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Article

PHOTO-BIO-MODULATION AND PATIENT'S COMPLIANCE WITH CLEAR ALIGNERS

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ABSTRACT

Photobiomodulation (PBM) stimulates orthodontic tooth movements since it increases alveolar bone turnover. The aim of this study is to evaluate how PBM can influence orthodontic treatment with a clear aligner. A sample of 21 subjects was divided into a laser group (10 patients) and a control group (11 patients). All subjects were instructed to wear each clear aligner 12 hours a day for 2 weeks. PBM was given in the laser group every second week. The laser group successfully finished the treatment, while at the 3rd to the 5th aligner, the control group did not finish the treatment. Laser treatment is better than treatment without laser. PBM combined with aligners determines in 12 hours the same tooth movement obtained by wearing the aligner 22 hours a day. This aspect could be useful for those patients who prefer not to use the aligners during the day. PBM makes clear aligner treatment more comfortable since patients must wear the aligners for fewer hours than subjects treated orthodontically without laser.

KEYWORDS: *photobiomodulation, low-level laser therapy, tooth movement, clear aligners, biostimulation, diode laser*

INTRODUCTION

Studies on the effects of orthodontic treatment associated with lasers have recently increased. Most likely, the laser will be employed more to biostimulate orthodontic movement, potentially reducing the treatment time. A literature review shows surgery is the most effective technical method to accelerate the orthodontic movement, followed by Photobiomodulation (PBM) (1). Another review reported that PBM can not only reduce the time of the treatment but also reduce orthodontic pain (2).

PBM uses low-power lasers, such as diode lasers, to stimulate cells. PBM is simple to use, painless, and does not present side effects. In order to achieve results, it is necessary to use the correct laser parameters (2). The quantity of

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tooth movement may vary depending on the type of laser and to parameters setting (such as wavelength, output and laser density) (3, 4). It is seen that the diode laser has a better penetration in human tissues and is an efficient device to be used in orthodontic clinical practice (5-6).

A correct energy density (Fluence= J/cm2) is of the utmost importance to obtain biological effects. The dosage of the laser's energy follows Arndt-Schulz's law: low dosages stimulate, and high dosages inhibit. However, if a too-low dosage is used, it cannot be compensated by increasing exposure time (7). Hence, the need was perceived to set the laser parameters correctly.

The laser effects applied in orthodontics are different and have been biologically demonstrated through studies on humans, animals and in vitro experiments. Various orthodontic biological laser effects have been demonstrated:

- stimulation of bone turnover (7-11)
- improvement in tooth movement (8, 10)
- reduction of post-orthodontic pain (2, 5)
- improvement in the production of keratinized gingiva (12)
- reduction of root resorption (6)
- stimulation of cell proliferation (13)
- stimulation of osteoblastic cells proliferation (11, 14)
- reduction of relapse (15)

Furthermore, no systemic side effects have been demonstrated for PBM.

PBM can stimulate bone turnover; therefore, it can also accelerate orthodontic movement without damaging either teeth or surrounding tissues (16).

Different studies clinically highlighted how PBM can accelerate orthodontic movement with fixed braces devices. On the other hand, none of the studies has highlighted PBM's effects on tooth movement in orthodontic treatments with invisible aligners. The null hypothesis of our study is that there is no difference between the laser treatment and the one without the laser. Since an increasing number of patients ask for aesthetic and less invasive treatments, we planned a study to verify if PBM, applied to the invisible aligners, can reduce the daily wearing of aligners. This study aims to check if PBM can accelerate tooth movement in orthodontic treatment with invisible clear aligners.

MATERIALS AND METHODS

Study design

The study was carried out in a private clinic in Bergamo, Italy. It was performed in accordance with the Declaration of Helsinki of 2013. The patients enrolled in the study received information, and they provided written consent. The standard protocol was to wear aligners for 22 (17, 18).

Inclusion and exclusion criteria

This pilot study allocated the patients into a laser group and a control group. Inclusion criteria were: vertebral maturation assessed on lateral cephalograms more advanced than CS4 (19), no previous orthodontic treatment, Class I malocclusion, permanent dentition completely erupted, incisal irregularity index from 4 mm to 6 mm (moderate crowding) in the mandibular arch.

The study sample comprised 21 patients (9 males and 12 females, ages 17 to 41) randomized into the study groups. The patient's clinical and demographic characteristics are reported in Table I.

Treatment protocol

All patients underwent a radiographic examination consisting of both orthopantomography and lateral cephalograms. Pre-treatment records consisted of initial dental casts and photos.

After 2 weeks, the same orthodontist who had carried out the baseline examinations examined patients in the laser and control groups. The dentist evaluated whether the aligner fitted properly and passively. If so, the subject was given the next aligner. If the aligner fitting was incorrect, the patient was instructed to wear the same aligner for 2 weeks, which continued until the aligner fitted correctly.

The laser group included 10 subjects (6 female, 4 male) who received external diode laser biostimulation (wavelength of 980 nm, continuous wave at 1 Watt output power) at each control visit. A flat-top optical fibre delivered the beam, and irradiation was administered by placing the beam outside the mouth, under the cheekbone at the level of the maxillary arch first, then at the level of the mandibular arch on the right and the left side. Finally, the flat top optical fibre was moved under the nose, firstly at the level of the maxillary arch, then at the level of the mandibular arch for 3 applications for each arch (Fig. 1).

The irradiation was performed for 50 seconds at each point (150 s for each arch). The energy density corresponding to an exposure time of 150 s per arch was 150J/cm2 (every second, the fluency was 1J/cm2). After the PBM, patients were instructed to wear each clear aligner 12 hours a day for 2 weeks.

The control group included 11 patients (6 female, 5 male) who were instructed to follow the same protocol: to wear each aligner 12 hours a day for 2 weeks.

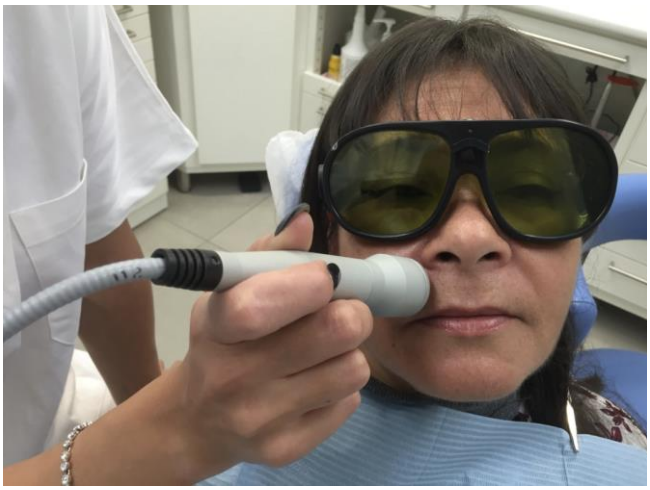

Fig. 1. *External laser bio stimulation with a flap top optical fiber. The beam was delivered by a flat-top optical fiber and irradiation was administered by placing the beam outside the mouth under the cheekbone at the level of the maxillary arch first, then at the level of the mandibular arch on the right and on the left side. Finally, the flat top optical fiber was moved under the nose, first at the level of the maxillary arch, then at the level of the mandibular arch for 3 applications for each arch.*

Clear Aligners were made of transparent pressure moulding sheets with thicknesses varying from 0.5 mm to 0.8 mm. Single resin attachments were used only for 25 canines that needed rotation (12 attachments in the control group, 13 in the laser group). The orthodontic factory made the Clin Check with "3Shape" software. Space analysis used Little's Irregularity index (20) in the lower arch from 3 to 3.

No patients received extraction orthodontic treatment or stripping. The expansion quantity was evaluated by measuring the inferior arch depth (distance between the most labial surface midpoint of the incisors to the mesial midpoint of the first molars) and the inferior arch perimeter (the sum of the individual segments: mesial of the left first molar to mesial of the left first premolar; width of left canine; distal of left lateral incisor to distal of right lateral incisor; width of right canine; mesial of right first premolar to mesial of the right first molar). Measures were carried out on digital models. The average of the increasing arch depth between the pre- and post-treatment was 1.76 mm, and the average of the increasing arch perimeter was 4.21 mm.

The mean of linear movement was 0.13±0.09 mm, while the mean rotational movement was 1.77±1.71°. The mean measurements of the two movements, linear and rotational, refer to all patients' anterior inferior arch from canine to canine.

Statistical analysis

Conventional descriptive statistics were carried out to analyze sample demographic and clinical characteristics. For comparisons between the two treatment groups, the t-test and the chi-squared test were used for numerical (age, crowding) and categorical (gender) characteristics.

In order to verify the null hypothesis, the P-value was calculated between the two treatment groups for the number of aligners correctly fitted at each follow-up visit.

RESULTS

Baseline findings

Descriptive statistics reported no differences between the two groups for age, gender and amount of crowding (Table I). Thus, the random assignment of participants to both treatment groups was validated.

The average age of the laser group was 26.6 years. The mean age of the control group was 25.5 years. The mean

crowding (Irregularity Index) in the laser group was 4.80 mm, and in the control group was 4.98 mm (Table II).

There were more females than males, but no significant differences were seen in gender distribution between the control group and the laser one. Females were older than males with a greater standard deviation, but there was no significant difference between groups (P-value=0.3796) (Table II).

Outcomes

In the control group, the third, fourth or fifth aligners did not fit correctly (mean= 3.6 aligners) (Table III). In the control group, the protocol of 12 hours a day failed, and we had to turn to the standard protocol of 22 hours a day to finish the treatment. All laser group patients successfully completed the treatment. The mean treatment duration with the 12-hour protocol was 7.2±1.6 weeks in the control group (at that point, treatment was discontinued) and 40±2 weeks in the laser group.

DISCUSSION

The effect of PBM on tooth movement during orthodontic treatment with clear aligners is investigated in this study. In 12 hours, PBM produces the same tooth movement obtained by wearing the clear aligner 22 hours a day without PBM; this agrees with those studies that have demonstrated a reduction in treatment time using PBM (2).

Different lasers can be used, but the diode laser seems the most effective in orthodontic biostimulation (3, 6, 7). The

Table. I. *Baseline findings: gender, age distribution, crowding.*

Variables	Total	Control Group	Laser Group	Significance*
Patients (n)	21	11	10	
Age (mean±SD)	26±5.4	25.5±4.9	26.6±6	NS
Range age	17-41	17-31	20-41	
Female, n (%)	12 (57)	6 (55)	6 (60)	NS
Male, n (%)	9 (43)	5 (45)	4 (40)	NS
Crowding (Irregularity Index), mean±SD	4.89±0.53	4.98±0.55	4.80±0.51	NS

(n= Number, SD= Standard Deviation, NS= not significant).
**Significance for comparison of group means calculated by paired t-test.*

Table II. *Baseline findings: age distribution.*

Variables	Female	Male	P-Value
Mean Age	26.9	24.8	0.3796
SD	6.4	3.6	

(SD= Standard Deviation)

Table III. *Outcomes: number of aligners fitted correctly for each treatment and number of treatments finished successfully.*

Variables	Laser Group	Control Group	P-Value
Total number aligner (mean±SD)	22.1±1	3.6±0.8	0.001
Success/Unsuccess (n)	10/0	0/11	

(n= Number, SD= Standard Deviation)

parameter set is fundamental to having clinical results (5, 6). The external laser biostimulation with a flat-top optical fibre (wavelength of 980 nm and continuous wave at 1-watt output power) seems to have predictable results. The protocol of 150 seconds of irradiation for each arch is clinically effective.

The improvement in tooth movement could be due to the biostimulation of bone turnover (7, 13, 14). The exact mechanism of the PBM on the bone is not yet fully understood. In vitro studies show that the light at a lower radiation dosage is absorbed by the intracellular chromophores in the mitochondria, thus increasing cell proliferation through photochemical alterations (6, 9, 21). This mechanism includes the promotion of angiogenesis (22), production of collagen (23), osteogenic cell proliferation and differentiation (24), mitochondrial oxidation and adenosine triphosphate synthesis (25, 26). PBM can enhance the local blood flow, increasing the supply of circulating cells, nutrition, oxygen, and inorganic salts to bone lesions (27); this had already been noted by Kobu (10), who showed that intraosseous blood flow increased by approximately 80% in tissues treated with PBM, and oxygen tension by approximately 15%. Kawasaki and Shimizu (28) showed that PBM increased the number of osteoclasts on the pressure side during experimental tooth movement in rats. PBM can achieve these cellular effects because the beam has a tissue penetration from 2.2 cm to 5.9 cm (29).

CONCLUSIONS

PBM is useful in orthodontic clinical practice, especially when patients lack compliance and do not wear the aligners 22 hours a day. PBM can produce an expected dental movement with a reduced wearing time.

Author Contributions

G.C. designed the research study; G.C. performed the research; P.C. and G.C. wrote the manuscript; all authors contributed to editorial changes; all authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Evaluation Study

INTRADISCAL INJECTION OF OXYGEN-OZONE GAS MIXTURE FOR THE TREATMENT OF CONTAINED CERVICAL DISC HERNIATIONS

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ABSTRACT

For disc herniations, open surgical approaches are reduced since new percutaneous methods allowing shrinkage of the disc and improvement of the radicular function are gaining interest. Studies on the spontaneous disappearance of disc fragments have demonstrated autoimmune responses with a chronic inflammatory reaction. Also, radicular pain is due mainly to biochemical mechanisms. Researchers in different fields surprisingly noticed that a brief, calculated oxidative stress by ozone administration might correct a persistent imbalance due to excessive, chronic oxidative injury. Oxygen-ozone gas injection in painful patients has a dramatic effect on clinical symptoms. On these bases, the intradiscal injection of oxygen-ozone gas has been conceived. We report the treatment on a series of patients affected by cervical disc pathology, treated by intradiscal injection of an oxygen-ozone gas mixture. The effects both on pain and on radicular dysfunction are impressive. The morphological effect of the treatment was also evaluated by pathological examination.

KEYWORDS: *intradiscal injection, contained disc herniation, oxygen-ozone, tinnitus*

INTRODUCTION

In cases of radicular dysfunction due to disco-radicular conflict, the treatment has evolved in the last two decades since methods that allow shrinkage of the herniated or protruded disc have been preferred to open tissue removal. Many percutaneous non-invasive techniques have been conceived. The common principle is to provoke morphological modification of the disc and its deformities. Simultaneously many studies have highlighted that pain may be due to biochemical mechanisms somehow independent of the mechanical problem. A situation of ischemia, acid intoxication of the

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nerve, and chronic inflammatory response are understood to combine causes of dysfunction. The spontaneous mechanisms of disc shrinkage and elimination of herniated fragments have been carefully studied, and a chronic inflammatory reaction has been developed (1). An autoimmune reaction may participate in physiopathology (2). Biochemical treatments may correct these problems, reducing the need for surgical intervention (3-7). The mixture of oxygen and ozone gases has been employed in medicine since the 30s to treat pain and dysfunction in patients affected by thrombotic and ischemic diseases. After experience in these fields, the empirical observations of powerful and long-lasting effects of this gas mixture injected in paravertebral muscles for treating pain and radicular dysfunction due to a disco-radicular conflict have led to detailed studies on the subject.

Working in different fields, researchers surprisingly noticed that a brief calculated oxidative stress achieved by ozone administration might correct a permanent imbalance caused by excessive or chronic oxidative injury. Bocci et al. have highlighted that modest, repeated ozone administration increases the activity of superoxide dismutase, catalase, and glutathione peroxidase, inducing a state of oxidative stress adaptation with very important therapeutic implications (8). The mixture is produced by an apparatus (ozone generator) that activates diatomic oxygen molecules in a voltaic arch. Ultraviolet spectrophotometry allows precise quantification of ozone percentages in the obtained mixture. The absence of side effects in over five million ozone therapy sessions for different pathologies has been reported since 1982 by Jacobs (9). The injection of an oxygen-ozone mixture in the intervertebral disc and the conjugation foramen (3, 4, 10) is combined with the paravertebral intramuscular treatment.

