

Original Article

ELECTROMYOGRAPHIC ANALYSIS OF PATIENTS DURING ORTHODONTIC TREATMENT WITH F22 ALIGNERS

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ABSTRACT

The aim of this research paper is to investigate the electromyographic behavior of the elevator, masseter and temporal muscles during the orthodontic treatment with aligners (F22). The subjects were 8 healthy adult patients between 20-40 years of age. EMG carried out at the beginning of the therapy (T0) and after each month (T1, T2, T3) with and without aligners (F22). In the statistical analysis, for each measure, a repeated measures ANOVA with post-hoc tests was used. The post-hoc tests show that the impact of the appliance is significant only at time T0. After an initial elevation, a subsequent lowering of the electromyographic values of the muscles Masseter and Temporal in patients wearing aligners (with F22 systematics) could suggest an adaptation of the entire stomatognathic system.

KEYWORDS: *clear aligners, F22, electromyography, EMG*

INTRODUCTION

Electromyography is a technique that deals with capturing, measuring and analyzing an electrical signal made by the muscles, in unfavorable conditions. The reference measurement inherent to this muscle “discharge” is expressed in microvolts (μ Volts) of very low intensity (1). Electromyography arises between biology and physics, and only the technological development of recent years has allowed it to become a discipline capable of contributing fully to the knowledge of neuromuscular physiology, so as to provide a valid aid for the diagnosis of numerous pathologies of nerves and muscles (2).

The theory of Neuromuscular Occlusion recognizes the role of muscles as *primum movens* in the functional physiology of the stomatognathic system, influences the muscle heads and actively uses TENS (Transcutaneous Electrical Nerve Stimulation) (3), in 1969 B. Jankelson began to use the patient’s muscles to be pointed the physiological centric position. In 1975, with the mandibular kinesiograph, he began to measure the different mandibular positions for the different functions and resting positions before and after TENS (4). The development of this tool further modified the diagnostic protocol developed by Jankelson over the years (5, 6).

Some studies (7-9) have shown that the use of these devices can reduce parafunctional activity, which often appears in times of stress. There is also a disparity of views regarding the influence of the type of material (rigid or resilient) on the neuromuscular response of individual patients (10,11).

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In the literature, studies have been reported to date concerning the muscular response of patients undergoing therapies carried out with rigid splints, or with disclusion plates, in any case devices that do not have a selectively orthodontic effect, but only of partial coverage or of the entire dental arch. As there is an increasing interest about esthetics and the use of these methods even in challenging cases (12-14), we tried to evaluate the behavior of these muscles during the first stages of orthodontic treatment with aligners (F22).

MATERIALS AND METHODS

From examining 8 patients from the Clinic of the School of Specialization in Orthodontics of the University of Ferrara, all adults, aged between 20 and 40 years, with the absence of periodontal, dental and joint temporomandibular and / or muscular pathologies in progress. Were used: K7 kinesiographic assessment system, produced by MYO-TRONICS inc., eight-channel electromyographic evaluation MYO-TRONICS inc., DUOTRODES dedicated bipolar electrodes, in silver/chloride for electromyography, pre-coated with gel, Aligners F22. Each session included 6 measurements (scans) of 15 seconds each, particularly 3 scans with aligners worn and 3 scans without. A statistical analysis was then performed on a data set consisting of 8 on which four electromyographic measurements relating to individual muscle were measured over four times (0, 1, 2, 3), Left Anterior Temporal Muscle (LTA), Temporal Right Anterior (RTA), Left Masseter (LMM) and Right Masseter (RMM), with or without the presence of appliance (NO / YES).

The analysis aims to verify whether and how the variations in measurements with or without a device change over time. To carry out the statistical analysis, a repeated measures ANOVA with post-hoc tests was used for each measure. Furthermore, the statistical software R (R Core Team 2016) and the packages lsmeans (Lenth 2016) and nlme (Pinheiro et al. 2016) were used. The reference significance thresholds used are the following: weak (p-value between 10% and 5%), standard (p-value between 5% and 1%), strong (p-value less than 0.001).

For each measure, the following are reported: the descriptive statistics (number of observations, mean, minimum, and maximum standard deviation) by combination of time and device, as well as the graph. The results of the multiple measures ANOVA test indicate, using the p-value, the significance of the time effect means that the measures change over time; appliance effect if generally, the presence (YES) or absence (NO) of the aligner changes the measurement; interaction effect if the appliance effect changes over time. The post hoc analysis means estimation of the YES / NO variation effect of the device on the measurement as time changes and related post-hoc confidence intervals.

RESULTS

Left temporal muscle (LTA) (Table I, II).

Table I. The table shows the statistical variable for the LTA. The average value of 8 patients for the left temporal muscle.

| Muscle | Time of treatment | Wearing aligners | Number of patients | Average | Standard deviation | Min | Max |
|--------|-------------------|------------------|--------------------|---------|--------------------|-----|------|
| LTA | 0 | NO | 8 | 3.6 | 1.7 | 1.5 | 7.1 |
| LTA | 0 | YES | 8 | 4.7 | 2.6 | 2.0 | 10.7 |
| LTA | 1 | NO | 8 | 3.7 | 1.6 | 1.5 | 7.2 |
| LTA | 1 | YES | 8 | 3.6 | 2.7 | 1.3 | 10.0 |
| LTA | 2 | NO | 8 | 3.7 | 1.7 | 1.4 | 7.3 |
| LTA | 2 | YES | 8 | 3.7 | 3.0 | 1.2 | 11.0 |
| LTA | 3 | NO | 8 | 3.7 | 1.6 | 1.5 | 7.2 |
| LTA | 3 | YES | 8 | 3.4 | 2.2 | 1.2 | 8.6 |

Table II. The post - hoc tests are reported below, showing that the impact of the appliance is only significant at time 0.

| Measure | Time | Difference | Estimate | p-value |
|---------|------|------------|-----------|----------|
| LTA | 0 | NO - YES | -1.15 | 0.002515 |
| LTA | 1 | NO - YES | 0.0625 | 0.8582 |
| LTA | 2 | NO - YES | -0.004167 | 0.9905 |
| LTA | 3 | NO - YES | 0.2458 | 0.4839 |

Left temporal muscle (RTA) (Table III, IV).

Table III. The table shows the statistic variable for the RTA. Average value of 8 patients for right temporal muscle.

| Muscle | Time of treatment | Wearing aligners | Number of patients | Average | Standard deviation | Min | Max |
|--------|-------------------|------------------|--------------------|---------|--------------------|-----|------|
| RTA | 0 | NO | 8 | 4.4 | 2.6 | 1.6 | 8.8 |
| RTA | 0 | YES | 8 | 6.1 | 4.2 | 2.0 | 14.1 |
| RTA | 1 | NO | 8 | 4.4 | 2.7 | 1.6 | 9.3 |
| RTA | 1 | YES | 8 | 4.4 | 3.9 | 1.3 | 13.0 |
| RTA | 2 | NO | 8 | 4.5 | 2.7 | 1.6 | 9.4 |
| RTA | 2 | YES | 8 | 4.4 | 3.8 | 1.3 | 13.1 |
| RTA | 3 | NO | 8 | 4.5 | 2.6 | 1.7 | 9.1 |
| RTA | 3 | YES | 8 | 4.4 | 3.5 | 1.5 | 11.8 |

Table IV. The post-hoc tests are reported below, showing that the impact of the appliance is only significant at time 0.

| Measure | Time | Difference | Estimate | p-value |
|---------|------|------------|----------|-----------|
| RTA | 0 | NO – YES | -1.712 | 0.0008049 |
| RTA | 1 | NO – YES | -25 | 0.9567 |
| RTA | 2 | NO – YES | 0.04167 | 0.9278 |
| RTA | 3 | NO – YES | 0.06667 | 0.8848 |

Left masseter muscle (LMM) (Table V).

Table V. The table shows the statistical variable for the LMM. The average value of 8 patients for the left temporal muscle.

| Muscle | Time of treatment | Wearing aligner | Number of patient | Average | Standard deviation | min | max |
|--------|-------------------|-----------------|-------------------|---------|--------------------|-----|-----|
| LMM | 0 | NO | 8 | 2.8 | 1.3 | 1.7 | 5.9 |
| LMM | 0 | YES | 8 | 3.6 | 2.0 | 2.2 | 8.3 |
| LMM | 1 | NO | 8 | 3.0 | 1.4 | 1.8 | 6.2 |
| LMM | 1 | YES | 8 | 2.9 | 2.1 | 1.5 | 7.9 |
| LMM | 2 | NO | 8 | 3.0 | 1.4 | 1.8 | 6.2 |
| LMM | 2 | YES | 8 | 3.1 | 2.4 | 1.5 | 8.8 |
| LMM | 3 | NO | 8 | 3.2 | 2.1 | 1.8 | 8.1 |
| LMM | 3 | YES | 8 | 3.3 | 2.7 | 1.4 | 9.8 |

The post-hoc tests are reported below, showing that the impact of the appliance is only significant at time 0 LMM, time average variation (Table VI).

Table VI. *Post-hoc tests.*

| Contrast | Estimate | SE | df | t.ratio | p-value |
|----------|----------|-------|----|---------|---------|
| 0 – 1 | 0.5312 | 0.245 | 21 | 2.168 | 0.1648 |
| 0 – 2 | 0.4854 | 0.245 | 21 | 1.981 | 0.2265 |
| 0 – 3 | 0.5979 | 0.245 | 21 | 2.44 | 0.0999 |
| 1 – 2 | -0.04583 | 0.245 | 21 | -0.1871 | 0.9976 |
| 1 – 3 | 0.06667 | 0.245 | 21 | 0.2721 | 0.9927 |
| 2 – 3 | 0.1125 | 0.245 | 21 | 0.4592 | 0.9671 |

Right masseter muscle (RMM) (Table VII).

Table VII. *The table shows the statistical variable for the RMM. Average value of 8 patients for right masseter muscle.*

| Muscle | Time of treatment | Wearing aligners | Number of patients | Average | Standard deviation | Min | Max |
|--------|-------------------|------------------|--------------------|---------|--------------------|-----|------|
| RMM | 0 | NO | 8 | 3.6 | 2.4 | 1.8 | 7.7 |
| RMM | 0 | YES | 8 | 4.7 | 3.5 | 2.3 | 10.7 |
| RMM | 1 | NO | 8 | 3.6 | 2.4 | 1.7 | 8.0 |
| RMM | 1 | YES | 8 | 3.4 | 3.1 | 1.5 | 10.4 |
| RMM | 2 | NO | 8 | 3.7 | 2.5 | 1.8 | 8.2 |
| RMM | 2 | YES | 8 | 3.6 | 3.4 | 1.4 | 11.3 |
| RMM | 3 | NO | 8 | 3.7 | 2.4 | 1.8 | 8.0 |
| RMM | 3 | YES | 8 | 3.6 | 3.3 | 1.4 | 11.1 |

The post-hoc tests are reported below, showing that the impact of the appliance is only significant at time 0 of RMM (Table VIII).

Table VIII. *Post-hoc tests.*

| Measure | Time | Difference | Estimate | p-value |
|---------|------|------------|----------|----------|
| RMM | 0 | NO - YES | -1.117 | 0.007261 |
| RMM | 1 | NO - YES | 0.1667 | 0.6689 |
| RMM | 2 | NO - YES | 0.05417 | 0.8893 |
| RMM | 3 | NO - YES | 0.02083 | 0.9573 |

DISCUSSION

At the beginning of the orthodontic treatment with aligners (F22), there was an increase in electromyographic values: at the beginning of therapy with aligners (T0), in the condition of the appliance worn (values in microvolts rise, from “Wearing aligner” NO to “Wearing aligner” YES, in T0). In the subsequent phases of therapy (T1, T2 and T3), there was a lowering of the electromyographic values, wearing the aligners (values that drop from “Wearing device” NO to “Wearing device” YES, in T1, T2 and T3).

In the past, other authors have investigated the electromyographic behavior of the mandibular muscles, with particular reference to the Masseter and Temporal muscles. The results of these investigations essentially indicate that a skeletal expansion in the transverse plane of the maxilla generally causes an increase in the electromyographic activity of the muscles mentioned above (15,16), while we note that there are different results in studies that consider the trend of these electromyographic values measured in growing patients treated with functional devices.

CONCLUSIONS

The present research wanted to highlight, within the proposed limits, after an initial elevation, a subsequent lowering of the electromyographic values of the muscles Masseter and Temporal in patients wearing aligners (with F22 systematics).

The very stable electromyographic picture in the months following the start of therapy, could suggest an adaptation of the entire stomatognathic system.

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Original Article

EVALUATION OF LONG-TERM STABILITY OF EDGEWISE ORTHODONTIC TECHNIQUE

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ABSTRACT

The relapse of teeth after orthodontic treatment has been a topic of ongoing interest for practitioners. The instability of the orthodontic treatment depends on different variables, such as the normal developmental maturation process of the maxillofacial complex. It is common to use post-treatment retentions to avoid a relapse, permanent or removable, depending on the previous malocclusion. The casts of 15 subjects treated with the edgewise technique between 1985 and 2000 were selected to evaluate dental arch changes among 17 years post-treatment. Time points were pre-treatment (T1), post-treatment (T2) and follow-up (T3). The parameters considered were intercanine, interpremolar and intermolar width, dental arch length and crowding. The collected data were submitted for statistical analysis. The intercanine width outcome is the most significant, with the greater changes observed during the treatment among T1 and T2, maintained over time as the difference between T3 and T1 is statistically significant. The other parameter values were not statistically significant. Within the field of the edgewise technique, the intercanine width outcome is the most significant: greater changes from the pre-treatment to the post-treatment condition led to more significant relapse.

