quality of life linked to a reduction in daytime sleepiness and mood changes.

Another improvement is a reduction in blood

pressure, with clear benefits on cardio-vascular complications. However, it is interesting to note that OATs or CPAP do not improve sleep architecture (percentage of time spent in REM phase) in a statistically significant way. Overall, CPAP shows better results, but the tolerability of OAs is significantly higher (35). Follow-up in the continuous use of MAD showed encouraging results (36, 37), but other studies demonstrated that after two years the adaptation of soft tissues to the new position may bring to a worsening of OSAS condition (Rose et al., 2002). National guidelines on the use of OAT refer primarily to the impressive work of the American Academy of Sleep Disorders (38). In these reviews and meta-analysis, various types of surgical, pharmacological, and dental treatment have been discussed, putting in evidence any successes and critical issues. Well-defined roles are also identified: specialist in sleep disorders will define diagnosis, degree of severity and any physiological contraindications. Role the dentist is to help the diagnosis through the observation of specific predictors and by submitting specific questionnaires, identify any other lesions, link different specialists and finally apply an OA, deciding the most suitable case by case. Follow up and recognition of any side effects will also be dentist responsibility. Various national associations, such as the Canadian (39) and the American (40) are on the same page. Similar guidelines have also been published in Italy, without substantial differences from the international vision (41, 44-54). For the Australian dentists association (42), on the other hand, there are no predictive parameters to predict therapeutic success of OATs: despite anthropometric measures, studies on air flow, scans, photographs, there are still no studies showing specific cases in which this type of device can be useful. The large presence of different devices available influences results of these meta-analyses: general recommendation is to try to standardize methods and applications to make field of study more uniform. Another class of products is used in OSAS, based on tongue retention (43): these are however, dated tools and almost completely supplanted by MAD.

OSAS is a serious and particularly widespread

disease. A well-trained dentist can represent a first line by playing a role in its identification and treating mild and more common cases, thanks to the use of special OA devices. However, general impression is that dentist role in OSAS syndrome is not well defined by publications of significant scientific impact. On the other hand, there are no centers dedicated to treatment of obstructive apneas in the dental field: therefore, collecting large numbers and being able to develop statistics with acceptable confidence margins can be difficult. Despite this, the impressive literature review by American Orthodontists Association seems to be going in right direction, to provide guidelines with strong scientific evidences. (44). However, greater multidisciplinary cohesion is necessary, creating teams made up of dentists, otolaryngologists and medical experts in sleep disorders, in order to develop a cooperation-based treatment plan for the pathology.

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HISTOMORPHOMETRIC EVALUATION OF BOVINE BONE AND EQUINE BONE MATRIX IN MAXILLARY SINUS FLOOR AUGMENTATION: A SYSTEMATIC REVIEW

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The aim of this study was to evaluate the efficiency of bovine and equine bone substitute materials used in sinus augmentation techniques. The research protocol was carried out using the PubMed database, including articles from October 2019 until February 2020 and those in English only. The search on PubMed (MED-LINE) was carried out with the following keywords: sinus augmentation OR sinus lift AND bovine bone graft OR equine bone matrix. The articles identified included randomised clinical trials, cross-sectional studies and case series. Outcomes were the percentage of newly formed bone, remaining graft particles and soft tissue in a follow-up at least of 5 months. The use of bovine bone graft (ABB) as a graft material in maxillary sinus elevation was evaluated in 9 studies. According to the quality assessment, 4 of the 9 studies were analysed and an arithmetic mean was performed showing the following results: newly formed bone (NFB 22.74%), residual grafts (RG 29.36%) and soft tissue percentage (SF 48.51%). The use of equine bone matrix (EBM) was assessed in four different studies including an RCT, a cross-sectional study and two case series; a mean percentage calculated by the extracted data of the four studies was estimated with newly formed bone (NFB 44.51%), residual grafts (RG 17.24%) and soft tissue percentage (SF 36.62%). EBM appears to be as effective as ABB for the maxillary sinus floor augmentation procedure.