Several techniques have been developed to treat disc herniation. Percutaneous techniques such as percutaneous discectomy, laser discectomy, and nucleoplasty have minimised the invasive nature of these surgeries and decreased complications such as postsurgical infection (5, 11, 12). Among these, only ozone treatment has the characteristic of acting on morphology and biochemical functions. We present the progress (4) of our experience by this method in a series of 1268 contained cervical disc herniations.

Specific attention is paid to dizziness and tinnitus, symptoms which have been observed in 317 out of our cervical herniation patients.

Tinnitus is the perception of sound without an external acoustic stimulus (13). Its prevalence is roughly estimated as being between 10% and 17% of the population, afflicting around a third of North Americans over 55.3 years. For many years, tinnitus was thought to arise almost exclusively out of abnormal neuronal activity within the auditory pathways. However, accumulated evidence suggests that tinnitus-related neural activity is much more complex and multimodal than previously thought.

More often than ever, researchers conclude that tinnitus can be evoked or modulated by inputs from the individual's somatosensory, somatomotor and visual–motor systems; this means that the psychoacoustic attributes of tinnitus (loudness and pitch) might be changed immediately –though only temporarily – by different stimuli, such as the following: forceful muscle contractions of head, neck and limbs; eye movements in the horizontal or vertical axis, pressure on myofascial trigger points; cutaneous stimulation of the hand/fingertip region and the face; electrical stimulation of the median nerve and hand; or finger movements as well as orofacial movements.

This specific subgroup is called somatosensory tinnitus, and it seems to be a good example of central integration of the central nervous system because an auditory symptom like tinnitus may be modulated immediately after various non-audiology stimuli are presented. The modulation phenomenon is yet to be fully understood, but there is scientific evidence of existing neural connections between somatosensory and auditory systems, and their "activation" may play a role in this type of tinnitus.

In clinical practice, tinnitus is still considered an untreatable symptom, and many professionals tell patients that "there is nothing to be done" or that "you have to learn to live with it".

MATERIALS AND METHODS

In eight years, from 2013 to 2020, 2417 patients were admitted to our Service because of clinical symptoms bespeaking a cervical disc pathology. These underwent clinical, electrophysiological, and neuroradiological investigations to establish a precise diagnosis.

Those 1149 patients who were affected by cervical spinal canal stenosis, discarthrose processes, osteophytes, myelopathy, or concomitant CSN pathologies were not included in the series.

In this paper, we present the series of 1268 cases in which a contained disc herniation has been demonstrated by CT scan or RMI and EMG has demonstrated radicular dysfunction corresponding to the metameric level.

The mean patient age was 38 years, and 47% were males. C3-C4 herniation was observed in 76 of the 1268 patients, C4-C5 in 88 cases, C5-C6 in 570, and C6-C7 in 531. Among these patients, multiple-level herniation was observed in 491 (38.7% of cases). The double level distribution was in 460 patients as C5-C6 and C6-C7 levels combination and 31 patients as C4-C5 and C6-C7 combination.

Patients enrolled had received pharmacological and physical therapy without remedial of the clinical picture. The perspective of solving the problem of reducing drug administration without conventional surgical treatment was offered to the patients, who consented after a detailed explanation. Dexamethasone administration, if pre-existing, was interrupted when starting O_2-O_3 injections. It was never associated with O_2-O_3 treatment. Non-steroid drugs were allowed if occasionally needed. The treatment - EUNI Method - consisted of an intradiscal injection of O_2-O_3 preceded and followed by 6 paravertebral injections.

– paravertebral injection consisted of administration of 20 ml of O_2-O_3 at 10 micrograms/ml concentration, divided into 2 sites of injection: 3 cm deep in the paravertebral muscles bilaterally, at the metameric level of the pathology.

– intradiscal injection: introducing the needle in the disc through the anterolateral approach corresponds to the classical open surgery approach. The procedure was carried out with a high-resolution C-arm. The procedure was performed with the patient in a supine position under mild sedation, with complete anesthesiological assistance and continuous vital signs recording. The procedure modalities are as follows: the patient's neck is extended, and a thin pillow is under the interscapular region. The approach is right anterolateral. The operator displaces the carotid sheath laterally and trachea-oesophagus medially between the second and third fingers of the left hand. The right hand then inserts the needle, passing between the two left fingers, though the soft tissue window between the carotid sheath and trachea-oesophagus. Local anaesthesia is not required in this location. 22G spinal needle with a length of 3.5 inches was used. The needle tip is directed toward the centre of the disc. The position of the needle is checked with anteroposterior and lateral views.

Three to five ml of gas are injected at 30 micrograms/ml concentration. The injected dose depends on the disc morphology: fissuration will allow gas mixture diffusion either along anterior or posterior longitudinal ligament or anterior epidural space.

RESULTS

Neck pain: among the 1268 patients, neck pain was abolished entirely in 1080 cases (85.1%); VAS reduction of 4 or more points was obtained in 156 cases (12.3 %), while the result was poor in 32 patients (2,52 %).

Radiated radicular pain was abolished entirely in 79.65% (1010 patients); among the 1268 patients, a VAS reduction of 4 or more points was obtained in 11.67 % (148 patients). The result was poor in 8,67 % (110 patients).

Dizziness and tinnitus were relevant symptoms in 317 out of our 1268 patients (25% of the entire series). A good improvement in these symptoms has been achieved with this treatment. The benefit was evident during outpatient therapies; in 58% of cases (184 patients): the observed benefit is generally long-lasting. The recurrence of the disorder at the end of the treatment was observed in 27% of cases.

Sensory dysfunction was abolished in 77,99% (989 patients) and improved in 15.8% (201 patients). Dysfunction remained unchanged in 78 cases.

Motor dysfunction such as M4 or M3 was present in 64.9% of our 1268 patients, i.e. 824 cases. An M3-level motor deficit was present in 228 patients (17.98%). M4 motor deficit was observed in 596, that is 47%. The motor defect pre-existed in our cure with a mean pre-existence time of 14 days. We observed complete regression of motor deficit in 97% of M4 patients (578 cases); recuperation was partial in the remaining 3%. Among M3 patients, recuperation was complete in 90% (205 patients).

Multiple-level disc pathology was present in 491 (38.7% of cases). The treatment was performed simultaneously in all pathological discs. The results obtained do not differ from those obtained for single-level pathology.

Patients underwent CT/MRI control 8 months after treatment. In 38,72% (491 cases), we observed a significant reduction in the volume of the hernia. The correlation with clinical signs was not statistically significant.

DISCUSSION

Mechanical compression of a nervous structure leads to a range of microvascular changes. Mild compression produces venous congestive nerve root oedema, severe compression results in arterial ischemia, and the root sets off sharp shooting pain along the dermatome (14, 15).

Experimental models suggest that material from the nucleus pulposus may act as a chemical or immunologic irritant to the nerve and that these mechanisms may produce an inflammatory response (2). When the disc ruptures, the immune-privileged nucleus displaces through a tear in the annulus fibrosus leading to direct exposure of the nucleus to our immune system, which, in turn, triggers the release of inflammatory mediators. These inflammatory mediators recruit monocytes from the immune system resulting in the chemotaxis of macrophages and angiogenesis. Subsequent lymphocyte activation with the secretion of interferon-gamma (IFN γ) and macrophage recruitment lead to one unfavourable effect of inflammation of nerve roots and dorsal root ganglia. Another favourable effect helps in the resorption of extruded nucleus pulposus. However, this natural resorption is a painful and slow phenomenon. Inflammatory markers such as interleukin-6 (IL-6), IL-12, IFN γ, and CD68 macrophages are more present in an extruded disc. There are two different types of macrophages seen in autoimmune-mediated inflammatory reactions.

M1 macrophages produce pro-inflammatory cytokines, and M2 macrophages produce anti-inflammatory cytokines. There is always a sequence in that activation of M1 is followed by M2. However, when and how activation of M1 switches to activation of M2 macrophages is unpredictable; the M1-mediated pro-inflammatory phase may last longer, leading to prolonged and painful illness. These inflammatory cascades of reactions are responsible for inflammatory radiculopathy with radiating pain along the course of the nerve (16). Tumor necrosis factor α and phospholipase A2 are significant in herniated nucleus pulposus. These are responsible for partial demyelination that increases nerve root sensitivity making them more susceptible to mechanical pressure (15). The mechanical compression due to herniated disc can trigger hyperexcitability leading to neuropathic paresthesia and pain.

Until now, studies have hypothesised that injection of such a powerful oxidant, such as ozone, induces overexpression of antioxidant enzymes, which neutralise excessive reactive oxygen species (ROS) formation (8). After intradiscal injection, ozone can accelerate the degradation of proteoglycans in the degenerated nucleus pulposus, leading to its reabsorption and dehydration with the consequent reduction of herniated material responsible for nerve root compression (6, 8).

In our opinion, the most important aspect is the biochemical modification of the medium in the epidural space. In epidural space, ozone acts as an anti-inflammatory agent modulating and hastening the switch from M1 to M2 macrophages, converting an inflammatory phase to a reparative one (16). Studies on pain, which often is disproportionate to the morphological evidence of discal-radicular conflict, have demonstrated that it is provoked by acid metabolites from the degenerative processes inside the disc and ischemia of the nerve root and the ganglion. In the 90s, attention was brought to A2 phospholipase. Saal et al. demonstrated that A2 phospholipase is the cause of radicular pain, independent of the immunological response or a direct inflammatory process (2). A2 phospholipase is responsible for the arachidonic acid liberation and hence prostaglandins. High levels of A2 phospholipase have been demonstrated in herniated discs. Ozone injected in the disc and in the epidural space of the conjugation foramen and along the posterior longitudinal ligament acts as a powerful stimulus to the activation of antioxidant defence, favouring the normalisation of redox balance with neutralisation of acidosis, increased synthesis of ATP, Ca2+ reuptake and resolution of oedema (1, 2, 8).

Thus, symptoms arising from disc herniation are due to the amalgamation of bio-chemico-mechanical factors (14-16). The complete biochemical reaction to an intradiscal injection of oxygen-ozone gas mixture for treating cervical disc herniations is not yet understood, but there is strong clinical evidence that the effect is dramatic and long-lasting. The benefit is rapidly obtained on pain and nerve dysfunction, with progressive reduction of tingling. EMG controls have confirmed the recuperation of nerve function. We presume that this is achieved by amelioration of nerve ischemia. The oxygen-ozone gas mixture at 10% ozone concentrations acts as immunomodulatory. At 25–30% concentration, it helps to dehydrate the disc nucleus. Outside this therapeutic window, ozone will be cytotoxic above 45%. Most trials prove

that the ozone concentration at 25%–30% will be optimum for therapeutic effects in disc herniation (15, 17, 18, 19). Ozone acts differently at different concentrations in different tissues. Mechanisms of intradiscal ozone injection involve fragmentation of glycosaminoglycans which are abundantly present in the nucleus pulposus, with subsequent release of water molecules; this leads to a small decrease in volume of the nucleus with a significantly greater decrease in pressure resulting in the recoil of the nucleus and restoration of the intervertebral disc. This is probably the case in contained disc herniations, where the nucleus pulpous is protected by the Fas-ligand which prevents infiltration of immunocytes. Proteoglycans present in the annulus also limit the inflammatory reaction. Here, dehydration of the nucleus and cytokine-mediated repair of the annulus is more dominant reactions.

About tinnitus, Alcantara et al. (20) described how chiropractic treatment could reduce tinnitus, vertigo and hearing loss in a patient with cervical subluxation and temporomandibular disorder. Symptoms eventually ceased after nine sessions. Kessinger et al. (10) documented clinical changes after chiropractic sessions in a geriatric patient with tinnitus, vertigo, hearing loss and cervical alterations from C3 to C7. The patient's symptoms were alleviated throughout the sessions, and structural/functional improvements were also evident through radiographic examination.

The observation that we are here reporting of clinical improvement of these symptoms by oxygen-ozone treatment is interesting because it brings back to the idea that these are functional alterations which may have anatomopathological bases but are possibly reduced or eliminated working on metabolism.

The possibility of treating patients by an easy method which is rapidly effective for solving clinical problems is at hand. This treatment is useful in patients who did not respond to physical and conventional pain therapy as a last step in conservative treatment before deciding on open surgery. Most of these patients will not need more surgery anymore since ozone may act directly on the cause eliminating clinical symptoms. This technique is simple, has no risks, and offers the patient a solution without the discomfort of surgery and its possible risks.

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Case report

VERTICAL AND HORIZONTAL GBR VIA A DEMINERALIZED XENOGENIC BONE CORTICAL LAMINA: A CASE REPORT

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ABSTRACT

Vertical and horizontal bone defect is a challenge in oral surgery. Alveolar bone reconstruction is needed before implant insertion. Several surgical techniques are available. Here a case of posterior alveolar ridge reconstruction is reported together with a histological evaluation of bone sampled after 6 months of healing. Histological results suggest the validity of surgical procedures for bone volume regeneration in the distal mandibular region. Furthermore, according to our clinical experience, both the pre-hydration and stabilization of the device, with fixation screws, are strongly recommended if vertical bone regeneration is needed.

KEYWORDS: *bone, regeneration, mandible, lamina, crest, reconstruction*

INTRODUCTION

It is known that GBR is carried out through a barrier device, also named membrane, which maintains a volumetrically stable space in the area to be regenerated, preventing the soft tissues from colonizing it for the entire period necessary for regeneration (1). Barrier devices can be resorbable or non-absorbable; the latter is made of PTFE, generally reinforced with a titanium framework, or can be made of a titanium sheet only (1). Non-resorbable barriers must be removed during second-stage surgery and are indicated in the case of vertical or combined vertical and horizontal bone regenerations (1, 2).

On the other hand, the resorbable membranes, being degraded by the host, do not require any removal and are indicated exclusively for horizontal bone regeneration due to their limited ability to maintain a stable volume over time in the area to be regenerated (3). Resorbable membranes are a merchandise-heterogeneous family of devices that differ both in the materials used (i.e., bio-polymers; collagen) and how these materials are processed (i.e., cross-linking of collagen) to maintain their persistence in the organism and action of colonization inhibition, by the soft tissues, for all the time required for bone regeneration (4).

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Here, a case of posterior alveolar ridge reconstruction is reported together with a histological evaluation of bone sampled after 6 months of healing.

MATERIALS AND METHODS

A 44-year-old nonsmoker came to our clinic because she was concerned about lower jaw edentulness. She had no previous pathologies, and neither had she taken pills.

The absence of molars and atrophy of the posterior right mandible was seen at the clinical evaluation. Alveolar crestal reduction was vertical and broad. Impression was taken to plan the correct position of prosthetic restoration, and then a radiological Dima with guttapercha references was produced. The patient did a panorex X-ray and a CBCT; in this latter the patient wore the Dima. CBCT showed vertical and width atrophy (Fig. 1). Thus, it was proposed to perform a bone regeneration to insert an implant in a second stage. The patient agreed and signed informed consent.

First-stage surgical procedure

Antimicrobial prophylaxis was administered with amoxicillin-clavulanate (Clavulin, Glaxo- SmithKline, Italy), 1 g every 8 h for 7 days, starting 3 h before the operation, after an initial 1 min rinse with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) to disinfect the mouth. In the surgical area, loco-regional anaesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100,000 (Citocartin, Molteni Dental, Italy).

Then a crestal full-thickness incision was performed in the edentulous area with a No. 15 surgical blade to split keratinized tissue equally.