KEYWORDS: *relapse, stability, edgewise technique, intercanine width*

INTRODUCTION

The achievement of a good result was investigated over time and led to an improvement in materials, procedures, vestibular fixed appliance technique, and more esthetic treatments like aligners (1) and lingual orthodontics; this made possible even complex cases' treatments (2). However, a stable result over time is today a real challenge for the entire professional category. Therefore, different systems that could lead to the required results have been tested during these years, even applying light and continuous forces (3, 4).

Considering that less than 30% of treatment presents a satisfactory clinical alignment after 10 years from the end of the therapy, the percentage decreases to 10% after 20 years (5). Relapse after orthodontic treatment is defined as an undesired resurgence of a previously adjusted malocclusion, and this has been a topic of ongoing interest throughout most of this century (6, 7). Nowadays, we can relate several causes for relapsing: the skeletal growth (8-11), the neuromuscular forces (12, 13), the inferior incisors inclination variation (14), the variation of the inter canine's inferior width and the third molar eruption (7, 15, 16). In addition, it has been proved that the whole maxillo-facial complex is subject to physiological changes. Development of the arches affects intercanine and intermolar width, dental arch length (17-19)

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and crowding (20, 21). Several types of permanent or removable systems are used to avoid relapse risk. Depending on the previous malocclusion, they can be positioned in the lower arch, the upper arch, or both (22). Finally, the purpose of this study was to evaluate the stability of edgewise treatments over time through the examination of the changes in both arches over 17 years from the end of the treatment.

MATERIALS AND METHODS

Patient sample

For this study, 15 subjects treated with edgewise technique between 1985 and 2000 were selected from doctors Calderone's storage in Palermo and Cefalù (PA) in 2017. The inclusion criteria were:

- presence of pre-treatment (T1) and post-treatment (2) dental casts in optimal condition;
- possibility for the patient to come for dental impressions;
- arches conditions not affected by trauma or periodontal disease;
- post-treatment retention suspended for 3 years at least.

Of those patients, 12 females and 3 males were treated between 12-35 years old. In addition, 8 cases were treated with extractions and 7 without them. Regarding Angle's classification, 7 presented a bilateral class I, 6 presented a bilateral class II and 2 a bilateral class III. However, the sample is not homogeneous for malocclusion variability due to the complexity of recovering long-time records, the current presence of retention and the unavailability of patients to come for dental impressions after 17 years.

Analysis of dental casts

Patients came for alginate dental impressions. Casts obtained (T3), along with pre-treatment (T1) and post-treatment (T2) casts, were scanned with Sirona inEos X5. For every couple of casts, .stl files were obtained and used for software 3Shape Orthoanalyzer measurement. The focus was on intercanine, interpremolar and intermolar width, dental arch length and crowding. Also, overjet and overbite were calculated from casts in occlusion.

Statistical analysis

The collected data were submitted to statistical analysis with average values and standard deviation. Fisher's F ANOVA test was used to compare these values for every parameter of the three different time points. The level of statistical significance was predetermined as $p < 0.05$. Finally, Bonferroni's post-hoc test was used to verify where were located the significant differences among average group values.

RESULTS

Upper intercanine width

Fig. 1 shows that the upper intercanine width average values increase along the second measurement and decrease along the third measurement. Blue circles represent average values that must be compared with those displayed on the left. Vertical lines represent the standard deviation. From Bonferroni's test, the ICD average upper at T2 (35.2) is significantly higher ($p = 0.001258$) than the ICD upper average at T1 (33.2). Unlike this, there is no significant difference between the ICD upper average at T2 and T3.

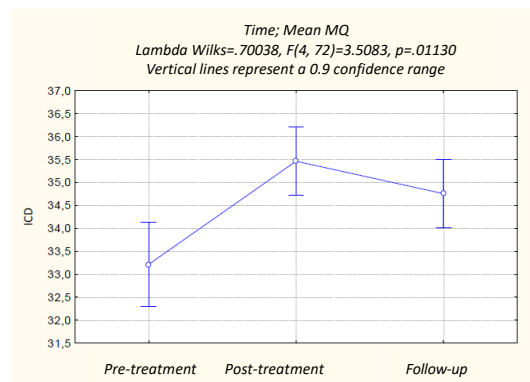


Fig. 1. Upper intercanine width ICD average values comparison.

Lower intercanine width

Fig. 2 shows that the average values increase along the second measurement and decrease along the third. The ICD lower average at T2 (27.0) is significantly higher ($p=0,001914$) than the ICD average at T1 (25.2). Otherwise, there is no statistically significant difference between the ICD lower average at T2 and T3, as there is no significant difference between the ICD average at T1 and T3.

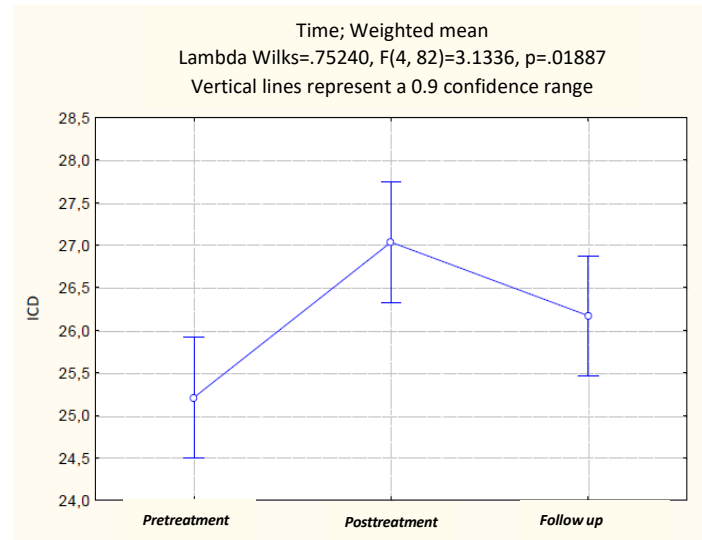


Fig. 2. Lower intercanine width ICD average values comparison.

Upper interpremolar width

The IPD upper average values increase from the T1 time point to T2 and decrease from T2 to T3. However, no significant differences were observed among the three time points ($p > 0.05$).

Lower interpremolar width

The IPD lower average values increase from T1 time point to T2 and decrease from T2 to T3. No significant differences were observed among the three time points ($p > 0.05$).

Upper intermolar width

The IMD upper average values increase from T1 to T2 and decrease from T2 to T3. No significant differences were observed among the three time points ($p > 0.05$).

Lower intermolar width

The IMD lower average values increase from T1 to T2 and decrease from T2 to T3. However, no significant differences were observed among the three time points ($p > 0.05$).

Dental arch length

The AL upper average values decrease from T1 to T2 but stay unvaried from T2 to T3; no significant differences were observed among the three time points ($p > 0.05$). The AL lower average values decrease from T1 to T2 but stay unvaried from T2 to T3; however, no significant differences were observed among the three time points ($p > 0.05$).

Upper crowding

The average values at T2 are significantly higher ($p=0,009958$) than the T1 average. Otherwise, there is no significant difference between T1, T2 and T3 values; likewise, there is no significant difference between the T1 and T3 average values.

Lower crowding

The average values at T2 are significantly higher than those at T1. However, there is no significant difference between T2 and T3 average values, likewise between T1 and T3 average values. For what concerns *Overjet* and *Overbite* analysis, obtained results are not statistically significant.

DISCUSSION

Based on the results, intercanine width outcomes are the most significant, with the greater changes observed during the treatment among T1 and T2. These are maintained over time due to the statistical difference between T3 and T1. Lower intercanine width changed mainly during treatment, while changes due to the relapse were not considerable. In the same way, upper and lower crowding average values suggest that the main change was during treatment, without relevant results about relapse. Regarding the other parameters analyzed, the interpremolar and intermolar width results were not statistically significant. Both outcomes showed an increase from T1 to T2 and a slight decrease from T2 to T3. Both upper and lower dental arch length tends to decrease from T1 to T2 but remains stable from T2 to T3. At last, overjet and overbite outcomes contrast with previous studies due to the variety of the sample.

Obtained results are, therefore, comparable to the primary studies carried out by Little at Washington University (6). It is interesting to notice that there are no significant differences for every parameter between the end of the therapy (T2) and the control over time (T3). The analyzed cases present excellent stability over time, proven by the 17 years of follow-up with no retention for at least 3 years. Tweed's edgewise technique can be important in achieving such a treatment goal. The diagnosis is obtained through the characteristic diagnostic triangle, and the therapy is carried out respecting the anterior limit of the dentition and without manipulating the occlusal plane inclination.

Nowadays, the innovation and improvement of the techniques applied have brought the possibility to improve the quality of the treatment and many clinical advantages, such as reduced chair time, less compliance requirement, less discomfort for the patient, and a shortened treatment time.

In order to obtain a satisfactory and stable result, it is mandatory to consider the anterior limit of the dentition, as well as the preservation of the patient's initial dental arch width and the achievement of an occlusion balanced with neuromuscular forces. In addition, it is essential to avoid rapid movements that do not allow the reshaping of periodontal fibres, which generally takes 4-6 months. Therefore, the need for a retention phase is considered primary, especially in parafunctional patients with muscle hypertonia.

CONCLUSIONS

This study reveals that, in edgewise treatment, the intercanine width outcome is the most significant; the results show considerable changes from the pre-treatment to the post-treatment condition without a substantial relapse. These findings highlight the importance of the clinical practice of a correct diagnosis to plan a stable treatment in time. The intercanine width expansion, in most cases, leads to a recovery of the preexisting condition, which is why the clinician shall preserve the initial dimensions. Future research should investigate the range in which it is safe to expand without risking relapse, and guidelines should be drawn to help the clinician in the treatment planning.

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Review

CORRELATION BETWEEN ORAL SURGERY AND MANDIBULAR FRACTURES IN OSTEOPOROSIS PATIENTS

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ABSTRACT

The purpose of this study was to investigate how osteoporosis can adversely affect one of the rarest, but now very common complications of surgery on the posterior jaw: the mandibular fracture. The term "mandibular fracture" refers to a broken jaw (mandible), while fractures of the upper jaw are sometimes called "jaw fractures", but are usually considered facial fractures (maxillofacial fracture). The fracture usually causes pain and swelling in the affected area and a feeling of misalignment of the teeth. Often, there is a narrowing of the opening of the mouth and a lateral displacement when opening or closing. The authors examined the literature to provide the scientific community with an etiological overview underlying this complication. Our analysis shows that although there are few articles in the indexed literature, this complication is quite common and often linked to the operator's inexperience but also to the systemic pathological influence of osteoporosis. For this reason, it would seem appropriate to prevent everything by using surgical protocols that reduce jaw fractures by assessing the risk that the disease may involve.

KEYWORDS: *third molar, mandibular fracture, oral surgery, impacted tooth, angle mandibular*

INTRODUCTION

The mandible is the only movable bone in the skull. It is of the dense and hard type and constitutes the lower third of the facial skeleton. Surgical extraction of the lower third molars is one of the most common procedures in oral surgery (1). This surgical procedure may be accompanied by intra- and post-operative complications such as pain, trismus, bleeding, infection, edema, lesions of the inferior alveolar nerve, displacement of teeth into adjacent spaces and mandibular fractures (Fig. 1).

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| The signs and symptoms of a mandibular fracture |
|---|
| Trismus |
| Bleeding from lacerated gingival or mucosal tissue |
| Ecchymosis/hematoma (at the fracture site or the sublingual space) |
| Loose, fractured, or displaced teeth |
| Palpable or visible "step" in the dental arch |
| Inability to chew or subjective (or obvious) altered bite |
| Paresthesia of the lip/chin |
| Lack of motion of the mandibular condyles with palpation through the external auditory canal |
| <i>From Viozzi CF. Maxillofacial and mandibular fractures in sports. Clin Sports Med 2017;36(2):355-68.</i> |

Fig. 1. The signs and symptoms of mandibular fracture.

Osteoporosis is a widespread metabolic disease affecting bone and is characterized by bone density collapse along with microarchitectural failure, leading to bone fragility and exposure to fracture risk. It affects one in three women and one in five men over 50. The female gender demonstrates declining bone loss as early as menopause, predominantly in trabecular bone, followed by slower trabecular and cortical bone loss. The mandible has some weak areas that are less resistant to fractures, such as the mandibular angle, the condyle, the mandibular symphysis, the body, and the coronoid process. The bony anatomy of the gonial angle, with its location between the ascending ramus and the mandibular body, as well as its association with the impaction of the lower third molar, makes it one of the most frequent fracture sites (40%). It is the most frequently fractured bone in the maxillofacial skeleton due to its prominence (2, 3). The jaw commonly fractures in the angle, condyle, and chin region.(4). The horizontal fracture of the mandible (Fig. 2) is very rare, and only a few cases have been reported in the literature (5).

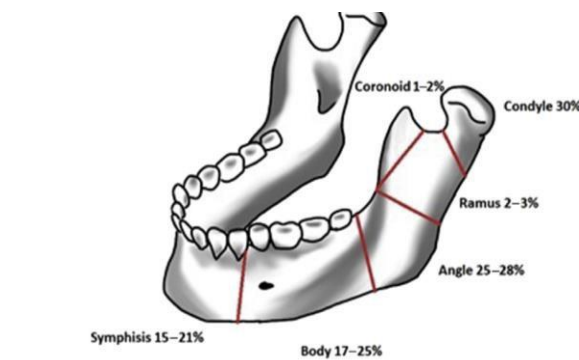


Fig. 2. Distribution of mandibular fractures (Mooney S, Gulati RD, Yusupov S, Butts SC. Mandibular Condylar fractures. *Facial Plast Surg Clin North Am.* 2022 Feb;30(1):85-98. doi: 10.1016/j.fsc.2021.08.007. PMID: 34809889).