Bone augmentation could be required for patients lacking an adequate quantity of bone for implant treatment (1). In vertical bone augmentation, a variety of graft materials such as autogenous bone, allografts, xenografts and alloplastic materials are often used (2, 3). Bovine bone substitute is frequently used for various augmentation procedures (4). A number of recently published studies have examined the safety and efficacy of equine derived bone graft substitutes in treating significant periodontal defects, in post-extraction ridge preservation procedures and in the augmentation of atrophied alveolar ridge (5, 44-49). The use of bone substitute reduces the entity of

intraoral harvesting, moreover, compared to the use of autologous bone alone it could express a larger bone volume for dental implant placement. In a systematic review, the mean implant survival rate for vertical and/or lateral ridge augmentation was 100% for bone substitute materials mixed with autologous bone and 98.6% for autologous bone alone, but a comparison with a meta-analysis was not possible due to missing data (6). Bone substitute materials are considered a scaffold for newly formed bone with an osteoconductive property; furthermore, the advantages of using a bone substitute, such as a bovine bone graft, include the long-term stability and low percentage of resorption

Key Words: sinus lift; sinus augmentation; bovine bone graft; equine bone matrix

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(7). Histomorphometric analysis is considered the gold-standard method for estimating the amount of newly formed bone, residual graft particles, and soft tissue components.(8, 28-43) A recent systematic review compared the histomorphometric effectiveness of various bone grafts (9) and no statistically significant differences were found in the percentage of new bone from a pairwise comparisons between any of the two substitutes. Treatment ranking based on the evidence network indicated that autografts presented the highest percentage of newly formed bone, followed by synthetic grafts, xenografts, and allografts (9). Regardless, bone substitute materials seemed to be good alternatives to autogenous bone grafts and were believed to avoid the disadvantages related to autografts, including morbidity rate, limited availability, and high volumetric changes (10). The purpose of this systematic review was to compare the predictability through a histomorphometric analysis of biomaterials of bovine and equine origins in sinus lift techniques.

METHODS

Search protocol

The search protocol was carried out using the PubMed database, with a limit time from October 2019 until February 2020, including English articles only. The search for articles included randomised clinical trials, case series and cross-sectional studies.

Focus question

According to PICO's guidelines (Table I), what is the effectiveness of equine and bovine bone substitute materials used for sinus floor elevation in terms of new bone volume, residual bone graft and connective tissue ratio?

Types of publication

The review included human prospective follow-up studies published in English. Letters, animal studies, case reports, reviews and systematic reviews were excluded.

Types of studies

The review included *in vivo* (human-trials) prospective studies, clinical trials and case series published from 1 January 2008 to 1 February 2020.

Search

The search on PUBMED (MED-LINE) was carried out with the following keywords: (sinus augmentation OR sinus lift AND bovine bone graft OR equine bone matrix).

Population

Men and woman between 20 and 79 years old were included in this study. Subjects included in the study were completely healthy with no systematic disease. Smoking patients were only included with a smoking rate below 5 cigarettes per day. In all the selected studies, patients had an atrophic maxillary posterior alveolar ridge treated with a sinus augmentation for implant treatment.

Inclusion criteria:

- Study performed with humans only
- Observation period of at least 5 months
- Only sinus augmentation via a lateral window approach
- Histomorphometric evaluation of augmented bone
- Exclusion criteria:
- Insufficient information regarding selected topic
- In vitro and animal studies
- Case reports
- Only clinical or radiologic evaluation of augmented bone changes.

Sequential search strategy:

All articles were screened and excluded following a PRISMA flowchart. Studies were excluded based on title check and abstract analysis. The final screening involved reading the full text of the articles (Fig. 1).