On the buccal side, the incision was intrasulcular extended to the adjacent premolars and canine without a vertical release incision while posteriorly the incision ended with a vestibular oblique incision (45°) at the level of the occlusal

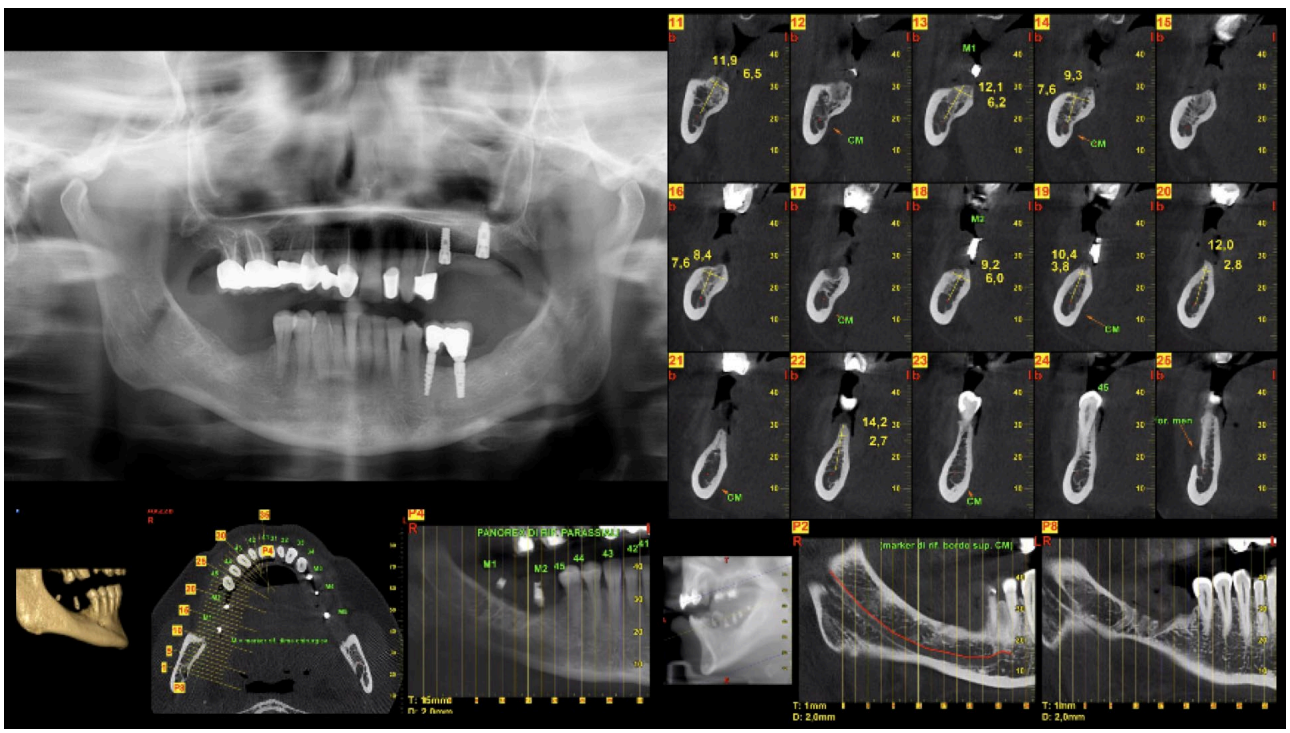

Fig. 1. *Pre-surgical RX, panorex and CBCT.*

plane. Lingually, to obtain an adequate length of the flap, a marginal incision was extended mesial to the adjacent premolars, avoiding vertical incisions (Fig. 2A, 2B).

To obtain passivation of the lingual flap, in the middle area, the superficial fibers of the mylohyoid muscle were carefully detached, by a periodontal probe (5). In the distal area, the adherent tissues, of the retro-molar pad, were full-thickness lifted by the use of a Prichard elevator (6). On the vestibular site, at the level of premolars, the flap was carefully full-thickness raised, to locate the mental foramen area and the neurovascular bundle. Then a shallow periosteum incision was made, from the distal to the medial portion of the buccal flap, by using a new No. 15 blade, then the inner surface of the flap was carefully "brushed", until the desired elongation was achieved (7).

The collection of autologous bone was performed principally from the homolateral ramus of the mandible and secondary from the recipient site by using a scraper (Safescraper® TWIST, META, Reggio Emilia, Italy). The bone scraping procedure was considered sufficient to activate the regional acceleratory phenomenon, at the recipient site (8) (Fig. 2B).

The device (Curved Soft Lamina, 1.0 mm: 35x35 mm, OsteoBiol, Tecnoss, Turin, Italy) was previously hydrated with sterile saline solution (Fig. 2C), then the device has been molded with a pair of curved surgical scissors. Once achieved the desired shape the device has been fixed on the vestibular site by means a Bone Fixation Screw (VM-01-003; FMD Dental, Rome, Italy) coupled with a titanium rondel so as to prevent the undesired laceration of the device, during the screwing procedure (Fig. 2E).

The autologous bone was then layered on the recipient site (Fig. 2F) followed by the placement of a layer of collagenated porcine xenograft (MP3, A3005FS, 1.0 cc, OsteoBiol, Tecnoss, Turin, Italy) (Fig. 2G). The device has been then reflected, over the graft and fixed, as previously described, on both the retro-molar area and the lingual side (Fig. 2H).

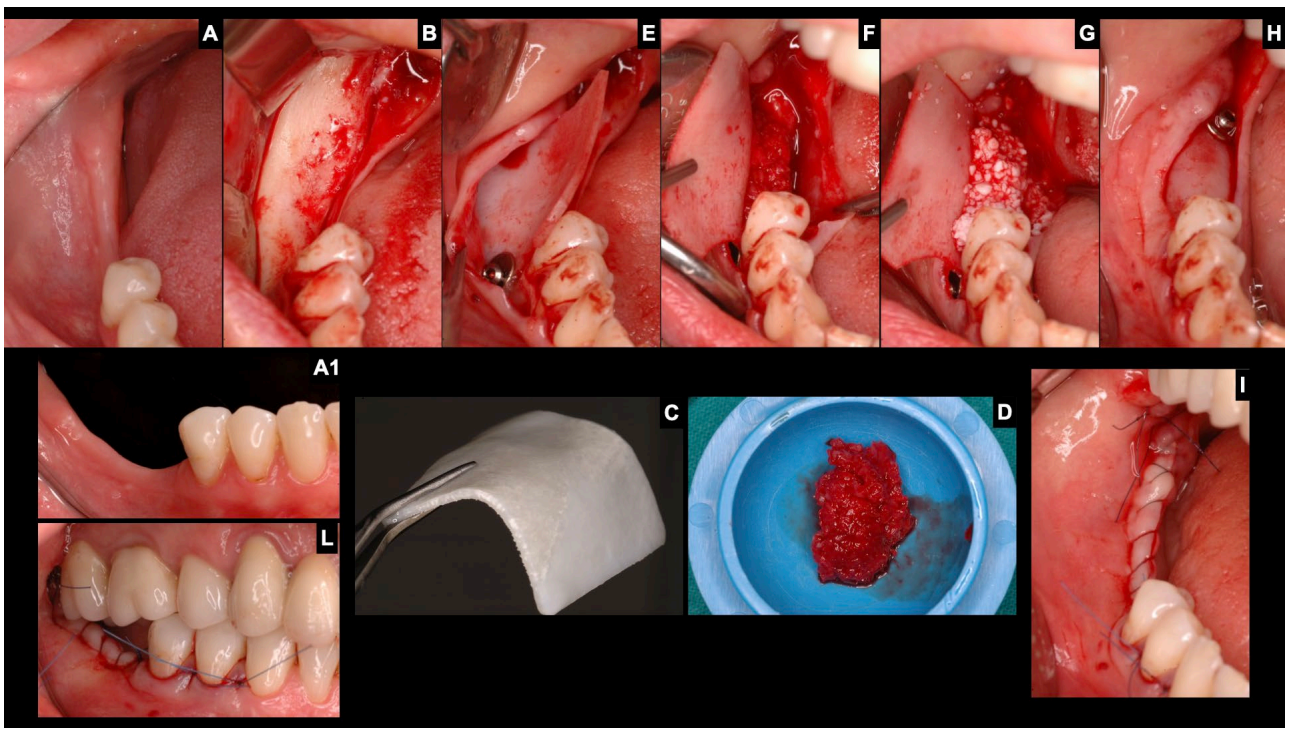

Fig. 2. ***A-A1)****: pre-surgical view;* ***B)****: muco-periosteal flap elevated;* ***C)****: cortical lamina is hydrated before modeling;* ***D)****: autologous bone is collected by means of a scraper from mandibular ramus;* ***E)****: lamina is fixed by means screws and titanium rondels; F: autologous bone is placed on mandible as first step;* ***G)****: then xenograft is placed on to the recipient site;* ***H)****: lamina is curved and fixed;* ***I-L)****: muco-periosteal flap is sutured.*

The free of tensions flap was thus sutured, with a double-layered continuous suture, by means of a 6-0 nylon wire, first deeper line was a continuous horizontal mattress, while the second line was a spiral continuous suture (Fig. 2I, 2L, 3).

The patient was discharged and oral hygiene and food instructions provided. Ibuprofen (Brufen 600 mg, Abbot, Italy), every 8-12 hours for 5 days was administered to control postoperative pain and edema. Rinses with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) were prescribed for the disinfection of the surgical wound, 2/3 times/day for 7 days. After 14 days the sutures were removed and new oral hygiene instructions were provided. The post-operative was uneventful, with exception of swelling and hematoma.

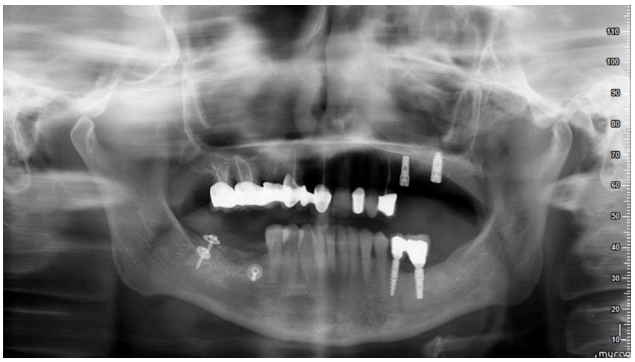

Fig. 3. *Post-operative panorex showing screws.*

Second-stage surgical procedure

After a suitable period, needed for the consolidation of the graft (6 months), the second stage surgery was performed. The re-entry (Fig. 4A, 4B) was executed with a full-thickness flap elevation approach, to remove the device, which had not been reabsorbed as expected (Fig. 4B, 4C). Prior to removing the coronal part of the device (Fig. 4D), by means of a No. 15 surgical blade, the fixation screws have been carefully pulled out. Then a two-piece cylindrical implant (I-Fix, FMD, Rome, Italy) was placed 2 mm under the free surface of the regenerated site. The implant site has been first prepared with a trephine drill (224RF, external diameter 2,7 mm, Hager & Meisinger GmbH, Neuss, Germany) so as to harvest a bone biopsy (Fig. 4E-L), then has been completed with dedicated cylindrical drills. Although the primary implant stability was greater than 50Ncm the fixture was placed submerged, in order to allow the periosteum-induced bone maturation (9). Thus, the flap was sutured, with the same continuous suture already described via a 4-0 silk wire (Fig. 4M, 5A). Drug prescriptions before and after surgery were identical to those of the first-stage surgery. The post-operative was limited to mild swelling, for 7 days.

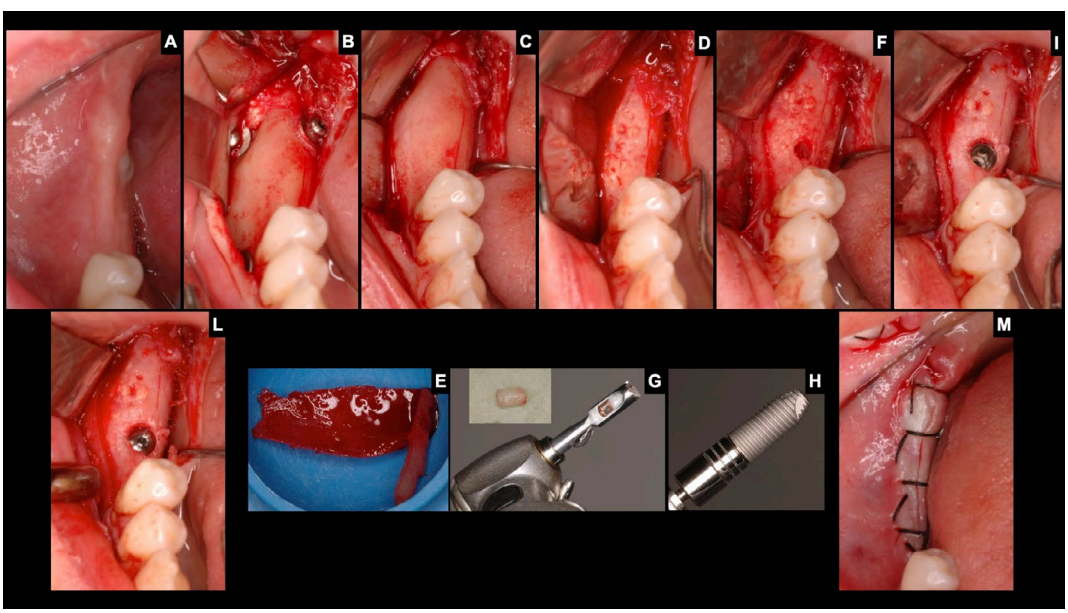

Fig. 4. **A**): *pre-surgical view before second stage surgery;* ***B****): a muco-periosteal flap is raised and lamina is visible;* ***C****): screws are removed;* ***D****): apical part of lamina is removed to find the underling regenerated bone;* ***E****): Fragment of removed lamina;* ***F****): initial implant tunnel preparation performed with a trephine drill to collect regenerated bone;* ***G****): trephine drill with biopsy;* ***H****): dental implant;* ***I****): implant insertion;* ***L****): implant connection is closed;* ***M****): muco-periosteal flap is sutured.*

Third-stage surgical procedure

After 4 months the implant was exposed with a full-thickness flap, dividing the band of keratinized gingiva into two halves, both the deepness of the fornix and the amount of keratinized gingiva were compliant with guidelines (10, 11) therefore additive soft tissue surgery was not needed. The flap was sutured with single sutures via a 4-0 silk wire. Drug prescriptions before and after surgery were identical to those of both first-stage and second-stage surgery. The post-operative was uneventful.

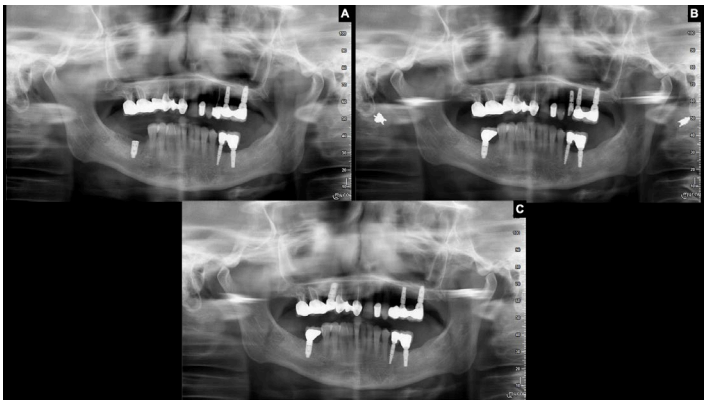

Fig. 5. RX – **A**): *when dental implant is inserted;* ***B****): after prosthetic placement;* ***C****): after 2 years from prosthetic placement.*

A month after surgery the case was finalized with a screw-retained metal-ceramic crown (Fig. 5B) and the hygiene instructions were delivered in the same stage. Follow-ups were performed at 3 and 6 months, so at 1 and 2 years (Fig. 5C), both the hard and soft tissues were healthy.

As in previous research (12, 13), the sample were fixed in 10% neutral buffered formalin and subsequently processed for analysis, by polarized transmitted light optical microscopy (Leitz Dialux, Germany). Thus, the sample was first demineralized, with a descaling solution containing EDTA (Kaltek, Padua, Italy), dehydrated on a scale of increasing alcohol content, embedded in paraffin and sectioned along its major axis, using a microtome (Leitz 1512, Germany). The sections, so performed, were then stained with hematoxylin-eosin.