The estimated incidence of mandibular fracture is 11.5 cases per 10,000 individuals (6). The mandibular angle is a frequent fracture site, accounting for 25-33% of all mandibular fractures.

In the literature, several variables influence the fracture: for example, the anatomical bone component, the masticatory forces, and the different dental occlusal loads.

A not very recent but quite simplistic study by Joshi et al. (7) reports that mandibular fractures are more frequent in regions where teeth are present rather than in the edentulous areas of the mandible. Bones often fracture at stress and tensile stress sites because their resistance to compressive forces is greater. Furthermore, Bodner et al. showed that the isolated mandible is subjected to non-equivalent tensile stress diffusion patterns when perpendicular forces are exerted on it (8).

MATERIALS AND METHODS

This study followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Fig. 3). The main research question was captured in the PICO (Population, Intervention, Comparison, Outcomes) format: "Can a less invasive operation during oral surgery in the jaw reduce the risk of fractures?"

The search strategy involved searching electronic databases: the PubMed (National Library of Medicine), Google Scholar, Scopus, Embase, Medline, and Cochrane Library databases were searched without time or language restriction to find articles describing the basic principles of HRW and its applications in dental practice (Fig. 3).

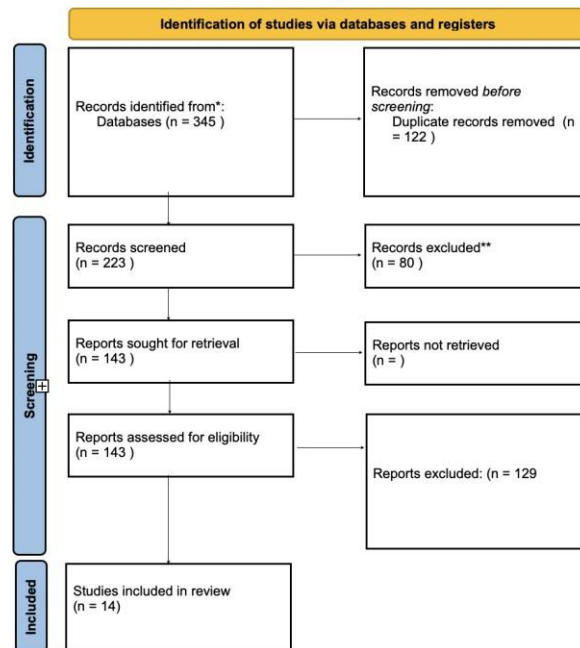


Fig. 3. Search strategy flow chart.

All studies reviewed were published between January 1, 2000, and December 30, 2022. The search strategy used a combination of different MESH terms and keywords on the six databases: “mandibular surgery”, “mandibular fractures”, “impacted tooth”, “bone fracture”, “mandibular cyst”, “wisdom tooth”, “osteoporosis”, “treatment” and “dysodontiasis”; the additional filter “Language: English” was used. The eligibility criteria for the following review include observational studies on patients after dental and cystic-type oral surgery in the mandible, iatrogenic fractures, traumatic fractures, all LeFort types, reviews, systematic reviews with or without meta-analysis, retrospective studies, RCTs, and case reports.

Studies on animal models, 3D models, and letters were excluded. The search strategy identified 345 references published between 2000 and 2022; 143 references were selected for eligibility, and only 14 were included in this review because they met the eligibility criteria.

RESULTS

Mandibular fracture and wisdom teeth surgery

In this review, the risk of mandibular fracture during the surgical procedure of extraction of the included eighths was analyzed, sometimes, although rare, as a post-operative complication. It is strictly necessary to include this complication in the pre-surgical informed consent to be submitted to patients by clearly explaining this eventuality. A study by Libersa et al. showed that intraoperative or post-operative mandible fracture incidence was reported to be 0.0049% (9). Osborn et al. unveiled in a major retrospective study the rate of intraoperative mandibular fracture is 1 in 30,583 patients, while the post-operative rate is 1 in 23,714 (10). Kunkel et al., along with other colleagues, showed in a review that mandibular fracture has an incidence of 1 in 29,000 cases (11).

Possible predisposing conditions were traced in this review: certainly advanced age of the patient, the presence of mandibular atrophic conditions, and patient-dependent systemic problems such as stages of medium to severe osteoporosis. The mandibular region is also significant in the risk of fracture during extraction of included or semi-erupted eighths. For example, the retromolar region is an area of lower resistance to fracture because it is thin in cross-section. In that area, the presence of an included and mesioverted tooth occupies a relatively significant space within the bone. All the more reason if we were faced with an osteoporotic bone with extractive surgical need that would involve removal of the surrounding bone to mobilize it. All this weakens this area mechanically. Studies by Hino et al. (12) retrospectively evaluated the clinical and radiographic data of 12 patients with 13 mandibular fractures after wisdom teeth removal. It

was observed that patients older than 30-40 years with tooth roots overlapping the lower alveolar canal or adjacent to the canal had a high risk of mandibular fracture. There were few intraoperative fractures, while slightly more late fractures, which occurred on average 6.6 days after surgery exclusively during chewing. Libersa et al. (9) evaluated 37 fractures in 750,000 extractions in a retrospective study and identified 17 intraoperative fractures and 10 late fractures. Of the 10 late fractures, 8 occurred in men and 6 occurred during mastication. Most of the late fractures occurred between 13 and 21 days after surgery, possibly caused by increased masticatory function and occlusal forces exceeding bone healing.

Differently, Krimmel and Reinert (13) retrospectively analyzed six patients who suffered a mandibular fracture following removal of a third molar. They showed that fractures occurred from 5 to 28 days (with an average of 14 days) after tooth removal. The patients ranged in age from 42 to 50 years and all had complete dentition. The authors of that study concluded that the main risk factor for mandibular fracture appears to have been the patient's advanced age in combination with a full dentition present in the arch.

Regardless of the mechanism, it was found that mandibular fractures occurring during or immediately after extraction of a mandibular third molar are not displaced. They generally radiate from an extraction site toward the weakest point.

In addition, the above review showed that the side of the fracture is less discussed as a risk factor. Wagner et al. (14) noted that fractures on the left side accounted for 70 percent of cases because right-handed surgeons have a better view of the right surgical field, which results in a less extensive osteotomy. Regarding angulation, the dystangular position is generally considered the most difficult (15). This is probably because mesioangular and vertical angulations are generally more common in patients.

In terms of the position of the impacted tooth, they were found to have higher incidences of mandibular fracture than the upper jaw. This is probably related to a higher degree of difficulty in extraction and more extensive bone removal (16). This review found a higher incidence of mandibular fracture for fully impacted teeth (64.8%) than for partially impacted teeth. This is because when the tooth is fully included in the bone you require more osteotomy access and bone removal for extraction. Post-operatively, this results in less cortical bone remaining and thus a more fragile mandibular angle, which can be an important causative factor for late fracture.

Another retrospective study found a relationship between pericoronitis cases on semi-included eighths (68.3%) and the incidence of late mandibular fracture (17). Recurrent infections may contribute to decalcification and thus to an increased likelihood of late fracture. Although the results are confounding enough to make a connection with mandibular fractures, clearer retrospective data would be needed. This review also observed how mandibular bone quality and density may affect fracture risk.

Pires et al. (18) showed that the period of greatest risk is the second and third post-operative weeks; what happens is that the newly formed granulation tissue in the postextraction socket is replaced by connective tissue and the strength of mandibular bone decreases during this period.

According to Bodner et al. (8), a delay in bone maturation during the regeneration period predisposes to weakening because two-thirds of the socket is not filled with osteoid material. Thus causing a decrease in mandibular bone strength.

Osteoporosis in elderly patients may be another highly predisposing reason. A major study (19) showed that elderly patients had thinning of the periodontal ligament and thus dental ankylosis, increasing the degree of extraction difficulty. All this leads to a significant need for osteotomies that facilitate the likelihood of a possible fracture.

Mandibular fracture and osteoporosis

Osteoporosis is a widespread metabolic disease affecting bone, is characterized by bone density collapse along with microarchitectural failure leading to bone fragility and exposure to fracture risk (20). It affects one in three women and one in five men over the age of 50. The female sex demonstrates declining bone loss as early as menopause, predominantly in trabecular bone, which is then followed by slower loss of trabecular and cortical bone (21).

Because osteoporotic fractures represent a worldwide health burden, it is important to prevent them. Recently, the literature has focused on the morphology of the inner part of the mandibular cortex below the mental foramen. Since then, and to date, several studies have demonstrated the usefulness of the mandibular cortical index as a predictive indication of osteoporosis (22).

Taguchi et al. suggested that bone findings of mandibular morphology would be useful in screening patients with osteoporosis in postmenopausal women (23). On the other hand, Yamada et al. reported in a study of 1021 Japanese men and women found that the mandibular cortical index was useful in identifying dental patients with osteoporosis, but not those with osteoporotic fractures (20).

According to Perry and Goldberg (24), the risk of mandibular fracture during lower third molar extraction in patients with osteoporosis is due to the creation of a bone area with a weakened structure that makes this type of complication more likely to occur. These changes may cause significant weakening of bone, particularly in the mandibular angle region. Therefore, it can be concluded that there is a relationship between the presence of pathological bone changes and the subsequent occurrence of fractures. Joshi et al. (7) pointed out the possibility that post-operative fractures may be incomplete intraoperative fractures, which may have exceeded stress tolerance limits in the weeks following extraction, as patients felt better and pain symptoms had almost disappeared by the end of the following week.

Recent pilot studies have shown that only alendronate and zoledronate have been shown to be less incident in the risk of jaw fractures (25, 26).

Risedronate has been shown to be more incident in susceptibility to mandibular fractures, McClung et al. shows in a specific study of elderly patients with osteoporosis diagnosed on the basis of bone mineral density rather than risk factors (27).

Some studies indicate that cranio-maxillofacial trauma in elderly women with osteoporosis is associated with falls. A recent systemic analysis showed that the number of maxillofacial fractures sustained in a series of 59 subjects older than 60 years was significantly related to the severity of osteoporosis as determined by a radiographic index of vertebral bone density. This association held for low-energy falls and motor vehicle accidents, observations taken as evidence of maxillofacial bone fragility in osteoporotic subjects (28). An analysis of 355 postmenopausal women showed that osteoporosis per se is associated with cranio-mandibular dysfunction (29). Some information is available on the risks of surgical failure in TMJ patients. A study of outcomes in a series of subjects undergoing arthroplasty or discectomy indicated that osteoporosis was the most significant risk factor for technical failure (30). Although mechanisms were neither indicated nor suggested by this study, it is clear that systemic factors must be considered in surgical planning. Although osteoporosis is a very common condition with a sometimes silent sometimes aggressive course, to date the literature does not present crisp guidelines to be followed to prevent this complication. More studies in this regard would be needed to have more scientific evidence

Treatment of mandibular fractures

The vast majority of mandibular fractures require surgical stabilization in order to obtain healing and correct occlusion which is lost after the trauma. In cases of a non-displaced fracture without obvious mobility on manual physical examination, a soft diet for 4 to 6 weeks is generally recommended (31). Displaced fractures or fractures that demonstrate mobility on physical examination are different. In this case, in fact, immobilization of the mandible is expected. Although mandibular fractures with good dentition on both sides of the fracture line can in some cases be treated with a period of intermaxillary fixation, most surgeons and patients prefer open reduction and internal fixation, which allows for a much quicker return to full pre-injury function and mobility (32). Patient demographics, comorbidities, dentition, and fracture characterization influence the treating surgeon's choice of fixation. Internal fixation for mandibular fracture can be divided into two categories: weight-bearing fixation and weight-bearing fixation denote a structure capable of withstanding all the load generated by the mandibular function (33). Typically, this requires the application of a large reconstructive plate to the lower margin of the mandible (Fig. 4).

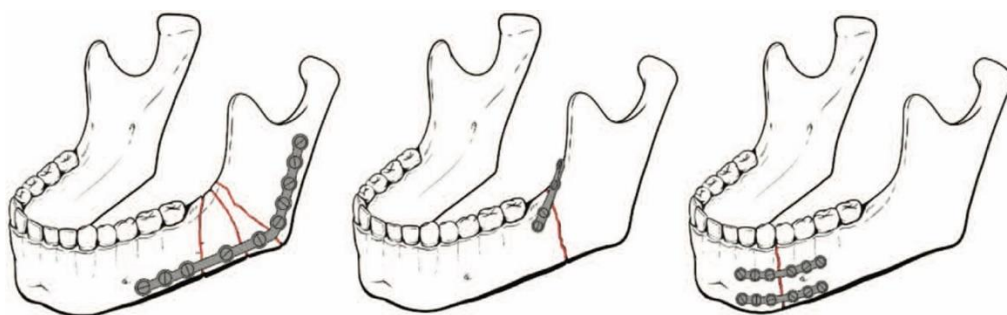


Fig. 4. *Different types of mandibular fixation (Rzewuska A, Kijak E, Halczy-Kowalik L. Rehabilitation in the treatment of mandibular condyle fractures. Dent Med Probl. 2021 Jan-Mar;58(1):89-96. doi: 10.17219/dmp/128092. PMID: 33847468).*

Other studies have demonstrated that load-sharing fixation characterizes a fixation scheme in which the functional load is shared between the base of fixation and the long fracture margin (34). Load-sharing fixation can be divided into rigid and non-rigid (functionally stable). In the past, surgeons often referred to plates by the size of the outer diameter of the screw used in the plate (e.g., 2.0 mm plate, 2.4 mm plate). Today, new reviews have highlighted the importance of more complex but more predictable plate systems (35, 36).

Ellis et al., in a randomized study, demonstrated that the vast majority of unilateral mandibular fractures with good occlusion can be treated in a closed manner (35).