Data extraction:

The data were included and arranged in the following characteristics (Table II):

- Author
- Year
- Type of publication
- Sample size
- Surgical sites
- Study groups
- Follow up
- Mean age
- Male and Female

Quality assessment

This was performed by using the risk of bias assessment tool, cited in the Cochrane handbook for the systematic review of intervention.(11) According to the Cochrane guidelines we have assessed a risk of bias also for case series using Joanna Briggs' Critical Appraisal Tool for Case-Series (12) (Table III).

Statistical analysis

No meta-analysis could be performed due to the heterogeneity between studies and the inclusion of clinical studies.

RESULTS

Bovine bone grafts

The use of bovine bone graft as a biomaterial in sinus augmentation was revealed in 9 studies

(13-21). According to the quality assessment, we analysed 4 of the included studies.(14, 17, 18, 20) A mean percentage calculated by the extracted data of the four studies was estimated with newly formed bone (NFB 22.7%), residual grafts (RG 29.3%) and soft tissue percentage (SF 48.5%). Schmitt et al. (17), in a comparison of different grafts materials such as autogenous bone, bovine bone grafts, beta tricalcium sulphate and mineralised cancellous bone allograft, evaluated the ABB group that showed a low percentage of newly formed bone (24.9%) and residual graft (21.36%). Torres et al. (18), in a split-mouth controlled study with a follow-up of six months, showed similar results (NFB 21.3%, RG 50% and ST 29.01%). According to Kim et al. (20), recombinant human bone morphogenetic protein 2 (ErhBMP-2) with a biphasic calcium phosphate (BCP) in comparison with ABB did not

Table I. Pico question table and outcome.

Component	Description
Problem (P)	Effectiveness of different bone substitute material used for Sinus floor elevation
Intervention (I)	Sinus floor elevation with two different bone substitutes
Comparison (C)	Comparison of two different bone substitute derived from bovine and equine
Outcome of interest (O)	Histomorphometric analysis of augmented area of sinus floor
Focus question	what is the effectiveness of equine and bovine bone substitute material used for sinus floor elevation in terms of new bone volume, residual bone graft volume and connective tissue ratio?
Pico Question Table	

Table II. Characteristics of the studies included in the systematic review.

Authors (year)	Study design	Effective population (n. of patients)	n. of surgical sites	Study group(s)	Follow up	Mean age (years)	n (M)	n (F)
Adiloglu S. (2019)	Cross-sectional study	19	30	EBB/ EBB+10% collagen gel	6	24-69	7	12
Cabbar F. (2011)	Controlled clinical trial	5	5	USB	6	53.7	7	3
Di Stefano D. (2015)	Randomized clinical trial	40	40	EBM/ABB	6	35-70	21	19
Kohal RJ.(2015)	Randomized clinical trial	25	25	ABB	6	38-75	-	-
Nevens M.(2013)	Case Series	10	12	EBM	6	20-65	5	5
Stacchi C.(2017)	Randomized clinical trial	9	18	ABB	6	39-79	?	?
Schmitt CM.(2013)	Randomized clinical trial	15	15	ABB	5	38-79	?	?
Kim MS.(2015)	Randomized clinical trial	21	21	ABB	6	52.37	13	8
Corinaldesi G.(2013)	Controlled clinical trial	9	9	ABB	4	39-61	3	9
Torres J.(2009)	Randomized clinical trial	5	10	ABB	6	55-67	2	3
Rivara F. (2017)	Case Series	17	17	EBM	6	?	?	?
Nizam N. (2018)	Controlled clinical trial	13	12	ABB	6	49.92	9	4

■ Equine studies; ABB: anorganic bovine bone; EBM:Equine derived bone mineral matrix; Gel: Equine bone mix + 10% collagen gel; USB:Unilab Surgibone(Rovine bone)

enhance bone regeneration (NFB was 20% for ABB and BCP group).

Equine bone grafts

The use of equine bone matrix (EBM) was assessed in four different studies, including an RCT, a cross-sectional study and two case series (14, 22-24).