RESULTS

From a clinical point of view, both the hard and soft tissues were healthy after 2 years follow-up. The histological examination, of the bone biopsy, shows in the upper portion a thick layer of connective (White Layer-WL). This soft tissue usually covers the regenerated bone and is considered a pseudo-periosteum (14). The WL wraps, in its lower portion, a wide area of amorphous material which is most likely represented by grafting material. Under the WL a spongy bone tissue, in various stages of remodeling, is found: there are areas of newly formed bone in which the cellular components are visibly active (Fig. 6A, 6B).

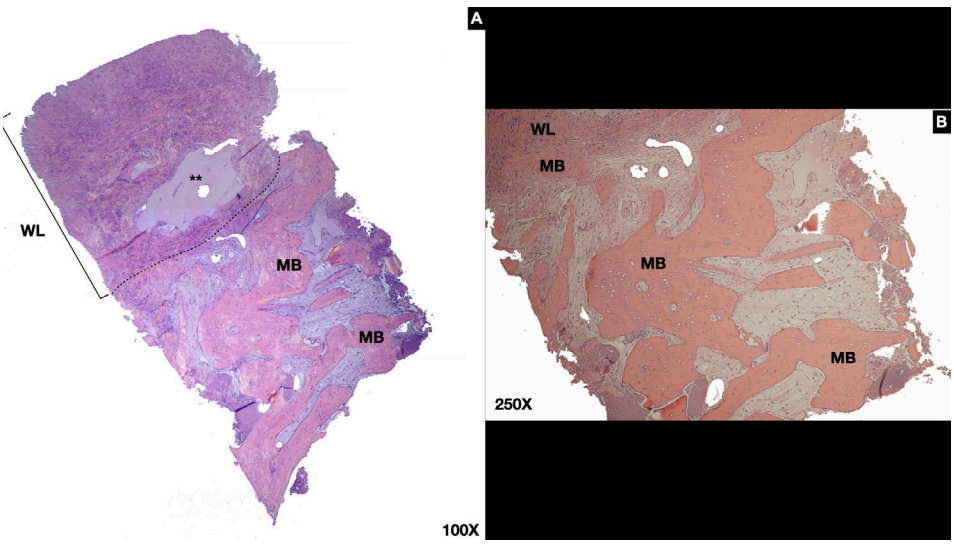

Fig. 6. *Histologic images.* ***A****): bone biopsy shows in the upper portion a thick layer of connective (White Layer-WL) by using polarized light, in the lower part there is amorphous material (**). Under WL (interrupt line) there is bi-refringent spongy bone (MB);* ***B****): higher magnification of bone biopsy.*

The mineralized bone is strongly birefringent, if observed with polarized light, due to its anisotropic structure (Fig. 6A). They are no visible cellular elements indicating inflammation or immune reaction, in the connective tissue of the marrow.

The histological examination of the device shows its strongly birefringent structure, if observed with polarized light, due to its anisotropic nature, of the collagen framework, traceable to haversian cortical bone (Fig. 7C). No cellular elements are identifiable in its structure (Fig. 7B)

DISCUSSION

Vertical and horizontal bone defect is a challenge in oral surgery. Alveolar bone reconstruction is needed before implant insertion. Several surgical techniques are available.

The barrier device proposed in this clinical case is the Curved Soft Lamina (CSL), which is a demineralized porcine cortical bone sheet, with a thickness of ~ 1.0 mm. Previous studies demonstrate its effectiveness in horizontal GBR (8-10). In some works, the device is passively applied to the recipient site (15, 16) in others it is immobilized using fixation screws (17). According to manufacturer instructions, CSL should be directly grafted without hydration. It can be particularly effective in association with pre-hydrated collagenated cortico-cancellous granules (CCG) of porcine xenograft. For this reason, CCG has been chosen, as graft material, due to its natural micro-porous consistency which facilitates new bone tissue formation, in defect sites (18, 19) and accelerates the regeneration process. Further studies show that it is gradually resorbable (20, 21), preserving the original graft shape and volume due to its osteoconductive property (22). Moreover, thanks to its collagen content, the product facilitates blood clotting and the subsequent invasion of repairing and regenerative cells. In this clinical case, CCG has been used in combination with autologous bone, harvested from the ramus, to give osteogenic properties to the graft, with a ratio of 50% for each one, as is usually done in vertical GBR with no resorbable devices (23).

In our previous clinical experiences, if lamina is passively placed on the recipient site without previous hydration a hygroscopic expansion in the following postoperative days can stretch the sutured flaps leading to the wound dehiscence, thus compromising the outcome of bone augmentation procedure. For this reason, the pre-hydration and subsequent fixation of lamina was adopted to achieve a stabilization of the underlining graft over the time, which is in mandatory in case of GBR (4, 6).

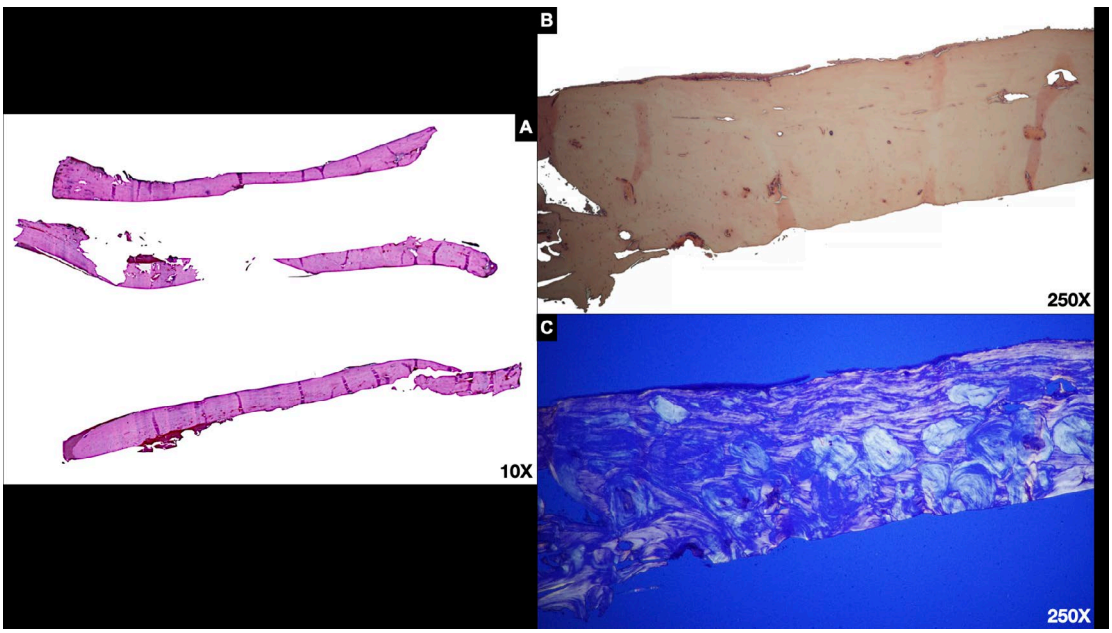

Fig. 7. *Histologic images – **A**): lamina a low magnification; **B**): lamina in standard light; **C**): lamina seen in polarized light. It is evident the anisotropic structure is due to collagen that is proper of harversian bone.*

CONCLUSIONS

Histological results suggest the validity of surgical procedure for bone volume regeneration in the distal mandibular region. Furthermore, according to our clinical experience, both the pre-hydration and stabilization of the device, with fixation screws, is strongly recommended, if vertical bone regeneration is needed.

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Case report

INCIDENTAL DIAGNOSIS OF MIKULICZ DISEASE: A CASE REPORT

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ABSTRACT

Mikulicz Disease (MD) is a chronic autoimmune condition considered part of the IgG4-related disease. Its aetiology is unknown. Furthermore, like all autoimmune diseases, it predominantly affects women. MD is characterized by the abnormal enlargement of salivary and lacrimal glands. In addition, it is characterized by significant plasma cell infiltration in lacrimal and salivary glands and high IgG4 serum levels. Therefore, ultrasound (US) examination, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) may be beneficial in the diagnosis of MD. In this case report, we describe an incidental diagnosis of MD after orbit magnetic resonance in a 46 years old female patient.

KEYWORDS: *Mikulicz syndrome, Mikulicz's disease, dacryosialoadenopathy, immunoglobulin G4, Mikulicz-Radecki syndrome*

INTRODUCTION

Mikulicz Disease (MD) was first described in 1888 by Johann von Mikulicz-Radecki, a German surgeon of Polish origins (1). He reported the case of a 42-year-old man with sudden enlargement of the lacrimal gland and subsequently of the parotid and submandibular glands (2). Mikulicz believed this disease was due to an infection, while others have described MD as possibly hereditary in aetiology. Later, in 1952, Godwin hypothesized that the swollen glands were due to an accumulation of lymphoplasmacytic infiltrate in the glands, and so proposed another name: benign lymphoepithelial lesion (3, 4) (Fig. 1).

In 1933, Sjogren described histological similarities between MD and Sjogren syndrome (SS) and suggested that

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MD was a subtype of SS (5). However, subsequent studies have demonstrated immune-histological differences between the due pathologies. In particular, the absence of Sjogren's syndrome-specific SS-A and SS-B antibodies in MD and the presence of infiltration of IgG4-positive plasma cells into the gland in MD (6-9).

Since 2003, MD has been considered part of the IgG4-related autoimmune disease spectrum, which includes fibro- inflammatory injury that involves different organs such as the pancreas, bile ducts, salivary glands, lacrimal gland, periorbital tissues, kidneys, lungs, lymph nodes, pachymeninges, breast, prostate, thyroid gland, retroperitoneum, pericardium, and skin (10-13). From an epidemiological point of view, MD predominantly affects women; these patients are interested in their middle age (14). In particular, MD is defined by the symmetrical and painless bilateral salivary gland (parotids, submandibular and sublingual) and lacrimal glands enlargement (15, 16). Moreover, this condition is characterized by poor lacrimal and salivary secretion. Some patients also complain of recurrent fever (15).

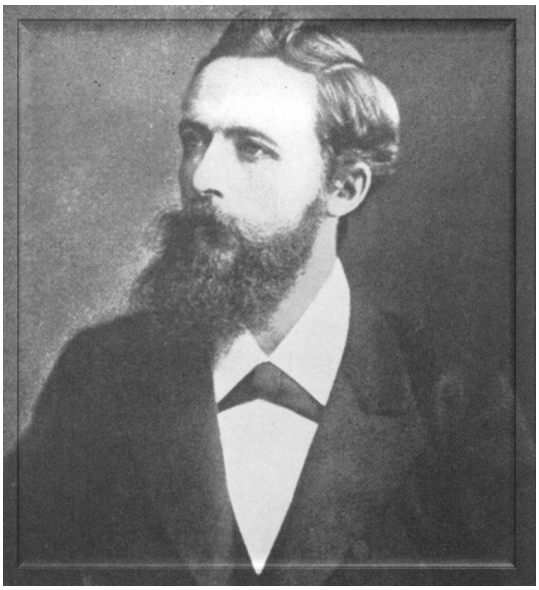

Fig. 1. *Johannes von Mikulicz-Radecki 16/05/1850 – 14/06/1905*

From a histological point of view, MD is characterized by glandular stromal hypertrophy and secretory parenchyma atrophy (17, 18). Consequently, this leads to fibrosis and hyalin infiltration. In addition, it is characterized by significant plasma cell infiltration in lacrimal and salivary glands and high IgG4 serum levels (19-21). Moreover, it is important to note that the involvement of these glands may also be found in other systemic pathologies: sarcoidosis, tuberculosis, leukaemia, and lymphosarcoma (22). Therefore, these diseases can be differentiated after the histological exam (22).

Ultrasound (US) examination of MD highlights the lacrimal and salivary glands with multiple hypoechoic areas in the enlarged glands (23). US can also be used to monitor the effectiveness of MD treatment (23). In addition, computed Tomography (CT) and Magnetic Resonance Imaging (MRI) may be beneficial in the diagnosis of MD (14, 18).

In CT, MD is characterized by bilateral lacrimal and salivary glands enlargement with no discrete lesion and homogenous enhancement. Instead, on MRI, MD is characterized by hypointense T2 and isointense T1 signals of glands (14, 18). Therefore, treatment is based on observation and systemic corticosteroid use (24, 25). In some cases, drainage and surgical removal are indicated in the specific case of strong pain (14).

The use of Glucocorticoids has been known for a long time. Its efficacy is in both reducing glandular enlargement and ameliorating glandular function. In research, it was underlined how the use of glucocorticoids markedly diminished the number of apoptotic cell s, interestingly more in MD than in SS (26); this is probably due to the characteristic reversibility process in this specific disease. Another treatment option is glandular needle aspiration (14). This procedure can be repeated multiple times and is usually the gold standard for patients with important comorbidities. Other treatments, such as conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) like methotrexate, azathioprine or mycophenolate mofetil, may be used to reduce adverse effects of long-term corticosteroids (27, 28). In addition, recent studies have shown significant efficacy of anti-CD20 monoclonal antibody rituximab in refractory patients (29). The prognosis of this condition is generally good, and it rarely evolves towards lymphoma (14). In this case report, authors describe an incidental diagnosis of MD after orbit magnetic resonance.

CASE REPORT

A 46-year-old female patient undergoes an orbit magnetic resonance (MRI) because of a sudden bilateral volumetric increase of both lacrimal glands. The MRI was performed with Siemens Magnetom AERA 1.5 T software SYNGO MR D13. Axial, sagittal and frontal planes had all been examined before and after gadolinium administration. At the MRI, a

bilateral volumetric increase of both lacrimals is visible (Fig. 2A-C). Therefore, the neuroradiologist decided to extend the exam by studying all salivary glands. They all appear symmetrically increased in volume (Fig. 3, 4A-B).