Differently, the situation is not so clear for bilateral fractures (37). This review considered the management of atrophic jaw fractures and how the literature expresses itself on the matter. Today, the most common form with the best results is the application of plates and lathes, so blocked systems are the first option. In the atrophic mandible, factors are considered that decrease the possibilities of consolidation of the low contact surface, poor vascular port, and patient morbidity (38).

DISCUSSION

The results obtained from this review lead to the rationale that the risk of post-extraction mandibular fracture is mainly linked to excessive osteotomy and/or local alterations. Patients at risk must be carefully informed about the importance of food choices in the post-operative period. Finally, the nonsurgical treatment plan appears to be the most suitable approach to nondisplaced fractures for cooperative patients. Technique comparisons addressed general aspects of surgical procedures for mandibular third molars, including the type of raised surgical flap, use of retractors, bone removal techniques, wound irrigation, wound closure, wound drainage, and complete/incomplete tooth removal. All studies analyzed by this review report evidence for each of these comparisons; however, due to the limited number of studies and patients and the high risk of bias, the evidence to make changes to surgical practice is therefore limited. However, this review helps describe the state of research evidence to support practice so that surgeons can make an informed choice in adopting new or continuing with established techniques.

Although today, the use of the mandibular cortical index appears to be quite predictive in evaluating total bone density in patients with bone demineralization, this study did not find great scientific evidence regarding the correlation between iatrogenic mandibular fractures and systemic bone conditions.

Furthermore, this review clarifies the need to carry out new clinical studies, such as randomized or prospective studies with longitudinal follow-up, since most of the available data comes from case series and retrospective studies. However, with the case evaluation of this review, it was possible to clearly identify no late post-operative fractures in patients younger than 20 years. This fact should be shared with third-party payers, who deny authorization to remove asymmetric third molars that will never function.

CONCLUSIONS

The data from this review also show that the patients at greatest risk for late post-third molar extraction fractures of the mandible are men aged 25 years or older, who have had a pre-operative or post-operative infection or menopausal woman suffering from osteomyelitis or taking some types of bisphosphonates. This group should be identified and educated before the intervention and receive unequivocal post-operative instructions that must be strictly respected to avoid late fractures.

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Case Report

HYALURONIC ACID ENRICHED WITH AMINOACID USED TO FILL BONE DEFECT AFTER CYST NASOPALATINE ENUCLEATION: A CASE REPORT

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ABSTRACT

In this study, we aim to evaluate the hyaluronic acid enriched with amino acids used to fill bone defects after cyst enucleation. A 56-year-old man was referred by the dentist to the Department of Oral Surgery, University of Chieti-Pescara, concerning a nasopalatine duct cyst. Hyaluronic acid was placed for better healing. The flap was sutured with interrupted suturing with 3-0. The cyst material was sent for histopathological examination. Microscopical examination revealed a cystic cavity covered by pseudostratified epithelium. The clinical, radiologic, and histopathological aspects suggested an infected nasopalatine duct cyst. No adverse reactions were recorded, and the postoperative course was characterized by the absence of pain. Clinical and radiographic controls were performed by digital dental X-ray at 2 and 4 months after cystic enucleation surgery. The X-ray showed increased bone mineralization. Within the limits of the present investigation, this case report mainly summarized the potential mechanism of HA in promoting bone regeneration and the application prospects of hyaluronic acid-based in bone regeneration.

KEYWORDS: *hyaluronic acid, cyst, nasopalatine duct, bone healing, bone graft, biomaterials*

INTRODUCTION

Cystic lesions are frequent in the oral and maxillofacial areas (1, 2). Nasopalatine duct cysts (NPDC), also known as incisive canal cysts, are the commonest developmental cysts in the jaws (3). The etiology is not certain, but mechanical trauma or bacterial infection could stimulate the proliferation of residual epithelial tissue in the nasopalatine duct. Genetic factors sometimes play a role (4). Secondary cysts are formed by mucus secretion from the retained epithelial cells. Males are affected 18–20 times more often than females. NPDC is suggested when the aspirate is clear and straw-colored (3, 4). A differential diagnosis must be made for lateral radicular cysts and cystic ameloblastoma (5). If the nasopalatine duct appears to be greater than 7 mm in diameter, the presence of a cyst should be suspected. Cyst enucleation and local curettage are a general treatment for nasopalatine cysts (6).

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Usually, the cysts remain asymptomatic. A common symptom is a recurrent swelling in the palatal aspect between the central incisors; at times, the cyst may extend labiopalatally, and fluctuation will be positive (7). The nasopalatine duct cyst is seen as a well-defined cystic outline between or above the apices of the maxillary first incisor teeth (8). Killey et al. reported that any radiograph that showed radiolucency less than 6 mm wide may be considered within normal limits as an incisive canal fossa without specific symptoms (9). Histopathology in the epithelium may be stratified squamous at a lower level; more superiorly, it may be pseudostratified columnar, cuboidal, and ciliated (10). The presence of mucous glands, goblet cells, and cilia highly indicates their origin within the incisive canal, as does the presence of nerves and blood vessels in the fibrous capsule (11).

Cystic contents are an important diagnostic aid to rule out normal incisive canal fossa radiolucency. The viscous fluid content may be mucoid material or even pus if the cyst has been infected (12). Surgical enucleation is the line of treatment of nasopalatine duct cysts, by raising a palatal flap from canine to canine. In the present case report, the residual bone defect after cyst enucleation was filled with hyaluronic acid (HA) enriched with amino acid (13).

CASE REPORT

A 56-year-old man was referred by the dentist to the Department of Oral Surgery, University of Chieti-Pescara, concerning a nasopalatine duct cyst. The swelling was initially small, but it gradually increased in size. No history of trauma was reported by patients. Intraoral examination revealed a pink-colored, well-defined swelling located between the roots of the central incisors. The panoramic radiograph and cone-beam computed tomography (CBCT) showed a well-defined unilocular radiolucent area beyond the nasal floor. The size evaluated by CBCT approximately was 4x3.5cm (Fig. 1).

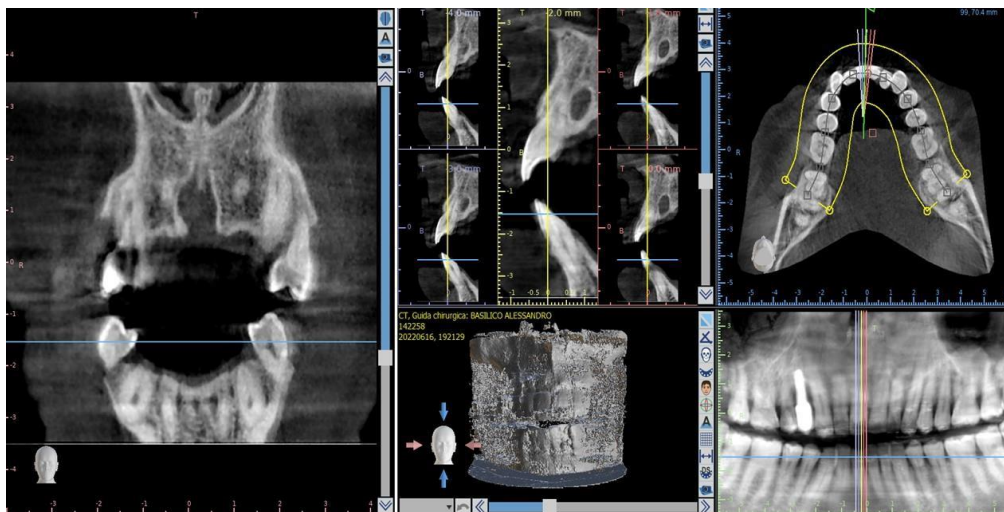


Fig. 1. A well-defined, unilocular radiolucent lesion in the maxillary anterior region on the CBCT was detected.

The patient was subjected to antibiotic treatment with Amoxicillin + Clavulanic acid (GlaxoSmithKline, UK) 2 gr/day for six days from the one before surgery. Disinfection of the oral cavity was achieved by rinsing with Chloroxidine digluconate at 0.2%. Conscious sedation was achieved by intravenous administration of benzodiazepines. After loco-regional infiltration anesthesia with Articain + Adrenaline 1/100.000 (Septodont, France), and a full-thickness of the palatal mucosa is engraved (Fig. 2).



Fig. 2. A): Intraoperative image showing the size of the cyst; **B):** Residual bone defect after cyst enucleation.

A palatal mucoperiosteal flap was reflected by a periosteal elevator to expose the cyst. The neurovascular bundle is salvaged and the cyst is carefully dissected free, from its bony bed. The inner lining of the cyst was scraped off and sent for microscopic evaluation. Hyaluronic acid (Italfarmacia, Rome, Italy) was placed to improve the healing response (14). The flap was sutured with interrupted suturing with a 3-0 polyamide (Assumid, Assut, Europe, Magliano dei Marsi, AQ Italy). The cyst material was sent for histopathological examination. Microscopical examination revealed a cystic cavity covered by pseudostratified epithelium. There is a fibrous connective tissue wall that is inflammatory in lymphocytes and plasma cells. The clinical, radiologic, and histopathological aspects suggest an infected nasopalatine duct cyst. No adverse reactions were recorded, and the postoperative course was characterized by the absence of pain. Clinical and radiographic controls were performed by digital dental X-ray at 2 and 4 months after cystic enucleation surgery. The X-ray showed increased bone mineralization (Fig. 3).

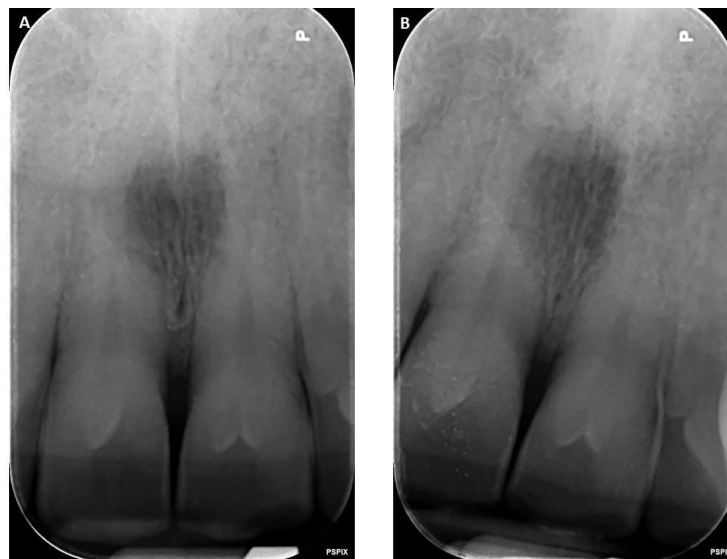


Fig. 3. A): Digital X-ray after 2 months. **B):** Digital x-ray after 4 months shows a bone defect reduction and an increase in mineralization.

DISCUSSION

The clinical results of this case report show the absence of pain and good soft tissue healing. Progressive healing has been recorded, and minor post-operative symptoms occurred, such as swelling, low pain, absence of sensitive alterations, and no hemorrhagic complications.

Bone healing involving a variety of cells, growth factors, cytokines, chemokines, and intracellular and extracellular signaling pathways and have a limited ability to self-heal after injury. When the length of the bone defect exceeds 2 to 2.5 times the diameter of the damaged bone, the self-healing ability of bone tissue alone is not enough (15, 16). For this reason, many biomaterials have been proposed such as, autologous bone, hydroxyapatite, porcine bone, bovine

bone etc. In recent years, hyaluronic acid-based hydrogels have received extensive attention in soft tissue augmentation regeneration and in bone regeneration (17). It is generally present in mammalian tissues and plays a critical role in cell differentiation, migration, proliferation, inflammation, angiogenesis, wound healing (18, 19).

In this case report, we described the use the HA for fill the cyst cavity, through clinical evaluation and reported a good healing without clinical sign. Also, the x-ray shows the radiopacity the bone defect residual after cysty and residual cavity volume reduction was recorded.

The clinical use of biomaterial with or without barrier membranes in bone defects resulting from cystic lesions is not completely clarified (20-25). Cystic cavities have been shown to heal well without the use of biomaterials, which could behave like foreign bodies. In this study, we used cross-linked high molecular weight hyaluronic acid enriched with amino acid.

In conclusion this case report mainly summarized the potential mechanism of HA in promoting bone regeneration and the application prospects of hyaluronic acid-based in bone regeneration.

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Comparative Study

PAIN-REDUCING EFFICACY OF ANESTHETIC SPRAY VERSUS COMPRESSION TO REDUCE THE PAIN DURING TOPICAL INJECTION IN THE PALATAL ZONE

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ABSTRACT

This study aims to evaluate the pain-reducing efficacy of xylocaine spray versus compression to reduce pain during topical anesthetic in the palatal zone. Thirty healthy individuals in the palatal area who needed local anesthesia participated in this comparative study. Male and female patients who consented to treatment between the age groups of 24–65 years with at least one posterior maxillary tooth extraction. All subjects provided signed informed consent. Exclusion criteria were as follows: smoking and malignant tumors. The subjects were randomly divided into two groups: Group A: 15 patients treated with xylocaine spray; Group B: 15 with local compression. Pain during injection and procedure satisfaction grades were recorded with a visual analog scale (VAS). The patients' perceptions were scored through the SEM score. No significant differences in patient perceptions and clinical pain were associated with the pre-anesthesia techniques ($p>0.05$). No differences regarding procedure satisfaction were detected between the xylocaine spray vs. pressure groups ($p>0.05$). Within the limits of the present investigation, the xylocaine spray and pressure procedure were effective for pain distress control during palatine local anesthesia.