Di Stefano et al. (14), in a randomised clinical trial, showed a greater quantity of newly formed bone in the EBM compared to the ABB control group (EBM 46.86% and ABB 25.12%). The residual graft was less in the EBM (11.05%) than the ABB (28.65%). Similar data were extracted by Adiloglu et al. (22), in a cross-sectional study; for the EBM group, newly formed bone (NFG) was estimated at a level of 63.6%

and the residual bone graft (RG) at 8.6% with respect to the soft tissue (ST 28.6%).

From two case series, Nevins (23) and Rivara (24), other concordant results were shown regarding the same parameters. Nevins et al. (23) revealed an NFG of 23.35%, an RG of 15.68% and an SF of 60.97%, which is in contrast with Rivara et al. (24), who showed an NFG of 39.84%, an RG of 33.24% and an SF of 26.91%. A mean percentage calculated by the extracted data of the four studies was estimated with newly formed bone (44.51%), residual grafts (17.24%) and soft tissue percentage (36.62%), as reported in Fig. 2. Results regarding the studies with a high and medium risk of bias that are not considered for the final evaluation are shown in Table IV.

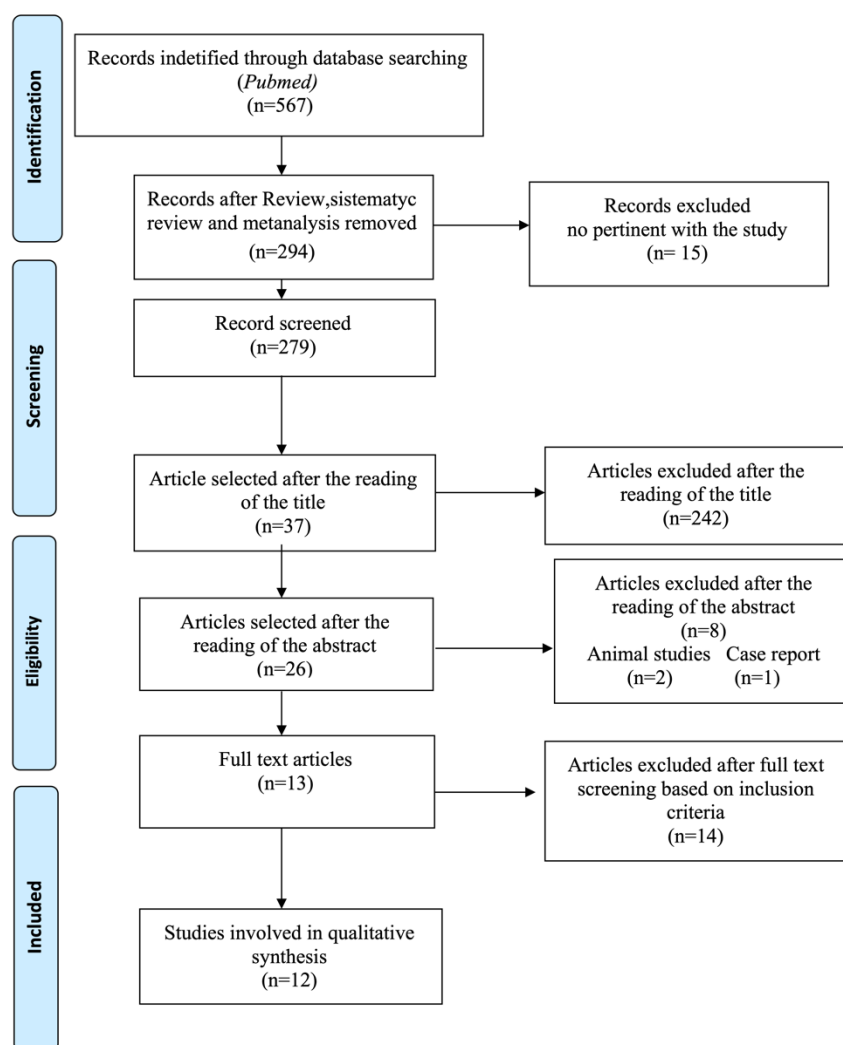


Fig. 1. *Prisma flowchart with the selection process.*

Table III. Risk of bias summary: review author's judgement about each risk of bias item presented across all included RCTs and Case Series.