The patient also underwent an ultrasound study of salivary glands (US machine: Hitachi-Aloka Arietta v70) which confirmed the diagnostic hypothesis of MD. Symmetrical and bilateral volumetric enlargement of both the lacrimal and

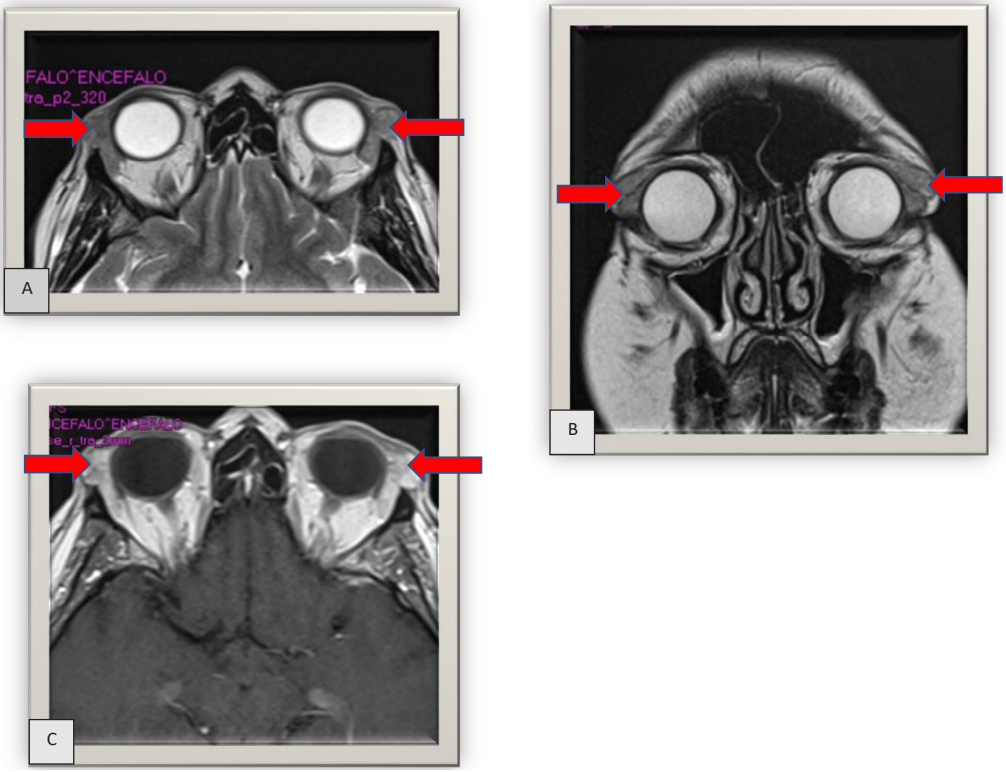

Fig. 2. ***A)****: T2 MR axial scan: volumetric increase of both lacrimal glands without alteration of their characteristic signal intensity (****arrows****);* ***B)****: T1 MR coronal scan: confirm the volumetric increase of both lacrimal glands (****arrows****);* ***C)****: T1 MR axial scan after injection of contrast medium: volumetric increase of the lacrimal glands showing intense and diffuse homogeneous enhancement after contrast injection medium (****arrows****).*

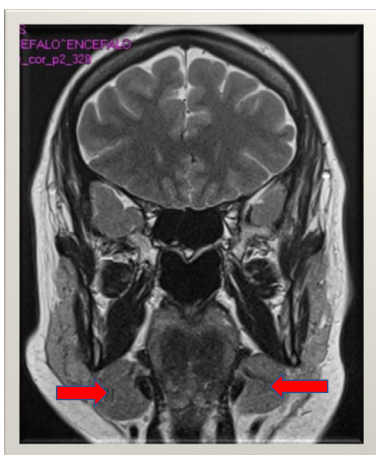

Fig 3. *RM axial scan demonstrates bilateral swelling of the submandibular glands (arrows).*

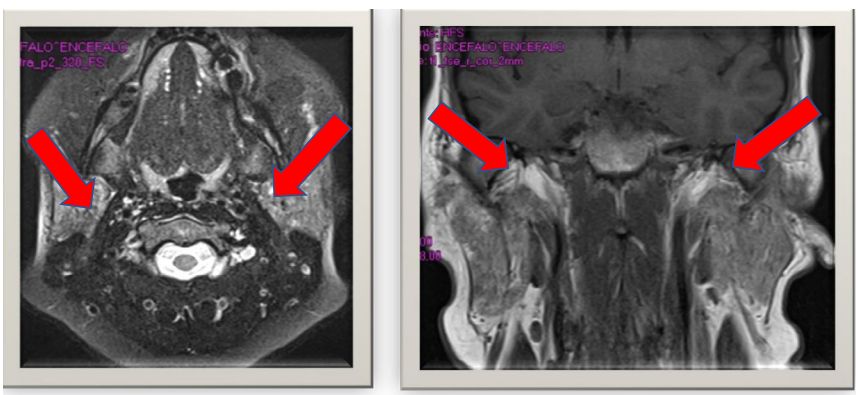

Fig 4. ***A)****: T1 MR axial scan: diffuse enlargement of the right and left parotid glands (****arrow****) with homogeneous and normal signal intensity without a focal parotid lesion;* ***B)****: T1 MR coronal scan: diffuse enlargement of the left parotid gland (****arrow****) with homogeneous and normal signal intensity without a focal parotid lesion, the right parotid also appears fairly enlarged with normal signal intensity.*

salivary glands characterizes the disease presented in this case report. Regarding the imaging, during the MRI, glands are visualized through enlarged; however, there is signal homogeneity in all sequences. More specifically, they are seen as hypointense in T2 sequences while isointense in T1 sequences. Therefore, no hyperintense areas are visualized in this kind of disease.

On the other hand, after gadolinium injection, the whole glands are homogeneously visualized as enhanced. Specifically: no lesions are visible in the part of the facial nerve crossing the parotid gland. Furthermore, no nervous or muscular structures are compromised despite the volumetric increase of lacrimal glands.

DISCUSSION

Immunoglobulin G4-related disease (IgG4-related disease) is a systemic immune-mediated fibroinflammatory pathology that involves various organs (30). Since its discovery in 2003, numerous conditions recognized as separate entities for decades, such as Mikulicz disease, are individual manifestations of IgG4-related disease based on shared histopathology (31). It is an autoimmune disease mainly affecting women between 40 and 60 (32).

The unique histopathological features of the IgG4-related disease are systemic inflammation, lymphoplasmacytic infiltration with IgG4-positive plasma cells, phlebitis and storiform fibrosis associated with elevated serum IgG4 concentrations in approximately 70% of patients (18).

Typical clinical presentation of MD is represented by symmetric enlargement of the lacrimal and glands associated with a reduction in the production of tears (xerophthalmia) and saliva (xerostomia) (30). Therefore, from a diagnostic point of view, IgG4 immunostaining should be performed in all tissue specimens where IgG4-RD is on the differential diagnosis (18, 30).

The 2019 American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) classification criteria for IgG incorporate histopathological findings and clinical context for diagnosis of this pathology (33). In addition, this clinical case underlines the importance of diagnostic tests such as US, CT and MRI to obtain an early diagnosis of this rare pathology (14, 23).

CONCLUSIONS

In the case discussed, the patient did not complain of any previously described symptoms except a sudden volumetric increase of lacrimal glands. Since it was also visible at the objective exam, the general physician prescribed her an Orbit MRI. The extension of the exam to salivary glands, the US exam and the elevated IgG4 serum allowed the formulation of a quick diagnosis and, therefore, to proceed to immediate treatment. The patient was treated with glucocorticoids. She recovered in a short time, and she then proceeded with her follow-up.

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Case report

FREE RADICALS, ANTIOXIDANTS, AND LIVER DISORDERS: SPECIAL EMPHASIS ON SILYBIN

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ABSTRACT

Free radicals, or reactive oxygen species (ROS), are unstable molecules that contain at least one unpaired electron in their outer orbit. They can cause chain reactions that damage cells and organs. In the presence of metals, the free radical superoxide anion O2 and hydrogen peroxide (H2O2) form the hydroxyl radical OH- that is responsible for lipid peroxidation, which is very toxic to the body. Free radicals cause oxidative stress and are generated in various conditions including chemical-physical environmental pollution, the intake of drugs, or a dysregulated metabolism. ROS transform DNA, resulting in ageing of the body, activation of carcinogenesis, neurodegeneration, and autoimmune, cardiovascular, and muscular diseases. Oxidative stress can be linked to various liver disorders as well, such as alcoholic and non-alcoholic fatty liver disease, liver intoxication due to free radicals, and hepatitis, including hepatitis C, which is very tedious and difficult to treat. Dysregulation of the balance between antioxidants and oxidants in the liver causes oxidative stress; antioxidants oppose the oxidation process and contrast the effects of free radicals. They are beneficial to human health, slowing down the ageing process and reducing the incidence of cancer, inflammation, neurological disorders, diabetes, and autoimmune, cardiovascular, and liver diseases. Pollutants can lead to liver disorders, while antioxidants have shown preventative and therapeutic effects in these diseases. In *in vivo* and *in vitro* studies, some drugs have been shown to have a protective effect on the liver by limiting chemical damage and fibrosis in the organ, as well as improving the lipid status. Many antioxidant substances such as silybin, resveratrol, curcumin, naringenin green tea, quercetin, and flavonoids in general, are taken with the diet by virtue of their benefits for the body, including the liver. Silybin is an active

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compound of Silybum marianum and has several interesting biological effects, including ones against cancer and liver diseases. When administered to mice, silybin is rapidly absorbed in both plasma and tissue and turns has an antioxidant and anti-inflammatory effect. It has been reported that this antioxidant has been used to prevent and combat liver damage. In light of this scientific evidence, it can be deduced that free radicals can be reduced with antioxidants, including silybin, which have a therapeutic effect on the whole organism and protect the liver.

KEYWORDS: *antioxidant, free radical, liver, flavonoids, therapy, natural compounds*

INTRODUCTION

Oxygen is important for life, but when it turns into free radicals it becomes toxic. Free radicals, or reactive oxygen species (ROS), are very unstable molecules because they have an unpaired (lost) electron. The missing electron is taken up into other molecules in the body, causing damage to the cells (Fig. 1). The substances that neutralize the free radicals and oppose these oxidation reactions are called antioxidants. Many antioxidants can be endogenous in nature and produced by the body, such as vitamins, but these and other compounds can be introduced with the diet as well (1). ROS are associated with liver diseases such as inflammation, steatosis, viral hepatitis, cirrhosis, jaundice, and hepatocellular carcinoma (2-5). Additionally, many chemical compounds such as alcohol and tobacco smoke can cause liver disease (6, 7).

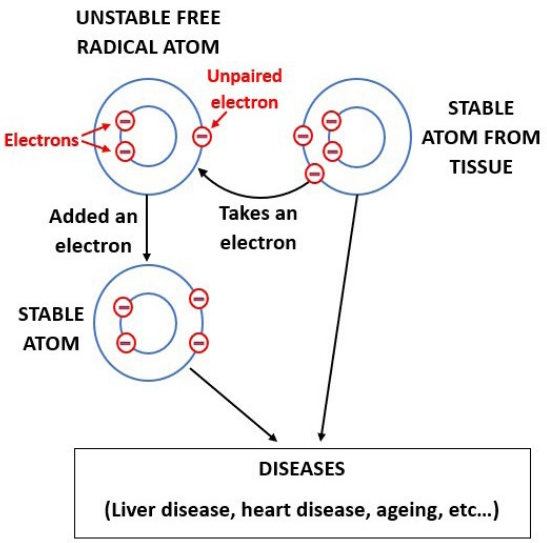

Fig. 1. *Free radicals increase in ageing and in various diseases. This figure shows a free radical with an unpaired electron which takes an electron from a stable atom in the tissue. This reaction can lead to cell death and cause various diseases.*

The correct physiological functioning of the body is regulated by the balance between ROS and antioxidants (8). When the levels of these free radicals increase, they lead to oxidative stress and inflammation, which are present in many human disorders (9, 10) (Table I). Liver inflammation can result in activation of vascular endothelial growth factor (VEGF) and cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF), with the priming of nuclear factor kappa B (NFkB) and nucleotide-binding leucine-rich repeat-containing protein (NLRP12) inflammasome (11, 12).

Many natural products, such as flavonoids, polyphenols, and resveratrol, have been proposed to alleviate the effects of ROS, mediating oxidative stress, metabolic disorders, and inflammation (12-14).

Flavonoids and their derivatives are antioxidant enzymes which modulate the immune response and have anti-inflammatory, anti-apoptotic, and anti-tumor activity (15). They inhibit lipid peroxidation and the oxidation of DNA and proteins, protecting many organs, including the brain and liver (16). Toxic insults to the liver can result in oxidative stress and the synthesis of high levels of free radicals which can lead to apoptosis and hepatocyte damage. Flavonoids exert antioxidant effects on cells by counteracting ROS after organ damage, including that of the liver.

Table I. *Some diseases induced by oxidative stress in humans.*

In humans, oxidative stress induces:		
Ageing	Depression	Liver damage
Alzheimer's disease	Diabetes	Memory loss
Arthritis	Heart failure	Parkinson's disease
Cancer	Infection	Retinal disorders
Cardiomyopathy	Inflammation	Rheumatism
Cataract	Ischemia	Stroke

The use of flavonoids such as silybin can help prevent damage caused by free radicals, such as peroxidation and inflammation (17). In addition, in cancer patients, silybin has a detoxifying power after chemotherapy and is recommended in anti-tumor treatments (18-19). In cases of poisoning, where the liver plays an important role, silybin appears to alleviate liver damage and increases the survival rate (20).

Silybin

Silybin represents about 50% to 70% of the silymarin extract, and it is the active compound of Silybum marianum, which possesses several interesting biological effects (20). Silybin can be distinguished in two forms, A and B, but it can also be found in combination of these two forms as isosilybin. Silybin is an extract of milk thistle, and it has been reported in diverse published articles that it is used against cancer and liver disease. It is poorly soluble in water; however, this drawback can be counteracted when it is used as a prodrug. Silybin is rapidly absorbed into both plasma and tissues when it is administered to mice in free and conjugated forms (20). It is used in acute and chronic liver injury as an antioxidant and anti-inflammatory compound due to its antifibrotic properties (21).

In experiments on rodents *in vivo*, silybin has shown effects on the liver, where there is an increase in glutathione-S-transferase with negligible side effects, such as a mild hyperbilirubinemia, which can regress after discontinuation of the administration (17). Silybin has a strong antioxidant effect, and acts as a scavenger by inhibiting the formation of free radicals and by modulating the permeability of cell membranes. In oxidative stress in the liver of mice, silybin inhibits nitric oxide (NO), glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, and contrarily, it increases ATP levels through the phosphorylation of ADP (22) (Fig. 2). Therefore, in damaged liver tissue, this antioxidant can improve lipid peroxidation, mitochondrial respiration, and cell death. Silybin shows a strong link with some inflammatory target proteins and is used in the prevention of ischemic damage in rats, where it increases the levels of superoxide dismutase and glutathione, while decreasing the levels of TNF and IL-6 both in the hippocampus and in the cortex cerebral (23).

Pro-inflammatory cytokines are produced by hepatocytes via NF-kB after cellular damage which may be physical, chemical, or biological. Silybin interferes with the transduction process controlled by the transcription factor NF-kB that is induced in inflammation (24). Silybin exerts its anti-inflammatory power by inhibiting the activation and translocation of NF-κB into the nucleus, suppressing phosphorylation and increasing IL-10 levels (17). Moreover, it inhibits cyclooxygenase (COX-2) expression, leukotriene formation, and protein kinase activation (20). In the liver, silybin also has antiviral activity, acting on the replication of the HCV virus in infected patients (25).

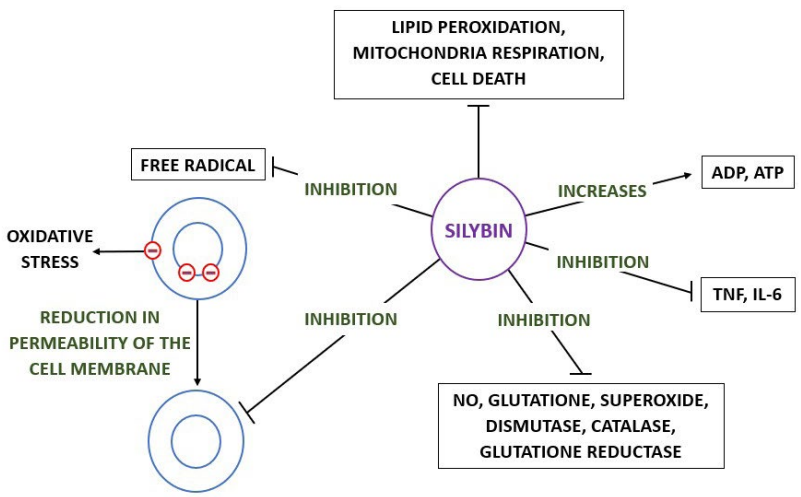

Fig. 2. *Silybin blocks free radicals, which leads to reduced permeability of the cell membrane. Silybin also inhibits lipid peroxidation, mitochondria respiration, cell death, nitric oxide (NO), glutatione, superoxide, dismutase, catalase, glutatione reductase, and tumor necrosis factor (TNF) and IL-6 cytokines. In addition, silybin increases levels of ADP and ATP.*

Silybin has been found to be beneficial in viral diseases such as COVID-19, where oxidative stress, immune system dysfunction, and inflammation are implicated (26). It has been shown to have an anti-inflammatory effect, probably by reducing cytokines such as IL-1, IL-6, and TNF, as well as to have an antiviral action by binding to SARS-COV-2 proteins, including the spike protein, and promoting the elimination of the virus (27) (Fig. 3).

Therefore, silybin proves to be an anti-inflammatory agent by acting as a protector against certain inflammatory liver disorders and diseases of the other organs (23).