KEYWORDS: *anesthetic spray, compression, anesthesia, xylocaine, pain, palate*

INTRODUCTION

Local anesthesia refers to a loss of sensation caused by a reversible nerve conduction blockade around the application site. In dentistry, local anesthetics are administered via a variety of anesthetic techniques that are classified according to their specific effects, such as (1) conduction anesthesia, (2) infiltration anesthesia, (3) topical anesthesia, or surface anesthesia (1). Although conduction anesthesia and infiltration anesthesia produce deep anesthesia, the use of needles may arouse fear and pain in patients.

The thought and performance of local anesthetic injection often provoke feelings of discomfort in the patient and have been described as one of the most anxiety-provoking procedures in dentistry (2). Acute pain depends on psychological factors, such as anxiety, fear, trust, and level of perception of the stimulus, which has put forward the use of dental topical anesthesia efficacy.

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On the other hand, although the intensity of the anesthesia is weak, topical anesthetics have little side effects with easy administration and reduce pain caused by needle injections and can thus generate positive responses towards dental treatment in patients (3).

Topical anesthetic gels are frequently used in dentistry to reduce or eliminate pain during the injection procedure (2). Topical anesthetics alter pain thresholds by controlling pain sensations through a blockade of signals that are transmitted from the peripheral sensory nerve fibers. However, they are only effective in blocking the pain stimuli in the superficial layer of the mucosa. Local anesthetics used for topical anesthesia must have superior mucosal permeability to easily reach free nerve terminals (4).

Vasoconstrictors are not added to topical local anesthetics because they undermine mucosal permeability. Furthermore, topical local anesthetics are typically more concentrated than injectable anesthetics in order to promote diffusion after passing through the mucosa (5).

In addition to topical anesthesia, there are some other simple methods to diminish pain during injection, for example, local pressure on the area before injection. According to the theory of gate control, which was first presented by Melzack and Wall, local pressure could reduce pain during injection. Stimulation of A beta fibers through pressure and vibration could regulate the medullary dorsal horn, resulting in a decrease in painful nerve inputs from peripheral tissues (6, 7).

The aim of this present study was to compare the effect of local pressure and topical anesthesia with xylocaine spray on pain during infiltration injection for topical anesthetic in dental nerve blocks.

MATERIALS AND METHODS

In this clinical randomized study, thirty patients were evaluated. Patients who agreed to participate in this study were randomly assigned to the study groups without considering their gender. A total of thirty healthy individuals needs local anesthesia in the posterior palatal area, participated in this comparative study. Male and female patients who consented for treatment between the age groups of 24–65 years with at least one posterior maxillary tooth extraction. All subjects provided signed informed consent. The subjects were randomly divided into two groups: Group A: 15 patients treated with Xylocaine spray; Group B: 15 with local compression. During injection, pain was recorded with a visual analog scale (VAS) (8, 9). Randomization was performed using the computer-generated random equal numbers of blinded packages containing either group code. Blinded packages were prepared by the nonclinical staff according to the generated random chart and were available to the investigator only after the child was recruited for the study.

Before the administration of anesthesia in each group, one side was randomly selected as experimental and the opposite as control. In group B, the pressure was applied with the mirror handle until the area was ischemic on the alveolar mucosa at the injection site (Fig. 1). In group A, the site was treated with xylocaine spray applied with xylocaine-soaked cotton for 5 minutes (Fig. 2).



Fig. 1. During the handle of the mirror the area was ischemic on the alveolar mucosa at the injection site.



Fig. 2. During the application of xylocaine-soaked cotton.

We followed the same protocol of asking the patient to keep the mouth open and using a suction apparatus to clear the pooling saliva, which helped to maintain the adhesiveness of cotton on the mucosa. In all the groups, palatal infiltration of 2% articaine with 1:200.000 adrenaline was carried out. All the injections were performed by the same surgery with a 25 mm and 27 gauge needle. During the insertion of the needle and during anaesthetic infiltration, the patient's behavior was evaluated for pain perception using sound, eye, motor (SEM) scale and visual analog scale (VAS) by the operator (Fig. 3).

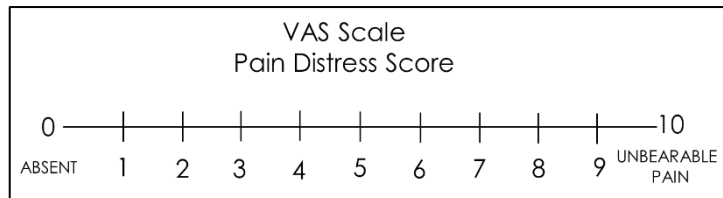


Fig. 3. Pain VAS: Are you having during palatine injection?

Immediately after the injection, the volunteers were asked to rate their pain during needle penetration and injection on the 10 mm VAS forms. On this scale, 0 was considered as no pain, 1 to 3 as mild pain, 4 to 6 as moderate pain, and 7 to 9 as severe pain. Patient satisfaction was validated using the VAS satisfaction score, which had two descriptors representing extremes of satisfaction; the patients rated their satisfaction by making a vertical mark on a scale of 0 to 10, where 0 stands for not satisfied all and 10 scores for completely satisfied (Fig. 4).

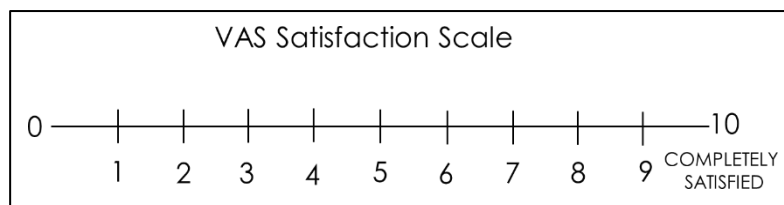


Fig. 4 Satisfaction VAS: Are you satisfied with the palatine block given during treatment?

The VAS was chosen because of its simplicity and as it is accepted as a standard scale for pain score. In the study, the patient's behavior was evaluated for pain perception using a SEM (sound, eye, motor) scale by the operator (Table I).

Table I. SEM scoring (sound, eye motor) during palatine injection.

| Parameter | Comfort (1) | Mild discomfort (2) | Moderate discomfort (3) | Severe discomfort (4) |
|-----------|------------------------------|---|--------------------------------|---|
| Sound | No sound | Non-specific sound | Verbal complaint, louder sound | Verbal complaint, shouting, crying |
| Eye | No sign | Dilated eyes without tears (anxiety sign) | Tears, sudden eye movements | Crying, tears covering the face |
| Motor | Relaxed body and hand status | Muscular contraction, hands contraction | Sudden body and hand movements | Hand movement for defence turning the head to opposite side |

Statistical analysis

The assessment was conducted using the statistical package GraphPad 8.0 (Prism, San Diego, CA, USA). The descriptive statistics were conducted by calculating the means, standard deviation, and 95% Confidence Intervals of the means. The Mann-Whitney test was applied to compare the study variables. The level of significance was considered for $p < 0.05$.

RESULTS

Pain score by VAS is a numerical rating scale where 0 stands for no pain and 10 represents the possible worst pain. Patient satisfaction score was assessed by VAS. At the beginning and the end of the scale are two descriptors

representing extremes of satisfaction, where 0 stands for not satisfied at all and 10 stands for completely satisfied. The exact questions that have been asked for Pain VAS and Satisfaction VAS has been mentioned.

Out of 30 patients included in the study, 14 were male and 16 were female in the age group of 24–65 years with a mean age of 6.27 years. Tables II and III show a comparison between both the test groups under VAS and SEM scales. The mean scores obtained for the group B were lower than the group A under both pain scales. However, the mean scores under both the pain scales were statistically not significant ($p > 0.05$).

Table II. Comparison of pain determined by the anaesthesia procedures by VAS.

| Group | N of patients | Mean \pm SD | 95% CI | P Value |
|---------------------|---------------|---------------|------------|---------|
| A – Xylocaine spray | 15 | 1 \pm 0.4 | (0.79-1.2) | p=0.827 |
| B – Pressure | 15 | 0.9 \pm 0.5 | (0.68-1.2) | |

Table III. Comparison between both test groups using SEM

| Group | N of patients | Mean \pm SD | 95% CI | P Value |
|---------------------|---------------|----------------|-----------|---------|
| A – Xylocaine spray | 15 | 3.7 \pm 0.13 | (3.4-3.9) | p=0.105 |
| B – Pressure | 15 | 4.1 \pm 0.19 | (3.7-4.5) | |

The pain distress associated to xylocaine spray vs. pressure group were respectively 1 \pm 0.4 (95% CI: 0.79-1.2) and 0.9 \pm 0.5 (95% CI: 0.68-1.2) ($p=0.827$). The SEM scale for xylocaine spray vs. pressure group were respectively 3.7 \pm 0.13 (95% CI: 3.4-3.9) and 4.1 \pm 0.19 (95% CI: 3.8-4.5) ($p=0.105$). The procedure satisfaction associated with xylocaine spray vs pressure were 5.5 \pm 1.9 and 4.9 \pm 2.0 ($p=0.447$) (Table IV) (Fig. 5).

Table IV. Comparison of satisfaction determined by the anaesthesia procedures by VAS.

| Group | N of patients | Mean \pm SD | 95% CI | P Value |
|---------------------|---------------|---------------|-----------|---------|
| A – Xylocaine spray | 15 | 5.5 \pm 1.9 | (4.4-6.5) | p=0.447 |
| B – Pressure | 15 | 4.9 \pm 2.0 | (3.8-6.0) | |

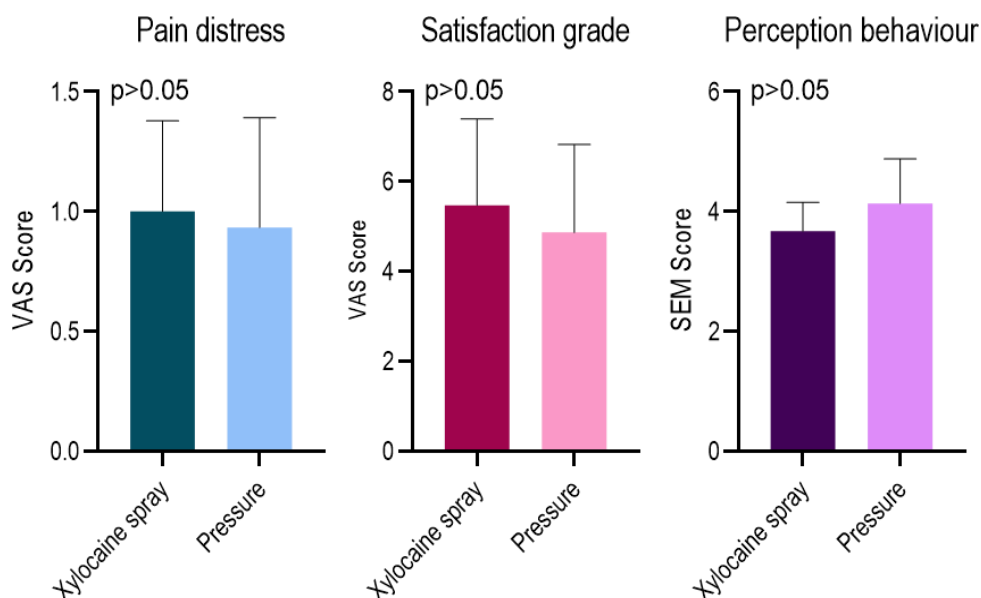


Fig. 5. Chart summary of the VAS and SEM scoring of the xylocaine spray vs pressure group ($p > 0.05$).

DISCUSSION

Topical anesthetics are highly useful for reducing discomfort, pain, and anxiety during dental procedures. Traditional topical anesthetic agents with benzocaine and lidocaine as active ingredients are available in various forms, and products should be selected based on their intended use (10). In this randomized clinical trial, we compared the effect of local pressure and xylocaine spray as a topical anesthetic agent on pain during infiltration injection for maxillary canine teeth. Topical anesthetics typically act for 10–15 min (11). When topical anesthetics are applied to the dried mucous membrane, they reversibly inhibit peripheral sensory nerve fibers, altering pain thresholds. Thus, the surface anesthetic action largely depends on the drug permeability (1). One method to improve the surface permeability is to alter the mode of drug delivery (12). In addition, dental anxiety and fear of needles is some of the most common problems encountered during dental extraction. Needle phobia is treated as one of the medical conditions affecting 10% of the population, which can result in physiological changes like blood pressure, heart rate, and stress hormone variations in the body (13).

According to the gate control theory, the rationale behind investigating the effect of local pressure on pain during infiltration injection in this study was that it could effectively reduce pain during injection (14). One of the most primitively used techniques, gaining popularity, is acupressure, which involves applying pressure at certain key points that stimulate the nervous system to initiate natural healing (15). It is a procedure that either involves the application of pressure directly by finger in a circular motion or the application of consistent and constant pressure through bead/pellet at the stipulated points. The myelinated nerve fibers in muscles are stimulated with the application of pressure at acupoints, which will activate the midbrain and pituitary-hypothalamus via the spinal cord. Various neurotransmitters, like Enkephalin, b-endorphin, Dynorphin, Serotonin, and Noradrenalin, play an important role by stimulating A δ fibers in the skin and muscles. The A δ fibers, which terminate in the second layer of the black horn, release the enkephalins, which inhibit the incoming painful sensations (16). In conclusion, the xylocaine spray and pressure are equally effective in controlling pain during the administration of local anesthesia.