Authors (year)	Random allocation	Inclusion/exclusion criteria clearly defined	Blinding of participants	Blinding of examiners	allocation concealment	outcome data	Reporting of follow-up	Total	RISK
Adiloglu S.(2019)	Y	Y	Y	N	Y	Y	Y	6 on 7	LOW RISK
Cabbar F.(2011)	N	Y	N	N	N	Y	Y	3 on 7	HIGH RISK
Di Stefano D.(2015)	Y	Y	Y	Y	Y	Y	Y	7 on 7	LOW RISK
Kohal R.J.(2015)	unclear	Y	Y	unclear	Y	Y	Y	5 on 7	MODERATE RISK
Stacchi C.(2017)	Y	Y	N	N	Y	Y	Y	5 on 7	MODERATE RISK
Schmitt CM.(2013)	Y	Y	Y	Y	Y	Y	Y	7 on 7	LOW RISK
Kim MS.(2015)	Y	Y	Y	Y	Y	Y	Y	7 on 7	LOW RISK
Corinaldesi G.(2013)	Unclear	Y	N	N	N	Y	Y	3 on 7	HIGH RISK
Torres J.(2009)	Y	Y	Y	Y	Y	Y	Y	7 on 7	LOW RISK
Nizam N.(2018)	Y	Y	Y	N	Y	Y	Y	5 on 7	MODERATE RISK

RTC, randomized clinical trials; one point was attributed when the answer was Yes. If the answer was No or Unclear, no more points were attributed.

Risk of Bias Assessment for Case-Series using Joanna Briggs' Critical Appraisal Tool for Case-Series

	Nevins M. (2013)	Rivara F.(2017)
Were there clear criteria for inclusion in the case series?	Y	Y
Was the condition measured in a standard, reliable way for all participants included in the case series?	Y	Y
Were valid methods used for identification of the condition for all participants included in the case series?	Y	Y
Did the case series have consecutive inclusion of participants?	Y	Y
Did the case series have complete inclusion of participants?	Y	Y
Was there clear reporting of the demographics of the participants in the study?	Y	Unclear
Was there clear reporting of clinical information of the participants?	Y	Y
Were the outcomes or follow up results of cases clearly reported?	Y	Y
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	Y	Y
Was statistical analysis appropriate?	Y	Y
Overall Appraisal	LOW RISK	LOW RISK

Available judgments for each supporting item were 'yes', 'no', and 'unclear'.

Tab. IV. Outcomes of the studies included in the systematic review.

Authors (year)	NFB	RG	ST	OP
Adiloglu S.(2019)	(EBM) =63.6±19.5% (EBM+GEL)=45.3±23%	(EBM)=8.6±6.3% (EBB+GEL)= 19±19.4%	(EBM)=28.6±18.9% (EBB+GEL)=35.7±14.9%	6
Cabbar F.(2011)	(USB)=15.8%	(USB)=21.9%	(USB)=59.9%	6
Di Stefano D.(2015)	(EBM) =46.86±12.81% (ABB)= 25.12±7.25%	(EBM)=11.05±9.27% (ABB)=28.65±9.70%	N.D.	6
Kohal R.J.(2015)	(ABB)=23.62%	(ABB)=33.90%	N.D.	6
Nevins M.(2013)	(EBM)=23.35%	(EBM)=15.68%	(EBM)=60.97%	6
Stacchi C.(2017)	(ABB)=38.5%	(ABB)=22.3%	(ABB)=43.5%	6
Schmitt CM.(2013)	(ABB)=24.90%	(ABB)=21.36%	(ABB)=53.74%	5
Kim MS.(2015)	(ABB)=19.67%	(ABB)=17.43%	(ABB)=62.8%	6
Corinaldesi G.(2013)	(ABB)=19.88%	(ABB)=43.0%	(ABB)=37.11%	4
Torres J.(2009)	(ABB)=21.3%	(ABB)=50%	(ABB)=29.01%	6
Rivara F.(2017)	(EBM) 39.84%	(EBM) 33.24%	(EBM)26.91%	6
Nizam N.(2018)	(ABB)=21.38%	(ABB)=25.95%	(ABB)=52.67%	6