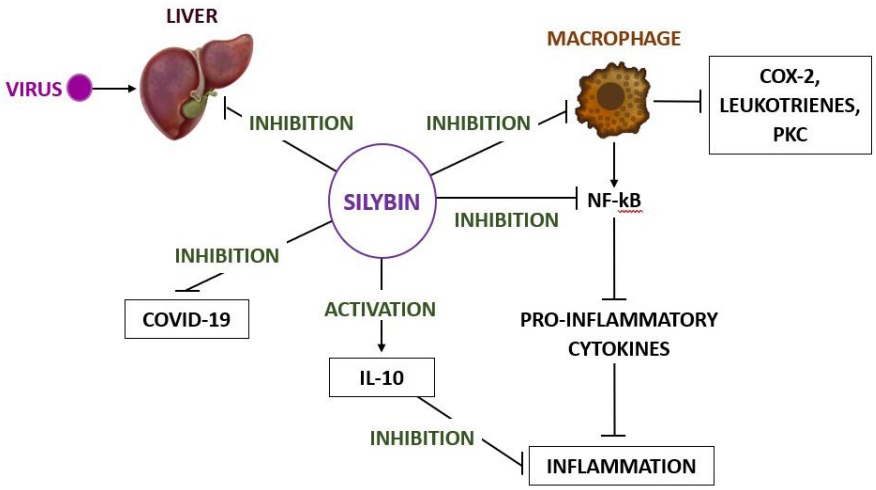

Fig. 3. *Figure showing the antiviral activity of silybin in the liver which inhibits COVID-19. Silybin also inhibits macrophages that produce COX-2, leukotrienes, and PKC. Moreover, silybin inhibits NF-kB, resulting in the decreased release of pro-inflammatory cytokines and reduced inflammation. There is also activation of IL-10, which is an inhibitor of the inflammatory response*.

CONCLUSIONS

In this paper, we report that free radicals are responsible for lipid peroxidation, which is toxic to the body. Oxidative stress can transform DNA, accelerate ageing of the body, and can activate carcinogenesis, neurodegeneration, and autoimmune, cardiovascular, muscular, and liver diseases. On the contrary, antioxidants such as silybin oppose the oxidation process and contrast the effects of free radicals, helping to prevent these disorders, particularly liver damage. The reduction of ROS by silybin and other antioxidants has a therapeutic effect for the entire body and has been shown to prevent and cure liver disease.

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Case Reports

HYDROXYAPATITE AND BETA-TRICALCIUM PHOSPHATE IN THE SOCKET PRESERVATION: PRESENTATION OF CLINICAL CASES

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ABSTRACT

The reduction in alveolar bone volume is a direct consequence of tooth loss. Hard and soft tissue remodelling is part of the normal physiological healing process of the residual edentulous ridge. This remodelling negatively affects the size of the ridge in the buccolingual and apicocoronal aspects. Ridge atrophy after tooth loss has been show to follow specific patterns. In the Maxilla, the alveolar buccal wall tends to reabsorb more rapidly after tooth extraction. The ridge is gradually represented by the palatal wall (centripetal resorption). The purpose of this article is to describe two clinical cases of preservation of the alveolar ridge with the use of a biomaterial and a membrane.

KEYWORDS: *bone, alveolus, ridge, tooth, regeneration*

INTRODUCTION

In a 12-month prospective study, Schropp et al. (1) analyzed 46 premolars and molars extraction sockets from 46 patients and found a 50% ridge loss with an average horizontal loss of 6.1 mm. Two-thirds of this loss of bone volume occurs within the first 3 months. This bone remodelling process may vary according to individual local and systemic factors, but it mainly affects the horizontal bucco-coronal thickness in the anterior sites (2-4). Su et al. observed an average loss of 3.87 mm in ridge width and 1.67 mm in ridge height in the anterior and premolar sites during the first three months after extraction

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(5). The ridge is gradually represented by the palatal wall (centripetal resorption), compromising the sagittal and axial intermaxillary relationship (6-8). This phenomenon appears progressive and often translates into aesthetic and functional challenges during rehabilitation with dental implants.

To minimize the need for bone regeneration during implant placement "Alveolar Socket Preservation" (ASP), has been described in recent decades. This technique was born as a procedure aimed at preventing, or more appropriately limiting, the alteration of the post-extraction bone crest to have an optimal implant-prosthetic rehabilitation. The different alveolar preservation techniques proposed in the literature involve biomaterials, bone grafts, their combination, and barrier membranes (9, 10). The biomaterial used to fill the alveolus should ideally inhibit the growth within the alveolus of the epithelial and connective tissue, stabilize the clot and limit bacterial contamination (11).

In addition to resorbable and non-resorbable membranes, various biomaterials used for soft tissue augmentation, such as autogenous free gingival grafts, dermal allografts and collagen matrix xenografts, were also used to seal the alveolus (12). The purpose of this article is to describe two clinical cases of preservation of the alveolar ridge with the use of a biomaterial and a membrane.

Clinical cases presentation

Both patients underwent a medical history and control of any active pathological process. All therapeutic options were examined with patients, and socket preservation and subsequent implant prosthetic rehabilitation were chosen. Considering the risks and benefits of the proposed procedure, the signature of informed consent was obtained.

Case 1:

A 69-year-old patient complained of pain and mobility of 1.4. Intraoral physical examination revealed grade 2 dental mobility, associated with mesial and distal periodontal probing of about 7 mm with bleeding. The periapical X-ray confirmed the bone loss, which would not have allowed us an immediate post-extraction implantology (Fig. 1).

Under local anaesthesia (Mepivacaine 2% Sain-Maurdes-Fosses, France), the dental element was extracted, and the post-extraction alveolus was thoroughly cleaned and irrigated with a saline solution. The walls of the alveolus were examined to determine the morphology of the defect and the presence of the vestibular bone wall.

The alveolus was filled with biomaterial (Rigenera Biotek S.R.L. Pavolaro di Dueville, Vicenza, Italy) composed of 30% of Slow Resorption Hydroxyapatite (HA) and 70% of Rapidly Resorption Beta-Tricalcium phosphate. To cover the

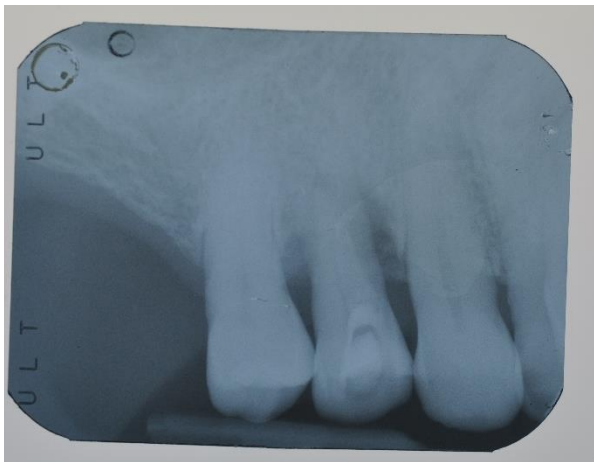

Fig. 1. *Case 1. Pre-operative X-ray.*

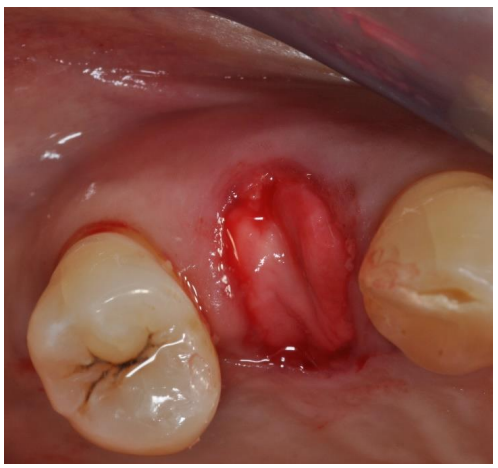

Fig. 2. *Case 1. An occlusal vision of the exposed membrane to obtain second-intention healing.*

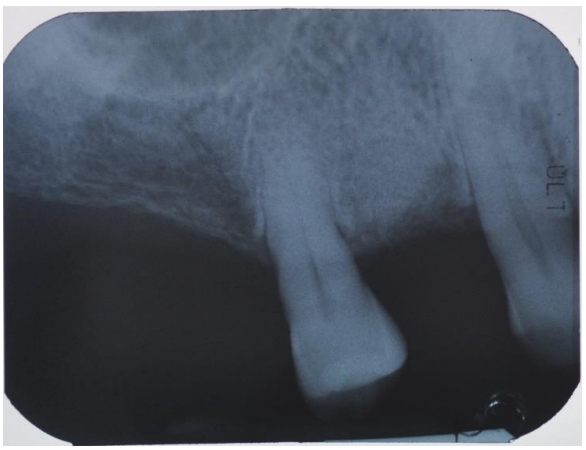

Fig. 3. *Case 1. Radiographic control of regeneration.*

biomaterial was inserted a resorbable membrane (Evolution Fine Tecnoss®, Giaveno, Italy) intentionally left exposed according to the socket Preservation technique. (Fig. 2). After 5 months of healing, clinically good preservation was evident. A control intraoral X-ray was performed (Fig. 3), and implant insertion was scheduled.

At the lifting of the flap, we noticed good preservation of the alveolus that allowed us to insert the implant 12 mm 3.75 Ø (Safe BT Biotec srl Povolaro di Dueville (VI) Italy) (Fig. 4, 5). Antibiotics (Amoxicillin 1 gr) were prescribed for 7 days. The patient underwent monthly check-ups to follow the recovery. After 4 months of osseointegration, a full-thickness flap was lifted, and the healing screw was placed (Fig. 6). Subsequently, prosthetic rehabilitation was carried out (Fig.7-9).

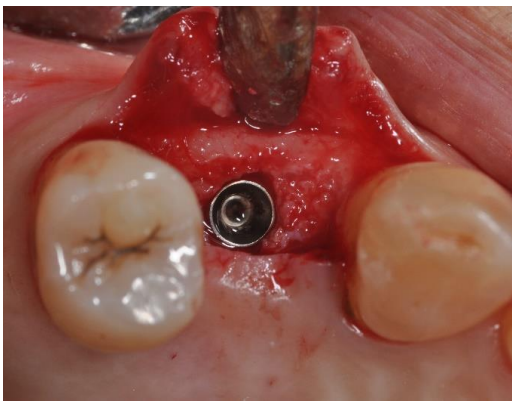

Fig. 4. *Case 1. Occlusal vision at the insertion of the implant (note the excellent preservation of the bone crest)*

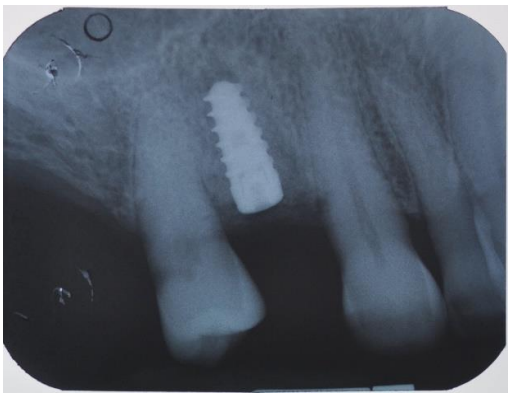

Fig. 5. *Case 1. Control X-ray at 4 months.*

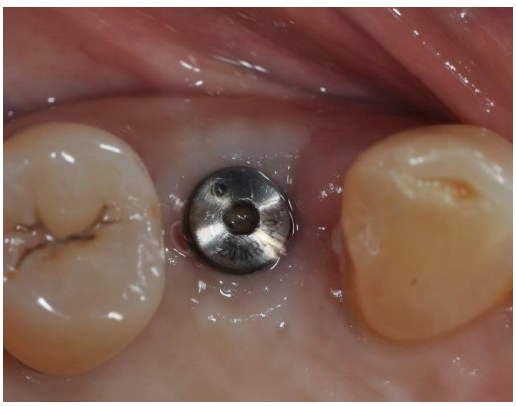

Fig. 6. *Case 1. Insertion of the healing screw (we note how the vestibular draft has been maintained).*

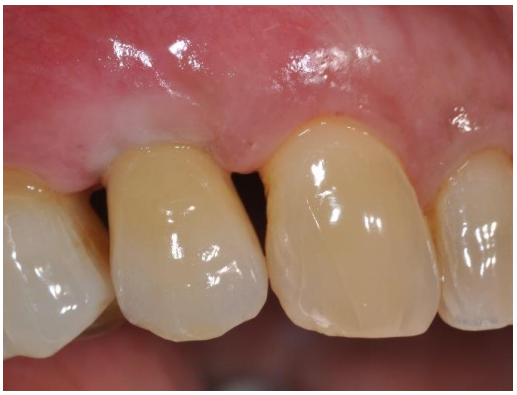

Fig. 7. *Case 1. Prosthetic rehabilitation.*

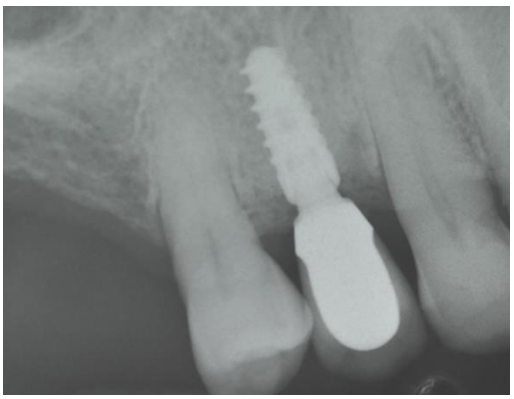

Fig. 8. *Case 1. X-ray post-rehabilitation control.*

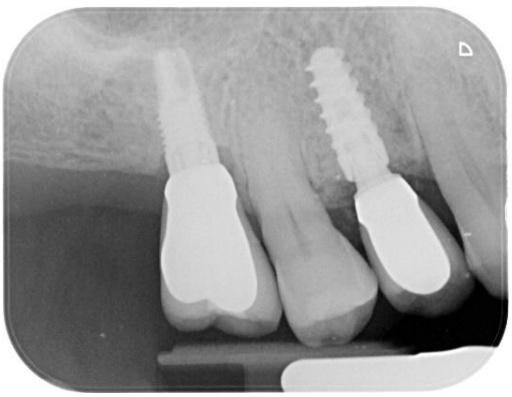

Fig. 9. *Case 1. X-ray follow-up of 2 years.*

Case 2

A 78-year-old man presented to our observation with a vestibular fistulized periodontal abscess of element 2.1 (Fig. 10). Periodontal probing and radiographic examination revealed mesial vertical bone resorption and the lack of part of the vestibular cortex of 2.1 (Fig. 11). With the patient's consent, it was decided to extract the dental element and carry out a subsequent implant-prosthetic rehabilitation.

As in the previous case, at the same time as the dental extraction, a reconstruction of the bone volume was carried out with a biomaterial (Rigenera Biotek S.R.L. Pavolaro di Dueville, Vicenza, Italy) and a resorbable membrane (Evolution Fine Tecnoss®, Giaveno, Italy) was inserted to cover the biomaterial. Due to the lack of much of the buccal plate, evidenced by the probing, a full-thickness pocket was prepared using a sharp blade and a microsurgical elevator. Subsequently, the membrane was shaped according to the "ice cream cone" technique (10) and partially inserted inside the pocket created previously (Fig. 12).

No primary healing intention was sought since the soft tissues would have migrated in the initial healing phase, creating a greater amount of keratinized gingiva. After 5 months, at the time of insertion of a 10 mm 3.75 Ø implant (Safe BT Biotec srl Povolaro di Dueville, Vicenza, Italy), the mucosa and the underlying bone were well represented. (Fig. 13-17). After osseointegration, at 4 months of healing, the prosthetic rehabilitation was carried out (Fig. 18-20).