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Original Article

EFFECT OF EQUINE XENOGRAFT ON GENE EXPRESSION OF DENTAL PULP STEM CELLS IN VITRO

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ABSTRACT

Bone grafting is a surgical procedure used to repair complex bone deficit. The damaged bone is replaced with a graft that supports bone regeneration and healing of bone defects. This technique is vital in orthopedics, dentistry, and trauma surgery, addressing the conditions that traditional methods cannot effectively treat. Bone grafts can originate from various sources, including the patient's body, donors, or synthetic materials, each with distinct advantages and challenges. In this study, we cultured dental pulp stem cells to determine whether equine xenograft (EQX) bones can promote osteoblast differentiation. The gene expression of a panel of 15 differentiation markers was analyzed at two-time points. After 24 h of treatment, FOSL1, SPP1, and MMP14 expression were upregulated. After four days, increased expression of SP7, COL1A1, and COL4A1 was observed. This study indicates that EQX could be considered a material that favors bone regeneration by promoting osteoblast differentiation.

KEYWORDS: *graft, bone, osteoblasts, stem, expression*

INTRODUCTION

Bone grafting is a surgical procedure that replaces missing or damaged bone to repair complex bone fractures, support bone regeneration, and facilitate the healing of bone defects (1). This technique is fundamental in orthopedics, dentistry, and trauma surgery, addressing conditions that traditional methods cannot effectively treat. Bone grafts facilitate healing through three primary mechanisms: osteogenesis, i.e., the process of new bone formation by osteoblasts contained within the graft material; osteoinduction, i.e., the recruitment and differentiation of progenitor cells into osteoblasts, promoted by growth factors such as bone morphogenetic proteins or osteoinductive materials, and osteoconduction, i.e., the process where bone tissue grows onto a surface, providing a scaffold for new bone formation.

Osteoconductive materials can act as a framework for bone ingrowth and support the migration and proliferation of bone-forming cells, facilitating bone regeneration.

Bone grafts can originate from various sources, including the patient's body, donors, or synthetic materials, each with distinct advantages and challenges (2). Autografts involve the patient's bone, typically harvested from the iliac crest, tibia, or ribs. This method is considered the gold standard due to its biocompatibility, osteogenic potential, and minimal

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risk of immune rejection. Autografts support bone healing through osteogenesis, osteoinduction, and osteoconduction. However, drawbacks include limited availability, potential donor site morbidity, and increased surgical time.

Allografts use processed bone derived from cadaveric donors to ensure sterility and reduce immunogenicity. These grafts provide a structural framework for new bone growth and can be stored in tissue banks for future use. Despite their convenience and availability, allografts carry risks of disease transmission and immune response and lack osteogenic properties, relying solely on osteoconductive and osteoinductive capabilities. Xenografts use bone from other species, commonly bovine. These grafts undergo extensive processing to remove all cellular components and reduce immunogenicity, leaving behind a mineralized matrix that supports bone ingrowth. Xenografts are readily available and avoid donor site complications (2). Synthetic bone substitutes include hydroxyapatite, tricalcium phosphate, and bioactive glass (3). These grafts are designed to mimic natural bone's physical and chemical properties, promoting osteoconduction and, in some cases, osteoinduction.

Synthetic materials eliminate risks of disease transmission and donor site morbidity, offering a customizable and readily available alternative.

In the present investigation, an *in vitro* model was used to test the effect of a xenograft material on undifferentiated cells. Osteoplant (Bioteck SRL, Vicenza, Italy) is an equine xenograft (EQX) of cortical and spongy bone tissue used to fill bone defects in orthopedic, maxillofacial, and oral surgery (4-6). To verify how EQX acts on stem cells to induce bone formation, we treated dental pulp stem cells (DPSCs) with Osteoplant to analyze gene expression.

MATERIALS AND METHODS

Dental Pulp Stem Cells (DPSCs) Isolation

Dental pulp was extracted from the third molars of healthy subjects and digested for 1 h at 37°C in a solution containing 1 mg/ml collagenase type I and 1 mg/ml dispase, dissolved in phosphate-buffered saline (PBS) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 500 µg/ml clarithromycin. The solution was then filtered using 70 µm Falcon strainers (Sigma Aldrich, St Louis, Mo, U.S.A.) to separate mesenchymal stem cells from fibroblasts. Stem cells were cultivated in α -MEM culture medium (Sigma Aldrich, St Louis, Mo, U.S.A.) supplemented with 20% Fetal Bovine Serum (FBS), 100 µM 2P-ascorbic acid, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma Aldrich, St Louis, Mo, U.S.A.). The flasks were incubated at 37°C and 5% CO₂, and the medium was changed twice weekly.

DPSCs were characterized by immunofluorescence for the positive mesenchymal stem cell marker, CD105, CD90, and CD73, and negative marker CD34, as described in Sollazzo et al. (7).

Cell treatment

DPSCs were maintained in a humidified atmosphere containing 5% CO₂ at 37°C. The cells were seeded at a concentration of 1.0×10^5 cells/ml with Osteoplant (Bioteck SRL, Vicenza, Italy) at the concentration of 1 mg/ml in 9 cm² (3 ml) wells containing DMEM supplemented with 10% serum and antibiotics. Another set of wells containing untreated cells was used as a control. The treatment was carried out at two time points: 24 h and 4 days. At the end of the treatment period, the cells were lysed and processed for total RNA extraction.

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

According to the manufacturer's instructions, total RNA was isolated from the cells using RNeasy Mini Kit (Qiagen, Hilden, Germany). The pure RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

cDNA synthesis was performed starting from 500 ng of total RNA using the PrimeScript RT Master Mix (Takara Bio Inc., Kusatsu, Japan). The reaction mixture was incubated at 37 °C for 15 min and inactivated by heating at 70 °C for 10 s. cDNA was amplified by real-time quantitative PCR using an ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). All PCR reactions were performed in a 20 µL volume. Each reaction contained 10 µl of 2x qPCRBIO SYGreen Mix Lo-ROX (PCR Biosystems, Ltd., London, UK), 400 nM of each primer, and cDNA.

Custom primers belonging to the “extracellular matrix, adhesion molecule” pathway, “osteoblast differentiation,” and “inflammation” pathway were purchased from Sigma Aldrich. The selected genes grouped by functional pathways are listed in Table I.

All experiments were performed using non-template controls to exclude reagent contamination. PCR was performed using two analytical replicates. The amplification profile started with 10 min at 95°C, followed by a two-step

amplification for 15'' at 95°C and 60'' at 60°C for 40 cycles. In the final step, a melting curve dissociation analysis was performed.

Table I. Selected genes used in Real-Time PCR grouped by functional pathway.

| Pathway | Gene |
|---|--|
| Osteoblast differentiation | RUNX2 (Runt-related transcription factor 2) ALP (Alkaline phosphatase) FOSL1 (FOS-like antigen 1) SP7 (Osterix) ENG (Endoglin) SPP1 (Osteopontin) SPARC (Osteonectin) |
| Extracellular matrix, adhesion molecule | COL1A1 (Collagen type I alpha1) COL3A1 (Collagen type III alpha 1) COL4A1 (Collagen type IV alpha 1) MMP VII (Matrix Metalloproteinase 7) MMP XII (Matrix Metalloproteinase 12) MMP XIV (Matrix Metalloproteinase 14) |
| Inflammation | IL1A (Interleukin 1 Alpha) IL1R (Interleukin 1 Receptor Type 1) IL6 (Interleukin 6) |
| Reference gene | RPL13 (Ribosomal protein L13) |

Statistical analysis

Quantification was performed using the delta-delta Ct method. The gene expression levels were normalized to the expression of the reference gene (RPL13) and expressed as fold changes relative to the expression in untreated cells.

RESULTS

The DPCSs were phenotypically characterized using immunofluorescence. Fig. 1a shows cytoskeletal filaments stained with vimentin. The cell surfaces were positive for mesenchymal stem cell markers CD90 (Fig. 1b) and CD73 (Fig. 1c) and negative for markers of hematopoietic origin CD34 (Fig. 1d).

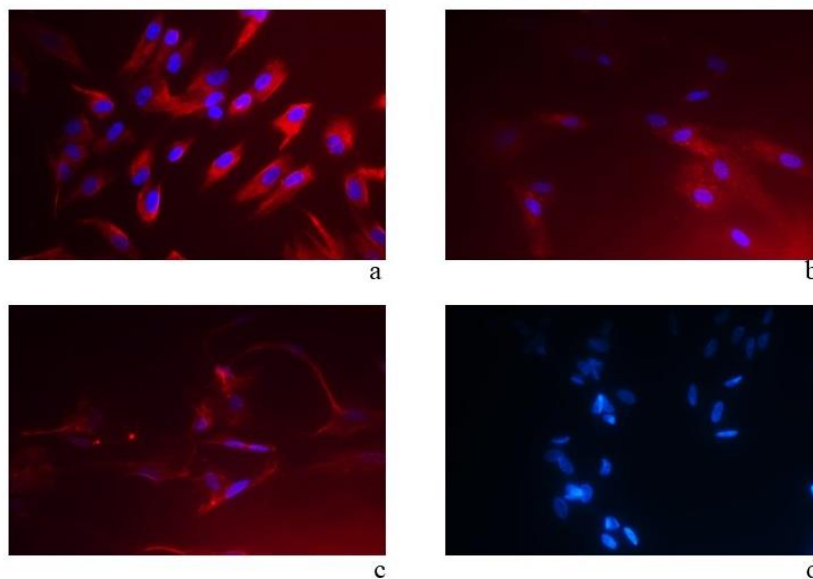


Fig. 1. DPCSs by indirect immunofluorescence (Rhodamine). Immunofluorescence staining of vimentin (a), mesenchymal stem cell marker CD73 (b), CD90 (c), and hematopoietic markers CD34 (d). Nuclei were stained with DAPI. Original magnification x40.

The effect of EQX treatment in the modulation of gene expression was analyzed by quantitative real-time RT-PCR; specifically, the expression levels of osteoblast-related genes, extracellular matrix, and inflammation pathways were measured. Table II reports the gene expression level variation obtained after 24 h and 4 days of cell treatment.

Following EQX treatment, several genes exhibited a significant increase in expression levels, exceeding a two-fold change compared to the untreated cells (Table II). Specifically, FOSL1, SPP1, and MMP14 were up-regulated shortly after only 24 h of treatment, while SP7, COL1A1, and COL4A1 were up-regulated after 4 days of treatment.

Table II. Gene expression in ADSCs after 24h and 4 days of treatment. Numbers express the fold changes of the de-regulated genes in treated cells vs. untreated cells. ND – not determined. In bold significant gene expression level.

| Gene | Protein | 24 h | 4 days |
|--------|-------------------------------------|------------|------------|
| RUNX2 | Runt-related transcription factor 2 | 1.7 | 1.0 |
| ALP | Alkaline phosphatase | 1.7 | 1.7 |
| FOSL1 | FOS-like antigen 1 | 2.9 | 1.5 |
| SP7 | Osterix | 0.7 | 2.2 |
| ENG | Endoglin | 1.9 | 1.2 |
| SPP1 | Osteopontin | 3.3 | nd |
| SPARC | Osteonectin | 1.6 | 1.2 |
| COL1A1 | Collagen type I alpha1 | 1.5 | 2.4 |
| COL3A1 | Collagen type III alpha 1 | 1.7 | 1.2 |
| COL4A1 | Collagen type IV alpha 1 | 1.7 | 2.5 |
| MMP7 | Matrix Metalloproteinase 7 | 0.9 | 1.7 |
| MMP12 | Matrix Metalloproteinase 12 | 0.1 | 1.2 |
| MMP14 | Matrix Metalloproteinase 14 | 5.1 | 1.6 |
| IL1A | Interleukin 1 Alpha | 1.3 | 1.1 |
| IL1R1 | Interleukin 1 Receptor type 1 | 1.0 | 1.0 |
| IL6 | Interleukin 6 | 1.5 | 0.6 |
| IL6R | Interleukin 6 Receptor | 2.0 | 1.2 |

DISCUSSION

DPSCs are a type of mesenchymal stem cell found within the dental pulp (8). DPSCs have gained attention for their remarkable regenerative capabilities, including the potential to differentiate into various cell types (9). For this reason, the DPSCs were considered a promising candidate for regenerative medicine and tissue engineering applications.

These cells are accessible, ethically non-controversial, and possess unique properties that distinguish them from other stem cells.

DPSCs are typically harvested from the pulp of extracted teeth, such as third molars (wisdom teeth) or deciduous teeth. The dental pulp tissue is subjected to enzymatic digestion or explant culture techniques to obtain the stem cells.

The isolation process is relatively straightforward and minimally invasive, making DPSCs a readily available source of stem cells compared to other MSCs derived from bone marrow or adipose tissue.

DPSCs exhibit several key properties that make them valuable for regenerative therapies like self-renewal and multipotency since they can differentiate into various cell types, including odontoblasts, osteoblasts, chondrocytes, adipocytes, and even neurons and myocytes, as well as immunomodulatory effects, i.e., the ability to modulate immune responses, reducing inflammation and promoting tissue repair (10).

Here, we investigated if EQX can induce DPSCs differentiation toward osteoblast lineage. Some genes related to bone formation are activated, including transcription factors FOS-like antigen 1 and Osterix, matrix proteins Osteopontin, Collagen type I and IV, and matrix the remodeling protein Matrix Metalloproteinase 14.

FOSL1, a member of the FOS family of transcription factors, also known as FRA1, plays a significant role in regulating cellular processes such as proliferation, differentiation, and apoptosis (11). Its involvement in osteogenesis, the process of bone formation, has emerged in recent years (12).

FOSL1 and the other FOS family members form heterodimers with members of the Jun family, constituting the AP-1 transcription complex (13). Its transcriptional activity is modulated by various signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway and the Wnt/ β -catenin pathway, which regulate FOSL1 expression and activity during osteogenesis.