■ Equine studies; ABB: anorganic bovine bone; EBM:Equine derived bone mineral matrix; Gel:Equine bone mix + 10% collagen gel; USB:Unilab Surgibone(Bovine bone); NFB:Newly formed bone ;RG:Residual grafts particles; OP:Observational Period; ST:Soft tissue.

DISCUSSION

The purpose of this review was to evaluate the efficiency and predictability demonstrated by two biomaterials used in maxillary sinus augmentation technique. The authors want to clarify that the analysis of equine biomaterials was performed by including RCTs, cross-sectional studies and case series. In addition, it should be noted that this evaluation was performed on a limited number of scientific studies, which is why the result, obtained from the behaviour of EBM, requires further randomised studies to be validated.

This revision takes into consideration the use of bovine and equine biomaterials for maxillary sinus augmentation, a confirmed technique for implant rehabilitation in atrophic maxilla. The result is derived from an average of the data reported by 9 studies that analysed the efficacy of ABB and 4 studies that analysed the effectiveness of EBM. The collected data led to a heterogeneous analysis that did not allow a direct comparison of the two materials due to the absence of RCTs, with tests and controls having equine bone and bovine bone, respectively. The only study that reported a direct comparison between ABB and EBM was that of Di Stefano et al. (14); homogeneous data were extracted showing a higher percentage of newly formed bone with equine substitutes compared to bovine grafts. These data reflect the average that has been highlighted by comparing the two materials separately.

The results obtained were predictable, and once

again confirm the predictability of the bovine bone in long-term maintenance through a good percentage of residual grafts. A greater amount of NFB at the time of implant placement was observed in sites grafted with EBM, and a greater amount of RB was observed in sites grafted with ABB. This can be explained by the different behaviour observed *in vitro* by the osteoclasts grown on the two materials (25, 26, 67-73) and this phenomenon could be attributed to the different manufacturing processes.

The histological evaluation of the transplanted sites showed that the sites with EBM were completely reabsorbed and the ABB grafting material was well integrated and in complete continuity with NFB. ABB is a non-absorbable bone substitute and has osteoconductive properties (26).

However, the use of non-resorbable bone substitutes produces a newly formed bone composite and substitute rather than a homogeneous bone structure (27). No evidence indicates whether this situation is an advantage or disadvantage but could require a multidisciplinary approach (50-66). As a possible advantage, this structure could represent protection against bone resorption, ensuring the long-term stability of the maxillary sinus, a high resistance from air pressure, ensuring sufficient volume for implant rehabilitation and allowing the formation of new bone and capillary infiltration (28). An analysis that has not been taken into consideration for all these studies, given the heterogeneity of the data, is the implant follow-up on bovine bone and

Mean differences		
	ABB	EBM
NFB	22.74%	44.51%
RG	29.36%	17.24%
ST	48,51%	36.62%
NFB:newly formed bone RG: Residual grafts particles ST:Soft tissue		

Fig. 2. Mean percentage of the two biomaterials.

equine bone. Di Stefano et al. (14), in their 3-year follow-up, showed 100% survival in both sites, while Adiloglu et al (22). On EBM a 6-year follow-up showed an implant failure of 3 (7.5%) cases during the prosthesis phase.

In this systematic review, the data collected with a follow up of 6 months after maxillary sinus floor augmentation showed a higher amount of NFB in EBM group, whereas ABB showed a higher level of residual grafts (RG). Within the limit of this review and the heterogeneity of the extracted data, EBM appears to be as effective as ABB for maxillary augmentation, but further studies with a clinical follow-up are needed to understand the long-term stability with a minimum of 5 years.

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