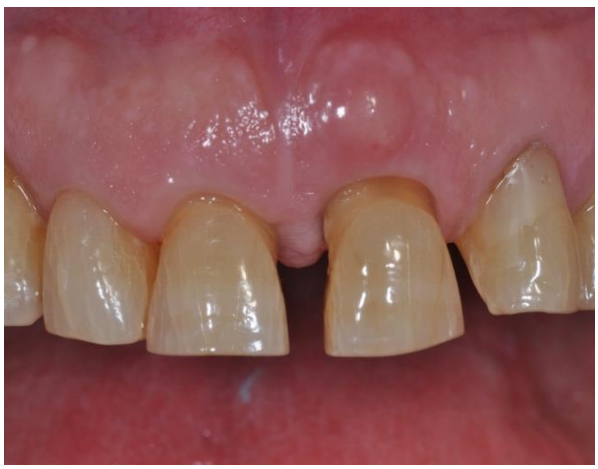

Fig. 10. *Case 2. Pre-operative image shows a buccal fistula of 2.1.*

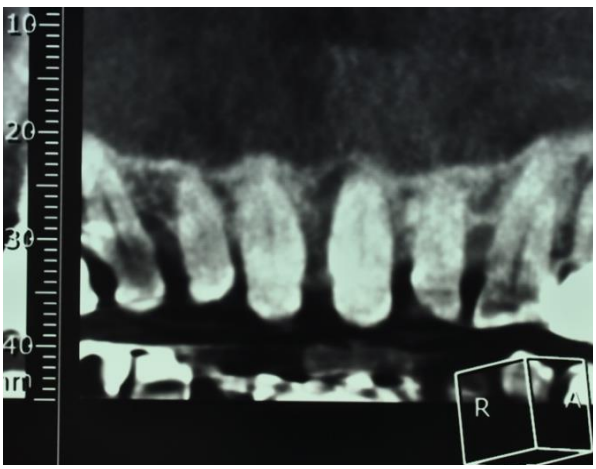

Fig. 11. *Case 2. Pre-operative CBCT shows bone loss mesially at 2.1 due to periodontal abscess.*

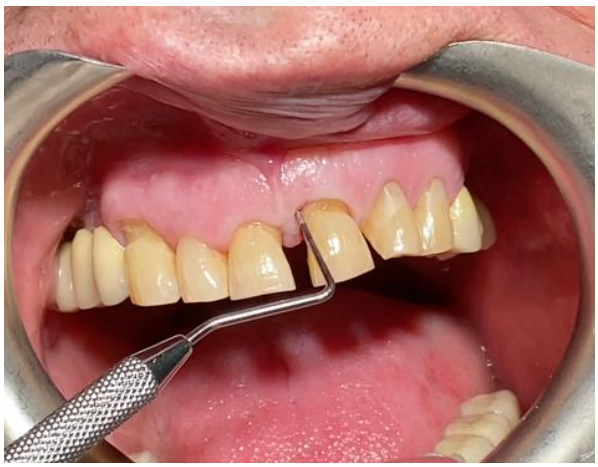

Fig. 12. *Case 2. Surgery-curettage of the alveolus and bone reconstruction.*

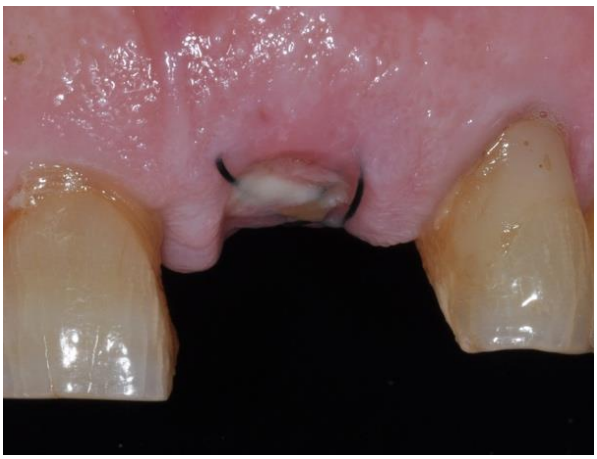

Fig. 13. *Case 2. Healing after one week.*

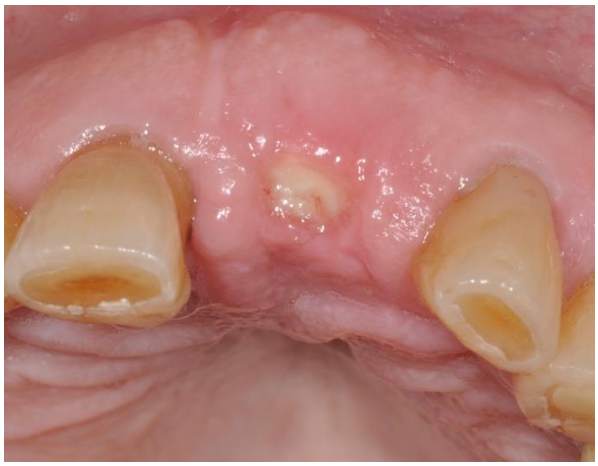

Fig. 14. *Case 2. Healing after 2 weeks, the almost complete mucosal closure and the absence of inflammation are evident.*

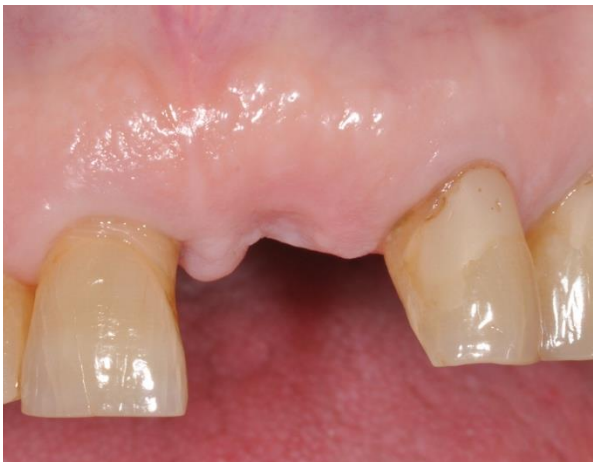

Fig. 15. *Case 2. The appearance of the completely healed ridge.*

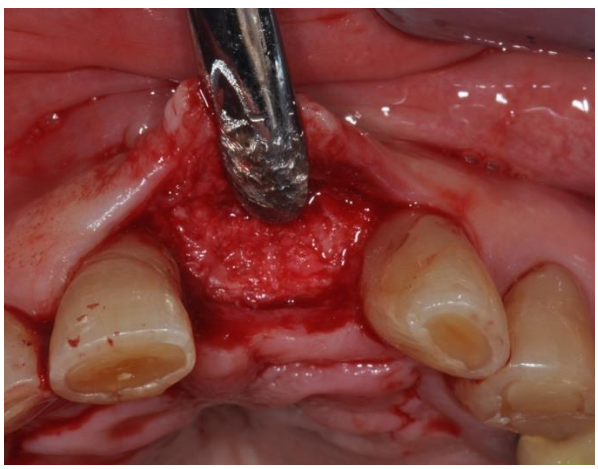

Fig. 16. *Case 2. When the flap is opened, excellent bone regeneration is evident which allowed us to insert an implant in the correct three-dimensional position.*

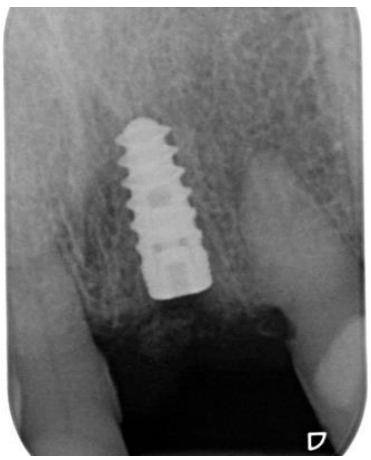

Fig. 17. *Case 2. Post-operative X-ray.*

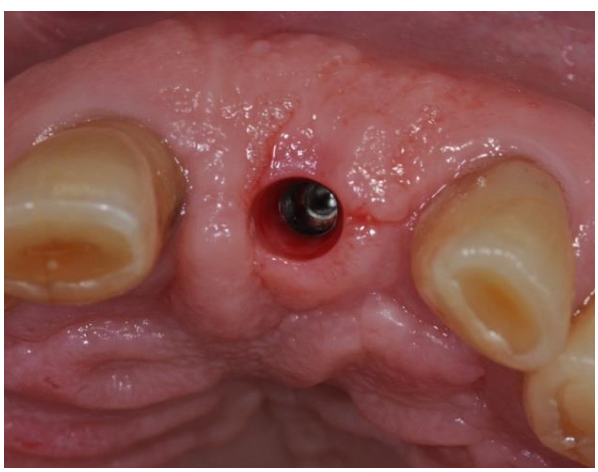

Fig. 18. *Case 2. Detail of the buccal plate and implant position.*

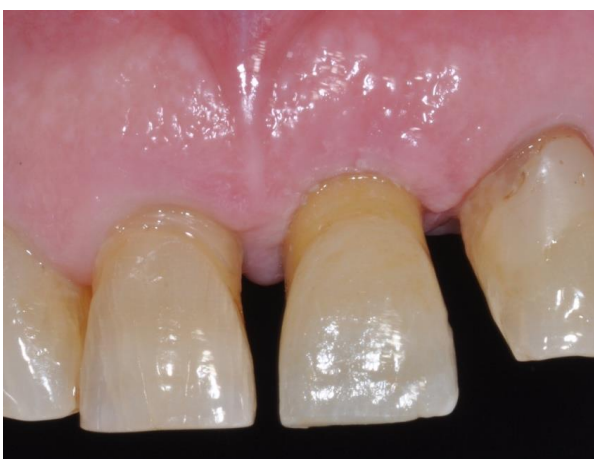

Fig. 19. *Case 2. Prosthetic rehabilitation.*

DISCUSSION

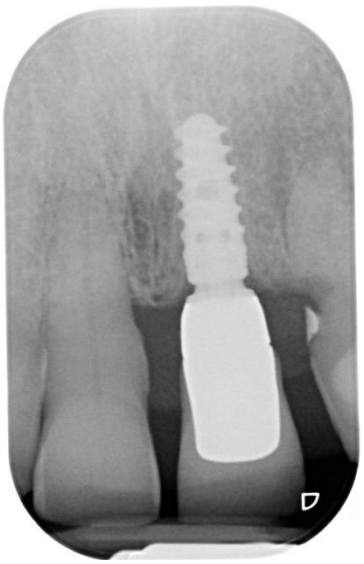

Fig. 20. *Case 2. Final X-ray.*

The alveolus is an anatomical entity linked to the presence of the tooth in the arch. After a dental extraction, physiological healing is followed by a three-dimensional reduction of the alveolar volume (13). The tooth and its attachment apparatus, the root cement, the periodontal ligament, and the alveolar bone establish a functional unit.

The forces developed during the masticatory activity are transmitted from the tooth crown, through the root and attachment apparatus, to the hard tissues of the alveolar process, where they will be dispersed. This alveolar atrophy is the consequence of the teeth loss and the change in conditions inside or around the alveolus, and it will result in a series of adaptive alterations of the edentulous portion of the alveolar ridge.

One research article points out that the cause of the greater degree of reabsorption of the vestibular side is to be connected to the anatomy typical of the alveolar process: the buccal cortex, in fact, essentially consists of fasciculate bone, while the lingual one has a smaller fraction of it (14). The presence of vestibular tissue is functionally related to the dental element itself, and it gradually disappears after extraction leading to the reduction of the vertical and horizontal dimensions of the alveolar ridge. The purpose of socket preservation is to maintain the original alveolar dimensional contours, limiting the natural process of post-extraction resorption. A multicenter study underlined the importance and advantages of the socket preservation technique after tooth extraction of the anterior upper arch (15). These areas frequently have a thin vestibular bone plate, with a small amount of bundle bone that is inevitably lost during tooth extraction resulting in an early collapse of the labial plate and consequent imperfection (16, 17).

In the past years and still today, socket preservation has been the object of multiple studies with the use of different alveolar filling materials. These fall into one of four categories: autogenous bone grafts, allogeneic bone grafts, xenogenic bone grafts and alloplasts. Depending on which of these is used, the process can be osteoconductive, osteopromotor or osteoinductive (18, 19). Among the bone grafts used for alveolar preservation, biomaterials of synthesis present a growing diffusion in dental surgery thanks to their excellent bio-compatibility and the ability to act as a scaffold, stabilizing the clot in the different stages of healing.

Hydroxyapatite is one of the most commonly used biomaterials in bone regeneration techniques. Some authors underlined that hydroxyapatite synthesized in nanomolecular form, with dimensional characteristics similar to the molecules deposited by human osteoblasts, has better bone resorption and better bone new formation than traditional biomaterials (20). Furthermore, if these hydroxyapatite nano molecules are combined with rapidly resorbing substances, better results are obtained in terms of resorption of the nanohydroxyapatite, leaving space for bone regeneration (21, 22).

The material used as a filler in the two clinical cases is Rigenera BCP in 0.25-1.00 mm granules (Biotec S.R.L. Povaloro di Dueville, Vicenza, Italy) composed of 30% slow resorption hydroxyapatite and 70% Beta-tricalcium rapid resorption phosphate. The nanostructured surface promotes blood absorption by entering the granules of proteins and growth factors and promotes cell differentiation (23).

In another study (24), histological examinations were performed 6 months after surgery on bone samples in regenerated sites with materials based on hydroxyapatite, an amount of newly formed bone of 41% indicated a residual biomaterial quantity of 27% and a presence of medullary spaces of about 30%.

A fundamental role is played by the membranes that cover the grafted material. In the last decade, non-absorbable membranes, resorbable membranes, collagen sponges and non-cross-linked xenogenic collagen matrices have been used. The resorbable and non-absorbable membranes can be totally covered with a coronally advanced flap to obtain the primary closure following the biological principles of guided bone regeneration. However, this procedure inevitably changes the gingival architecture and location of the mucogingival junction. To avoid this, today, we tend to use crosslinked collagen membranes with different periods of resorption that can be intentionally left exposed to provide a transient barrier function (25). Histological studies of non-cross-linked collagen matrices in non-submerged or submerged environments revealed

complete integration with mature mucosal and submucosal tissues and membrane revascularization after 3 months (26).

The membrane used (Evolution Fine, Tecnoss®, Giaveno, Italy) is obtained from heterologous mesenchymal tissue and is completely resorbable. Experimental studies have shown histological evidence of the prolonged barrier effect of this membrane, which lasts at least eight weeks, protecting the graft from external agents (27).

The possible beneficial effect on the amount of bone resorption that occurs after tooth extraction with flap or flapless is controversial. Although some studies have shown slightly less pronounced bone remodelling of the alveolar crest after flapless extraction (28), other studies have failed to find significant differences between the flap and flapless tooth extractions (29).

Although it is suggested that wound dehiscence and membrane exposure may cause infection and lack of bone formation, recent studies show that intentional exposure to bioresorbable barriers does not adversely affect alveolar preservation procedures (30, 31).

A study in 2016 showed that the dense collagen matrix Evolution protects the graft from infection in case of membrane exposure, which does not become infected, and the wound heals by second intention (32). In these two clinical cases, we used a flapless approach and left the membrane exposed during healing. Lifting and advancing a full-thickness flap can cause a marginal recession to adjacent teeth, alteration of the shape of the papillae, loss of keratinized mucosa, and greater post-operative discomfort for the patient.

In this study, we observed no adverse effects where membranes were left exposed to the oral cavity and sutures were mainly used to hold the membranes in place.

CONCLUSIONS

Oral rehabilitation with dental implants requires sufficient bone architecture in both vertical and horizontal dimensions. The socket preservation after extraction plays a fundamental role in maintaining the alveolar ridge. The clinical results obtained in these two cases seem to indicate that the use of Beta-tricalcium phosphate as a filler, and a membrane of completely resorbable heterologous mesenchymal tissue, are able to convey and promote bone regeneration.