Several loss- and gain-of-function studies in mice have demonstrated that Fos family members play specific roles in osteogenesis and bone remodeling. Transgenic mice overexpressing Fra-1 display severe osteosclerosis, a bone disorder characterized by increased bone mass caused by increased osteoblast differentiation and function (14). Mice lacking Fra-1 develop osteopenia, a low bone mass disease, proving that Fra1 is an important regulator of bone mass by affecting bone matrix production and maintaining osteoblast activity (15).

FOSL1 plays diverse roles in osteogenesis, influencing osteoblast differentiation and modulating gene expression in extracellular matrix synthesis and remodeling.

SP7, also known as osterix, is a zinc finger-containing transcription factor that plays a critical role in bone formation and osteoblast differentiation (16, 17). Osterix is required to induce osteoblast-specific genes, such as osteonectin, osteopontin, osteocalcin, and alkaline phosphatase, crucial for osteoblast differentiation and bone mineralization (18, 19).

Identified as a key regulator of the genetic network controlling osteogenesis, SP7 functions downstream of Runx2, another essential transcription factor in bone development (19, 20). Other findings suggest that Osterix is regulated via both Runx2-dependent and -independent mechanisms and that Osterix controls osteoblast differentiation, at least in part, by regulating the expression of genes not controlled by Runx2 (21).

Given its pivotal role in bone formation, mutations in or dysregulation of SP7 are associated with various bone disorders. Common SP7 polymorphisms are associated with bone mineral density variation and fracture risk, rare SP7 mutations cause skeletal dysplasia, and SP7 may contribute to bone metastasis (22).

Osteogenesis imperfecta, a genetic disorder characterized by brittle bones, has been linked to mutations in SP7 (23). Patients with these mutations exhibit symptoms such as frequent fractures, bone deformities, and growth deficiencies, reflecting impaired osteoblast function and bone matrix production (24).

Osteoporosis is a condition characterized by reduced bone mass and increased fracture risk. Dysregulation of SP7 expression or activity can disrupt the balance between bone formation and resorption, contributing to the development of osteoporosis (22).

In addition to its role in osteoblasts, SP7 also influences the differentiation of chondrocytes, which are responsible for cartilage formation. Although primarily known for its osteogenic functions, emerging evidence suggests that SP7 may play a role in regulating the balance between osteogenesis and chondrogenesis, which is crucial for endochondral ossification, a process by which long bones are formed (25).

SPP1 gene codes for Osteopontin, a prominent bone matrix protein expressed by preosteoblastic cells early in bone formation. Still, the highest expression is observed in mature osteoblasts at sites of bone remodeling (26). It plays a crucial role in bone mineralization and in the attachment of osteoclasts to the mineral matrix. Osteopontin is involved in various physiological and pathological processes, including bone remodeling, immune response, and inflammation (27).

Collagen Type I is the main structural protein in bone extracellular matrix. Although the production of type I collagen is not exclusive to the differentiating osteoblast but is also produced by fibroblasts, type I collagen is considered a useful osteoblast differentiation marker when expressed with other bone markers (28).

Collagen Type IV is a structural component of the extracellular matrix and a major basement membrane component that separates epithelial and endothelial cells from the connective tissue (29). Studies have shown that Collagen Type IV is not only a structural protein but is also involved in tissue genesis, differentiation, homeostasis, and remodeling (30). This role appears to be not limited to epithelial cells but involves additional cell types, including mesenchymal stem cells. Collagen Type IV seems to play a significant role in the differentiation of stem cells towards osteoblasts and adipoblasts. Indeed, Li et al. (31) demonstrated that the inhibition of miR-214-5p promotes the cell survival of osteoblasts and extracellular matrix production by targeting COL4A1. Later, it was demonstrated that miR-214-5p may weaken osteogenic differentiation of bone marrow stem cells by downregulating COL4A1. Indeed, miR-214-5p may promote adipogenic differentiation downregulating the TGF- β /Smad2/COL4A1 signaling pathway (32). A genome-wide linkage scan found a genomic region at 13q34, including COL4A1 and COL4A2 (collagen type IV alpha-1 and alpha-2 subunits) significantly linked with forearm bone mineral density (33). A significant COL4A1 gene

expression level was found in human osteoporosis fracture bone, compared with bone from individuals with osteoarthritis and individuals without bone pathology (34).

The matrix metalloproteinase protein (MMP) family is involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development and tissue remodeling. Although MMP14 is not considered a classic marker of osteoblast differentiation, MMP14 appears to play a multifaceted role, influencing various signaling pathways and cell fate decisions critical for bone formation and remodeling (35). Deletion of the membrane-anchored Mmp14 in mesenchymal progenitors, but not in committed osteoblasts, redirects cells' fate decisions from osteogenesis to adipo- and chondrogenesis (36).

The collagenolytic activity of MMP14 also regulates the differentiation of mesenchymal stem cells into bone-producing osteoblasts in 3-dimensional (3D) collagen matrices (37). Furthermore, MMP14 is essential for osteoblast survival during the osteoblast/osteocyte transition and is required for proper lacunae formation in osteocytes (38). The parathyroid hormone stimulates osteocyte proliferation by activating the Wnt pathway and increasing the MMP14 expression level, which appears to control bone resorption by regulating soluble RANKL production (39). Furthermore, MMP14 has been implicated in osteoclastogenesis regulation. Indeed, the suppression of MMP14 in osteoblasts increased osteoclastogenesis (39, 40).

CONCLUSIONS

Bone grafting is a crucial technique in modern medicine, offering solutions for complex bone defects and promoting effective healing. Continued advancements in biomaterials, biological enhancements, and regenerative medicine are poised to overcome current limitations, expanding potential applications and success rates of bone grafts.

Through ongoing research and innovation, bone grafting will continue to evolve, enhancing patient care and surgical outcomes in orthopedic and reconstructive procedures. EQX is currently used in dental practice. DPSCs represent a promising frontier in regenerative medicine, offering potential solutions for various dental and orthopedic conditions. Their ease of access, multipotency, and immunomodulatory properties position them as a valuable resource for developing innovative therapies. We demonstrated that EQX could stimulate DPSCs to differentiate into the osteoblast lineage. We understand that further research is necessary to comprehend the mechanism by which EQX influences stem cells fully.

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Case report

GRAFT OF THE ILIAC CREST AND TOTAL IMPLANT FULL-ARCH REHABILITATION: A CASE REPORT

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ABSTRACT

The purpose of this study was to assess changes in bone volume after the block bone increase and placement of dental implants and further assess the aesthetic outcome. This case highlights the success of implant osseointegration and aesthetic oral rehabilitation 5 months after the maxillary graft with a corticocancellous block obtained from the iliac crest. This article was conducted through a literature review based on international data. This study presents the case of a patient who benefits from this method of treatment, which leads to jaw reconstruction and dental implant placement. The postoperative evolution of the patient was favorable, with the integration collected from grafts of the iliac ridge and dental implant insertion. After the prosthetic loading, the masticatory and aesthetic function of the patient were restored. This jaw reconstruction method has proven effective, with a high degree of reliability and a significant improvement in patient's quality of life.

KEYWORDS: *bone regeneration, iliac crest graft, dental implants, maxillary reconstruction, full arch*

INTRODUCTION

Bone grafts are indicated in several cases: congenital and/or acquired defects, lack of teeth, post-traumatic resorption or loss of teeth, infections, and cancer. Due to inherent genetic, inductive, and conductive qualities, self-transplants present the best material for grafts (1). Although current literature indicates several suitable sites for explanting (jaw bone and extra-oral sources such as ribs, skull, and tibia), most authors prefer explanting bone from the iliac crest for four significant reasons:

1. abundance of both sponge and cortical bone, easily adaptable to implant site;
2. ease and subsequent speed of explantation;
3. the curvature of the iliac crest is very adaptable to the maxillary facial area;
4. infrequent diseases.

The loss of the teeth causes bone resorption of the jaw, creating aesthetic and rehabilitative problems. Important

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atrophies can compromise facial aesthetics, particularly in cases where the smile reveals an uneven rapport between arches (2). Moreover, in This case, a non-harmonious rapport between teeth and gums or a limited opening of the oral cavity renders hygiene difficult and causes several problems.

Over the years, numerous techniques for treating maxillary atrophies, using grafts and transplants, have been proposed.

In 1980, Breine et al. (3) were the first to study the effects of these procedures. Later, Mish et al. (4) corrected three-dimensional maxillary defects with explant and grafting of cortical bone from the anterior-medial area of the iliac crest to elevate the maxillary sinus. Mish et al. (4) obtained a 99% survival rate after 8 years with implants inserted in grafts. After that, many others successfully proposed auto-explants from the iliac crest, with or without the maxillary sinus elevation or subsequent implant insertion. This later research revealed that the procedure is safe (4).

TECHNIQUES

Deep incision explant

Many authors use this method when collecting large quantities of bone is necessary. The procedure requires the detachment of the gluteal muscles and the muscle of the medial hip wall (5). In the iliac crest, two incisions, one frontal and one posterior, are performed, followed by collecting bone. Undoubtedly, this procedure provides large quantities of bone; however, it is not free of important complications:

- abdominal hernias;
- bleeding;
- in situ e peritoneal vascular damage.

Worthy of note is the risk of fracture of the iliac crest and subsequent aesthetic problems. Several variants of this technique aim to limit muscle detachment and maintain the crest's conformation (6).

Lateral explants

This procedure requires the detachment of the gluteal muscles from the hip and four bone explants of a thinner section that may or may not interest the iliac crest (sub-crest). The surgery is relatively easy and rapid. Minor aesthetic problems are produced if the iliac crest is maintained. Postoperative pain, however, and impairment of deambulation are evident and long-lasting due to the detachment of the gluteal muscle and the tensor sideband. The risk of perforating the medial cortical hip wall and subsequent vascular damage, peritonitis, and intestinal fissures or occlusions should not be underestimated (7).

Medial explant

Similar to the lateral explant technique, the procedure involves postural muscles. The involvement of a postural muscle and not the gluteus (necessary for deambulation) makes many authors prefer this technique. Due to the minor adhesion of the periosteum compared to that of the lateral hip wall, the detachment of the postural muscles is easier. The same authors also report fewer complications, such as peritoneal bleeding, loss of sensibility, and aesthetic problems (8). They note that pain and deambulatory problems are less severe and require less recovery time. The theoretical risk of a lesion of the lateral femoral cutaneous nerve is possible because of its location on the medial iliac. The repositioning of both periosteum and abdominal muscles seems to reduce hemorrhaging and edema due to the pressure exerted by the intestines (9).

Bicortical explant with maintenance in situ of flat spongy bone

Periosteum and medial and lateral muscles are disassembled, and four osteotomies are performed on each side to isolate two specular bone blocks, leaving a spongiosa lamina in situ (10). This procedure leaves the iliac crest intact, even if reduced in size. Deambulatory problems remain because of the detachment of the periosteum and muscle. The risk of fracture of the remaining iliac crest, however, exists.

The oblique explant of only spongy bone segments from the crest and repositioning the crest without muscle detachment is another technique that avoids complications (11). The two incisions are divergent; one incision starts from the crest and is executed towards the bottom of the hip bone, and a second begins laterally and extends outwards. These two incisions allow the explant of marrow bone while maintaining the residual fragments of the crest, primarily cortical, integral through bone synthesis.

Explant of lateral cortical marrow bone

This surgical procedure calls for a crosswise, full-length incision of the hip bone starting laterally under the crest. This procedure leaves the bony cap crest attached to the periosteum and abdominal muscles. To effect the incision, scalpels, and spoons are required to explant the cortical marrow segment. After the explant, the crest is returned to its original position. The surgery facilitates access to the hip, reduces postoperative complications, and preserves the crest. The detachment of the gluteo muscle often compromises deambulation (12).

Explant of cortical marrow medial bone with rotation of medial crest

This procedure begins with an incision in the iliac crest membrane and continues laterally and medially to create a bony cap attached to the abdominal muscles. The cap is rotated to detach the membrane and the iliac muscle from the medial side of the ileum. A successive incision is made from a bone segment containing both cortical and marrow bone. This segment is shaped by cortical and marrow flaps (results of cortical hip disk) (13). The membrane and muscle are repositioned, and the iliac crest is repositioned and secured. This procedure, sparing the gluteal and abdominal muscles from detachment and sensibly reducing problems of postoperative pain and deambulation, provides large quantities of bone and preservation of the crest. According to several authors, this surgery also avoids the necessity of drainage as the entire area of the operation is closed.

The possible complications cited by the various authors are the fracture of the residual cortical bone and the subsequent formation of retroperitoneal bleeding and hematomas (14).

EXPLANT SURGERY: MONO-BICORTICAL EXPLANT

After sterilizing with iodopovidone, the operating site is delimited with sterile bandages, anesthesia, and vasoconstrictor infiltration (15).

An incision along the iliac crest is made for a length of 5-8 cm, starting at least 1 cm from the anterior point of the iliac crest and continuing toward the posterior iliac crest.

The subcutaneous incision includes the fascia lata and periosteum, using clamps to stop bleeding. The detachment of the periosteum exposes the explant site, giving particular attention to the iliac muscle.

The cortical bone segments are created with manual and oscillating saw blades, cutting the vertical incisions and then two parallel incisions to limit a box. The bone segment graft consists of the marrow section along the straight bone cuts. The segments should be stored in a physiologic solution before implantation. From the exposed area, spongy bone material can be collected using a surgical spoon. Intra-osseo bleeding is controlled with collagen sponges or shaped fibrin and, if required, orthopaedic wax.

After drainage placement, the suturing follows the anatomical levels inversely, starting at the periosteum and fascia lata using resorbable sutures and separate stitches. The suturing continues on the subcutaneous level with rapid absorption sutures and finally on the cutaneous level with separate or continuous stitches (16, 17). It is crucial to observe several parameters prior to the first incision (Fig. 1):

- a distance of 1 cm from the anterior point of the iliac crest to avoid damage to the inguinal canal and the insertion of the sartorius muscle;
- safety of the ileum-gastric nerve, assuring that it lays sideways on the crest;
- the length of the incision is proportional to the size of the explant, usually 5-8 cm; longer incisions must always follow the vertical direction.