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Evaluation Study

EFFECTIVENESS OF A DIODE LOW-LEVEL LASER THERAPY ON TOOTH SENSITIVITY RELATED TO IN-OFFICE BLEACHING: A CLINICAL STUDY

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ABSTRACT

The aim of this study was to investigate the effectiveness of low-level laser therapy (LLLT) on tooth sensitivity post-in-office bleaching. Sixty patients were selected for a randomised clinical trial. After the in-office bleaching procedures with 38 % hydrogen peroxide, the participants were randomly divided into two groups of 30 subjects each. In the LLLT group, patients received LLLT through an 810 nm diode laser with 0.5W for 30 sec at a density of 15 J/cm2, while participants in the placebo group were subjected to an LLLT with similar conditions but without any energy output. The intensity of tooth sensitivity was recorded at 1, 24, and 48 h after bleaching using a visual analogue scale (VAS). The intensity of tooth sensitivity was not significantly different between groups at 1h after bleaching (p=0.593). At 24 h and 48 h after the bleaching procedure, the pain level was significantly lower in the LLLT group compared to the placebo groups ($p < 0.0001$). The LLLT with a diode laser could be used as a suitable strategy to reduce the intensity of tooth sensitivity after in-office bleaching.

KEYWORDS: *bleaching, tooth, sensitivity, low-level laser therapy, pain*

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INTRODUCTION

Bleaching is one of the most frequently performed procedures in esthetic dentistry and can significantly impact the look of the teeth by removing extrinsic and intrinsic stains. Several techniques have been proposed to provide efficient tooth whitening, including in-office bleaching, night guard and home-applied bleaching. Although the use of home bleaching techniques has considerably increased during the last few years, in-office bleaching continues to have a great demand for patients requesting immediate results, intolerant to wearing trays or worried about ingesting bleaching products (1, 2). The reactive oxygen species released by bleaching materials oxidise dentin and enamel chromogens, producing the whitening effect. It has been demonstrated that hydrogen peroxide (HP) and its derivatives could easily reach the pulp tissue through enamel and dentine, causing structural damage and inflammatory reactions (3). Adverse effects of bleaching agents, including cytotoxicity and DNA modification, have been reported in several in vitro studies (4). Tooth sensitivity is the most usual clinical side effect of in-office treatment with 35 % HP. The frequency and degree of tooth sensitivity depend on the patient's pain threshold, the concentration of the bleaching agent and eventually, the heat used for accelerating the proceedings (5).

Several studies reported an incidence rate of 65–85 % for tooth sensitivity after the in-office bleaching treatment using high concentrations of HP alone or associated with heat. Usually, pain and discomfort are generally mild and transient. However, it may sometimes be severe and irritating as it requires the withdrawal of whitening treatment. In order to reduce teeth sensitivity from whitening, some researchers have proposed the application of agents containing potassium nitrate and fluoride or casein-phosphopeptide amorphous calcium phosphate before, during or after the ending of the whitening treatment. Several studies confirm the success of desensitising agents in reducing tooth sensitivity after bleaching (6-9).

Low-level laser therapy (LLLT) has become more significant in medicine and dentistry due to its anti-inflammatory, analgesic and biostimulating effects (10, 11). These excellent properties suggest that LLLT may decrease the damage and inflammation induced by in-office bleaching products in the pulp tissue, thus reducing the risk and intensity of tooth sensitivity caused by the bleaching (12, 13). Considering that the smile aesthetic is a hot topic today, as well as the use of low-level laser (14, 15), this study was conducted to evaluate the effectiveness of a diode laser to low power on tooth sensitivity arising from in-office bleaching.

MATERIALS AND METHODS

The samples used for this study came from 60 subjects treated at the Dental Clinic of the University of Campania "Luigi Vanvitelli". The study was conducted in agreement with a randomised double-blind and placebo-controlled clinical trial and in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. All patients were in good general health with maxillary and mandibular anterior teeth without caries, visible defects or any restoration. Teeth with negative responses to the vitality test were excluded from the study. Patients were at least 18 years old with central incisors of a shade darker than C2 using the shade guide of Vita Classical A1-D4® (Germany). Subjects who used analgesics, anti-inflammatory or antioxidant medicine and those with smoking habits were excluded from the study. Patients during orthodontic treatment with temporomandibular disorders, bruxism habits or any medical and pathologic defect such as gingival recession or dentin exposure that could cause tooth involvement were also excluded from the study (16-19). None of the patients recruited reported symptoms attributable to tooth sensitivity. Two weeks before the bleaching treatment, the participants underwent professional oral hygiene and received oral hygiene instructions to brush their teeth twice per day with a similar toothbrush and toothpaste during the experiment. All patients were informed about the treatment procedures and asked to sign an informed consent document before the beginning of the study.

The in-office bleaching procedure

Before the bleaching, patients were randomly divided into two groups (n=30) using software for casual distribution by a not-clinician researcher. Then, bleaching was carried out by a single clinician operator. Initially, the gingival tissues of the teeth to be bleached were isolated from the bleaching gel using a light-cured resin dam (OpalDam Kit, Ultradent;

Corsico- MI, Italy). Next, a 38% ready-to-use HP-containing gel (The Smile® Strong, Italy) was applied from the canine to the canine tooth of both jaws for a total period of 20 minutes for a 1 single whitening session, according to the manufacturer's instructions. After the bleaching treatment, the gel was rinsed off, and the participants underwent two different treatments for each of the 2 study groups:

LLLT group (n=30): the whitened teeth received an irradiation treatment utilising a diode laser (Soft Touch; 810 nm, 5 W, Creation). The laser probe (a fibre of 400 μm diameter) was positioned in contact mode with the cervical enamel of the tooth with irradiation for 30 sec in continuous-wave using 0.5 W, with horizontal and vertical moving to cover the whole area. As a result, each tooth received an energy density of 15 J/cm2.

Placebo group (n=30): the teeth whitened underwent the same procedure but with the device switched off.

Subsequently, the participants were asked to record the degree of tooth sensitivity perceived at 1 h, from 1 to 24 h, and from 24 to 48 h at the end of the bleaching treatment. A visual analogue scale (VAS) was employed to objectify the degree of pain intensity, consisting of a 100-mm horizontal line with 0-10 points where the ends were represented from 0 (the left side), indicating no pain and 10 (the right side) representing the worst possible pain never warned. The participant and the clinician operator who collected the VAS data were blinded to the assignment groups. During this research, all operators wore surgical masks to prevent the spread of respiratory system viruses and maintain office hygiene (20, 21).

Statistical analysis

The total VAS scores obtained at each measurement point for each study group were considered for the statistical analysis. The VAS values were compared between the different evaluation times in each group and between the single values of each study group. The statistical analysis was performed with GraphPad Prism version 9 (by Dotmatics, San Diego, CA, USA). The significance level for all tests was predetermined at $p < 0.05$.

RESULTS

All 60 participants completed the study. The mean age of the patients was similar in the study groups (LLLT group 26.4 ± 6.8 and placebo group 27.8 ± 9.2), and there were no significant gender differences among the groups (LLLT 18 females and placebo 16 females) ($p = 0.854$). Table I shows the value VAS (total, mean and standard deviation, SD) for each study group at different time points during the clinical trial. The comparison of the VAS scores at different time points for each participant belonging to the same group revealed statistically significant differences with $p < 0.0001$ using the Wilcoxon signed-rank test comparing A *vs* C and B *vs* C for the LLLT group and A *vs* B and B *vs* C for the placebo group, while it was reported no statistically significant differences after comparing A *vs* B of LLLT group and A *vs* C of the placebo group, with $p= 0.2885$ and $p= 0.1009$, respectively. Fig. 1 shows the changes in dental sensitivity reported

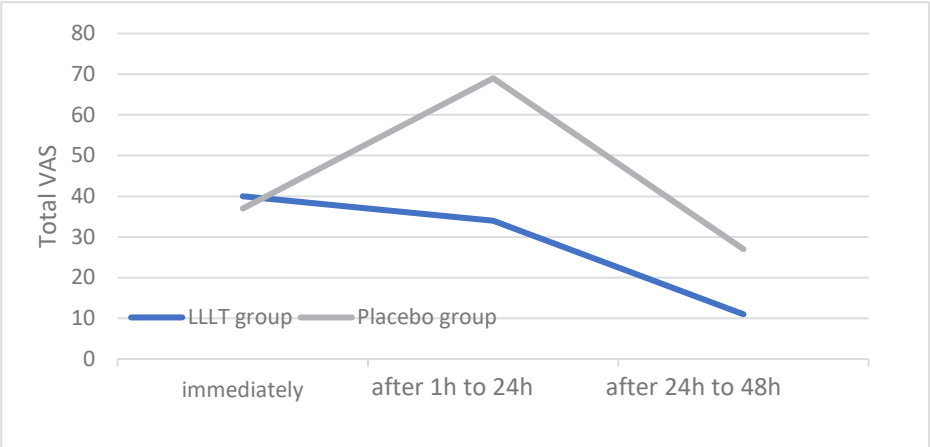

Fig. 1. *Comparison of total VAS from total VAS during the observation period.*

by patients of both groups during the entire observation period. The unpaired t-test was used to compare pain intensity reported by each subject at different time points for each study group. The results showed no statistical differences in the intensity of tooth sensitivity among groups within the first 1 h after bleaching (p = 0.593). In contrast, significance was highlighted between both group differences at 24 h (p< 0.0001) and 48 h (p <0.0001) after whitening treatment (Table I).

DISCUSSION

This study determined the effects of LLLT on the decrease of tooth sensitivity related to in-office bleaching. Most sensitivity complaints were reported within the first 24 h after bleaching, in agreement with the results from other authors (22-26). Both groups recorded tooth sensitivity within 1 hour immediately after whitening with overlapping values (total VAS score 40 and 37 for LLLT and placebo group, respectively, with p= 0.593). These results indicate that LLLT cannot reduce the incidence of pain and discomfort for tooth sensitivity after in-office bleaching from immediately to 1 h post-bleaching. No significant differences were found among the groups, implying that LLLT had no immediate effects on reducing pain and discomfort perceived by the patients. Within 24 h after bleaching, pain level increased in the placebo group (total VAS of 69) while it decreased in the individuals from the LLLT group (total VAS of 34); this means that the laser treatment can reduce the painful experiences of dental sensitivity that are statistically reported in patients within the 24 hours post whitening 8 (p <0.0001). The intensity of referred tooth sensitivity at 48 h after in-office bleaching was slight for both groups but especially for participants of the LLLT group (total VAS of 11 and 27, respectively, for LLLT and placebo groups, p<0.0001). Finally, the intensity of the dental sensitivity returns to the baseline values for the LLLT group, while it remains above the initial values in the placebo group from 24 h to 48 h after whitening. Thus, we can confirm that the use of LLLT can limit tooth sensitivity, reported as pain/discomfort, using a VAS score, in patients from 1 hour up to 48 hours post-in-office whitening, while it does not have any effect on the symptoms reported by patients within 1 hour after the item procedure. These findings indicate that LLLT should be considered an effective strategy for alleviating pain and discomfort after in-office bleaching procedures. Since the maximal pain level generally occurs within 24 h after bleaching, irradiation with LLLT can be included in the current strategies. When pain degree at different measurement points was compared within each group, the intensity of tooth sensitivity in the placebo group reached the peak value at 24 h after bleaching (total VAS of 69) and reduced after that (p <0.0001). In the placebo group, the total VAS score at 48 h was comparable to that recorded at 1 h after bleaching (total VAS score 27 vs 37 with p= 0.1009), while the laser therapy in the LLLT group was effective, reducing the pain level at 48 h in comparison with that of the 1 h interval (total VAS score 11 *vs* 40 with p <0.0001). On the contrary, patients in the laser group perceived higher tooth sensitivity immediately after treatment, although they experienced a progressive reduction of tooth sensitivity throughout the treatment protocol.

To determine the effectiveness of LLLT on the potential onset of pain derived from tooth sensitivity was necessary to include a placebo group. The assignment of a placebo group is necessary when assessing the analgesic effects of LLLT; this is essential to consider the influence on pain relief of the psychological impact of a treatment that utilises a high-technology apparatus; for this reason, a control group without laser and placebo was not inserted (27, 28). The search shows a significant improvement in tooth sensitivity in subjects belonging to the placebo group over the study period. This trend represents a custom and should be considered since post-bleaching sensitivity has a limited duration and, in most cases,

Table I. *VAS scores (mm) with means and standard deviation (SD) for each study group at different time points.*

Groups	Immediately to 1h			After 1h to 24h			After 24h to 48 h		
VAS	Total	Mean	SD	Total	Mean	SD	Total	Mean	SD
LLLT	**40**(a)	1.3	0.76	**34**(b)	1.3	0.50	**11**(c)	0.37	0.49
Placebo	**37**(a)	1.23	0.68	**69**(b)	2.3	0.75	**27**(c)	0.9	0.66
	p= 0.5925			p< 0.0001			p< 0.0001		

P value for LLLT group: a vs b (p= 0.2885); a vs c (p < 0.0001); b vs c (p< 0.0001)
P value for placebo group: a vs b (p= < 0.0001); a vs c (p= 0,1009); b vs c (p< 0.0001)

it does not last more than 2 days after in-office bleaching. The efficacy of LLLT in reducing post-bleaching sensitivity could be assigned to the bio-stimulating effects, analgesic, and anti-inflammatory effects of low-power lasers (28). Laser therapy may recover the cell damage and modulate the inflammatory process induced by HP products in the pulp tissue, reducing the nerve transmission of pain for depolarisation of the membrane of the nerve endings and suppressing the passage of neurosensory impulses. These effects probably occur after several hours after laser treatment. The difference in pain scores (VAS) among the study groups was not significant within 1 h after bleaching. The effectiveness of the diode laser 810 nm in reducing post-bleaching sensitivity could be related to its ability to penetrate in depth, reaching the pulp chamber (29-32). The energy density employed in this study for a diode laser treatment was 15 J/cm2.

There are few studies regarding the influence of LLLT on the viability of cells exposed to bleaching agents, with controversial outcomes. Dantas et al. (33) indicated that irradiation by a low-power 780 nm laser at an energy density of 10 J/cm2 compensated for the cytotoxic effects of 35% HP on human pulp fibroblasts. Pereira et al. (34) showed that LLLT influenced the behaviour of odontoblast-like cells by irradiating with a diode laser 830 nm with an energy density of 85 mW/cm2 of for 10 sec 0.8 J/cm2, thus promoting the expression of the odontoblastic phenotype in a more significant way compared to longer time/highest laser energy density. On the contrary, Lima et al. (35) concluded that both HP and carbamide peroxide reduced the cell activity of odontoblasts and their injurious properties, whose effects could not be offset by LLLT at the defined parameters. Few studies have performed randomised clinical trials investigating the effects of LLLT on reducing post-bleaching sensitivity. However, several studies focused on the effectiveness of diode LLLT on dentine hypersensitivity by non-carious cervical lesions and on pain deep of cavity preparations post-dental restoration. Therefore, a direct comparison of our outcomes with previous results from other authors is impossible. There are conflicting reports regarding the effectiveness of LLLT in alleviating dentin hypersensitivity in patients with non-carious cervical lesions or deep from cavity preparations (36, 37), even if the mechanism of tooth sensitivity after in-office bleaching is assumed to be different from that of dentin hypersensitivity. The tooth sensitivity post-bleaching could be related to inflammatory mechanisms directed to the dental pulp and not to the exposure of the dentinal tubules. The limitation of this study was the subjective nature of VAS questionnaires, the difference in pain thresholds of the subjects and, above all, the impossibility to standardise a home technique to create a standard stimulus that could stimulate the tooth sensitivity symptoms (38-40).

The present study indicated that irradiation from a low-power diode laser could effectively reduce the intensity of tooth sensitivity after in-office bleaching; this makes it a viable alternative to conventional methods for controlling post-bleaching sensitivity. However, further studies with a larger patient group will be required to evaluate different parameters both for applied energy power and for the activity times to reduce dental sensitivity after in-office bleaching, which will allow comparing these results with those obtained with other agents used for counteracting dental sensitivity.

The use of diode LLLT cannot reduce the intensity of tooth sensitivity referred immediately to 1 h after in-office bleaching, while it is a valuable aid to decrease pain sensations reported by the patients from 1 to 48 h after application.

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