Fig. 1. *Anesthesia and first incision.*

With adequate clamping, the lower-level incisions are executed. Without damaging the iliac and gluteal muscles, the periosteum of the crest is carefully detached from the lateral and medial sections of the hip. It is useful for performing wide divarication and tissue protection (Fig. 2, 3), followed by a manual evaluation of the graft (18).



Fig. 2. Photos showing detachment of muscle from the iliac crest.



Fig. 3. Photos showing and clamping of the iliac crest.

The osteotomy can be made with manual or oscillating saws below the crest. The area is then delimited with two incisions of the desired width perpendicular to the iliac crest and terminates with the caudal portion resection to free the flap from the surrounding bone. A surgical spoon can be employed in the exposed area if more marrow bone is necessary. The explanted segment is preserved in a physiologic solution. The crest fragments are recomposed with plates and screws. Vascular intra-osseo hemostasis is done with collagenous sponges and compressed, and when necessary, orthopaedic wax (Fig. 4, 5) (19).



Fig. 4. Osteotomy and harvesting of the autologous graft.

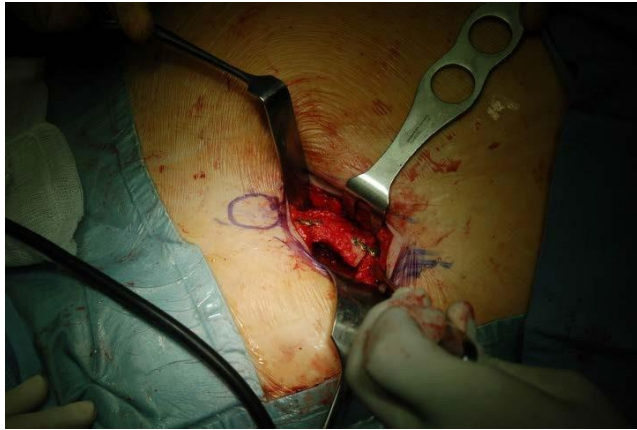


Fig. 5. Osteotomy and harvesting of the autologous graft.

Before closing the exposed area, it is paramount to inspect accurately for bleeding to avoid complications; the suturing proceeds from the lowest level, periosteum and fascia lata, with separate stitches using slow absorption filament. The subcutaneous sutures should use rapidly absorbable filament (20). The cutaneous suturing can be either continuous or separate stitching, preferably intracutaneous, for better aesthetic results. After suturing, compressive dressing is removed after 24 hours, and ice packings are recommended.

Complications

Although relatively safe, this surgery carries both minor and major complications. The minor complications include pain, infections, accumulation of liquid, and difficulty walking (21). Among the severe complications are nerve and vascular damage, fractures, and hernias. The nerve damage usually interests three structures:

- the lateral branch of the cutaneous intercostal nerve;
- the lateral cutaneous branch of the iliohypogastric nerve;
- the lateral cutaneous femoral nerve.

This last complication generally occurs in those cases where the nerve is situated along the medial anterior iliac crest and below the inguinal ligament, even if in 2,7% of the cases, the nerve runs above the inguinal ligament. This position of the nerve increases the risk of nerve damage, which, if permanent, causes muscle pain.

Postoperative pain and difficulty in walking are to be expected (22). The return to normal deambulation varies with the size of the explant, and the pain may vary from a few days to 2 weeks.

BONE GRAFT OPERATION

Once the explant is completed, the subsequent step is grafting at the recipient site. After preparing the site with chlorhexidine and sterile cloths, anesthesia, and vasoconstrictors to control bleeding and postoperative pain, an incision is made along the mucosa (Fig. 6), extending it perpendicularly and outwards to the free mucous to detach the periosteum and expose the bone.

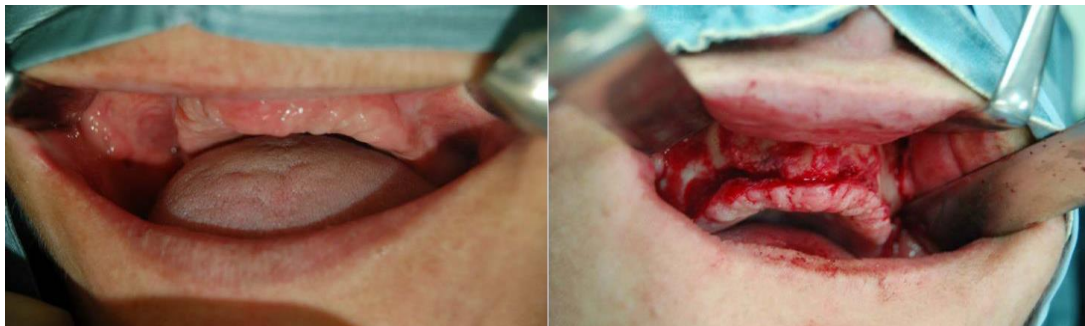


Fig. 6. Preparation of the maxillary site.

The graft is secured location using screws (Fig. 7). Sometimes, dividing the collected graft into smaller segments may be necessary to facilitate the reconstruction of irregular surfaces. Bone spurs and rough edges can be smoothed with a hand drill. The eventual spaces between the alveolar and the graft should be filled with spongy bone segments (23).

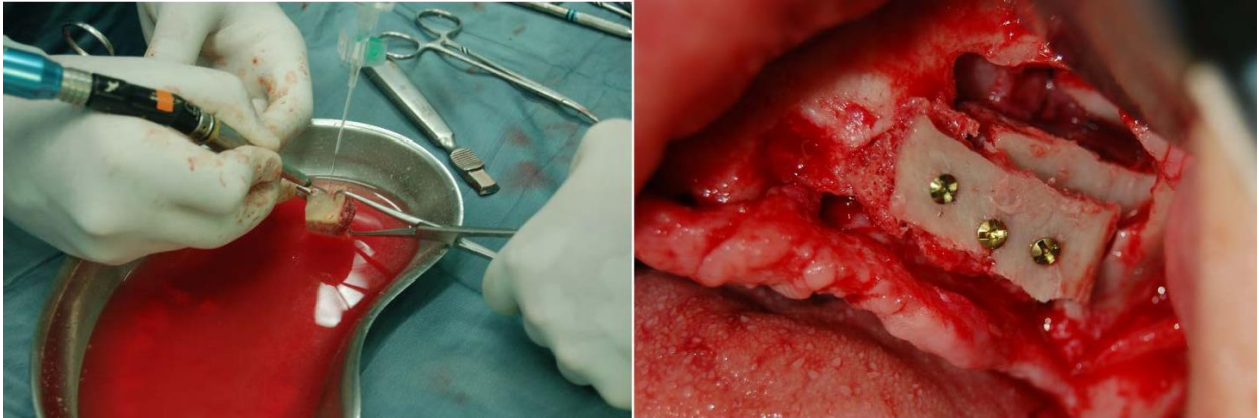


Fig. 7. *Modelling of bone graft and fixation with screw.*

Extending the incision to have sufficient tissue to close it may be necessary. The incision of the mucous membrane is closed with resorbable sutures (Fig. 8, 9) (24).



Fig. 8. *Suture of the flap and spongy bone in syringes.*

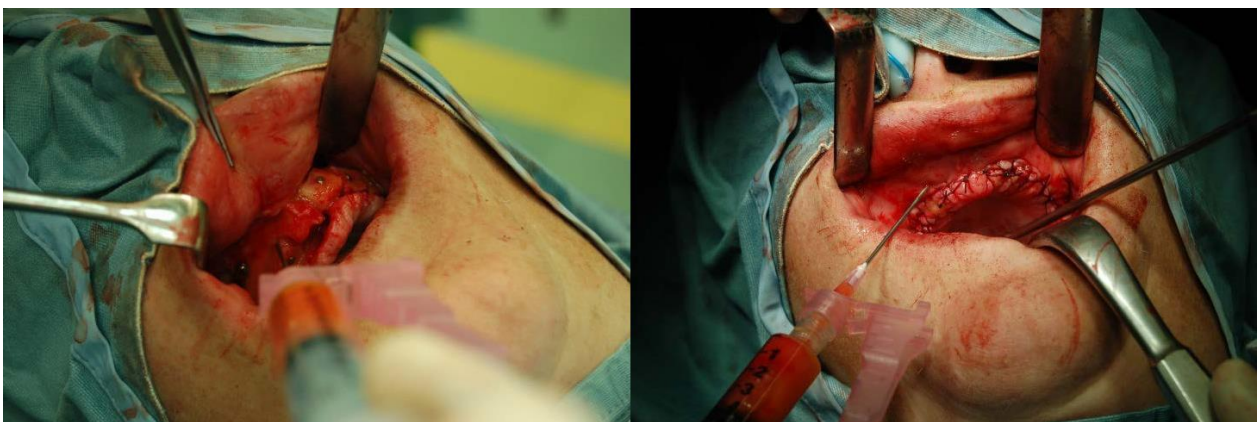


Fig. 9. *Suture of the flap.*

INSERTION OF THE DENTAL IMPLANTS

After 4 months without complications, the graft's integration and volume maintenance are verified with X-rays (Fig. 10, 11). In order to insert the dental implants, it is necessary to prepare a wax model and a surgical bite plate.

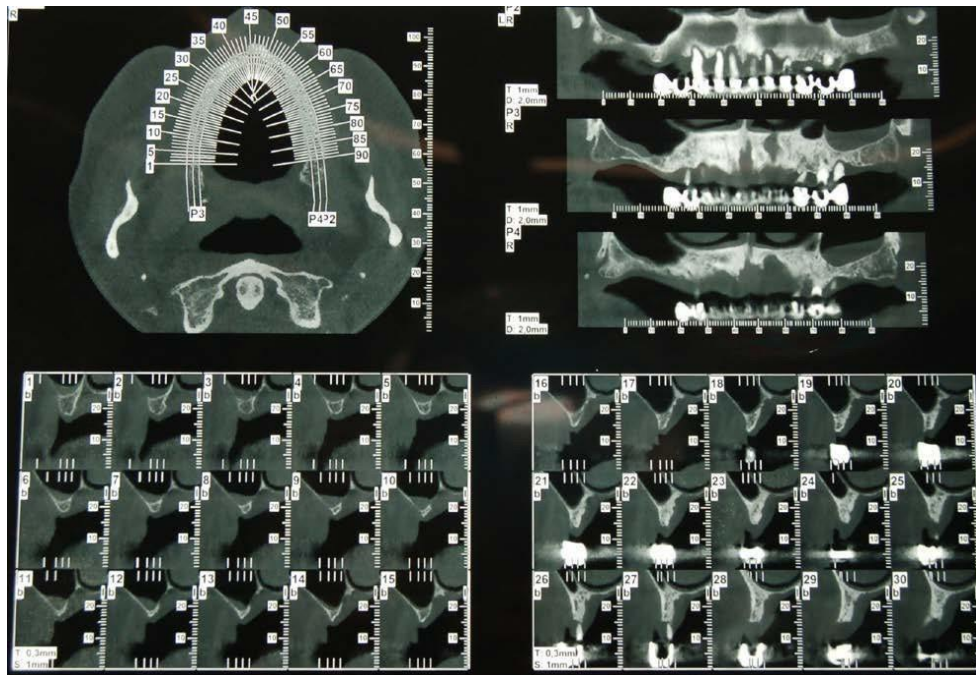


Fig. 10. Case 1: preoperative Tc Conebeam.



Fig. 11. Coronal section of CBCT, which demonstrated the fixed graft.

The insertion can be done in the dentist's office. The oral cavity is rinsed with a 0.20% chlorhexidine solution, followed by an anesthesia and vasoconstrictors injection. A flap of the muco-periosteum is lifted in the grafted area, and the screws used to fix the graft are removed. At the same time, it is useful to check the graft's consolidation and state of maintenance.

The implant site is prepared using a drill that increases the diameter and irrigates it with a physiologic solution. When the dental cavity is ready, the implant is screwed into place (Fig. 12) using a rotating instrument and finished manually. Before suturing, a plug screw should be used to protect the thread of the dental implant (25).

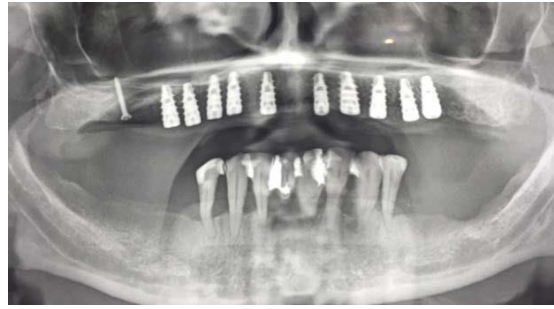


Fig.12. *Rx-Orthopantomogram postoperative with multi-implants inserted in graft.*

Healing and maturation of bone require about 3 months. The prosthesis phase can then begin. When the complete adaption of the peri-implant tissue and a satisfactory aesthetic result is reached, the procedure may be considered optimally terminated (26) (Fig. 13, 14).



Fig. 13. *Extra-oral view of full-arch rehabilitation.*



Fig. 14. *Intra-oral view of full-arch rehabilitation.*

CONCLUSIONS

Our case report and the literature review highlight that therapy for the reconstruction of important maxillary defects should be thoroughly studied and planned before selecting the best therapeutic options for each case. In our option, the most important parameters to consider are:

- the extension and form of the defect;
- the subsequent determination of the site to collect the graft;

We have not experienced a significant loss of bone graft volume during the healing process; therefore, iliac crest grafts are a valid and successful solution today.

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